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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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Manuscripts

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3 **Intravesical recombinant BCG followed by perioperative chemo-immunotherapy**
4 **for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single**
5 **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

1
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3 muscle-invasive, resectable, urothelial cancer, Bacillus Calmette-Guérin, neo-
4 adjuvant, adjuvant, chemotherapy, checkpoint inhibition, radical cystectomy, pelvic
5 lymphadenectomy
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10 **Strengths and limitations of this study**

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

25 Beside bladder sparing chemoradiation therapy, radical cystectomy is the accepted
26 standard curative treatment modality for patients with muscle invasive bladder cancer
27 (MIBC) without evidence of metastatic disease (cM0) (1). Despite the radical surgical
28 approach, stage independent cure rates are however only around 50% at 5 years. Two
29 phase III trials using cisplatin-based neoadjuvant chemotherapy demonstrated a
30 significant improvement of overall survival of muscle-invasive bladder cancer of
31 approximately 5% compared to radical cystectomy alone (2-3). These results were
32 confirmed in a meta-analysis demonstrating that the addition of neoadjuvant cisplatin-
33 based chemotherapy can improve overall survival (OS) by around 5% (4). Therefore,
34 according to international guidelines, the use of cisplatin-based neoadjuvant
35 chemotherapy is considered standard of care in all patients with localized MIBC with
36 planned curative local treatment (1).
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46 For a long time, there was no consensus which cisplatin-combination regimen
47 (cisplatin/gemcitabine vs dose dense MVAC [ddMVAC, MVAC: methotrexate,
48 vinblastine, adriamycin and cisplatin]) should be administered in the neoadjuvant
49 setting. Recently, a phase III clinical trial (VESPER) suggested improved OS for the
50 ddMVAC regimen compared to cisplatin/gemcitabine (5).
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56 There remains a high unmet need to improve the cure rate for patients with localized
57 MIBC. Moreover, establishment of a treatment with high local control omitting the need
58 for either complete resection or irradiation of the bladder would substantially improve
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3 quality of life for those patients. Early results from clinical trials support the feasibility
4 of bladder preserving approaches after immune-chemo-therapy (HCRN GU16-257) (6)
5 In recent years, immunotherapy using PD-1 or PD-L1 targeting immune checkpoint
6 inhibitors (ICI) proved to be beneficial for patients with metastatic bladder cancer and
7 a significant improvement in OS was shown for pembrolizumab in the second-line
8 setting (7). The first results have been presented and published using ICIs as
9 neoadjuvant treatment for localized MIBC. Two monotherapy studies using either
10 pembrolizumab (PURE-01) or atezolizumab (ABACUS) demonstrated pCR of 30-40%
11 (8, 9).

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

33
34 The combination of cisplatin/gemcitabine chemotherapy with atezolizumab has been
35 demonstrated to be effective and safe in a large phase III trial (15). The trial was
36 positive for the primary endpoint of progression free survival (PFS) without unexpected
37 toxicity from the chemo-immunotherapy combination.

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

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17 A far developed and promising new BCG-derived vaccine is the recombinant
18 Mycobacterium bovis (M. bovis) BCG Δ ureC::hly. rBCG Δ ureC::hly which was
19 formulated as VPM1002BC for intravesical immunotherapy against NMIBC. This
20 recombinant BCG (rBCG) VPM1002BC leads to translocation of proteins to the cytosol
21 of infected host or cancer cells by perforation of the phagosome (21, 22). In preclinical
22 models, these changes induce macrophage apoptosis, T cell priming, and
23 proinflammatory cytokine expression, leading to CD4+ and CD8+ T cell responses that
24 are superior compared to the parental BCG subtype Prague. These observations are
25 potentially leading to an improved immune response. VPM1002BC has been used for
26 intravesical therapy in patients with BCG refractory NMIBC in a clinical phase I/II trial
27 (SAKK06/14). The phase I part demonstrated very good tolerance of the compound
28 without need for dose modifications or grade 3 or 4 adverse events (23). The phase II
29 part including 42 patients clearly met the primary endpoint resulting in a recurrence-
30 free survival (RFS) rate in the bladder at 60 weeks in 49.3% of patients (24), while
31 historical data from second-line treatment with conventional BCG results in a RFS rate
32 of 12.5% (25). Only two patients (5%) did tolerate less than 5 instillations and this was
33 not directly related to VPM1002BC. Over the whole course of therapy, treatment
34 related grade 1, 2 and 3 adverse events (AEs) were observed in 14.3%, 54.8%, and
35 4.8% of the patients, respectively.

56 57 58 **Methods/Design**

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3 The trial aims to implement a new immuno-immuno-chemotherapy induction therapy
4 for MIBC combining rBCG intravesical installations and ICI followed by neo-adjuvant
5 ICI in combination with chemotherapy followed by radical cystectomy and adjuvant ICI
6 (figure 1).
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10 The trial is a single arm phase 2 trial including patients with histologically proven
11 urothelial cell carcinoma of the bladder (pT2 or cT2, cT3 or cT4a and \leq cN1 (defined
12 as a solitary lymph node \leq 2 cm in the greatest dimension) and cM0 and be considered
13 suitable for curative multimodality treatment including radical cystectomy by a
14 multidisciplinary tumor board. Furthermore, location of tumor must allow placement of
15 catheter without risk of bleeding. All histological subtypes are eligible with the
16 exception of small cell neuroendocrine carcinoma. The renal function must be
17 estimated to reach a glomerular filtration rate of (eGFR) $>$ 50 mL/min/1.73m² to allow
18 the use of cisplatin. Patients with prior intravesical BCG, with macrohematuria and
19 those unable to retain BCG instillation for less than 1 hour are excluded.
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27 The protocol includes additional research questions such as preoperative assessment
28 of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and
29 correlation with the pathological outcome, the tumor immunome before and after
30 neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation
31 to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy
32 endpoints, the effect of the gut microbiota on the response to immunotherapy, immune
33 parameters in urine samples and their relation to efficacy endpoints.
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39 Patients receive intravesical rBCG (VPM1002BC) by 3 weekly instillations of rBCG
40 with single dose of VPM1002BC, live, $1-19.2 \times 10^8$ colony forming units (CFU) on day
41 1, 8 and 15 of the protocol. Atezolizumab 1200mg fixed dose is started with the first
42 instillation of rBCG (1/-1 day) and continued in combination with the chemotherapy
43 every 3 weeks (q3w) for 4 cycles. Chemotherapy consists of cisplatin and gemcitabine
44 for 4 cycles and is started on day 22 after the first rBCG instillation. Cisplatin is used
45 at a dose of 70mg/m² iv on d1 q3w and gemcitabine is used at a dose of 1000mg/m²
46 iv on d1 and d8 q3w. Radical cystectomy is performed 4 to 8 weeks after completion
47 of the last chemo-immunotherapy cycle. Adjuvant atezolizumab is given 1200mg fixed
48 dose q3w for 13 cycles starting 4-16 weeks after date of surgery.
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58 **Endpoints**

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

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13 Event-free survival (EFS)

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

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

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

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

- 40 • Recurrence of locoregional disease
- 41 • Appearance of metastases at any localization
- 42 • Death

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46 Patients without event at the time of analysis and patients starting a subsequent
47 treatment in the absence of an event will be censored at the date of the last available
48 assessment showing no event before the start of the subsequent treatment, if any.

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50 This endpoint will only be calculated for patients in the R0 resection set.
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55 Overall survival (OS)

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57 OS is defined as the time from treatment start until death from any cause. Patients not
58 experiencing an event will be censored at the last date they were known to be alive.
59 This endpoint will be calculated for patients in the full analysis set (FAS).
60

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.

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

20 The sample size is based on the primary endpoint pCR. The null hypothesis is a pCR
21 rate $\leq 35\%$ (based on reference 26) and the alternative hypothesis a pCR rate $\geq 55\%$.
22 Using Simon's minimax two-stage design with a type I error of 5% and a power of 80%,
23 39 resected patients are needed. With an estimated drop-out rate of 15% (7 patients),
24 we plan to recruit a total of 46 patients.
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29 After the first 12 patients have completed neoadjuvant treatment, an interim safety
30 analysis will be performed. AEs and SAEs will be analyzed descriptively. Special focus
31 will be given to CTCAE grade ≥ 3 directly related to intravesical rBCG.
32
33

34 After neoadjuvant therapy and resection of the first 21 patients an interim efficacy
35 analysis will be performed. If the number of patients with pCR is 8 or less, the trial will
36 be stopped for futility. If, however, the number of patients with pCR is 9 or more, the
37 trial will continue to stage 2.
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41 The primary analysis will take place after all patients have completed neoadjuvant
42 therapy and had surgery, if applicable. The secondary analysis will be performed when
43 all patients have reached a follow-up of at least 2 years.
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46 For the primary endpoint, the point estimate of the pCR rate will be calculated using
47 the uniformly minimum variance unbiased estimator (UMVUE) and the corresponding
48 two-sided 90% confidence interval will be calculated using the "stage-wise ordering"
49 based-method. If the lower bound of the confidence interval is above 35%, the null
50 hypothesis can be rejected.
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54 For all other binary endpoints the point estimate and exact 95% Clopper-Pearson
55 confidence interval of the proportion will be calculated.
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58 For the primary analysis of the primary endpoint the results from the central pathology
59 review will be used. Supportive analyses are planned based on the following results:
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- 1
- 2
- 3 • Local pathology
- 4
- 5 • MRI (local and central assessment) before surgery
- 6
- 7 • Cystoscopy and biopsy before surgery
- 8
- 9 • ctDNA

10 The following subgroup analyses are planned for the primary endpoint:

- 11
- 12 • high PD-L1 expression (assessed by standardized immunohistochemistry on
- 13 tumor cells (TC) and tumor-associated immune cells (IC) using a $\geq 5\%$ positivity
- 14 on IC (i.e. IC2) as cutoff) versus no or low expression
- 15
- 16
- 17 • ypT0 vs rest
- 18
- 19 • ypN0 vs rest
- 20
- 21 • resection status of TUR-B (complete versus incomplete)
- 22

23 All time-to-event endpoints will have the median value estimated using the Kaplan-

24 Meier method. The number and type of events of each endpoint will be presented

25 descriptively by frequency and percentage.

26

27 Categorical variables will be summarized with frequency and percentage. The

28 denominator for percentages will be the number of patients within the set of interest,

29 unless otherwise specified. Continuous variables will be summarized using median

30 and range.

31

32 Laboratory values will be expressed as the absolute values and as grading according

33 to NCI CTCAE v5.0. AE grading will be presented by type, grade, and relation showing

34 frequency and percentage of the within-patient worst grade. In addition, grade ≥ 3 AEs

35 and AEs with relation to treatment ≥ 3 will be summarized separately.

36

37 **Patient and Public Involvement**

38 The protocol was developed within the SAKK network involving multiple stakeholders

39 including physicians specialized in uro-oncology, nurses and the patient advisory

40 board. The design of the trial is aimed to improve cure rates and to pave a scientific

41 way to avoid radical cystectomy in the future, both clear aims to improve quality of live.

42 Patients will be recruited within the SAKK network and the trial is accessible to the

43 public via the SAKK webpage ([https://www.sakk.ch/en/news/new-trial-patients-](https://www.sakk.ch/en/news/new-trial-patients-bladder-cancer-sakk-0619)

44 bladder-cancer-sakk-0619). After closing and analysis of the trial results will be

45 published in scientific journals. A lay abstract will be uploaded on the SAKK webpage.

46

47 **Discussion**

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3 The herein presented clinical trial SAKK 06/19 is the further development of immuno-
4 chemotherapy for MIBC within the SAKK network. SAKK has performed a
5 predecessor single arm phase II trial using neoadjuvant chemo-immunotherapy with
6 cisplatin/gemcitabine in combination with the PD-L1 inhibitor durvalumab (SAKK
7 06/17). In this trial a total of 61 patients were included in Switzerland and in one
8 German center between 5/2018 and 9/2019. We presented the primary analysis at
9 ASCO 2022 (26) as first trial in MIBC to report a primary endpoint of EFS (manuscript
10 in preparation).

11
12 The rationale of the SAKK 06/17 trial was the addition of neo-adjuvant chemotherapy
13 with cisplatin and gemcitabine to checkpoint inhibition to support the development of a
14 therapeutic immune response by reducing the influence of the chronic inflammation
15 caused by the immune suppressive innate cell network. Predominantly myeloid
16 derived suppressor cells (MDSCs, including macrophages and neutrophils) are
17 responsible for chronic inflammation hampering the immune response. Gemcitabine is
18 known to reduce MDSCs and is therefore the ideal partner for an immuno-
19 chemotherapy (29). As a consequence of immune activation, IFN-gamma is released
20 resulting in TH1 T cell response. However, IFN-gamma also induces PD-1 expression
21 on TH1 T cells leading to adaptive immune suppression aiming to stop the T-cell
22 response (30). The use of ICIs is intended to block this negative feedback loop to allow
23 a prolonged T-cell response.

24
25 Several similar neo-adjuvant studies in MBIC using immunotherapy or the combination
26 of immuno-chemotherapy have reported pCR rates in the same range of 30-40% and
27 in addition, residual NMIBC can be found in approximately 15-20% (8, 9, 27, 28).
28 Therefore there is hardly any improvement in the pCR rate compared to cisplatin-
29 based chemotherapy, especially when compared to the more active regimen of
30 ddMVAC (5).

31
32 In view of these rather modest results so far, strategies to further augment the immune
33 response need to be evaluated. Beside concomitant application of radiotherapy and
34 immune checkpoint blockade, BCG appears to be a promising combination partner.
35 BCG has been used for treatment of NMIBC for decades with very good success. It
36 induces initial CR in 70-75% of patients with CIS and prevents recurrence in 55-65%
37 of patients with high-risk papillary tumors (16, 17). However, 25-45% of patients don't
38 respond initially and up to 40% experience relapse after initial response. BCG induces
39 an intense local inflammatory response that mediates tumor immunity. Several steps
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3 are involved in mounting the inflammatory response including attachment to the
4 urothelium with uptake by antigen presenting cells (APC) and putative internalization
5 into urothelial cells followed by a boost of the innate immune response and induction
6 of adaptive responses (18). Preclinical experiments demonstrated that intravesical
7 BCG instillations induce a robust infiltration of T cells (CD4+ and CD8+) in the bladder
8 wall (31). Moreover, a systemic immune response arises following intravesical BCG
9 demonstrated by increased levels of different cytokines and chemokines including
10 IFN γ , IL-1, IL-2, IL-8, TNF, CCL2, CCL5 (32).

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

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50 This trial tests the hypothesis if a new recombinant BCG can enhance the local and
51 systemic immune response in the context of immune checkpoint inhibition and
52 chemotherapy and thereby increase pCR rate and consequently also event-free
53 survival. Improving pCR rate would be a next step to the ultimate goal of omitting
54 radical surgery or extensive local radiotherapy to the bladder for these patients.
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3 Trial status

4 Recruitment started May 2022, estimated closure of accrual April 2025.
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8 List of abbreviations
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10	APC	Antigen presenting cells
11	BCG	Bacillus Calmette-Guerin
12	CIS	Carcinoma in situ
13	CR	Complete response
14	EFS	Event-free survival
15	eGFR	Estimated glomerular filtration rate
16	mAB	Monoclonal antibody
17	MDSC	Myeloid-derived suppressor cells)
18	MIBC	Muscle invasive bladder cancer
19	NCI	
20	CTCAE	NCI Common Terminology Criteria for Adverse Events
21	NMIBC	Non-Muscle invasive bladder cancer
22	OS	Overall survival
23	PaR	Pathological response
24	pCR	Pathological complete remission
25	PD-L1	Programmed cell death-ligand 1
26	PD1	Programmed cell death protein 1
27	PFS	Progression-free survival
28	RFS	Recurrence-free survival
29	SAKK	Schweizerische Arbeitsgemeinschaft für Klinische 30 Krebsforschung (Swiss Group for Clinical Cancer Research) 31 32 33 34 35 36 37 38 39

40 Ethics approval

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58 Conflict of interest declaration

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60 Merck, Pfizer, Roche, MSD, Janssen, Novartis

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51 Author contributions

52 UP study design & manuscript writing, RC study design & manuscript writing, MS study
53 design, SH study design and statistical planning, CR study design, SR translational
54 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 IFN γ 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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References:

1. Witjes JA, Bruins HM, Cathomas R, Compérat EM, Cowan NC, Gakis G, Hernández V, Linares Espinós E, Lorch A, Neuzillet Y, Rouanne M, Thalmann GN, Veskimäe E, Ribal MJ, van der Heijden AG. European Association of Urology Guidelines on Muscle-invasive and Metastatic Bladder Cancer: Summary of the 2020 Guidelines. *Eur Urol* 2021, 79(1):82-104.
2. Grossman HB, Natale RB, Tangen CM, Speights VO, Vogelzang NJ, Trump DL, deVere White RW, Sarosdy MF, Wood DP, Jr., Raghavan D et al: Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. *N Engl J Med* 2003, 349(9):859-866.
3. Griffiths G, Hall R, Sylvester R, Raghavan D, Parmar MK. International phase III trial assessing neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder cancer: long-term results of the BA06 30894 trial. *J Clin Oncol* 2011, 29(16):2171-2177.
4. Advanced Bladder Cancer (ABC) Meta-analysis Collaboration. Neoadjuvant chemotherapy in invasive bladder cancer: update of a systematic review and meta-

1
2
3 analysis of individual patient data advanced bladder cancer (ABC) meta-analysis
4 collaboration. *Eur Urol*. 2005 Aug;48(2):202-5; discussion 205-6.

5
6 5. Pfister C, Gravis G, Fléchon A, Chevreau C, Mahammedi H, Laguerre B, Guillot A,
7 Joly F, Soulié M, Allory Y, Harter V, Culine S. Dose-Dense Methotrexate, Vinblastine,
8 Doxorubicin, and Cisplatin or Gemcitabine and Cisplatin as Perioperative
9 Chemotherapy for Patients With Nonmetastatic Muscle-Invasive Bladder Cancer:
10 Results of the GETUG-AFU V05 VESPER Trial, *J Clin Oncol*. 2022 Jun
11 20;40(18):2013-2022.

12
13 6. Galsky MD, Daneshmand S, Chan KG, Dorff TB, Cetnar JP et al. Phase 2 trial of
14 gemcitabine, cisplatin, plus nivolumab with selective bladder sparing in patients with
15 muscle- invasive bladder cancer (MIBC): HCRN GU 16-257. *J Clin Oncol* 2021, 39;
16 no.15_suppl (May 20, 2021) 4503.

17
18 7. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, Vogelzang NJ,
19 Climent MA, Petrylak DP, Choueiri TK et al: Pembrolizumab as Second-Line Therapy
20 for Advanced Urothelial Carcinoma. *N Engl J Med* 2017, 376(11):1015-1026.

21
22 8. Necchi A, Anichini A, Raggi D, Briganti A, Massa S, Lucianò R, Colecchia M,
23 Giannatempo P, Mortarini R, Bianchi M, Farè E, Monopoli F, Colombo R, Gallina A,
24 Salonia A, Messina A, Ali SM, Madison R, Ross JS, Chung JH, Salvioni R, Mariani L,
25 Montorsi F. Pembrolizumab as Neoadjuvant Therapy Before Radical Cystectomy in
26 Patients With Muscle-Invasive Urothelial Bladder Carcinoma (PURE-01): An Open-
27 Label, Single-Arm, Phase II Study. *J Clin Oncol*. 2018 Dec 1;36(34):3353-3360.

28
29 9. Powles T, Kockx M, Rodriguez-Vida A, Duran I, Crabb SJ, Van Der Heijden MS,
30 Szabados B, Pous AF, Gravis G, Herranz UA, Protheroe A, Ravaud A, Maillet D,
31 Mendez MJ, Suarez C, Linch M, Prendergast A, van Dam PJ, Stanoeva D,
32 Daelemans S, Mariathasan S, Tea JS, Mousa K, Banchereau R, Castellano D.
33 Clinical efficacy and biomarker analysis of neoadjuvant atezolizumab in operable
34 urothelial carcinoma in the ABACUS trial. *Nat Med*. 2019 Nov;25(11):1706-1714.

35
36 10. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, Bellmunt J, Burris HA,
37 Petrylak DP, Teng SL et al: MPDL3280A (anti-PD-L1) treatment leads to clinical
38 activity in metastatic bladder cancer. *Nature* 2014, 515(7528):558-562.

39
40 11. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV,
41 Necchi A, Dawson N, O'Donnell PH, Balmanoukian A, Loriot Y et al: Atezolizumab in
42 patients with locally advanced and metastatic urothelial carcinoma who have
43
44
45
46
47
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51
52
53
54
55
56
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58
59
60

1
2
3 progressed following treatment with platinum-based chemotherapy: a single-arm,
4 multicentre, phase 2 trial. *Lancet* 2016, 387(10031):1909-1920.

5
6 12. Powles T, Duran I, van der Heijden MS, Loriot Y, Vogelzang NJ, De Giorgi U,
7 Oudard S, Retz MM, Castellano D, Bamias A et al: Atezolizumab versus
8 chemotherapy in patients with platinum-treated locally advanced or metastatic
9 urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised
10 controlled trial. *Lancet* 2018, 391(10122):748-757.

11
12 13. Sternberg CN, Loriot Y, James N, Choy E, Castellano D, Lopez-Rios F, Banna
13 GL, De Giorgi U, Masini C, Bamias A et al. Primary Results from SAUL, a
14 Multinational Single-arm Safety Study of Atezolizumab Therapy for Locally Advanced
15 or Metastatic Urothelial or Nonurothelial Carcinoma of the Urinary Tract. *Eur Urol*
16 2019, 76(1):73-81.

17
18 14. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, Loriot
19 Y, Necchi A, Hoffman-Censits J, Perez-Gracia JL et al. Atezolizumab as first-line
20 treatment in cisplatin-ineligible patients with locally advanced and metastatic
21 urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* 2017,
22 389(10064):67-76.

23
24 15. Galsky MD, Arija JAA, Bamias A, Davis ID, De Santis M, Kikuchi E, Garcia-Del-
25 Muro X, De Giorgi U, Mencinger M, Izumi K et al. Atezolizumab with or without
26 chemotherapy in metastatic urothelial cancer (IMvigor130): a multicentre,
27 randomised, placebo-controlled phase 3 trial. *Lancet* 2020, 395(10236):1547-1557.

28
29 16. Babjuk M, Burger M, Comperat EM, Gontero P, Mostafid AH, Palou J, van Rhijn
30 BWG, Roupret M, Shariat SF, Sylvester R et al. European Association of Urology
31 Guidelines on Non-muscle-invasive Bladder Cancer (TaT1 and Carcinoma In Situ) -
32 2019 Update. *Eur Urol* 2019, 76(5):639-657.

33
34 17. Herr HW, Schwalb DM, Zhang ZF, Sogani PC, Fair WR, Whitmore WF, Jr.,
35 Oettgen HF: Intravesical bacillus Calmette-Guerin therapy prevents tumor
36 progression and death from superficial bladder cancer: ten-year follow-up of a
37 prospective randomized trial. *J Clin Oncol* 1995, 13(6):1404-1408.

38
39 18. Pettenati C, Ingersoll MA: Mechanisms of BCG immunotherapy and its outlook
40 for bladder cancer. *Nat Rev Urol* 2018, 15(10):615-625.

41
42 19. Biot C, Rentsch CA, Gsponer JR, Birkhauser FD, Jusforgues-Saklani H,
43 Lemaitre F, Auriau C, Bachmann A, Bousso P, Demangel C et al: Preexisting BCG-

- 1
2
3 specific T cells improve intravesical immunotherapy for bladder cancer. *Sci Transl*
4 *Med* 2012, 4(137):137ra172.
- 5
6 20. Rentsch CA, Birkhauser FD, Biot C, Gsponer JR, Bisiaux A, Wetterauer C,
7 Lagranderie M, Marchal G, Orgeur M, Bouchier C et al: Bacillus Calmette-Guerin
8 strain differences have an impact on clinical outcome in bladder cancer
9 immunotherapy. *Eur Urol* 2014, 66(4):677-688.
- 10
11 21. Grode L, Seiler P, Baumann S, Hess J, Brinkmann V, Nasser Eddine A, Mann P,
12 Goosmann C, Bander mann S, Smith D et al: Increased vaccine efficacy against
13 tuberculosis of recombinant *Mycobacterium bovis* bacille Calmette-Guerin mutants
14 that secrete listeriolysin. *J Clin Invest* 2005, 115(9):2472-2479.
- 15
16 22. Nieuwenhuizen NE, Kulkarni PS, Shaligram U, Cotton MF, Rentsch CA, Eisele B,
17 Grode L, Kaufmann SHE: The Recombinant Bacille Calmette-Guerin Vaccine
18 VPM1002: Ready for Clinical Efficacy Testing. *Front Immunol* 2017, 8:1147.
- 19
20 23. Rentsch CA, Bosshard P, Mayor G, Rieken M, Puschel H, Wirth G, Cathomas R,
21 Parzmair GP, Grode L, Eisele B et al: Results of the phase I open label clinical trial
22 SAKK 06/14 assessing safety of intravesical instillation of VPM1002BC, a
23 recombinant mycobacterium *Bacillus Calmette Guerin* (BCG), in patients with non-
24 muscle invasive bladder cancer and previous failure of conventional BCG therapy.
25 *Oncoimmunology* 2020, 9(1):1748981.
- 26
27 24. Rentsch CA, Thalmann GN, Lucca I, Kwiatkowski M, Wirth GJ, Strebel RT,
28 Engeler D, Pedrazzini A, Hüttenbrink C, Schultze-Seemann W, Torpai R, Bubendorf
29 L, Wicki A, Roth B, Bosshard P, Püschel H, Boll DT, Hefermehl L, Roghmann F,
30 Gierth M, Ribl K, Schäfer S, Hayoz S. A Phase 1/2 Single-arm Clinical Trial of
31 Recombinant *Bacillus Calmette-Guérin* (BCG) VPM1002BC Immunotherapy in Non-
32 muscle-invasive Bladder Cancer Recurrence After Conventional BCG Therapy:
33 SAKK 06/14. *Eur Urol Oncol* 2022, 5(2):195-202.
- 34
35 25. Di Lorenzo, G., et al., Gemcitabine versus bacille Calmette-Guerin after initial
36 bacille Calmette-Guerin failure in non-muscle-invasive bladder cancer: a multicenter
37 prospective randomized trial. *Cancer*, 2010. 116(8): p. 1893-900.
- 38
39 26. Cathomas R, Rothschild S I, Hayoz S, Spahn M, Oezdemir B, Kiss B., Erdmann
40 A, Aeppli S, Mach N, Strebel R T, Hadaschik B A, Berthold D R, Pless M, Zihler D,
41 Schmid M, Schneider M, Musilova J, Petrusch U. Perioperative
42 chemoimmunotherapy with durvalumab for operable muscle-invasive urothelial
43
44
45
46
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50
51
52
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59
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2
3 carcinoma (MIUC): Primary analysis of the single arm phase II trial SAKK 06/17.
4 Journal of Clinical Oncology 2022 40:16_suppl, 4515-4515
5
6 27. Funt SA, Lattanzi M, Whiting K, Al-Ahmadie H, Quinlan C, Teo MY, Lee CH, Aggen
7 D, Zimmerman D, McHugh D, Apollo A, Durdin TD, Truong H, Kamradt J, Khalil M,
8 Lash B, Ostrovnaya I, McCoy AS, Hettich G, Regazzi A, Jihad M, Ratna N, Boswell A,
9 Francese K, Yang Y, Folefac E, Herr HW, Donat SM, Pietzak E, Cha EK, Donahue TF,
10 Goh AC, Huang WC, Bajorin DF, Iyer G, Bochner BH, Balar AV, Mortazavi A,
11 Rosenberg JE. Neoadjuvant Atezolizumab With Gemcitabine and Cisplatin in Patients
12 With Muscle-Invasive Bladder Cancer: A Multicenter, Single-Arm, Phase II Trial. *J Clin*
13 *Oncol.* 2022 Apr 20;40(12):1312-1322.
14
15 28. Rose TL, Harrison MR, Deal AM, Ramalingam S, Whang YE, Brower B, Dunn M,
16 Osterman CK, Heiling HM, Bjurlin MA, Smith AB, Nielsen ME, Tan HJ, Wallen E,
17 Woods ME, George D, Zhang T, Drier A, Kim WY, Milowsky MI. Phase II Study of
18 Gemcitabine and Split-Dose Cisplatin Plus Pembrolizumab as Neoadjuvant Therapy
19 Before Radical Cystectomy in Patients With Muscle-Invasive Bladder Cancer.
20 *J Clin Oncol.* 2021 Oct 1;39(28):3140-3148.
21
22 29. Eriksson, E., et al., Gemcitabine reduces MDSCs, tregs and TGFbeta-1 while
23 restoring the teff/treg ratio in patients with pancreatic cancer. *J Transl Med*, 2016.
24 14(1): p. 282.
25
26 30. Inman, B.A., et al., PD-L1 (B7-H1) expression by urothelial carcinoma of the
27 bladder and BCG induced granulomata: associations with localized stage progression.
28 *Cancer*, 2007. 109(8): p.1499-505.
29
30 31. Biot, C., et al., Preexisting BCG-specific T cells improve intravesical
31 immunotherapy for bladder cancer. *Sci Transl Med*, 2012. 4(137): p.137ra72.
32
33 32. Taniguchi, K., et al., Systemic immune response after intravesical instillation of
34 bacilli Calmette-Guerin (BCG) for superficial bladder cancer. *Clin Exp Immunol*, 1999.
35 115(1): p. 131-5.
36
37 33. Bacon JW, Müller DC, Ritch E, Annala M, Dugas SG, Herberts C, Vandekerkhove
38 G, Seifert H, Zellweger T, Black PC, Bubendorf L, Wyatt AW, Rentsch CA. Somatic
39 Features of Response and Relapse in Non-muscle-invasive Bladder Cancer Treated
40 with Bacillus Calmette-Guérin Immunotherapy. *Eur Urol Oncol.* 2021 Dec 8:S2588-
41 9311(21)00191-7.
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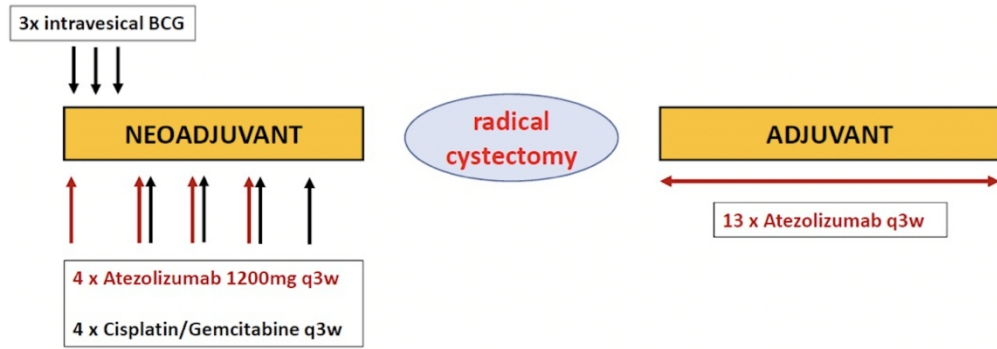


figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

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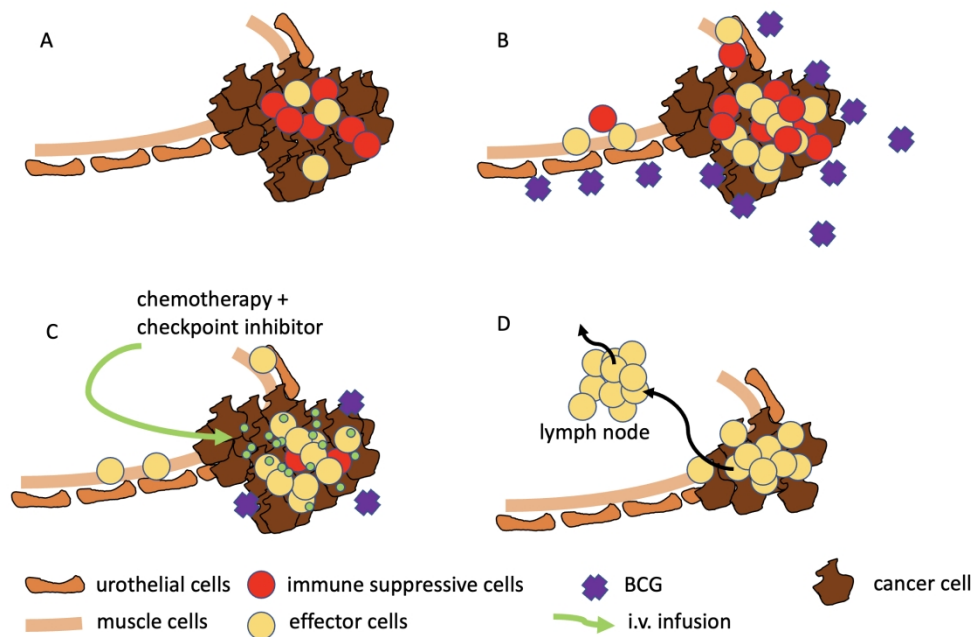


figure 2: A: the urothelial cancer (brown) is infiltrated by immune cells (yellow and red), B: BCG enhances the local inflammation by IFN γ release resulting in increased number of immune suppressive immune cells (MDSC), and upregulation of PD-L1, C: chemotherapy diminishes MDSC, checkpoint inhibition 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 chemotherapy, radical cystectomy plus pelvic lymphadenectomy, and adjuvant immunotherapy. Protocol of a multicenter, single arm phase 2 trial (SAKK 06/19).

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Manuscripts

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3 **A novel sequential treatment strategy for patients with muscle-invasive bladder**
4 **cancer (MIBC): intravesical recombinant BCG, followed by neoadjuvant chemo-**
5 **immunotherapy, radical cystectomy plus pelvic lymphadenectomy, and**
6 **adjuvant immunotherapy. Protocol of a multicenter, single arm phase 2 trial**
7 **(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).

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3 There remains a high unmet need to improve the cure rate for patients with localized
4 MIBC. Moreover, establishment of a treatment with high local control omitting the need
5 for either complete resection or irradiation of the bladder would substantially improve
6 quality of life for those patients. Early results from clinical trials support the feasibility
7 of bladder preserving approaches after immune-chemo-therapy (HCRN GU16-257) (6)
8 In recent years, immunotherapy using PD-1 or PD-L1 targeting immune checkpoint
9 inhibitors (ICI) proved to be beneficial for patients with metastatic bladder cancer and
10 a significant improvement in OS was shown for pembrolizumab in the second-line
11 setting (7). The first results have been presented and published using ICIs as
12 neoadjuvant treatment for localized MIBC. Two monotherapy studies using either
13 pembrolizumab (PURE-01) or atezolizumab (ABACUS) demonstrated pCR of 30-40%
14 (8, 9).

15 Atezolizumab is a human monoclonal antibody (mAb) of the immunoglobulin G (IgG)
16 1 kappa subclass that inhibits binding of PD-L1. Atezolizumab was the first ICI to be
17 tested in patients with urothelial carcinoma (UC). The published study program of
18 atezolizumab in UC is broad, comprising phase I to phase IV trials in metastatic
19 pretreated patients (10 - 13) and a phase II trial in metastatic treatment naïve cisplatin-
20 ineligible patients (14). In the phase I trial, 95 pretreated metastatic UC patients
21 received atezolizumab achieving a 40% response rate (10). The phase II trial included
22 310 platinum-pretreated patients and achieved a response rate of 15% including 5%
23 complete remissions (CR) (11). 931 patients were randomized in the phase III trial
24 comparing atezolizumab against chemotherapy of physician's choice (either
25 docetaxel, paclitaxel or vinflunine). While the primary endpoint of improved OS for
26 patients with high PD-L1 expression was not reached, the OS was numerically higher
27 in the intention to treat population (12). Atezolizumab had a better safety profile than
28 chemotherapy with 20% grade 3/4 toxicity as compared to 43% on chemotherapy. The
29 efficacy and safety were confirmed in a large real-world population (N=1004) safety
30 trial also including patients usually not eligible for immunotherapy trials such as
31 patients with brain metastasis, autoimmune disease, renal insufficiency, HIV positivity
32 as well as frail patients (13). Moreover, atezolizumab monotherapy demonstrated
33 interesting efficacy in the first line treatment of cisplatin-ineligible patients with a
34 response rate of 23% (9% CR) and an OS of 15.9 months (14).

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

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

18
19 A far developed and promising new BCG-derived vaccine is the recombinant
20 Mycobacterium bovis (*M. bovis*) BCG Δ ureC::hly. rBCG Δ ureC::hly which was
21 formulated as VPM1002BC for intravesical immunotherapy against NMIBC. This
22 recombinant BCG (rBCG) VPM1002BC leads to translocation of proteins to the cytosol
23 of infected host or cancer cells by perforation of the phagosome (21, 22). In preclinical
24 models, these changes induce macrophage apoptosis, T cell priming, and
25 proinflammatory cytokine expression, leading to CD4+ and CD8+ T cell responses that
26 are superior compared to the parental BCG subtype Prague. These observations are
27 potentially leading to an improved immune response. VPM1002BC has been used for
28 intravesical therapy in patients with BCG refractory NMIBC in a clinical phase I/II trial
29 (SAKK06/14). The phase I part demonstrated very good tolerance of the compound
30 without need for dose modifications or grade 3 or 4 adverse events (23). The phase II
31 part including 42 patients clearly met the primary endpoint resulting in a recurrence-
32 free survival (RFS) rate in the bladder at 60 weeks in 49.3% of patients (24), while
33 historical data from second-line treatment with conventional BCG results in a RFS rate
34 of 12.5% (25). Only two patients (5%) did tolerate less than 5 instillations and this was
35 not directly related to VPM1002BC. Over the whole course of therapy, treatment
36 related grade 1, 2 and 3 adverse events (AEs) were observed in 14.3%, 54.8%, and
37 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 \leq cN1 (defined as a solitary lymph node \leq 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, $1-19.2 \times 10^8$ 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/m² iv on d1 q3w and gemcitabine is used at a dose of 1000mg/m² 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)

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

4 This endpoint will be calculated for patients in the safety set.
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8 The protocol includes additional research questions such as preoperative assessment
9 of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and
10 correlation with the pathological outcome, the tumor immunome before and after
11 neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation
12 to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy
13 endpoints, the effect of the gut microbiota on the response to immunotherapy, immune
14 parameters in urine samples and their relation to efficacy endpoints.
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23 **Statistics**

24 The sample size is based on the primary endpoint pCR. The null hypothesis is a pCR
25 rate $\leq 35\%$ (based on reference 26) and the alternative hypothesis a pCR rate $\geq 55\%$.
26 Using Simon's minimax two-stage design with a type I error of 5% and a power of 80%,
27 39 resected patients are needed. With an estimated drop-out rate of 15% (7 patients),
28 we plan to recruit a total of 46 patients.
29

30 After the first 12 patients have completed neoadjuvant treatment, an interim safety
31 analysis will be performed. AEs and SAEs will be analyzed descriptively. Special focus
32 will be given to CTCAE grade ≥ 3 directly related to intravesical rBCG.
33

34 After neoadjuvant therapy and resection of the first 21 patients an interim efficacy
35 analysis will be performed. If the number of patients with pCR is 8 or less, the trial will
36 be stopped for futility. If, however, the number of patients with pCR is 9 or more, the
37 trial will continue to stage 2.
38

39 The primary analysis will take place after all patients have completed neoadjuvant
40 therapy and had surgery, if applicable. The secondary analysis will be performed when
41 all patients have reached a follow-up of at least 2 years.
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43 For the primary endpoint, the point estimate of the pCR rate will be calculated using
44 the uniformly minimum variance unbiased estimator (UMVUE) and the corresponding
45 two-sided 90% confidence interval will be calculated using the "stage-wise ordering"
46 based-method. If the lower bound of the confidence interval is above 35%, the null
47 hypothesis can be rejected.
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3 For all other binary endpoints the point estimate and exact 95% Clopper-Pearson
4 confidence interval of the proportion will be calculated.

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

- 8 • Local pathology
- 9 • MRI (local and central assessment) before surgery
- 10 • Cystoscopy and biopsy before surgery
- 11 • ctDNA

12
13 The following subgroup analyses are planned for the primary endpoint:

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

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

24
25 Categorical variables will be summarized with frequency and percentage. The
26 denominator for percentages will be the number of patients within the set of interest,
27 unless otherwise specified. Continuous variables will be summarized using median
28 and range.

29
30 Laboratory values will be expressed as the absolute values and as grading according
31 to NCI CTCAE v5.0. AE grading will be presented by type, grade, and relation showing
32 frequency and percentage of the within-patient worst grade. In addition, grade ≥ 3 AEs
33 and AEs with relation to treatment ≥ 3 will be summarized separately.

34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 **Patient and Public Involvement**

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

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10 The herein presented clinical trial SAKK 06/19 is the further development of immuno-
11 chemotherapy for MIBC within the SAKK network. SAKK has performed a
12 predecessor single arm phase II trial using neoadjuvant chemo-immunotherapy with
13 cisplatin/gemcitabine in combination with the PD-L1 inhibitor durvalumab (SAKK
14 06/17). In this trial a total of 61 patients were included in Switzerland and in one
15 German center between 5/2018 and 9/2019. We presented the primary analysis at
16 ASCO 2022 (26) as first trial in MIBC to report a primary endpoint of EFS (manuscript
17 in preparation).
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21 The rationale of the SAKK 06/17 trial was the addition of neo-adjuvant chemotherapy
22 with cisplatin and gemcitabine to checkpoint inhibition to support the development of a
23 therapeutic immune response by reducing the influence of the chronic inflammation
24 caused by the immune suppressive innate cell network. Predominantly myeloid derived
25 suppressor cells (MDSCs, including macrophages and neutrophils) are responsible for
26 chronic inflammation hampering the immune response. Gemcitabine is known to
27 reduce MDSCs and is therefore the ideal partner for an immuno-chemotherapy (27).
28 As a consequence of immune activation, IFN-gamma is released resulting in TH1 T
29 cell response. However, IFN-gamma also induces PD-1 expression on TH1 T cells
30 leading to adaptive immune suppression aiming to stop the T-cell response (28). The
31 use of ICIs is intended to block this negative feedback loop to allow a prolonged T-cell
32 response. Furthermore, the ddMVAC protocol was avoided to not allow methotrexate
33 to built up its known T cell suppressive capacity counteracting the immune activating
34 intention of this protocol.
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38 Several similar neo-adjuvant studies in MBIC using immunotherapy or the combination
39 of immuno-chemotherapy have reported pCR rates in the same range of 30-40% and
40 in addition, residual NMIBC can be found in approximately 15-20% (8, 9, 29, 30).
41 Therefore there is hardly any improvement in the pCR rate compared to cisplatin-
42 based chemotherapy, especially when compared to the more active regimen of
43 ddMVAC (5).
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47 In view of these rather modest results so far, strategies to further augment the immune
48 response need to be evaluated. Beside concomitant application of radiotherapy and
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3 immune checkpoint blockade, BCG appears to be a promising combination partner.
4 BCG has been used for treatment of NMIBC for decades with very good success. It
5 induces initial CR in 70-75% of patients with CIS and prevents recurrence in 55-65%
6 of patients with high-risk papillary tumors (16, 17). However, 25-45% of patients don't
7 respond initially and up to 40% experience relapse after initial response. BCG induces
8 an intense local inflammatory response that mediates tumor immunity. Several steps
9 are involved in mounting the inflammatory response including attachment to the
10 urothelium with uptake by antigen presenting cells (APC) and putative internalization
11 into urothelial cells followed by a boost of the innate immune response and induction
12 of adaptive responses (18). Preclinical experiments demonstrated that intravesical
13 BCG instillations induce a robust infiltration of T cells (CD4+ and CD8+) in the bladder
14 wall (31). Moreover, a systemic immune response arises following intravesical BCG
15 demonstrated by increased levels of different cytokines and chemokines including
16 IFN γ , IL-1, IL-2, IL-8, TNF, CCL2, CCL5 (32).

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18 Resistance mechanisms to BCG are not entirely understood but interestingly,
19 granulomata found in patients not responding to BCG were found to be highly
20 expressing PD-L1 (28) suggesting a T-cell exhaustion resulting from checkpoint
21 activation. Patients with ARIDA1A mutation and CCNE1 amplification also appear to
22 be at higher risk of relapse after BCG treatment (33). The immune response induced
23 by intravesical BCG is, however, not solely restricted to the superficial urothelial layer
24 but affects the whole bladder wall and also induces a systemic immune response (20).
25 Therefore, the next logical step appears to use intravesical BCG also in patients with
26 muscle-invasive bladder cancer as an adjuvant to prime and boost the immune
27 response (both innate and adaptive) when using systemic immunotherapy with
28 checkpoint inhibitors (figure 2). To avoid clinically relevant delay three installations of
29 BCG were considered to be enough to prime and boost. This intended priming of the
30 immune system might be better achieved by using the novel rBCG strain VPM1002BC
31 which appears to have improved safety (21) immunogenicity (22). This is mediated by
32 the exchange of the urease C gene with the Isteriolysin gene in rBCG VPM1002BC
33 leading to a stronger adoptive and innate immune response. Furthermore, increased
34 autophagy likely contributes to more rapid elimination of rBCG in the host and because
35 listeriolysin is only active at acidic pH it is rapidly degraded in the cytosol of the host
36 cell and it's effects are short-lived.
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3 This trial tests the hypothesis if a new recombinant BCG can enhance the local and
4 systemic immune response in the context of immune checkpoint inhibition and
5 chemotherapy and thereby increase pCR rate and consequently also event-free
6 survival. Improving pCR rate would be a next step to the ultimate goal of omitting
7 radical surgery or extensive local radiotherapy to the bladder for these patients.
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13 Ethics and dissemination: The study has received approval by ethical committee
14 Zurich, Switzerland, BASEC-No. 2021-01872. Results will be made available by
15 publication. Trial registration number: NCT04630730
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19 20 21 22 Trial status

23 Recruitment started May 2022, estimated closure of accrual April 2025.
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27 28 List of abbreviations

29	APC	Antigen presenting cells
30	BCG	Bacillus Calmette-Guerin
31	CIS	Carcinoma in situ
32	CR	Complete response
33	EFS	Event-free survival
34	eGFR	Estimated glomerular filtration rate
35	mAB	Monoclonal antibody
36	MDSC	Myeloid-derived suppressor cells)
37	MIBC	Muscle invasive bladder cancer
38	NCI	
39	CTCAE	NCI Common Terminology Criteria for Adverse Events
40	NMIBC	Non-Muscle invasive bladder cancer
41	OS	Overall survival
42	PaR	Pathological response
43	pCR	Pathological complete remission
44	PD-L1	Programmed cell death-ligand 1
45	PD1	Programmed cell death protein 1
46	PFS	Progression-free survival
47	RFS	Recurrence-free survival
48	SAKK	Schweizerische Arbeitsgemeinschaft für Klinische 49 Krebsforschung (Swiss Group for Clinical Cancer Research) 50 51 52 53 54 55 56 57 58 59 60

Ethics approval

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3 Ethics approval 25.03.2022, ethical committee Zurich, Switzerland, BASEC-No.
4 2021-01872
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8 Consent for publication

9 All authors have no objections for publication.
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13 Availability of data and materials

14 Not applicable
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18 Conflict of interest declaration

19 UP: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS,
20 Merck, Pfizer, Roche, MSD, Janssen, Novartis
21
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23 RC: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS,
24 Merck, Pfizer, Roche, MSD, Ipsen, Janssen, Novartis; Honoraria (compensated,
25 institutional) for Janssen, Astellas
26
27

28 MS: None

29 SH: None

30 CR: None
31
32

33 SR: Honoraria (compensated, institutional) from Roche, Astra Zeneca, BMS,
34 Boehringer Ingelheim, MSD, Novartis, Amgen, Eli Lilly, Eisai, Merck Serono, Pfizer,
35 Takeda, Bayer, Janssen, Otsuka, PharmaMar, Sanofi; Advisory role
36 (institutional, compensated): Astea Zeneca, Boehringer Ingelheim, BMS, Pfizer, Eisai,
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39 Serono, Roche
40
41

42 MS: None

43 AO: Advisory role (compensated, institutional): Astra Zeneca, Astellas, Bayer,
44 Janssen, Molecular Partners, MSD, Pfizer, Roche, Sanofi Aventis (compensated,
45 institutional). Novartis, Janssen, Bayer, MSD, AstraZeneca, Merck, Astellas
46 (compensated). Research support (institutional): TEVA, Janssen. Travel support
47 Astellas, Bayer, Janssen, Sanofi Aventis. Speakers Bureau (compensated,
48 institutional): Astellas, Bayer, Janssen
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Provision of drugs

Atezolizumab by Roche

VPM1002BC by VPM/BBIO/SIPL

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 $IFN\gamma$ 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.

References:

1. Witjies JA, Bruins HM, Cathomas R, Compérat EM, Cowan NC, Gakis G, Hernández V, Linares Espinós E, Lorch A, Neuzillet Y, Rouanne M, Thalmann GN, Veskimäe E, Ribal MJ, van der Heijden AG. European Association of Urology Guidelines on Muscle-invasive and Metastatic Bladder Cancer: Summary of the 2020 Guidelines. *Eur Urol* 2021, 79(1):82-104.
2. Grossman HB, Natale RB, Tangen CM, Speights VO, Vogelzang NJ, Trump DL, deVere White RW, Sarosdy MF, Wood DP, Jr., Raghavan D et al: Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. *N Engl J Med* 2003, 349(9):859-866.
3. Griffiths G, Hall R, Sylvester R, Raghavan D, Parmar MK. International phase III trial assessing neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder cancer: long-term results of the BA06 30894 trial. *J Clin Oncol* 2011, 29(16):2171-2177.
4. Advanced Bladder Cancer (ABC) Meta-analysis Collaboration. Neoadjuvant chemotherapy in invasive bladder cancer: update of a systematic review and meta-analysis of individual patient data advanced bladder cancer (ABC) meta-analysis collaboration. *Eur Urol*. 2005 Aug;48(2):202-5; discussion 205-6.
5. Pfister C, Gravis G, Fléchon A, Chevreau C, Mahammedi H, Laguerre B, Guillot A, Joly F, Soulié M, Allory Y, Harter V, Culine S. Dose-Dense Methotrexate, Vinblastine, Doxorubicin, and Cisplatin or Gemcitabine and Cisplatin as Perioperative Chemotherapy for Patients With Nonmetastatic Muscle-Invasive Bladder Cancer: Results of the GETUG-AFU V05 VESPER Trial, *J Clin Oncol*. 2022 Jun 20;40(18):2013-2022.
6. Galsky MD, Daneshmand S, Chan KG, Dorff TB, Cetnar JP et al. Phase 2 trial of gemcitabine, cisplatin, plus nivolumab with selective bladder sparing in patients with muscle-invasive bladder cancer (MIBC): HCRN GU 16-257. *J Clin Oncol* 2021, 39; no.15_suppl (May 20, 2021) 4503.
7. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, Vogelzang NJ, Climent MA, Petrylak DP, Choueiri TK et al: Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma. *N Engl J Med* 2017, 376(11):1015-1026.
8. Necchi A, Anichini A, Raggi D, Briganti A, Massa S, Lucianò R, Colecchia M, Giannatempo P, Mortarini R, Bianchi M, Farè E, Monopoli F, Colombo R, Gallina A, Salonia A, Messina A, Ali SM, Madison R, Ross JS, Chung JH, Salvioni R, Mariani L,

- 1
2
3 Montorsi F. Pembrolizumab as Neoadjuvant Therapy Before Radical Cystectomy in
4 Patients With Muscle-Invasive Urothelial Bladder Carcinoma (PURE-01): An Open-
5 Label, Single-Arm, Phase II Study. *J Clin Oncol*. 2018 Dec 1;36(34):3353-3360.
6
7
8 9. Powles T, Kockx M, Rodriguez-Vida A, Duran I, Crabb SJ, Van Der Heijden MS,
9 Szabados B, Pous AF, Gravis G, Herranz UA, Protheroe A, Ravaud A, Maillet D,
10 Mendez MJ, Suarez C, Linch M, Prendergast A, van Dam PJ, Stanoeva D,
11 Daelemans S, Mariathasan S, Tea JS, Mousa K, Banchereau R, Castellano D.
12 Clinical efficacy and biomarker analysis of neoadjuvant atezolizumab in operable
13 urothelial carcinoma in the ABACUS trial. *Nat Med*. 2019 Nov;25(11):1706-1714.
14
15 10. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, Bellmunt J, Burris HA,
16 Petrylak DP, Teng SL et al: MPDL3280A (anti-PD-L1) treatment leads to clinical
17 activity in metastatic bladder cancer. *Nature* 2014, 515(7528):558-562.
18
19 11. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV,
20 Necchi A, Dawson N, O'Donnell PH, Balmanoukian A, Loriot Y et al: Atezolizumab in
21 patients with locally advanced and metastatic urothelial carcinoma who have
22 progressed following treatment with platinum-based chemotherapy: a single-arm,
23 multicentre, phase 2 trial. *Lancet* 2016, 387(10031):1909-1920.
24
25 12. Powles T, Duran I, van der Heijden MS, Loriot Y, Vogelzang NJ, De Giorgi U,
26 Oudard S, Retz MM, Castellano D, Bamias A et al: Atezolizumab versus
27 chemotherapy in patients with platinum-treated locally advanced or metastatic
28 urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised
29 controlled trial. *Lancet* 2018, 391(10122):748-757.
30
31 13. Sternberg CN, Loriot Y, James N, Choy E, Castellano D, Lopez-Rios F, Banna
32 GL, De Giorgi U, Masini C, Bamias A et al. Primary Results from SAUL, a
33 Multinational Single-arm Safety Study of Atezolizumab Therapy for Locally Advanced
34 or Metastatic Urothelial or Nonurothelial Carcinoma of the Urinary Tract. *Eur Urol*
35 2019, 76(1):73-81.
36
37 14. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, Loriot
38 Y, Necchi A, Hoffman-Censits J, Perez-Gracia JL et al. Atezolizumab as first-line
39 treatment in cisplatin-ineligible patients with locally advanced and metastatic
40 urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* 2017,
41 389(10064):67-76.
42
43 15. Galsky MD, Arija JAA, Bamias A, Davis ID, De Santis M, Kikuchi E, Garcia-Del-
44 Muro X, De Giorgi U, Mencinger M, Izumi K et al. Atezolizumab with or without
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3 chemotherapy in metastatic urothelial cancer (IMvigor130): a multicentre,
4 randomised, placebo-controlled phase 3 trial. *Lancet* 2020, 395(10236):1547-1557.
- 5
6 16. Babjuk M, Burger M, Comperat EM, Gontero P, Mostafid AH, Palou J, van Rhijn
7 BWG, Roupret M, Shariat SF, Sylvester R et al. European Association of Urology
8 Guidelines on Non-muscle-invasive Bladder Cancer (TaT1 and Carcinoma In Situ) -
9 2019 Update. *Eur Urol* 2019, 76(5):639-657.
- 10
11 17. Herr HW, Schwalb DM, Zhang ZF, Sogani PC, Fair WR, Whitmore WF, Jr.,
12 Oettgen HF: Intravesical bacillus Calmette-Guerin therapy prevents tumor
13 progression and death from superficial bladder cancer: ten-year follow-up of a
14 prospective randomized trial. *J Clin Oncol* 1995, 13(6):1404-1408.
- 15
16 18. Pettenati C, Ingersoll MA: Mechanisms of BCG immunotherapy and its outlook
17 for bladder cancer. *Nat Rev Urol* 2018, 15(10):615-625.
- 18
19 19. Biot C, Rentsch CA, Gsponer JR, Birkhauser FD, Jusforgues-Saklani H,
20 Lemaitre F, Auriau C, Bachmann A, Bouso P, Demangel C et al: Preexisting BCG-
21 specific T cells improve intravesical immunotherapy for bladder cancer. *Sci Transl*
22 *Med* 2012, 4(137):137ra172.
- 23
24 20. Rentsch CA, Birkhauser FD, Biot C, Gsponer JR, Bisiaux A, Wetterauer C,
25 Lagranderie M, Marchal G, Orgeur M, Bouchier C et al: Bacillus Calmette-Guerin
26 strain differences have an impact on clinical outcome in bladder cancer
27 immunotherapy. *Eur Urol* 2014, 66(4):677-688.
- 28
29 21. Grode L, Seiler P, Baumann S, Hess J, Brinkmann V, Nasser Eddine A, Mann P,
30 Goosmann C, Bander mann S, Smith D et al: Increased vaccine efficacy against
31 tuberculosis of recombinant Mycobacterium bovis bacille Calmette-Guerin mutants
32 that secrete listeriolysin. *J Clin Invest* 2005, 115(9):2472-2479.
- 33
34 22. Nieuwenhuizen NE, Kulkarni PS, Shaligram U, Cotton MF, Rentsch CA, Eisele B,
35 Grode L, Kaufmann SHE: The Recombinant Bacille Calmette-Guerin Vaccine
36 VPM1002: Ready for Clinical Efficacy Testing. *Front Immunol* 2017, 8:1147.
- 37
38 23. Rentsch CA, Bosshard P, Mayor G, Rieken M, Puschel H, Wirth G, Cathomas R,
39 Parzmair GP, Grode L, Eisele B et al: Results of the phase I open label clinical trial
40 SAKK 06/14 assessing safety of intravesical instillation of VPM1002BC, a
41 recombinant mycobacterium Bacillus Calmette Guerin (BCG), in patients with non-
42 muscle invasive bladder cancer and previous failure of conventional BCG therapy.
43 *Oncoimmunology* 2020, 9(1):1748981.
- 44
45
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3 24. Rentsch CA, Thalmann GN, Lucca I, Kwiatkowski M, Wirth GJ, Strebel RT,
4 Engeler D, Pedrazzini A, Hüttenbrink C, Schultze-Seemann W, Torpai R, Bubendorf
5 L, Wicki A, Roth B, Bosshard P, Püschel H, Boll DT, Hefermehl L, Roghmann F,
6 Gierth M, Ribi K, Schäfer S, Hayoz S. A Phase 1/2 Single-arm Clinical Trial of
7 Recombinant Bacillus Calmette-Guérin (BCG) VPM1002BC Immunotherapy in Non-
8 muscle-invasive Bladder Cancer Recurrence After Conventional BCG Therapy:
9 SAKK 06/14. *Eur Urol Oncol* 2022, 5(2):195-202.
- 10 25. Di Lorenzo, G., et al., Gemcitabine versus bacille Calmette-Guerin after initial
11 bacille Calmette-Guerin failure in non-muscle-invasive bladder cancer: a multicenter
12 prospective randomized trial. *Cancer*, 2010. 116(8): p. 1893-900.
- 13 26. Cathomas R, Rothschild S I, Hayoz S, Spahn M, Oezdemir B, Kiss B., Erdmann
14 A, Aeppli S, Mach N, Strebel R T, Hadaschik B A, Berthold D R, Pless M, Zihler D,
15 Schmid M, Schneider M, Musilova J, Petrusch U. Perioperative
16 chemoimmunotherapy with durvalumab for operable muscle-invasive urothelial
17 carcinoma (MIUC): Primary analysis of the single arm phase II trial SAKK 06/17.
18 *Journal of Clinical Oncology* 2022 40:16_suppl, 4515-4515
- 19 27. Eriksson, E., et al., Gemcitabine reduces MDSCs, tregs and TGFbeta-1 while
20 restoring the teff/treg ratio in patients with pancreatic cancer. *J Transl Med*, 2016.
21 14(1): p. 282.
- 22 28. Inman, B.A., et al., PD-L1 (B7-H1) expression by urothelial carcinoma of the
23 bladder and BCG induced granulomata: associations with localized stage progression.
24 *Cancer*, 2007. 109(8): p.1499-505.
- 25 29. Funt SA, Lattanzi M, Whiting K, Al-Ahmadie H, Quinlan C, Teo MY, Lee CH, Aggen
26 D, Zimmerman D, McHugh D, Apollo A, Durdin TD, Truong H, Kamradt J, Khalil M,
27 Lash B, Ostrovnaya I, McCoy AS, Hettich G, Regazzi A, Jihad M, Ratna N, Boswell A,
28 Francese K, Yang Y, Folefac E, Herr HW, Donat SM, Pietzak E, Cha EK, Donahue TF,
29 Goh AC, Huang WC, Bajorin DF, Iyer G, Bochner BH, Balar AV, Mortazavi A,
30 Rosenberg JE. Neoadjuvant Atezolizumab With Gemcitabine and Cisplatin in Patients
31 With Muscle-Invasive Bladder Cancer: A Multicenter, Single-Arm, Phase II Trial. *J Clin*
32 *Oncol*. 2022 Apr 20;40(12):1312-1322.
- 33 30. Rose TL, Harrison MR, Deal AM, Ramalingam S, Whang YE, Brower B, Dunn M,
34 Osterman CK, Heiling HM, Bjurlin MA, Smith AB, Nielsen ME, Tan HJ, Wallen E,
35 Woods ME, George D, Zhang T, Drier A, Kim WY, Milowsky MI. Phase II Study of

1
2
3 Gemcitabine and Split-Dose Cisplatin Plus Pembrolizumab as Neoadjuvant Therapy
4 Before Radical Cystectomy in Patients With Muscle-Invasive Bladder Cancer.

5
6 J Clin Oncol. 2021 Oct 1;39(28):3140-3148.

7
8 31. Biot, C., et al., Preexisting BCG-specific T cells improve intravesical
9 immunotherapy for bladder cancer. Sci Transl Med, 2012. 4(137): p.137ra72.

10
11 32. Taniguchi, K., et al., Systemic immune response after intravesical instillation of
12 bacilli Calmette-Guerin (BCG) for superficial bladder cancer. Clin Exp Immunol, 1999.
13 115(1): p. 131-5.

14
15 33. Bacon JW, Müller DC, Ritch E, Annala M, Dugas SG, Herberts C, Vandekerkhove
16 G, Seifert H, Zellweger T, Black PC, Bubendorf L, Wyatt AW, Rentsch CA. Somatic
17 Features of Response and Relapse in Non-muscle-invasive Bladder Cancer Treated
18 with Bacillus Calmette-Guérin Immunotherapy. Eur Urol Oncol. 2021 Dec 8:S2588-
19 9311(21)00191-7.
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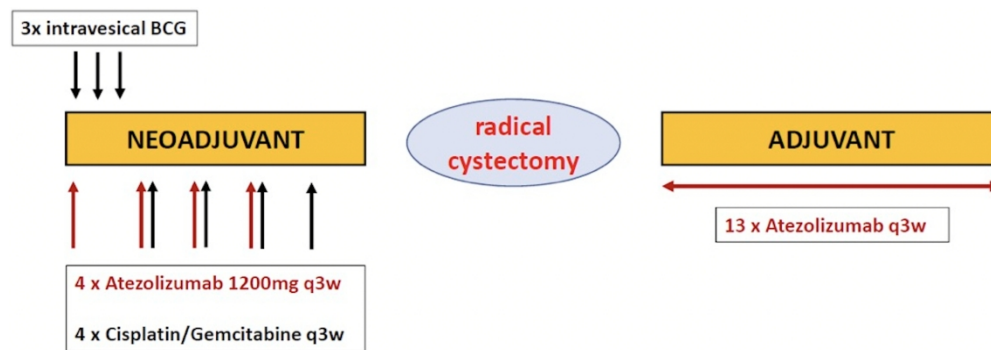


figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

480x167mm (144 x 144 DPI)

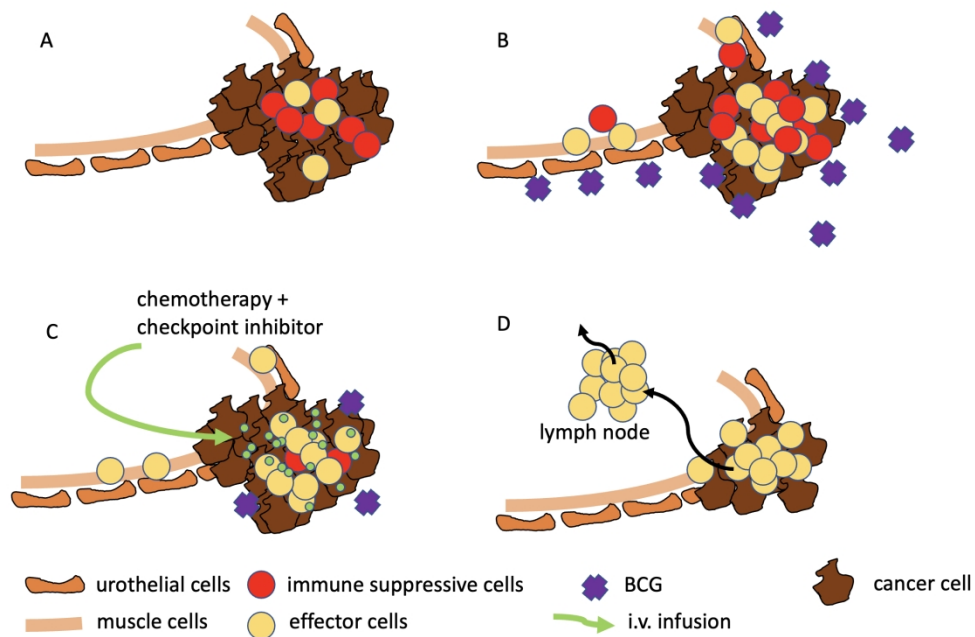


figure 2: A: the urothelial cancer (brown) is infiltrated by immune cells (yellow and red), B: BCG enhances the local inflammation by IFN γ release resulting in increased number of immune suppressive immune cells (MDSC), and upregulation of PD-L1, C: chemotherapy diminishes MDSC, checkpoint inhibition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

1	Introduction			
2				
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	25-27 of 85
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
5				
6		6b	Explanation for choice of comparators	63 of 85
7				
8	Objectives	7	Specific objectives or hypotheses	28 of 85
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	63 of 85
12				
13				
14	Methods: Participants, interventions, and outcomes			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	76 of 85
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	30-31 of 85
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	37-42 of 85
23			administered	
24				
25		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	41 of 85
26			change in response to harms, participant request, or improving/worsening disease)	
27				
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	55-59 & 78 of 85
29			(eg, drug tablet return, laboratory tests)	
30				
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	59 of 85
32				
33	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	
34			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	31 of 85
35			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
36			efficacy and harm outcomes is strongly recommended	
37				
38	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for	32 of 85
39			participants. A schematic diagram is highly recommended (see Figure)	
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1	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
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	22 of 85
5				

6 **Methods: Assignment of interventions (for controlled trials)**

7 Allocation:

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10	Sequence	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
11	generation			
12				
13				
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16	Allocation	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
17	concealment			
18	mechanism			
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n.a. phase II
21				
22				
23				
24	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
25				
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27		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
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31 **Methods: Data collection, management, and analysis**

32				
33	Data collection	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
34	methods			
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38		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
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1 Data management 19 Plans for data entry, coding, security, and storage, including any related processes to promote data quality 62 of 85
 2 (eg, double data entry; range checks for data values). Reference to where details of data management
 3 procedures can be found, if not in the protocol
 4
 5 Statistical methods 20a Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the 63-64 of 85
 6 statistical analysis plan can be found, if not in the protocol
 7
 8 20b Methods for any additional analyses (eg, subgroup and adjusted analyses) 63-64 of 85
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 10 20c Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any
 11 statistical methods to handle missing data (eg, multiple imputation) 64 of 85
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14 **Methods: Monitoring**

15
 16 Data monitoring 21a Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of 74-75 of 85
 17 whether it is independent from the sponsor and competing interests; and reference to where further details
 18 about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not
 19 needed
 20
 21 21b Description of any interim analyses and stopping guidelines, including who will have access to these interim 63 of 85
 22 results and make the final decision to terminate the trial
 23
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 25 Harms 22 Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse 43-54 of 85
 26 events and other unintended effects of trial interventions or trial conduct
 27
 28 Auditing 23 Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent 74 of 85
 29 from investigators and the sponsor
 30
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32 **Ethics and dissemination**

33
 34 Research ethics 24 Plans for seeking research ethics committee/institutional review board (REC/IRB) approval 1 of 85
 35 approval
 36
 37 Protocol 25 Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, 76 of 85
 38 amendments analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals,
 39 regulators)
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1	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
2				
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4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	73-74 of 85
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7	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
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10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____
11				
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13	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
14				
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17	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
18				
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20	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
21				
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	77 of 85
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n.a.
27				
28				
29	Appendices			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Separate file
32				
33				
34	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
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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”](https://creativecommons.org/licenses/by-nc-nd/3.0/) license.

BMJ Open

A novel sequential treatment strategy for patients with muscle-invasive bladder cancer (MIBC): intravesical recombinant BCG, followed by neoadjuvant chemotherapy, radical cystectomy plus pelvic lymphadenectomy, and adjuvant immunotherapy. Protocol of a multicenter, single arm phase 2 trial (SAKK 06/19).

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Secondary Subject Heading:	Urology
Keywords:	IMMUNOLOGY, Urological tumours < UROLOGY, CHEMOTHERAPY, Urological tumours < ONCOLOGY, ONCOLOGY

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Manuscripts

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3 **A novel sequential treatment strategy for patients with muscle-invasive bladder**
4 **cancer (MIBC): intravesical recombinant BCG, followed by neoadjuvant chemo-**
5 **immunotherapy, radical cystectomy plus pelvic lymphadenectomy, and**
6 **adjuvant immunotherapy. Protocol of a multicenter, single arm phase 2 trial**
7 **(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).

1
2
3 There remains a high unmet need to improve the cure rate for patients with localized
4 MIBC. Moreover, establishment of a treatment with high local control omitting the need
5 for either complete resection or irradiation of the bladder would substantially improve
6 quality of life for those patients. Early results from clinical trials support the feasibility
7 of bladder preserving approaches after immune-chemo-therapy (HCRN GU16-257) (6)
8 In recent years, immunotherapy using PD-1 or PD-L1 targeting immune checkpoint
9 inhibitors (ICI) proved to be beneficial for patients with metastatic bladder cancer and
10 a significant improvement in OS was shown for pembrolizumab in the second-line
11 setting (7). The first results have been presented and published using ICIs as
12 neoadjuvant treatment for localized MIBC. Two monotherapy studies using either
13 pembrolizumab (PURE-01) or atezolizumab (ABACUS) demonstrated pCR of 30-40%
14 (8, 9).

15 Atezolizumab is a human monoclonal antibody (mAb) of the immunoglobulin G (IgG)
16 1 kappa subclass that inhibits binding of PD-L1. Atezolizumab was the first ICI to be
17 tested in patients with urothelial carcinoma (UC). The published study program of
18 atezolizumab in UC is broad, comprising phase I to phase IV trials in metastatic
19 pretreated patients (10 - 13) and a phase II trial in metastatic treatment naïve cisplatin-
20 ineligible patients (14). In the phase I trial, 95 pretreated metastatic UC patients
21 received atezolizumab achieving a 40% response rate (10). The phase II trial included
22 310 platinum-pretreated patients and achieved a response rate of 15% including 5%
23 complete remissions (CR) (11). 931 patients were randomized in the phase III trial
24 comparing atezolizumab against chemotherapy of physician's choice (either
25 docetaxel, paclitaxel or vinflunine). While the primary endpoint of improved OS for
26 patients with high PD-L1 expression was not reached, the OS was numerically higher
27 in the intention to treat population (12). Atezolizumab had a better safety profile than
28 chemotherapy with 20% grade 3/4 toxicity as compared to 43% on chemotherapy. The
29 efficacy and safety were confirmed in a large real-world population (N=1004) safety
30 trial also including patients usually not eligible for immunotherapy trials such as
31 patients with brain metastasis, autoimmune disease, renal insufficiency, HIV positivity
32 as well as frail patients (13). Moreover, atezolizumab monotherapy demonstrated
33 interesting efficacy in the first line treatment of cisplatin-ineligible patients with a
34 response rate of 23% (9% CR) and an OS of 15.9 months (14).

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

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

18
19 A far developed and promising new BCG-derived vaccine is the recombinant
20 Mycobacterium bovis (*M. bovis*) BCG Δ ureC::hly. rBCG Δ ureC::hly which was
21 formulated as VPM1002BC for intravesical immunotherapy against NMIBC. This
22 recombinant BCG (rBCG) VPM1002BC leads to translocation of proteins to the cytosol
23 of infected host or cancer cells by perforation of the phagosome (21, 22). In preclinical
24 models, these changes induce macrophage apoptosis, T cell priming, and
25 proinflammatory cytokine expression, leading to CD4+ and CD8+ T cell responses that
26 are superior compared to the parental BCG subtype Prague. These observations are
27 potentially leading to an improved immune response. VPM1002BC has been used for
28 intravesical therapy in patients with BCG refractory NMIBC in a clinical phase I/II trial
29 (SAKK06/14). The phase I part demonstrated very good tolerance of the compound
30 without need for dose modifications or grade 3 or 4 adverse events (23). The phase II
31 part including 42 patients clearly met the primary endpoint resulting in a recurrence-
32 free survival (RFS) rate in the bladder at 60 weeks in 49.3% of patients (24), while
33 historical data from second-line treatment with conventional BCG results in a RFS rate
34 of 12.5% (25). Only two patients (5%) did tolerate less than 5 instillations and this was
35 not directly related to VPM1002BC. Over the whole course of therapy, treatment
36 related grade 1, 2 and 3 adverse events (AEs) were observed in 14.3%, 54.8%, and
37 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 \leq cN1 (defined as a solitary lymph node \leq 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, $1-19.2 \times 10^8$ 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/m² iv on d1 q3w and gemcitabine is used at a dose of 1000mg/m² 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)

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

4 This endpoint will be calculated for patients in the safety set.
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8 The protocol includes additional research questions such as preoperative assessment
9 of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and
10 correlation with the pathological outcome, the tumor immunome before and after
11 neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation
12 to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy
13 endpoints, the effect of the gut microbiota on the response to immunotherapy, immune
14 parameters in urine samples and their relation to efficacy endpoints.
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23 **Statistics**

24 The sample size is based on the primary endpoint pCR. The null hypothesis is a pCR
25 rate $\leq 35\%$ (based on reference 26) and the alternative hypothesis a pCR rate $\geq 55\%$.
26 Using Simon's minimax two-stage design with a type I error of 5% and a power of 80%,
27 39 resected patients are needed. With an estimated drop-out rate of 15% (7 patients),
28 we plan to recruit a total of 46 patients.
29

30 After the first 12 patients have completed neoadjuvant treatment, an interim safety
31 analysis will be performed. AEs and SAEs will be analyzed descriptively. Special focus
32 will be given to CTCAE grade ≥ 3 directly related to intravesical rBCG.
33

34 After neoadjuvant therapy and resection of the first 21 patients an interim efficacy
35 analysis will be performed. If the number of patients with pCR is 8 or less, the trial will
36 be stopped for futility. If, however, the number of patients with pCR is 9 or more, the
37 trial will continue to stage 2.
38

39 The primary analysis will take place after all patients have completed neoadjuvant
40 therapy and had surgery, if applicable. The secondary analysis will be performed when
41 all patients have reached a follow-up of at least 2 years.
42

43 For the primary endpoint, the point estimate of the pCR rate will be calculated using
44 the uniformly minimum variance unbiased estimator (UMVUE) and the corresponding
45 two-sided 90% confidence interval will be calculated using the "stage-wise ordering"
46 based-method. If the lower bound of the confidence interval is above 35%, the null
47 hypothesis can be rejected.
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3 For all other binary endpoints the point estimate and exact 95% Clopper-Pearson
4 confidence interval of the proportion will be calculated.

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

- 8 • Local pathology
- 9 • MRI (local and central assessment) before surgery
- 10 • Cystoscopy and biopsy before surgery
- 11 • ctDNA

12
13 The following subgroup analyses are planned for the primary endpoint:

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

20
21 All time-to-event endpoints will have the median value estimated using the Kaplan-
22 Meier method. The number and type of events of each endpoint will be presented
23 descriptively by frequency and percentage.

24
25 Categorical variables will be summarized with frequency and percentage. The
26 denominator for percentages will be the number of patients within the set of interest,
27 unless otherwise specified. Continuous variables will be summarized using median
28 and range.

29
30 Laboratory values will be expressed as the absolute values and as grading according
31 to NCI CTCAE v5.0. AE grading will be presented by type, grade, and relation showing
32 frequency and percentage of the within-patient worst grade. In addition, grade ≥ 3 AEs
33 and AEs with relation to treatment ≥ 3 will be summarized separately.

34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 **Patient and Public Involvement**

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

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10 The herein presented clinical trial SAKK 06/19 is the further development of immuno-
11 chemotherapy for MIBC within the SAKK network. SAKK has performed a
12 predecessor single arm phase II trial using neoadjuvant chemo-immunotherapy with
13 cisplatin/gemcitabine in combination with the PD-L1 inhibitor durvalumab (SAKK
14 06/17). In this trial a total of 61 patients were included in Switzerland and in one
15 German center between 5/2018 and 9/2019. We presented the primary analysis at
16 ASCO 2022 (26) as first trial in MIBC to report a primary endpoint of EFS (manuscript
17 in preparation).
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21 The rationale of the SAKK 06/17 trial was the addition of neo-adjuvant chemotherapy
22 with cisplatin and gemcitabine to checkpoint inhibition to support the development of a
23 therapeutic immune response by reducing the influence of the chronic inflammation
24 caused by the immune suppressive innate cell network. Predominantly myeloid derived
25 suppressor cells (MDSCs, including macrophages and neutrophils) are responsible for
26 chronic inflammation hampering the immune response. Gemcitabine is known to
27 reduce MDSCs and is therefore the ideal partner for an immuno-chemotherapy (27).
28 As a consequence of immune activation, IFN-gamma is released resulting in TH1 T
29 cell response. However, IFN-gamma also induces PD-1 expression on TH1 T cells
30 leading to adaptive immune suppression aiming to stop the T-cell response (28). The
31 use of ICIs is intended to block this negative feedback loop to allow a prolonged T-cell
32 response. Furthermore, the ddMVAC protocol was avoided to not allow methotrexate
33 to built up its known T cell suppressive capacity counteracting the immune activating
34 intention of this protocol.
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38 Several similar neo-adjuvant studies in MBIC using immunotherapy or the combination
39 of immuno-chemotherapy have reported pCR rates in the same range of 30-40% and
40 in addition, residual NMIBC can be found in approximately 15-20% (8, 9, 29, 30).
41 Therefore there is hardly any improvement in the pCR rate compared to cisplatin-
42 based chemotherapy, especially when compared to the more active regimen of
43 ddMVAC (5).
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47 In view of these rather modest results so far, strategies to further augment the immune
48 response need to be evaluated. Beside concomitant application of radiotherapy and
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3 immune checkpoint blockade, BCG appears to be a promising combination partner.
4 BCG has been used for treatment of NMIBC for decades with very good success. It
5 induces initial CR in 70-75% of patients with CIS and prevents recurrence in 55-65%
6 of patients with high-risk papillary tumors (16, 17). However, 25-45% of patients don't
7 respond initially and up to 40% experience relapse after initial response. BCG induces
8 an intense local inflammatory response that mediates tumor immunity. Several steps
9 are involved in mounting the inflammatory response including attachment to the
10 urothelium with uptake by antigen presenting cells (APC) and putative internalization
11 into urothelial cells followed by a boost of the innate immune response and induction
12 of adaptive responses (18). Preclinical experiments demonstrated that intravesical
13 BCG instillations induce a robust infiltration of T cells (CD4+ and CD8+) in the bladder
14 wall (31). Moreover, a systemic immune response arises following intravesical BCG
15 demonstrated by increased levels of different cytokines and chemokines including
16 IFN γ , IL-1, IL-2, IL-8, TNF, CCL2, CCL5 (32).

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

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3 SAKK Schweizerische Arbeitsgemeinschaft für Klinische
4 Krebsforschung (Swiss Group for Clinical Cancer Research)
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9 Ethics approval

10 Ethics approval 25.03.2022, ethical committee Zurich, Switzerland, BASEC-No.
11 2021-01872
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16 Consent for publication

17 All authors have no objections for publication.
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21 Availability of data and materials

22 Not applicable
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24
25

26 Conflict of interest declaration

27 UP: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS,
28 Merck, Pfizer, Roche, MSD, Janssen, Novartis
29

30 RC: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS,
31 Merck, Pfizer, Roche, MSD, Ipsen, Janssen, Novartis; Honoraria (compensated,
32 institutional) for Janssen, Astellas
33
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35 MS: None
36

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53 Janssen, Molecular Partners, MSD, Pfizer, Roche, Sanofi Aventis (compensated,
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3 Astellas, Bayer, Janssen, Sanofi Aventis. Speakers Bureau (compensated,
4 institutional): Astellas, Bayer, Janssen
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15 Provision of drugs

16 Atezolizumab by Roche

17 VPM1002BC by VPM/BBIO/SIPL
18
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22 Author contributions

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24
25 Ulf Petrusch performed the study design and wrote the manuscript, Martin Spahn
26 performed the study design, Martina Schneider submitted the protocol to authorities
27 and ethical committee, Stefanie Hayoz performed the study design and did all
28 statistical planning, Cyrill A. Rentsch performed the study design, Sacha I. Rothschild
29 planned all translational research and will perform the analysis, Aurelius Omlin
30 performed the study design and coordinated all centers for patient accrual, and
31 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 IFN γ 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.

References:

1. Witjes JA, Bruins HM, Cathomas R, Compérat EM, Cowan NC, Gakis G, Hernández V, Linares Espinós E, Lorch A, Neuzillet Y, Rouanne M, Thalmann GN, Veskimäe E, Ribal MJ, van der Heijden AG. European Association of Urology Guidelines on Muscle-invasive and Metastatic Bladder Cancer: Summary of the 2020 Guidelines. *Eur Urol* 2021, 79(1):82-104.
2. Grossman HB, Natale RB, Tangen CM, Speights VO, Vogelzang NJ, Trump DL, deVere White RW, Sarosdy MF, Wood DP, Jr., Raghavan D et al: Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. *N Engl J Med* 2003, 349(9):859-866.
3. Griffiths G, Hall R, Sylvester R, Raghavan D, Parmar MK. International phase III trial assessing neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder cancer: long-term results of the BA06 30894 trial. *J Clin Oncol* 2011, 29(16):2171-2177.
4. Advanced Bladder Cancer (ABC) Meta-analysis Collaboration. Neoadjuvant chemotherapy in invasive bladder cancer: update of a systematic review and meta-analysis of individual patient data advanced bladder cancer (ABC) meta-analysis collaboration. *Eur Urol*. 2005 Aug;48(2):202-5; discussion 205-6.
5. Pfister C, Gravis G, Fléchon A, Chevreau C, Mahammedi H, Laguerre B, Guillot A, Joly F, Soulié M, Allory Y, Harter V, Culine S. Dose-Dense Methotrexate, Vinblastine, Doxorubicin, and Cisplatin or Gemcitabine and Cisplatin as Perioperative Chemotherapy for Patients With Nonmetastatic Muscle-Invasive Bladder Cancer: Results of the GETUG-AFU V05 VESPER Trial, *J Clin Oncol*. 2022 Jun 20;40(18):2013-2022.

- 1
2
3 6. Galsky MD, Daneshmand S, Chan KG, Dorff TB, Cetnar JP et al. Phase 2 trial of
4 gemcitabine, cisplatin, plus nivolumab with selective bladder sparing in patients with
5 muscle- invasive bladder cancer (MIBC): HCRN GU 16-257. *J Clin Oncol* 2021, 39;
6 no.15_suppl (May 20, 2021) 4503.
- 7
8
9 7. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, Vogelzang NJ,
10 Climent MA, Petrylak DP, Choueiri TK et al: Pembrolizumab as Second-Line Therapy
11 for Advanced Urothelial Carcinoma. *N Engl J Med* 2017, 376(11):1015-1026.
- 12
13 8. Necchi A, Anichini A, Raggi D, Briganti A, Massa S, Lucianò R, Coecchia M,
14 Giannatempo P, Mortarini R, Bianchi M, Farè E, Monopoli F, Colombo R, Gallina A,
15 Salonia A, Messina A, Ali SM, Madison R, Ross JS, Chung JH, Salvioni R, Mariani L,
16 Montorsi F. Pembrolizumab as Neoadjuvant Therapy Before Radical Cystectomy in
17 Patients With Muscle-Invasive Urothelial Bladder Carcinoma (PURE-01): An Open-
18 Label, Single-Arm, Phase II Study. *J Clin Oncol*. 2018 Dec 1;36(34):3353-3360.
- 19
20 9. Powles T, Kockx M, Rodriguez-Vida A, Duran I, Crabb SJ, Van Der Heijden MS,
21 Szabados B, Pous AF, Gravis G, Herranz UA, Protheroe A, Ravaud A, Maillet D,
22 Mendez MJ, Suarez C, Linch M, Prendergast A, van Dam PJ, Stanoeva D,
23 Daelemans S, Mariathasan S, Tea JS, Mousa K, Banchereau R, Castellano D.
24 Clinical efficacy and biomarker analysis of neoadjuvant atezolizumab in operable
25 urothelial carcinoma in the ABACUS trial. *Nat Med*. 2019 Nov;25(11):1706-1714.
- 26
27 10. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, Bellmunt J, Burris HA,
28 Petrylak DP, Teng SL et al: MPDL3280A (anti-PD-L1) treatment leads to clinical
29 activity in metastatic bladder cancer. *Nature* 2014, 515(7528):558-562.
- 30
31 11. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV,
32 Necchi A, Dawson N, O'Donnell PH, Balmanoukian A, Loriot Y et al: Atezolizumab in
33 patients with locally advanced and metastatic urothelial carcinoma who have
34 progressed following treatment with platinum-based chemotherapy: a single-arm,
35 multicentre, phase 2 trial. *Lancet* 2016, 387(10031):1909-1920.
- 36
37 12. Powles T, Duran I, van der Heijden MS, Loriot Y, Vogelzang NJ, De Giorgi U,
38 Oudard S, Retz MM, Castellano D, Bamias A et al: Atezolizumab versus
39 chemotherapy in patients with platinum-treated locally advanced or metastatic
40 urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised
41 controlled trial. *Lancet* 2018, 391(10122):748-757.
- 42
43 13. Sternberg CN, Loriot Y, James N, Choy E, Castellano D, Lopez-Rios F, Banna
44 GL, De Giorgi U, Masini C, Bamias A et al. Primary Results from SAUL, a
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2
3 Multinational Single-arm Safety Study of Atezolizumab Therapy for Locally Advanced
4 or Metastatic Urothelial or Nonurothelial Carcinoma of the Urinary Tract. *Eur Urol*
5 2019, 76(1):73-81.

6
7
8 14. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, Loriot
9 Y, Necchi A, Hoffman-Censits J, Perez-Gracia JL et al. Atezolizumab as first-line
10 treatment in cisplatin-ineligible patients with locally advanced and metastatic
11 urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* 2017,
12 389(10064):67-76.

13
14
15 15. Galsky MD, Arija JAA, Bamias A, Davis ID, De Santis M, Kikuchi E, Garcia-Del-
16 Muro X, De Giorgi U, Mencinger M, Izumi K et al. Atezolizumab with or without
17 chemotherapy in metastatic urothelial cancer (IMvigor130): a multicentre,
18 randomised, placebo-controlled phase 3 trial. *Lancet* 2020, 395(10236):1547-1557.

19
20
21 16. Babjuk M, Burger M, Comperat EM, Gontero P, Mostafid AH, Palou J, van Rhijn
22 BWG, Roupert M, Shariat SF, Sylvester R et al. European Association of Urology
23 Guidelines on Non-muscle-invasive Bladder Cancer (TaT1 and Carcinoma In Situ) -
24 2019 Update. *Eur Urol* 2019, 76(5):639-657.

25
26
27 17. Herr HW, Schwalb DM, Zhang ZF, Sogani PC, Fair WR, Whitmore WF, Jr.,
28 Oettgen HF: Intravesical bacillus Calmette-Guerin therapy prevents tumor
29 progression and death from superficial bladder cancer: ten-year follow-up of a
30 prospective randomized trial. *J Clin Oncol* 1995, 13(6):1404-1408.

31
32
33 18. Pettenati C, Ingersoll MA: Mechanisms of BCG immunotherapy and its outlook
34 for bladder cancer. *Nat Rev Urol* 2018, 15(10):615-625.

35
36
37 19. Biot C, Rentsch CA, Gsponer JR, Birkhauser FD, Jusforgues-Saklani H,
38 Lemaitre F, Auriat C, Bachmann A, Bouso P, Demangel C et al: Preexisting BCG-
39 specific T cells improve intravesical immunotherapy for bladder cancer. *Sci Transl*
40 *Med* 2012, 4(137):137ra172.

41
42
43 20. Rentsch CA, Birkhauser FD, Biot C, Gsponer JR, Bisiaux A, Wetterauer C,
44 Lagranderie M, Marchal G, Orgeur M, Bouchier C et al: Bacillus Calmette-Guerin
45 strain differences have an impact on clinical outcome in bladder cancer
46 immunotherapy. *Eur Urol* 2014, 66(4):677-688.

47
48
49 21. Grode L, Seiler P, Baumann S, Hess J, Brinkmann V, Nasser Eddine A, Mann P,
50 Goosmann C, Bander mann S, Smith D et al: Increased vaccine efficacy against
51 tuberculosis of recombinant Mycobacterium bovis bacille Calmette-Guerin mutants
52 that secrete listeriolysin. *J Clin Invest* 2005, 115(9):2472-2479.

- 1
2
3 22. Nieuwenhuizen NE, Kulkarni PS, Shaligram U, Cotton MF, Rentsch CA, Eisele B,
4 Grode L, Kaufmann SHE: The Recombinant Bacille Calmette-Guerin Vaccine
5 VPM1002: Ready for Clinical Efficacy Testing. *Front Immunol* 2017, 8:1147.
6
7
8 23. Rentsch CA, Bosshard P, Mayor G, Rieken M, Puschel H, Wirth G, Cathomas R,
9 Parzmair GP, Grode L, Eisele B et al: Results of the phase I open label clinical trial
10 SAKK 06/14 assessing safety of intravesical instillation of VPM1002BC, a
11 recombinant mycobacterium *Bacillus Calmette Guerin* (BCG), in patients with non-
12 muscle invasive bladder cancer and previous failure of conventional BCG therapy.
13 *Oncoimmunology* 2020, 9(1):1748981.
14
15
16 24. Rentsch CA, Thalmann GN, Lucca I, Kwiatkowski M, Wirth GJ, Strebel RT,
17 Engeler D, Pedrazzini A, Hüttenbrink C, Schultze-Seemann W, Torpai R, Bubendorf
18 L, Wicki A, Roth B, Bosshard P, Püschel H, Boll DT, Hefermehl L, Roghmann F,
19 Gierth M, Ribi K, Schäfer S, Hayoz S. A Phase 1/2 Single-arm Clinical Trial of
20 Recombinant *Bacillus Calmette-Guérin* (BCG) VPM1002BC Immunotherapy in Non-
21 muscle-invasive Bladder Cancer Recurrence After Conventional BCG Therapy:
22 SAKK 06/14. *Eur Urol Oncol* 2022, 5(2):195-202.
23
24
25 25. Di Lorenzo, G., et al., Gemcitabine versus bacille Calmette-Guerin after initial
26 bacille Calmette-Guerin failure in non-muscle-invasive bladder cancer: a multicenter
27 prospective randomized trial. *Cancer*, 2010. 116(8): p. 1893-900.
28
29
30 26. Cathomas R, Rothschild S I, Hayoz S, Spahn M, Oezdemir B, Kiss B., Erdmann
31 A, Aeppli S, Mach N, Strebel R T, Hadaschik B A, Berthold D R, Pless M, Zihler D,
32 Schmid M, Schneider M, Musilova J, Petrausch U. Perioperative
33 chemoimmunotherapy with durvalumab for operable muscle-invasive urothelial
34 carcinoma (MIUC): Primary analysis of the single arm phase II trial SAKK 06/17.
35 *Journal of Clinical Oncology* 2022 40:16_suppl, 4515-4515
36
37
38 27. Eriksson, E., et al., Gemcitabine reduces MDSCs, tregs and TGFbeta-1 while
39 restoring the teff/treg ratio in patients with pancreatic cancer. *J Transl Med*, 2016.
40 14(1): p. 282.
41
42
43 28. Inman, B.A., et al., PD-L1 (B7-H1) expression by urothelial carcinoma of the
44 bladder and BCG induced granulomata: associations with localized stage progression.
45 *Cancer*, 2007. 109(8): p.1499-505.
46
47
48 29. Funt SA, Lattanzi M, Whiting K, Al-Ahmadie H, Quinlan C, Teo MY, Lee CH, Aggen
49 D, Zimmerman D, McHugh D, Apollo A, Durdin TD, Truong H, Kamradt J, Khalil M,
50 Lash B, Ostrovnaya I, McCoy AS, Hettich G, Regazzi A, Jihad M, Ratna N, Boswell A,
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1
2
3 Francese K, Yang Y, Folefac E, Herr HW, Donat SM, Pietzak E, Cha EK, Donahue TF,
4 Goh AC, Huang WC, Bajorin DF, Iyer G, Bochner BH, Balar AV, Mortazavi A,
5 Rosenberg JE. Neoadjuvant Atezolizumab With Gemcitabine and Cisplatin in Patients
6 With Muscle-Invasive Bladder Cancer: A Multicenter, Single-Arm, Phase II Trial. *J Clin*
7 *Oncol*. 2022 Apr 20;40(12):1312-1322.

8
9
10
11 30. Rose TL, Harrison MR, Deal AM, Ramalingam S, Whang YE, Brower B, Dunn M,
12 Osterman CK, Heiling HM, Bjurlin MA, Smith AB, Nielsen ME, Tan HJ, Wallen E,
13 Woods ME, George D, Zhang T, Drier A, Kim WY, Milowsky MI. Phase II Study of
14 Gemcitabine and Split-Dose Cisplatin Plus Pembrolizumab as Neoadjuvant Therapy
15 Before Radical Cystectomy in Patients With Muscle-Invasive Bladder Cancer.
16 *J Clin Oncol*. 2021 Oct 1;39(28):3140-3148.

17
18 31. Biot, C., et al., Preexisting BCG-specific T cells improve intravesical
19 immunotherapy for bladder cancer. *Sci Transl Med*, 2012. 4(137): p.137ra72.

20
21 32. Taniguchi, K., et al., Systemic immune response after intravesical instillation of
22 bacilli Calmette-Guerin (BCG) for superficial bladder cancer. *Clin Exp Immunol*, 1999.
23 115(1): p. 131-5.

24
25 33. Bacon JW, Müller DC, Ritch E, Annala M, Dugas SG, Herberts C, Vandekerkhove
26 G, Seifert H, Zellweger T, Black PC, Bubendorf L, Wyatt AW, Rentsch CA. Somatic
27 Features of Response and Relapse in Non-muscle-invasive Bladder Cancer Treated
28 with Bacillus Calmette-Guérin Immunotherapy. *Eur Urol Oncol*. 2021 Dec 8:S2588-
29 9311(21)00191-7.

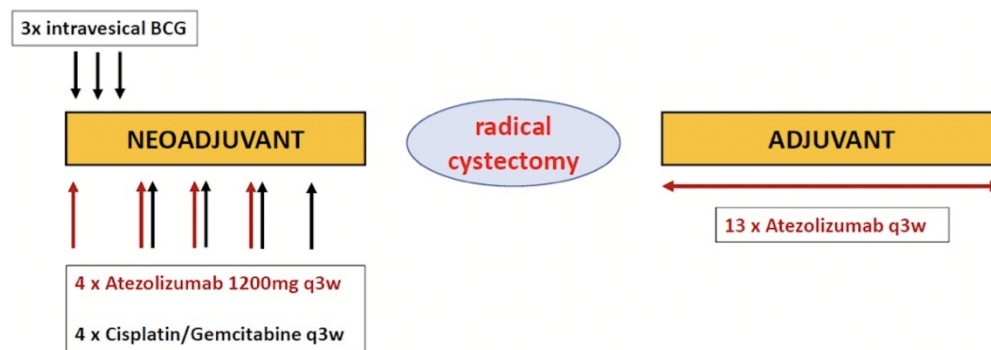


figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

480x167mm (144 x 144 DPI)

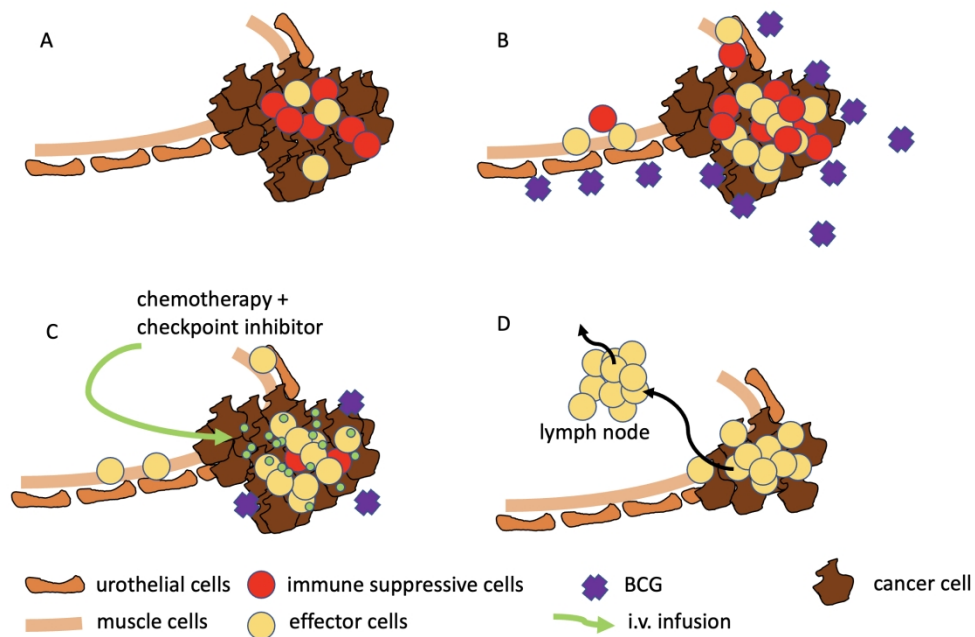


figure 2: A: the urothelial cancer (brown) is infiltrated by immune cells (yellow and red), B: BCG enhances the local inflammation by IFN γ release resulting in increased number of immune suppressive immune cells (MDSC), and upregulation of PD-L1, C: chemotherapy diminishes MDSC, checkpoint inhibition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

1	Introduction			
2				
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	25-27 of 85
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
5				
6		6b	Explanation for choice of comparators	63 of 85
7				
8	Objectives	7	Specific objectives or hypotheses	28 of 85
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	63 of 85
12				
13				
14	Methods: Participants, interventions, and outcomes			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	76 of 85
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	30-31 of 85
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	37-42 of 85
23			administered	
24				
25		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	41 of 85
26			change in response to harms, participant request, or improving/worsening disease)	
27				
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	55-59 & 78 of 85
29			(eg, drug tablet return, laboratory tests)	
30				
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	59 of 85
32				
33	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	31 of 85
34			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	
35			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
36			efficacy and harm outcomes is strongly recommended	
37				
38	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for	32 of 85
39			participants. A schematic diagram is highly recommended (see Figure)	
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1	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
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	22 of 85
5				

6 **Methods: Assignment of interventions (for controlled trials)**

7 Allocation:

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9				
10	Sequence	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
11	generation			
12				
13				
14				
15				
16	Allocation	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
17	concealment			
18	mechanism			
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n.a. phase II
21				
22				
23				
24	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
25				
26				
27		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
28				
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31 **Methods: Data collection, management, and analysis**

32				
33	Data collection	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
34	methods			
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39		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
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1	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
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5	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
6				
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8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	63-64 of 85
9				
10		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
11				
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14 **Methods: Monitoring**

15				
16	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
17				
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22		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
23				
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25	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
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28	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
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32 **Ethics and dissemination**

33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	1 of 85
35				
36				
37	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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1	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
2				
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4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	73-74 of 85
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6				
7	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
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10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____
11				
12				
13	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
14				
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17	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
18				
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20	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
21				
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	77 of 85
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n.a.
27				
28				
29	Appendices			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Separate file
32				
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34	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
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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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

BMJ Open

A novel sequential treatment strategy for patients with muscle-invasive bladder cancer (MIBC): intravesical recombinant BCG, followed by neoadjuvant chemotherapy, radical cystectomy plus pelvic lymphadenectomy, and adjuvant immunotherapy. Protocol of a multicenter, single arm phase 2 trial (SAKK 06/19).

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Keywords:	IMMUNOLOGY, Urological tumours < UROLOGY, CHEMOTHERAPY, Urological tumours < ONCOLOGY, ONCOLOGY

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Manuscripts

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3 **A novel sequential treatment strategy for patients with muscle-invasive bladder**
4 **cancer (MIBC): intravesical recombinant BCG, followed by neoadjuvant chemo-**
5 **immunotherapy, radical cystectomy plus pelvic lymphadenectomy, and**
6 **adjuvant immunotherapy. Protocol of a multicenter, single arm phase 2 trial**
7 **(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).

1
2
3 There remains a high unmet need to improve the cure rate for patients with localized
4 MIBC. Moreover, establishment of a treatment with high local control omitting the need
5 for either complete resection or irradiation of the bladder would substantially improve
6 quality of life for those patients. Early results from clinical trials support the feasibility
7 of bladder preserving approaches after immune-chemo-therapy (HCRN GU16-257) (6)
8 In recent years, immunotherapy using PD-1 or PD-L1 targeting immune checkpoint
9 inhibitors (ICI) proved to be beneficial for patients with metastatic bladder cancer and
10 a significant improvement in OS was shown for pembrolizumab in the second-line
11 setting (7). The first results have been presented and published using ICIs as
12 neoadjuvant treatment for localized MIBC. Two monotherapy studies using either
13 pembrolizumab (PURE-01) or atezolizumab (ABACUS) demonstrated pCR of 30-40%
14 (8, 9).

15 Atezolizumab is a human monoclonal antibody (mAb) of the immunoglobulin G (IgG)
16 1 kappa subclass that inhibits binding of PD-L1. Atezolizumab was the first ICI to be
17 tested in patients with urothelial carcinoma (UC). The published study program of
18 atezolizumab in UC is broad, comprising phase I to phase IV trials in metastatic
19 pretreated patients (10 - 13) and a phase II trial in metastatic treatment naïve cisplatin-
20 ineligible patients (14). In the phase I trial, 95 pretreated metastatic UC patients
21 received atezolizumab achieving a 40% response rate (10). The phase II trial included
22 310 platinum-pretreated patients and achieved a response rate of 15% including 5%
23 complete remissions (CR) (11). 931 patients were randomized in the phase III trial
24 comparing atezolizumab against chemotherapy of physician's choice (either
25 docetaxel, paclitaxel or vinflunine). While the primary endpoint of improved OS for
26 patients with high PD-L1 expression was not reached, the OS was numerically higher
27 in the intention to treat population (12). Atezolizumab had a better safety profile than
28 chemotherapy with 20% grade 3/4 toxicity as compared to 43% on chemotherapy. The
29 efficacy and safety were confirmed in a large real-world population (N=1004) safety
30 trial also including patients usually not eligible for immunotherapy trials such as
31 patients with brain metastasis, autoimmune disease, renal insufficiency, HIV positivity
32 as well as frail patients (13). Moreover, atezolizumab monotherapy demonstrated
33 interesting efficacy in the first line treatment of cisplatin-ineligible patients with a
34 response rate of 23% (9% CR) and an OS of 15.9 months (14).

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

5
6 Intravesical instillation of Bacillus Calmette Guérin (BCG) is the recommended
7 standard of care treatment for patients with intermediate/high risk for progression non-
8 muscle invasive bladder cancer (NMIBC) after complete transurethral resection of the
9 bladder tumor (TURB) (16). BCG was shown to cure carcinoma in situ (CIS) and
10 prevent recurrence of high grade NMIBC and to prolong survival compared to TURB
11 alone (16, 17). While the exact mechanism of BCG effect is not entirely understood, it
12 is clear that intravesical BCG induces a local inflammation leading to induction of the
13 innate immune system allowing for a tumor-specific immunity (adaptive immune
14 response (18, 19). Several different BCG strains have been developed and used for
15 intravesical therapy. It has been recognized that there might be differences in terms of
16 immunogenicity and efficacy between strains (20). This has increased interest in
17 developing novel BCG formulations.

18
19 A far developed and promising new BCG-derived vaccine is the recombinant
20 Mycobacterium bovis (*M. bovis*) BCG Δ ureC::hly. rBCG Δ ureC::hly which was
21 formulated as VPM1002BC for intravesical immunotherapy against NMIBC. This
22 recombinant BCG (rBCG) VPM1002BC leads to translocation of proteins to the cytosol
23 of infected host or cancer cells by perforation of the phagosome (21, 22). In preclinical
24 models, these changes induce macrophage apoptosis, T cell priming, and
25 proinflammatory cytokine expression, leading to CD4+ and CD8+ T cell responses that
26 are superior compared to the parental BCG subtype Prague. These observations are
27 potentially leading to an improved immune response. VPM1002BC has been used for
28 intravesical therapy in patients with BCG refractory NMIBC in a clinical phase I/II trial
29 (SAKK06/14). The phase I part demonstrated very good tolerance of the compound
30 without need for dose modifications or grade 3 or 4 adverse events (23). The phase II
31 part including 42 patients clearly met the primary endpoint resulting in a recurrence-
32 free survival (RFS) rate in the bladder at 60 weeks in 49.3% of patients (24), while
33 historical data from second-line treatment with conventional BCG results in a RFS rate
34 of 12.5% (25). Only two patients (5%) did tolerate less than 5 instillations and this was
35 not directly related to VPM1002BC. Over the whole course of therapy, treatment
36 related grade 1, 2 and 3 adverse events (AEs) were observed in 14.3%, 54.8%, and
37 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 \leq cN1 (defined as a solitary lymph node \leq 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, $1-19.2 \times 10^8$ 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/m² iv on d1 q3w and gemcitabine is used at a dose of 1000mg/m² 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)

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

4 This endpoint will be calculated for patients in the safety set.
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8 The protocol includes additional research questions such as preoperative assessment
9 of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and
10 correlation with the pathological outcome, the tumor immunome before and after
11 neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation
12 to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy
13 endpoints, the effect of the gut microbiota on the response to immunotherapy, immune
14 parameters in urine samples and their relation to efficacy endpoints.
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23 **Statistics**

24 The sample size is based on the primary endpoint pCR. The null hypothesis is a pCR
25 rate $\leq 35\%$ (based on reference 26) and the alternative hypothesis a pCR rate $\geq 55\%$.
26 Using Simon's minimax two-stage design with a type I error of 5% and a power of 80%,
27 39 resected patients are needed. With an estimated drop-out rate of 15% (7 patients),
28 we plan to recruit a total of 46 patients.
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34 After the first 12 patients have completed neoadjuvant treatment, an interim safety
35 analysis will be performed. AEs and SAEs will be analyzed descriptively. Special focus
36 will be given to CTCAE grade ≥ 3 directly related to intravesical rBCG.
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39 After neoadjuvant therapy and resection of the first 21 patients an interim efficacy
40 analysis will be performed. If the number of patients with pCR is 8 or less, the trial will
41 be stopped for futility. If, however, the number of patients with pCR is 9 or more, the
42 trial will continue to stage 2.
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46 The primary analysis will take place after all patients have completed neoadjuvant
47 therapy and had surgery, if applicable. The secondary analysis will be performed when
48 all patients have reached a follow-up of at least 2 years.
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50 For the primary endpoint, the point estimate of the pCR rate will be calculated using
51 the uniformly minimum variance unbiased estimator (UMVUE) and the corresponding
52 two-sided 90% confidence interval will be calculated using the "stage-wise ordering"
53 based-method. If the lower bound of the confidence interval is above 35%, the null
54 hypothesis can be rejected.
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3 For all other binary endpoints the point estimate and exact 95% Clopper-Pearson
4 confidence interval of the proportion will be calculated.

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

- 8 • Local pathology
- 9 • MRI (local and central assessment) before surgery
- 10 • Cystoscopy and biopsy before surgery
- 11 • ctDNA

12
13 The following subgroup analyses are planned for the primary endpoint:

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

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

24
25 Categorical variables will be summarized with frequency and percentage. The
26 denominator for percentages will be the number of patients within the set of interest,
27 unless otherwise specified. Continuous variables will be summarized using median
28 and range.

29
30 Laboratory values will be expressed as the absolute values and as grading according
31 to NCI CTCAE v5.0. AE grading will be presented by type, grade, and relation showing
32 frequency and percentage of the within-patient worst grade. In addition, grade ≥ 3 AEs
33 and AEs with relation to treatment ≥ 3 will be summarized separately.

34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 **Patient and Public Involvement**

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

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10 The herein presented clinical trial SAKK 06/19 is the further development of immuno-
11 chemotherapy for MIBC within the SAKK network. SAKK has performed a
12 predecessor single arm phase II trial using neoadjuvant chemo-immunotherapy with
13 cisplatin/gemcitabine in combination with the PD-L1 inhibitor durvalumab (SAKK
14 06/17). In this trial a total of 61 patients were included in Switzerland and in one
15 German center between 5/2018 and 9/2019. We presented the primary analysis at
16 ASCO 2022 (26) as first trial in MIBC to report a primary endpoint of EFS (manuscript
17 in preparation).
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21 The rationale of the SAKK 06/17 trial was the addition of neo-adjuvant chemotherapy
22 with cisplatin and gemcitabine to checkpoint inhibition to support the development of a
23 therapeutic immune response by reducing the influence of the chronic inflammation
24 caused by the immune suppressive innate cell network. Predominantly myeloid derived
25 suppressor cells (MDSCs, including macrophages and neutrophils) are responsible for
26 chronic inflammation hampering the immune response. Gemcitabine is known to
27 reduce MDSCs and is therefore the ideal partner for an immuno-chemotherapy (27).
28 As a consequence of immune activation, IFN-gamma is released resulting in TH1 T
29 cell response. However, IFN-gamma also induces PD-1 expression on TH1 T cells
30 leading to adaptive immune suppression aiming to stop the T-cell response (28). The
31 use of ICIs is intended to block this negative feedback loop to allow a prolonged T-cell
32 response. Furthermore, the ddMVAC protocol was avoided to not allow methotrexate
33 to built up its known T cell suppressive capacity counteracting the immune activating
34 intention of this protocol.
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38 Several similar neo-adjuvant studies in MBIC using immunotherapy or the combination
39 of immuno-chemotherapy have reported pCR rates in the same range of 30-40% and
40 in addition, residual NMIBC can be found in approximately 15-20% (8, 9, 29, 30).
41 Therefore there is hardly any improvement in the pCR rate compared to cisplatin-
42 based chemotherapy, especially when compared to the more active regimen of
43 ddMVAC (5).
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47 In view of these rather modest results so far, strategies to further augment the immune
48 response need to be evaluated. Beside concomitant application of radiotherapy and
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3 immune checkpoint blockade, BCG appears to be a promising combination partner.
4 BCG has been used for treatment of NMIBC for decades with very good success. It
5 induces initial CR in 70-75% of patients with CIS and prevents recurrence in 55-65%
6 of patients with high-risk papillary tumors (16, 17). However, 25-45% of patients don't
7 respond initially and up to 40% experience relapse after initial response. BCG induces
8 an intense local inflammatory response that mediates tumor immunity. Several steps
9 are involved in mounting the inflammatory response including attachment to the
10 urothelium with uptake by antigen presenting cells (APC) and putative internalization
11 into urothelial cells followed by a boost of the innate immune response and induction
12 of adaptive responses (18). Preclinical experiments demonstrated that intravesical
13 BCG instillations induce a robust infiltration of T cells (CD4+ and CD8+) in the bladder
14 wall (31). Moreover, a systemic immune response arises following intravesical BCG
15 demonstrated by increased levels of different cytokines and chemokines including
16 IFN γ , IL-1, IL-2, IL-8, TNF, CCL2, CCL5 (32).

17
18 Resistance mechanisms to BCG are not entirely understood but interestingly,
19 granulomata found in patients not responding to BCG were found to be highly
20 expressing PD-L1 (28) suggesting a T-cell exhaustion resulting from checkpoint
21 activation. Patients with ARIDA1A mutation and CCNE1 amplification also appear to
22 be at higher risk of relapse after BCG treatment (33). The immune response induced
23 by intravesical BCG is, however, not solely restricted to the superficial urothelial layer
24 but affects the whole bladder wall and also induces a systemic immune response (20).
25 Therefore, the next logical step appears to use intravesical BCG also in patients with
26 muscle-invasive bladder cancer as an adjuvant to prime and boost the immune
27 response (both innate and adaptive) when using systemic immunotherapy with
28 checkpoint inhibitors (figure 2). To avoid clinically relevant delay three installations of
29 BCG were considered to be enough to prime and boost. This intended priming of the
30 immune system might be better achieved by using the novel rBCG strain VPM1002BC
31 which appears to have improved safety (21) immunogenicity (22). This is mediated by
32 the exchange of the urease C gene with the Isteriolysin gene in rBCG VPM1002BC
33 leading to a stronger adoptive and innate immune response. Furthermore, increased
34 autophagy likely contributes to more rapid elimination of rBCG in the host and because
35 listeriolysin is only active at acidic pH it is rapidly degraded in the cytosol of the host
36 cell and it's effects are short-lived.
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Our trial includes a broad translational research program evaluating different possible markers of treatment efficacy. We hope to help identify molecular predictive 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

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3 SAKK Schweizerische Arbeitsgemeinschaft für Klinische
4 Krebsforschung (Swiss Group for Clinical Cancer Research)
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9 Ethics approval

10 Ethics approval 25.03.2022, ethical committee Zurich, Switzerland, BASEC-No.
11 2021-01872
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16 Consent for publication

17 All authors have no objections for publication.
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21 Availability of data and materials

22 Not applicable
23
24
25

26 Conflict of interest declaration

27 UP: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS,
28 Merck, Pfizer, Roche, MSD, Janssen, Novartis
29

30 RC: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS,
31 Merck, Pfizer, Roche, MSD, Ipsen, Janssen, Novartis; Honoraria (compensated,
32 institutional) for Janssen, Astellas
33
34

35 MS: None
36

37 SH: None
38

39 CR: None
40

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42 Boehringer Ingelheim, MSD, Novartis, Amgen, Eli Lilly, Eisai, Merck Serono, Pfizer,
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53 Janssen, Molecular Partners, MSD, Pfizer, Roche, Sanofi Aventis (compensated,
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3 Astellas, Bayer, Janssen, Sanofi Aventis. Speakers Bureau (compensated,
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13
14

15 Provision of drugs

16 Atezolizumab by Roche

17 VPM1002BC by VPM/BBIO/SIPL
18
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22 Author contributions

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24
25 Ulf Petrusch performed the study design and wrote the manuscript, Martin Spahn
26 performed the study design, Martina Schneider submitted the protocol to authorities
27 and ethical committee, Stefanie Hayoz performed the study design and did all
28 statistical planning, Cyrill A. Rentsch performed the study design, Sacha I. Rothschild
29 planned all translational research and will perform the analysis, Aurelius Omlin
30 performed the study design and coordinated all centers for patient accrual, and
31 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 IFN γ 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.

References:

1. Witjes JA, Bruins HM, Cathomas R, Compérat EM, Cowan NC, Gakis G, Hernández V, Linares Espinós E, Lorch A, Neuzillet Y, Rouanne M, Thalmann GN, Veskimäe E, Ribal MJ, van der Heijden AG. European Association of Urology Guidelines on Muscle-invasive and Metastatic Bladder Cancer: Summary of the 2020 Guidelines. *Eur Urol* 2021, 79(1):82-104.
2. Grossman HB, Natale RB, Tangen CM, Speights VO, Vogelzang NJ, Trump DL, deVere White RW, Sarosdy MF, Wood DP, Jr., Raghavan D et al: Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. *N Engl J Med* 2003, 349(9):859-866.
3. Griffiths G, Hall R, Sylvester R, Raghavan D, Parmar MK. International phase III trial assessing neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder cancer: long-term results of the BA06 30894 trial. *J Clin Oncol* 2011, 29(16):2171-2177.
4. Advanced Bladder Cancer (ABC) Meta-analysis Collaboration. Neoadjuvant chemotherapy in invasive bladder cancer: update of a systematic review and meta-analysis of individual patient data advanced bladder cancer (ABC) meta-analysis collaboration. *Eur Urol*. 2005 Aug;48(2):202-5; discussion 205-6.
5. Pfister C, Gravis G, Fléchon A, Chevreau C, Mahammedi H, Laguerre B, Guillot A, Joly F, Soulié M, Allory Y, Harter V, Culine S. Dose-Dense Methotrexate, Vinblastine, Doxorubicin, and Cisplatin or Gemcitabine and Cisplatin as Perioperative Chemotherapy for Patients With Nonmetastatic Muscle-Invasive Bladder Cancer: Results of the GETUG-AFU V05 VESPER Trial, *J Clin Oncol*. 2022 Jun 20;40(18):2013-2022.

- 1
2
3 6. Galsky MD, Daneshmand S, Chan KG, Dorff TB, Cetnar JP et al. Phase 2 trial of
4 gemcitabine, cisplatin, plus nivolumab with selective bladder sparing in patients with
5 muscle- invasive bladder cancer (MIBC): HCRN GU 16-257. *J Clin Oncol* 2021, 39;
6 no.15_suppl (May 20, 2021) 4503.
- 7
8
9
10 7. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, Vogelzang NJ,
11 Climent MA, Petrylak DP, Choueiri TK et al: Pembrolizumab as Second-Line Therapy
12 for Advanced Urothelial Carcinoma. *N Engl J Med* 2017, 376(11):1015-1026.
- 13
14
15 8. Necchi A, Anichini A, Raggi D, Briganti A, Massa S, Lucianò R, Coecchia M,
16 Giannatempo P, Mortarini R, Bianchi M, Farè E, Monopoli F, Colombo R, Gallina A,
17 Salonia A, Messina A, Ali SM, Madison R, Ross JS, Chung JH, Salvioni R, Mariani L,
18 Montorsi F. Pembrolizumab as Neoadjuvant Therapy Before Radical Cystectomy in
19 Patients With Muscle-Invasive Urothelial Bladder Carcinoma (PURE-01): An Open-
20 Label, Single-Arm, Phase II Study. *J Clin Oncol*. 2018 Dec 1;36(34):3353-3360.
- 21
22
23 9. Powles T, Kockx M, Rodriguez-Vida A, Duran I, Crabb SJ, Van Der Heijden MS,
24 Szabados B, Pous AF, Gravis G, Herranz UA, Protheroe A, Ravaud A, Maillet D,
25 Mendez MJ, Suarez C, Linch M, Prendergast A, van Dam PJ, Stanoeva D,
26 Daelemans S, Mariathasan S, Tea JS, Mousa K, Banchereau R, Castellano D.
27 Clinical efficacy and biomarker analysis of neoadjuvant atezolizumab in operable
28 urothelial carcinoma in the ABACUS trial. *Nat Med*. 2019 Nov;25(11):1706-1714.
- 29
30
31 10. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, Bellmunt J, Burris HA,
32 Petrylak DP, Teng SL et al: MPDL3280A (anti-PD-L1) treatment leads to clinical
33 activity in metastatic bladder cancer. *Nature* 2014, 515(7528):558-562.
- 34
35
36 11. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV,
37 Necchi A, Dawson N, O'Donnell PH, Balmanoukian A, Loriot Y et al: Atezolizumab in
38 patients with locally advanced and metastatic urothelial carcinoma who have
39 progressed following treatment with platinum-based chemotherapy: a single-arm,
40 multicentre, phase 2 trial. *Lancet* 2016, 387(10031):1909-1920.
- 41
42
43 12. Powles T, Duran I, van der Heijden MS, Loriot Y, Vogelzang NJ, De Giorgi U,
44 Oudard S, Retz MM, Castellano D, Bamias A et al: Atezolizumab versus
45 chemotherapy in patients with platinum-treated locally advanced or metastatic
46 urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised
47 controlled trial. *Lancet* 2018, 391(10122):748-757.
- 48
49
50 13. Sternberg CN, Loriot Y, James N, Choy E, Castellano D, Lopez-Rios F, Banna
51 GL, De Giorgi U, Masini C, Bamias A et al. Primary Results from SAUL, a
52
53
54
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56
57
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60

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2
3 Multinational Single-arm Safety Study of Atezolizumab Therapy for Locally Advanced
4 or Metastatic Urothelial or Nonurothelial Carcinoma of the Urinary Tract. *Eur Urol*
5 2019, 76(1):73-81.

6
7
8 14. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, Loriot
9 Y, Necchi A, Hoffman-Censits J, Perez-Gracia JL et al. Atezolizumab as first-line
10 treatment in cisplatin-ineligible patients with locally advanced and metastatic
11 urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* 2017,
12 389(10064):67-76.

13
14
15 15. Galsky MD, Arija JAA, Bamias A, Davis ID, De Santis M, Kikuchi E, Garcia-Del-
16 Muro X, De Giorgi U, Mencinger M, Izumi K et al. Atezolizumab with or without
17 chemotherapy in metastatic urothelial cancer (IMvigor130): a multicentre,
18 randomised, placebo-controlled phase 3 trial. *Lancet* 2020, 395(10236):1547-1557.

19
20
21 16. Babjuk M, Burger M, Comperat EM, Gontero P, Mostafid AH, Palou J, van Rhijn
22 BWG, Roupert M, Shariat SF, Sylvester R et al. European Association of Urology
23 Guidelines on Non-muscle-invasive Bladder Cancer (TaT1 and Carcinoma In Situ) -
24 2019 Update. *Eur Urol* 2019, 76(5):639-657.

25
26
27 17. Herr HW, Schwalb DM, Zhang ZF, Sogani PC, Fair WR, Whitmore WF, Jr.,
28 Oettgen HF: Intravesical bacillus Calmette-Guerin therapy prevents tumor
29 progression and death from superficial bladder cancer: ten-year follow-up of a
30 prospective randomized trial. *J Clin Oncol* 1995, 13(6):1404-1408.

31
32
33 18. Pettenati C, Ingersoll MA: Mechanisms of BCG immunotherapy and its outlook
34 for bladder cancer. *Nat Rev Urol* 2018, 15(10):615-625.

35
36
37 19. Biot C, Rentsch CA, Gsponer JR, Birkhauser FD, Jusforgues-Saklani H,
38 Lemaitre F, Auriou C, Bachmann A, Bouso P, Demangel C et al: Preexisting BCG-
39 specific T cells improve intravesical immunotherapy for bladder cancer. *Sci Transl*
40 *Med* 2012, 4(137):137ra172.

41
42
43 20. Rentsch CA, Birkhauser FD, Biot C, Gsponer JR, Bisiaux A, Wetterauer C,
44 Lagranderie M, Marchal G, Orgeur M, Bouchier C et al: Bacillus Calmette-Guerin
45 strain differences have an impact on clinical outcome in bladder cancer
46 immunotherapy. *Eur Urol* 2014, 66(4):677-688.

47
48
49 21. Grode L, Seiler P, Baumann S, Hess J, Brinkmann V, Nasser Eddine A, Mann P,
50 Goosmann C, Bander mann S, Smith D et al: Increased vaccine efficacy against
51 tuberculosis of recombinant Mycobacterium bovis bacille Calmette-Guerin mutants
52 that secrete listeriolysin. *J Clin Invest* 2005, 115(9):2472-2479.

- 1
2
3 22. Nieuwenhuizen NE, Kulkarni PS, Shaligram U, Cotton MF, Rentsch CA, Eisele B,
4 Grode L, Kaufmann SHE: The Recombinant Bacille Calmette-Guerin Vaccine
5 VPM1002: Ready for Clinical Efficacy Testing. *Front Immunol* 2017, 8:1147.
6
7
8 23. Rentsch CA, Bosshard P, Mayor G, Rieken M, Puschel H, Wirth G, Cathomas R,
9 Parzmair GP, Grode L, Eisele B et al: Results of the phase I open label clinical trial
10 SAKK 06/14 assessing safety of intravesical instillation of VPM1002BC, a
11 recombinant mycobacterium *Bacillus Calmette Guerin* (BCG), in patients with non-
12 muscle invasive bladder cancer and previous failure of conventional BCG therapy.
13 *Oncoimmunology* 2020, 9(1):1748981.
14
15
16 24. Rentsch CA, Thalmann GN, Lucca I, Kwiatkowski M, Wirth GJ, Strebel RT,
17 Engeler D, Pedrazzini A, Hüttenbrink C, Schultze-Seemann W, Torpai R, Bubendorf
18 L, Wicki A, Roth B, Bosshard P, Püschel H, Boll DT, Hefermehl L, Roghmann F,
19 Gierth M, Ribi K, Schäfer S, Hayoz S. A Phase 1/2 Single-arm Clinical Trial of
20 Recombinant *Bacillus Calmette-Guérin* (BCG) VPM1002BC Immunotherapy in Non-
21 muscle-invasive Bladder Cancer Recurrence After Conventional BCG Therapy:
22 SAKK 06/14. *Eur Urol Oncol* 2022, 5(2):195-202.
23
24
25 25. Di Lorenzo, G., et al., Gemcitabine versus bacille Calmette-Guerin after initial
26 bacille Calmette-Guerin failure in non-muscle-invasive bladder cancer: a multicenter
27 prospective randomized trial. *Cancer*, 2010. 116(8): p. 1893-900.
28
29
30 26. Cathomas R, Rothschild S I, Hayoz S, Spahn M, Oezdemir B, Kiss B., Erdmann
31 A, Aeppli S, Mach N, Strebel R T, Hadaschik B A, Berthold D R, Pless M, Zihler D,
32 Schmid M, Schneider M, Musilova J, Petrausch U. Perioperative
33 chemoimmunotherapy with durvalumab for operable muscle-invasive urothelial
34 carcinoma (MIUC): Primary analysis of the single arm phase II trial SAKK 06/17.
35 *Journal of Clinical Oncology* 2022 40:16_suppl, 4515-4515
36
37
38 27. Eriksson, E., et al., Gemcitabine reduces MDSCs, tregs and TGFbeta-1 while
39 restoring the teff/treg ratio in patients with pancreatic cancer. *J Transl Med*, 2016.
40 14(1): p. 282.
41
42
43 28. Inman, B.A., et al., PD-L1 (B7-H1) expression by urothelial carcinoma of the
44 bladder and BCG induced granulomata: associations with localized stage progression.
45 *Cancer*, 2007. 109(8): p.1499-505.
46
47
48 29. Funt SA, Lattanzi M, Whiting K, Al-Ahmadie H, Quinlan C, Teo MY, Lee CH, Aggen
49 D, Zimmerman D, McHugh D, Apollo A, Durdin TD, Truong H, Kamradt J, Khalil M,
50 Lash B, Ostrovnaya I, McCoy AS, Hettich G, Regazzi A, Jihad M, Ratna N, Boswell A,
51
52
53
54
55
56
57
58
59
60

1
2
3 Francese K, Yang Y, Folefac E, Herr HW, Donat SM, Pietzak E, Cha EK, Donahue TF,
4 Goh AC, Huang WC, Bajorin DF, Iyer G, Bochner BH, Balar AV, Mortazavi A,
5 Rosenberg JE. Neoadjuvant Atezolizumab With Gemcitabine and Cisplatin in Patients
6 With Muscle-Invasive Bladder Cancer: A Multicenter, Single-Arm, Phase II Trial. *J Clin*
7 *Oncol*. 2022 Apr 20;40(12):1312-1322.

8
9
10
11 30. Rose TL, Harrison MR, Deal AM, Ramalingam S, Whang YE, Brower B, Dunn M,
12 Osterman CK, Heiling HM, Bjurlin MA, Smith AB, Nielsen ME, Tan HJ, Wallen E,
13 Woods ME, George D, Zhang T, Drier A, Kim WY, Milowsky MI. Phase II Study of
14 Gemcitabine and Split-Dose Cisplatin Plus Pembrolizumab as Neoadjuvant Therapy
15 Before Radical Cystectomy in Patients With Muscle-Invasive Bladder Cancer.
16 *J Clin Oncol*. 2021 Oct 1;39(28):3140-3148.

17
18 31. Biot, C., et al., Preexisting BCG-specific T cells improve intravesical
19 immunotherapy for bladder cancer. *Sci Transl Med*, 2012. 4(137): p.137ra72.

20
21 32. Taniguchi, K., et al., Systemic immune response after intravesical instillation of
22 bacilli Calmette-Guerin (BCG) for superficial bladder cancer. *Clin Exp Immunol*, 1999.
23 115(1): p. 131-5.

24
25 33. Bacon JW, Müller DC, Ritch E, Annala M, Dugas SG, Herberts C, Vandekerkhove
26 G, Seifert H, Zellweger T, Black PC, Bubendorf L, Wyatt AW, Rentsch CA. Somatic
27 Features of Response and Relapse in Non-muscle-invasive Bladder Cancer Treated
28 with Bacillus Calmette-Guérin Immunotherapy. *Eur Urol Oncol*. 2021 Dec 8:S2588-
29 9311(21)00191-7.

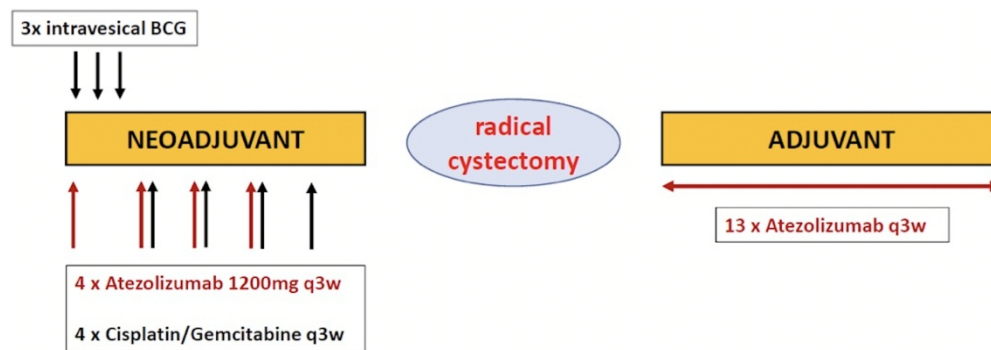


figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

480x167mm (144 x 144 DPI)

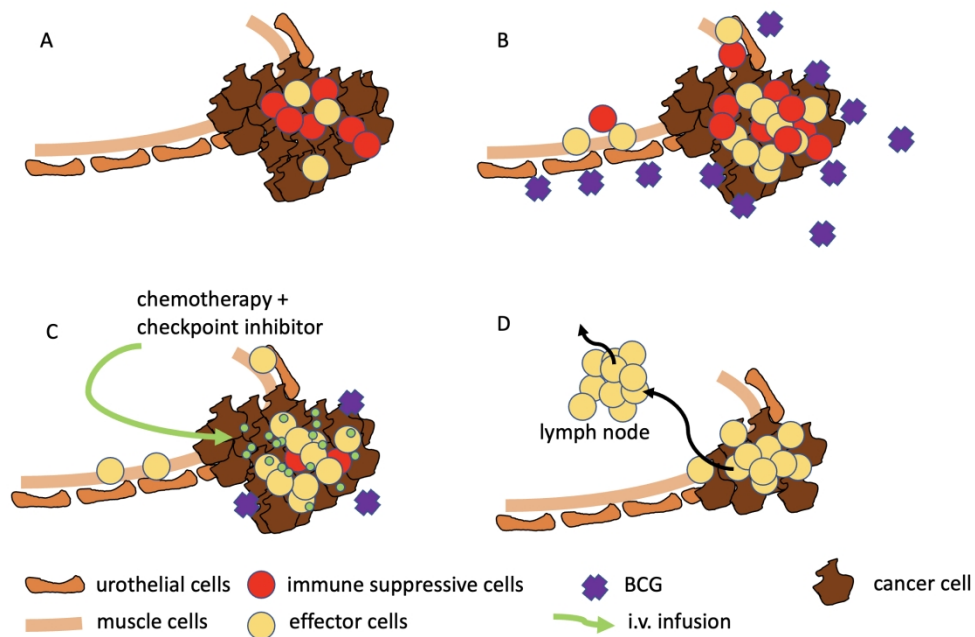


figure 2: A: the urothelial cancer (brown) is infiltrated by immune cells (yellow and red), B: BCG enhances the local inflammation by IFN γ release resulting in increased number of immune suppressive immune cells (MDSC), and upregulation of PD-L1, C: chemotherapy diminishes MDSC, checkpoint inhibition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

1	Introduction			
2				
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	25-27 of 85
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
5				
6		6b	Explanation for choice of comparators	63 of 85
7				
8	Objectives	7	Specific objectives or hypotheses	28 of 85
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	63 of 85
12				
13				
14	Methods: Participants, interventions, and outcomes			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	76 of 85
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	30-31 of 85
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	37-42 of 85
23			administered	
24				
25		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	41 of 85
26			change in response to harms, participant request, or improving/worsening disease)	
27				
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	55-59 & 78 of 85
29			(eg, drug tablet return, laboratory tests)	
30				
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	59 of 85
32				
33				
34	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	
35			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	31 of 85
36			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
37			efficacy and harm outcomes is strongly recommended	
38				
39				
40	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for	32 of 85
41			participants. A schematic diagram is highly recommended (see Figure)	
42				

1	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
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	22 of 85
5				

6 **Methods: Assignment of interventions (for controlled trials)**

7 Allocation:

8				
9				
10	Sequence	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
11	generation			
12				
13				
14				
15				
16	Allocation	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
17	concealment			
18	mechanism			
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n.a. phase II
21				
22				
23				
24	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
25				
26				
27		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
28				
29				
30				

31 **Methods: Data collection, management, and analysis**

32				
33	Data collection	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
34	methods			
35				
36				
37				
38		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
39				
40				
41				
42				

1 Data management 19 Plans for data entry, coding, security, and storage, including any related processes to promote data quality 62 of 85
 2 (eg, double data entry; range checks for data values). Reference to where details of data management
 3 procedures can be found, if not in the protocol
 4
 5 Statistical methods 20a Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the 63-64 of 85
 6 statistical analysis plan can be found, if not in the protocol
 7
 8 20b Methods for any additional analyses (eg, subgroup and adjusted analyses) 63-64 of 85
 9
 10 20c Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any
 11 statistical methods to handle missing data (eg, multiple imputation) 64 of 85
 12
 13

14 **Methods: Monitoring**

15
 16 Data monitoring 21a Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of 74-75 of 85
 17 whether it is independent from the sponsor and competing interests; and reference to where further details
 18 about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not
 19 needed
 20
 21 21b Description of any interim analyses and stopping guidelines, including who will have access to these interim 63 of 85
 22 results and make the final decision to terminate the trial
 23
 24
 25 Harms 22 Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse 43-54 of 85
 26 events and other unintended effects of trial interventions or trial conduct
 27
 28 Auditing 23 Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent 74 of 85
 29 from investigators and the sponsor
 30
 31

32 **Ethics and dissemination**

33
 34 Research ethics 24 Plans for seeking research ethics committee/institutional review board (REC/IRB) approval 1 of 85
 35 approval
 36
 37 Protocol 25 Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, 76 of 85
 38 amendments analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals,
 39 regulators)
 40
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1	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
2				
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4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	73-74 of 85
5				
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7	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
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____
11				
12				
13	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
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17	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
18				
19				
20	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
21				
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23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	77 of 85
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n.a.
27				
28				
29	Appendices			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Separate file
32				
33				
34	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
35				
36				

*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”](https://creativecommons.org/licenses/by-nc-nd/3.0/) license.