On preoperative systemic treatment of muscle-invasive bladder cancer

Dorp, J. van

CHAPTER 7

Summary and general discussion

SUMMARY AND GENERAL DISCUSSION

This thesis focuses on systemic preoperative treatment strategies for muscle-invasive bladder cancer (BC). In **Chapter 2** we reviewed the recent advancements in treatment with immune checkpoint inhibitors (ICI) in BC and the relation between its relation with the tumor immune micro-environment (TIME). In **Chapter 3** we have looked primarily at predicting pathological response after neoadjuvant platinum-based chemotherapy based on genomic biomarkers. In **Chapter 4** we retrospectively investigated the effects of neoadjuvant platinum-based chemotherapy on the tumor-immune microenvironment in muscle-invasive BC patients. In **Chapter 5** we have looked in detail at the results from cohort 2 of the NABUCCO trial, where patients were randomized to receive either of two different dosing regimens of ipilimumab and nivolumab. In addition, we set out to predict pathological response and clinical outcome based on circulating tumor DNA (ctDNA) in plasma and urine. In **Chapter 6** we retrospectively assessed the prostate tissue that was part of the radical cystoprostatectomy specimens from NABUCCO cohort 1.

CHAPTER 2 – THE BLADDER CANCER IMMUNE MICRO-ENVIRONMENT IN THE CONTEXT OF RESPONSE TO IMMUNE CHECKPOINT INHIBITION

In Chapter 2 we reviewed the current scientific progress on the BC immune micro-environment in the context of response to ICI. We evaluated recent and current clinical trials that are using ICI and included various aspects and parameters of the tumor immune micro-environment in our review. In addition, we reviewed different methods to modulate the tumor-immune microenvironment to potentially improve the effect of ICI, including the encouraging results with enfortumab vedotin, an antibody-drug conjugate.

Enfortumab vedotin is directed against nectin-4, a protein which is highly expressed in urothelial cancer cells[1]. Encouraging results have been observed when this drug was used as monotherapy in pretreated mUC in the EV-301 trial[2]. In addition, it was recently shown in the EV-302 trial that enfortumab vedotin combined with pembrolizumab led to a significant increase in PFS and OS compared to standard first-line platinum-based chemotherapy in previously untreated mUC patients. Median PFS was 12.5 months for patients treated with enfortumab vedotin combined with pembrolizumab and only 6.3 months for patients treated with platinum-based chemotherapy. Likewise, median OS improved from 16.1 months to 31.5 months[3]. In the preoperative setting, it was shown in the EV-103 trial that patients treated with enfortumab vedotin followed by radical surgery had a pathological complete response rate (ypT0N0) of 34% and pathological downstaging (ypT0/Tis/Ta/T1N0) in 42% of patients [4]. These data support the ongoing phase 3 programs evaluating enfortumab vedotin in combination with pembrolizumab in muscle-invasive BC (KEYNOTE-905/EV-303, KEYNOTE-B15/EV304)[5,6]. Taken together, enfortumab vedotin has

shown some very promising results and it will be very interesting to witness how these treatments will eventually influence the landscape of BC.

CHAPTER 3 – ASSESSMENT OF PREDICTIVE GENOMIC BIOMARKERS FOR RESPONSE TO CISPLATIN-BASED NEO-ADJUVANT CHEMOTHERAPY IN BLADDER CANCER

In Chapter 3 we have looked primarily at predicting pathological response after neoadjuvant cisplatin-based chemotherapy based on genomic biomarkers. We assessed a large cohort of 165 patients treated in 5 different hospitals and found primarily that deleterious mutations in *ERCC2* are associated with a pathological response after neoadjuvant treatment with cisplatin-based chemotherapy. However, mutations in other genes were not related to pathological response. While the conclusion from this work is robust, there are some methodological caveats that require some discussion.

Firstly, it should be noted that a different sequencing approach has been used in the different hospitals where the samples were analyzed. In the NKI-AVL, we performed panel-based deep sequencing and shallow whole-genome sequencing. In Vancouver, whole-exome sequencing was performed (Chapter 3, Supplementary Figure 1). The two cannot be combined without further consideration, with the risk of over- or underestimating the frequency of certain genomic alterations. We have chosen to present the data separately for the NKI-AVL patients including the shallow whole-genome sequencing data in Chapter 3, Supplementary Figure 4, which we believe is the most appropriate approach. Preferably, all samples should have been processed and sequenced in the same center to avoid potential discrepancies and batch effects.

The frequency of genomic alterations in the genes that were assessed in this study are comparable to what has been observed in other cohorts (Chapter 3, Supplementary Table 2).[7,8] However, the absolute number of genomic alterations is relatively low. Potentially, this could lead to a lack of statistical power to assess correlations between genomic alterations and outcome after cisplatin-based chemotherapy. Indeed, we observed a numerically higher mutation rate for *ERBB2*, *ATM* and *RB1* in responders compared to non-responders (Chapter 3, Figure 1). We cannot exclude that mutations in these genes were significantly associated with pathological response if we had assessed a sufficiently large number of patients.

The trend for improved progression-free survival (PFS) and overall survival (OS) for mutations in *ERCC2* is compelling and it would be interesting to assess a larger number of patients to see if this trend is significant. However, a possible prognostic effect of *ERCC2* cannot be excluded here. To further investigate this potential prognostic effect, it would be necessary to compare PFS and OS for patients with mutations in *ERCC2* that are either treated with cisplatin-based chemotherapy

followed by radical cystectomy or with a direct radical cystectomy (and no neoadjuvant cisplatin-based chemotherapy).

Finally, with no approved alternative preoperative treatment options currently available, it is debatable what the current clinical impact is of the findings described in this paper. The main case where it could be useful is when there is doubt about whether to treat a patient with neoadjuvant chemotherapy and sequencing data is available for *ERCC2* (or, to a lesser degree, any of the other markers that were investigated). When a relevant mutation is found, this would be an argument in favor of pre-treating patients with neoadjuvant chemotherapy, if the mutation is absent, the choice should be based on clinical parameters instead, as patients without an *ERCC2* mutation still can respond to neoadjuvant cisplatin-based chemotherapy. However, with new encouraging neoadjuvant treatment strategies becoming available, there is an unmet need to select the optimal treatment for every individual patient. To help in this endeavor, (genomic) biomarkers to predict pathological response and clinical outcome might become more appealing.

CHAPTER 4 – PLATINUM-BASED CHEMOTHERAPY INDUCES OPPOSING EFFECTS ON IMMUNOTHERAPY RESPONSE-RELATED SPATIAL AND STROMAL BIOMARKERS IN THE BLADDER CANCER MICROENVIRONMENT

In Chapter 4 we collected paired tumor tissue before and after neoadjuvant platinum-based chemotherapy from 116 muscle-invasive BC patients. We used RNA sequencing, multiplex immunofluorescence and immunohistochemistry on the pre- and post-treatment tissue samples to assess the effect of platinum-based chemotherapy on the TIME in a comprehensive manner. Primarily, we were interested in potential implications for subsequent response to ICI, as the results of the CheckMate 274 trial suggested that BC patients treated with neoadjuvant platinum-based chemotherapy and radical surgery benefit from adjuvant nivolumab[9]. We found that the percentage of PD-L1^{+} immune cells and the percentage of intratumoral CD8^{+} T-cells increased after neoadjuvant treatment. Conversely, we also observed an increase in fibroblast-based TGF-β signaling and an increase in distance from immune cells to the nearest cancer cell after treatment.

We have included patients with ypT1-4aNx after treatment with neoadjuvant chemotherapy and radical surgery. We opted to exclude patients without invasive BC after treatment, as the TIME in those patients would per definition not contain any vital tumor cells and would consist of only necrotic tissue, fibrosis and immune cells. Consequently, we have only included non-responding patients in our cohort and based our conclusions on this subpopulation of patients. However, this population is particularly relevant as it could potentially benefit from adjuvant nivolumab as has been shown in the CheckMate 274.

There are multiple parameters in the manuscript that are used as surrogate markers for potential benefit of subsequent ICI treatment, mostly based on historic data. Unfortunately, there are very few patients in our cohort that have received adjuvant treatment with ICI. It would be of interest to assess the patients that have been treated with neoadjuvant chemotherapy and adjuvant nivolumab in the CheckMate 274 and compare the patients that responded to adjuvant nivolumab to those patients that did not respond. However, defining response for adjuvant treatment is not as straightforward as it is for neoadjuvant treatment. Disease-free survival is presumably the most sensible outcome measure but this is also dependent on pathological response after radical surgery. Regardless, when comparing radical surgery specimens from patients that responded to nivolumab versus those that did not respond to nivolumab, biomarkers could emerge that are truly predictive of response to adjuvant treatment. It would be especially interesting if tissue parameters can be identified in transurethral resection of the bladder tumor (TUR-BT) samples obtained prior to neoadjuvant platinum-based chemotherapy that correlate with immune-induction and would benefit from adjuvant nivolumab. One could imagine a treatment-naïve phenotype that is not necessarily prone to respond to neoadjuvant platinum-based chemotherapy, but is indeed 'pushed' by this treatment towards a phenotype that is prone to respond to adjuvant nivolumab.

It is currently still unclear how platinum-based chemotherapy and ICI interact with each other in the context of bladder cancer. In this study we have found arguments that support combining platinum-based chemotherapy, such as increased intratumoral $CD8^+$ T-cells and increased expression of PD-L1 on immune cells. However, we have also found an increase in TGF-β signaling and an increase in distance from immune cells to the nearest cancer cell after treatment, which could suggest there is an antagonistic relationship between these two treatment strategies. These observations may explain conflicting results in first-line metastatic studies, where positive results for the addition of nivolumab to cisplatin-based chemotherapy in the CheckMate 901[10] were recently published after two earlier negative trials. It is likely that we should be specific when discussing the interaction between chemotherapy and ICI, as not all chemotherapy and ICI treatments are interchangeable and show the same results when used in combination. Nivolumab (PD-1 inhibitor) in particular seems to synergize with prior cisplatin-based chemotherapy specifically[9,10], whereas atezolizumab and pembrolizumab did not synergize with carboplatin-based regimens[11,12]. The next challenge will be to differentiate the different treatment strategies and find out why certain specific combinations work better when combined.

CHAPTER 5 – HIGH- OR LOW-DOSE PREOPERATIVE IPILIMUMAB PLUS NIVOLUMAB AND PREDICTING OUTCOME USING CTDNA IN THE NABUCCO TRIAL

In Chapter 5 we have looked in detail at the results from cohort 2 of the NABUCCO trial. In this trial, thirty patients were randomized to receive either two cycles of ipilimumab 3 mg/kg plus

nivolumab 1 mg/kg (cohort 2A) or two cycles of ipilimumab 1 mg/kg plus nivolumab 3 mg/kg (cohort 2B), followed in both cohorts by a third cycle of nivolumab 3 mg/kg. We found that 6/14 (43%) patients treated in cohort 2A had a pathological complete response, whereas only 1/14 (7%) patient in cohort 2B had a pathological complete response. In addition, we set out to predict pathological response and clinical outcome based on ctDNA in plasma and urine in patients treated in NABUCCO cohort 1 and 2 and found a strong correlation between the absence of ctDNA in plasma before radical surgery and pathological response and OS in all cohorts.

OPTIMAL DOSE OF COMBINED IPILIMUMAB PLUS NIVOLUMAB

When comparing the efficacy between the three separate NABUCCO cohorts, we observe a similar pathological complete response rate of 46% in cohort 1 and 43% in cohort 2A (*P*=1.00, Chapter 5, Figure 1). In contrast, cohort 2B only shows a complete pathological response rate of 7%, which is statistically different compared to cohort 1 (*P*=0.03, Chapter 5, Figure 1). However, we did not observe a statistically significant difference in pathological response rate between cohort 2A and 2B (*P*=0.08). The apparent difference in efficacy between the three cohorts could be explained by the higher dose of ipilimumab (3 mg/kg) that is used in cohort 1 and 2A, whereas a lower dose of ipilimumab (1 mg/kg) is used in cohort 2B. This would then suggest that a higher dose of ipilimumab (combined with nivolumab) leads to a higher efficacy and better overall results compared to a lower dose of ipilimumab (combined with nivolumab) in muscle-invasive BC. However, as mentioned before, there is no statistically significant difference when comparing pathological complete response rates between cohort 2A and 2B. In addition, while the high dose of ipilimumab is a common denominator between cohorts 1 and 2A, the two cohorts are not identical, with the main difference being the addition of nivolumab 1 mg/kg to the first treatment cycle in cohort 2A. Regardless, we believe differences in immunotherapy-related toxicity and observations from other studies support our hypothesis that a higher dose of ipilimumab (combined with nivolumab) leads to a higher efficacy and better overall results compared to a lower dose of ipilimumab (combined with nivolumab) in muscle-invasive BC.

CORRELATION BETWEEN DOSE, TOXICITY AND EFFICACY FOR COMBINED IPILIMUMAB PLUS NIVOLUMAB

We observed a numerically higher rate of all grade immunotherapy-related adverse events in cohort 1 and 2A in compared to cohort 2B (cohort 1: 96%; cohort 2A: 100%; cohort 2B: 73%, Chapter 5, Supplementary Table 2). Likewise, we also observed a numerically higher rate of grade ≥3 immunotherapy-related adverse events (cohort 1: 58%; cohort 2A: 40%; cohort 2B: 20%; Chapter 5, Supplementary Table 2). A dose-efficacy as well as a dose-toxicity relation has been described for ipilimumab as monotherapy in metastatic melanoma[13,14]. These data suggest that there would also be a correlation between toxicity and efficacy. A similar dose-toxicity relation has been described for ipilimumab in combination with nivolumab in multiple studies that tested the efficacy and/or tolerability of ipilimumab 3 mg/kg plus nivolumab 1 mg/kg compared to ipilimumab 1 mg/kg plus nivolumab 3 mg/kg directly[15-22]. However, the dose-efficacy relation of

ipilimumab in combination with nivolumab is less clear and is potentially dependent on tumor type and disease stage. However, regardless of tumor type, ipilimumab 3 mg/kg was at least as efficious compared to ipilimumab 1 mg/kg in all studies mentioned. This could suggest a correlation between efficacy and toxicity for ipilimumab in combination with nivolumab.

OPTIMAL DOSE OF IPILIMUMAB AND NIVOLUMAB IN OTHER STUDIES

In the CheckMate 032 trial, patients with advanced BC were treated with either nivolumab monotherapy, or in combination with ipilimumab with different dose combinations[20]. While this trial was not properly powered to detect a statistical difference in objective response rate or OS, both these outcome measures were numerically better in patients treated with plus 3 mg/kg ipilimumab plus 1 mg/kg nivolumab compared to patients treated with 1 mg/kg ipilimumab plus 3 mg/kg nivolumab or nivolumab monotherapy[20]. Notably, a similar trend was also observed in esophageal cancer and recurrent small-cell lung carcinoma in the same trial[17,18]. However, no difference in efficacy was observed in stage III melanoma patients that were treated with either a high dose of ipilimumab (3 mg/kg) plus nivolumab or a low dose of ipilimumab (1mg/kg) plus nivolumab in the OpACIN-neo trial[16]. Thus, it should be concluded that the trend for increased efficacy for a high dose of ipilimumab compared to a low dose (in combination with nivolumab) does not hold true for all cancer types and/or disease stages. While the precise underlying mechanism is not completely understood, we hypothesize that a higher dose of ipilimumab helps to recruit additional naïve T-cells to the tumor micro-environment, which are subsequently activated due to the effect of nivolumab. Potentially, tumors like stage III melanoma that are prone to respond to preoperative ipilimumab 1 mg/kg (in combination with nivolumab) are already prone to respond to checkpoint inhibitors to such an extent that a higher dose of ipilimumab (in combination with nivolumab) confers no additional benefit in terms of efficacy.

PREOPERATIVE COMBINED IMMUNOTHERAPY VERSUS NEOADJUVANT CISPLATIN-BASED CHEMOTHERAPY

The results from the NABUCCO trial and from other preoperative immunotherapy trials suggest that preoperative (ICI) followed by radical surgery could be a promising alternative for patients with muscle-invasive BC unfit to receive cisplatin-based chemotherapy, for whom there are currently no approved preoperative treatment strategies available. However, with pathological complete response rates approaching 50% for patients treated with high dose ipilimumab combined with nivolumab, it would be interesting to directly compare this treatment to standard cisplatin-based neoadjuvant chemotherapy in a randomized trial.

Previously, we retrospectively analyzed all patients with muscle-invasive BC (cT3-4aNx or cT1-4aN1-3) that were treated in the NKI-AvL during the period of inclusion of the first cohort of the NABUCCO trial[23]. We compared patients that were treated with preoperative ipilimumab plus nivolumab in NABUCCO to patients that were treated with neoadjuvant (or induction) cisplatin-based chemotherapy. Pathological complete response rate in the latter group was 22% (compared

to 46% in NABUCCO). Both PFS and OS was superior for the patients treated in NABUCCO[23]. As discussed prior, cT2-4aN0 BC patients were treated with dose-dense MVAC in the VESPER trial leading to a pathological complete response in 42% of patients[24]. However, the study population consisted primarily of cT2N0 patients (197/218), and patients with lymph node metastases were excluded[24]. Another study investigated cT3-4aN0 patients treated with dose-dense MVAC and observed a pathological complete response rate of 28%[25].

In the CheckMate 274 trial, improved disease-free survival was observed in patients treated with adjuvant nivolumab, in particular in patients that were previously treated with neoadjuvant cisplatin-based chemotherapy and with a high expression of PD-L1 on tumor cells[9]. This has led to approval of nivolumab as adjuvant treatment for muscle-invasive BC by the FDA in 2021. At an extended data analysis presented at ASCO GU 2023 it was shown that the 24-month disease-free survival rate for patients treated with adjuvant nivolumab was 48.4% compared to 38.8% for the placebo group. By comparison, the 24-months disease-free survival rate for NABUCCO cohort 1 is 92%. Note that patients with a complete pathological response after neoadjuvant treatment were not included in the CheckMate 274, which would probably have improved the disease-free survival rates presented in this trial. Moreover, it should be noted that there are currently no published data on OS for the CheckMate 274 trial. This is important, as disease-free survival does not necessarily translates to an OS benefit, potentially only delaying recurrence. If patients recur, they might have fewer treatment options in the metastatic setting. Despite this caveat, the combination of dose-dense MVAC for muscle-invasive BC, followed by radical cystectomy and adjuvant nivolumab for patients with residual muscle-invasive disease or lymph node metastases can be considered the current optimal treatment for patients with muscle-invasive BC. It should be noted that trials assessing novel treatments such as enfortumab vedotin as preoperative treatment for muscle-invasive BC are expected to impact the treatment landscape[26]. For that reason, it is difficult to define a robust state-of-the-art treatment to compare novel treatments against that will not be outdated when a new trial has run its course. However, this should not be a reason to disregard findings from new encouraging developments.

MACBETH TRIAL: A PHASE III TRIAL OF PREOPERATIVE DOSE-DENSE METHOTREXATE, VINBLASTINE, DOXORUBICIN, AND CISPLATIN FOLLOWED BY ADJUVANT NIVOLUMAB COMPARED TO PREOPERATIVE IPILIMUMAB PLUS NIVOLUMAB FOR LOCALLY ADVANCED UROTHELIAL CANCER

As part of the discussion of this thesis, I would like to propose a randomized phase 3 trial where we compare preoperative dose-dense MVAC followed by adjuvant nivolumab (standard arm) versus combined preoperative ipilimumab (3 mg/kg) plus nivolumab as is described in NABUCCO cohort 2A, with adjuvant cisplatin-based chemotherapy in case of residual muscle-invasive disease or lymph node metastases after radical surgery (experimental arm, Figure 1). The recommended standard-of-care for eligible patients is cisplatin-based neoadjuvant chemotherapy followed by radical surgery, however, especially in the context of ICI, we believe adding adjuvant nivolumab

for eligible patients is presumably the optimal choice, as discussed prior[27]. The main inclusion criteria will include a WHO performance score 0-1, cT3-4aN0M0 or cT1-4aN1-3M0 urothelial cancer, including upper tract tumors. All patients should be cisplatin-eligible and be eligible to receive ipilimumab and nivolumab. Primary outcome will be pathological complete response after radical surgery for the intention-to-treat population and OS after 24 months. Secondary outcomes will include complete pathological downstaging after surgery (ypTa/Tis/T1N0), toxicity and extended OS and PFS.

Based on the results of the trials that tested dose-dense MVAC, we assume a pathological complete response rate of 30% for the standard arm. This is lower than the 42% observed in the VESPER trial, however, as discussed prior, this trial mainly included cT2N0 patients[24]. We estimate >28% pathological complete response rate (as was observed in the retrospective trial in cT3-4a patients) since our patients will be relatively good condition, WHO 0-1 and eligible to receive either combination treatment. We assume a pathological complete response rate of 43% for the patients treated with combined ipilimumab plus nivolumab, as was also observed in NABUCCO cohort 2A. We also assume an α of 0.05 and a power of 80% ($\beta = 0.2$). This results in a total population size of N=428, divided equally over 2 cohorts.

The 24-month PFS and OS rates for patients treated in NABUCCO cohort 1 is 92%. The 24-month PFS and OS data for patients treated in NABUCCO cohort 2A is still currently being analyzed. Three cases of disease progression and three cases of death have been reported as of 31 March 2022, the data cut-off date used for the manuscript (Chapter 5, Extended Data Figure 2). Taken together, we conservatively estimate that the eventual PFS and OS will be around 82% for both cohort 1 and 2A combined after 24 months. The 24-month PFS rate for patients treated with adjuvant nivolumab in the CheckMate 274 is 48.4%. When we proportionally include follow-up for fictive patients with locally advanced disease that would have had a pathological complete response or complete pathological downstaging after neoadjuvant or induction cisplatin-based chemotherapy, we get to a total of 62% PFS after 24 months (based on the data presented by Einerhand *et al*., assuming 34% pathological complete downstaging of which 88% will have shown no progression and adjuvant therapy for all patients with residual muscle-invasive disease)[23]. As no OS data is available for the CheckMate 274, and similar rates for PFS and OS have been found in both NABUCCO cohort 1 and 2a, we will assume 62% and 82% as surrogate numbers for the sake of addressing whether we would have sufficient statistical power in our study to detect a difference. Based on an α of 0.05 and a power of 80% ($\beta = 0.2$), this results in a total required number of participants of N=156, divided equally over 2 cohorts. In conclusion, a total study population of 428 will have the statistical power to address both a difference in pathological complete response and an eventual difference in OS.

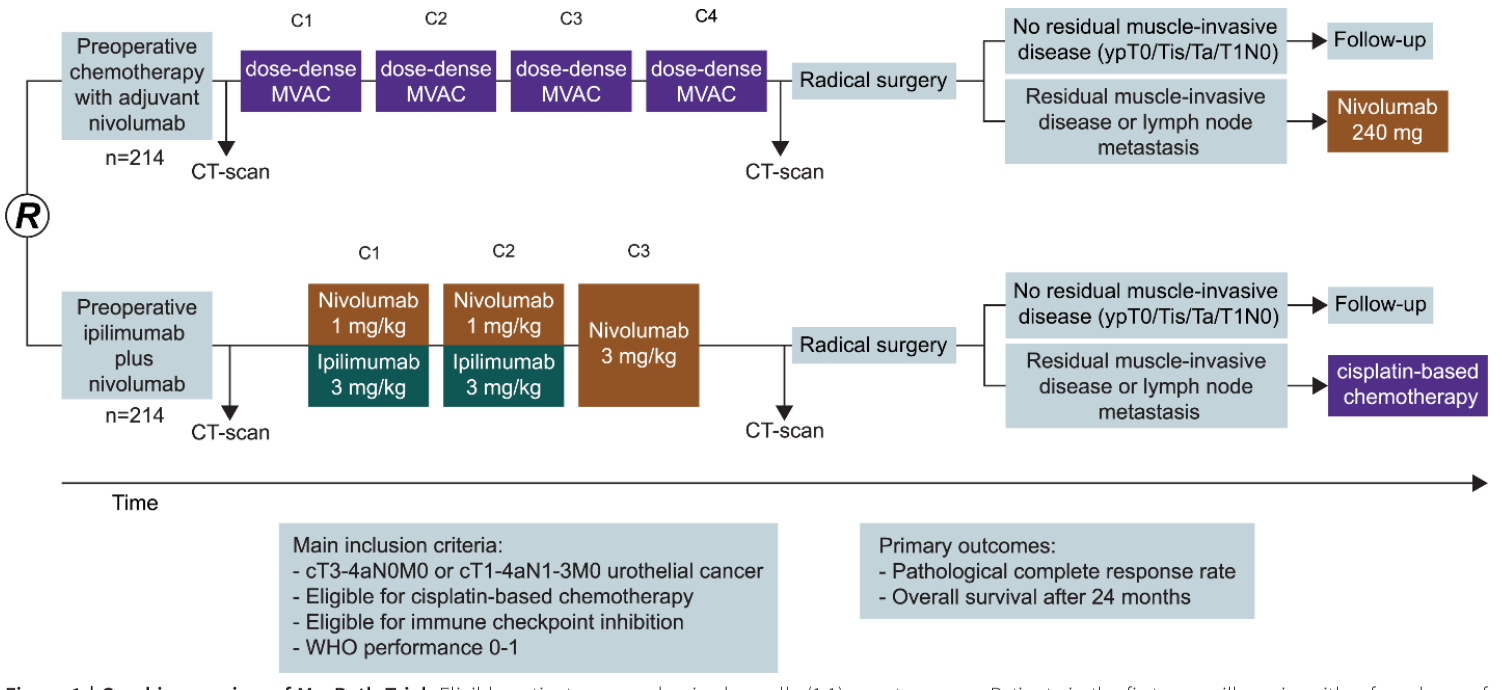

Figure 1 | Graphic overview of MacBeth Trial. Eligible patients are randomized equally (1:1) over two arms. Patients in the first arm will receive either four doses of dose-dense MVAC chemotherapy followed by radical surgery and adjuvant nivolumab for up to one year, as described for the CheckMate 274 trial. In the experimental arm, patients will receive ipilimumab and nivolumab as described for cohort 2A in the NABUCCO trial, followed by adjuvant cisplatin-based chemotherapy according to standard practices.

CTDNA IN PLASMA TO PREDICT PATHOLOGICAL RESPONSE AND CLINICAL OUTCOME

We found a strong correlation between the presence of ctDNA in plasma prior to radical surgery and pathological response and clinical outcome. In patients in cohort 1 and 2 of NABUCCO combined we found that ctDNA was no longer detectable in the last plasma measurement prior to surgery in seventeen (94%) patients that had no residual muscle-invasive disease after surgery (responders). In contrast, ctDNA was no longer detectable in the last plasma measurement prior to surgery in only six (29%) patients that had residual muscle-invasive disease or lymph node metastases after treatment (non-responders). While statistically significant, the clinical relevance of this biomarker is particularly apparent by looking at the outliers. There was no plasma sample available prior to surgery for one responder. The final available sample (taken before the third treatment cycle) still showed detectable ctDNA. However, there are four other responders that still did show detectable ctDNA prior to the third treatment cycle, of which all had no detectable ctDNA prior to surgery. In addition, none of the non-responders that had detectable ctDNA prior to the third cycle had subsequent undetectable ctDNA prior to surgery. Following this trend, it could be expected that the patient with detectable ctDNA prior to the third treatment cycle would have shown undetectable ctDNA if a sample prior to surgery would have been available.

When looking specifically at the six non-responders that have no detectable ctDNA prior to surgery, we find that four of these patients did not show any signs of disease recurrence during follow-up. Three of these patients had a single lymph node micrometastasis, and one other had residual muscle-invasive disease. However, two other patients with residual muscle-invasive disease did show disease recurrence, which are shown as events in patients with no detectable ctDNA prior to surgery (blue line) in Chapter 5, Figure 2a. Overall, the positive predictive value for the presence of ctDNA and disease recurrence is 59% (10/17). The negative predictive value, ie. the number of patients with absence of ctDNA prior to surgery and no disease recurrence is 92% (22/24). We can compare this to the predictive value of pathological response after surgery. For this comparison we can include the patients from other centers which were not included in the ctDNA analysis as these samples had not been analyzed. When interpreting these numbers, it should be noted that radical surgery is not a diagnostic procedure by itself and very likely influences the chance of disease recurrence, which is in contrast to blood withdrawal for the analysis of ctDNA in plasma. Overall, the positive predictive value for the presence of muscle-invasive disease and/or lymph node metastasis (non-responders) and disease recurrence is 42% (11/26). The negative predictive value for absence of muscle-invasive disease and/or lymph node metastasis (responders) and no disease recurrence is 96% (25/26). Based on these results, we could argue that the presence of ctDNA rivals pathological response after radical surgery to predict PFS after preoperative treatment with combined ipilimumab and nivolumab in muscle-invasive BC. Indeed, a recent study on ctDNA in urothelial cancer patients treated with neoadjuvant chemotherapy followed by surgery also concluded that ctDNA status before surgery and ctDNA dynamics during neoadjuvant chemotherapy both outperformed pathologic downstaging in predicting treatment efficacy and patient outcomes after radical cystectomy[28].

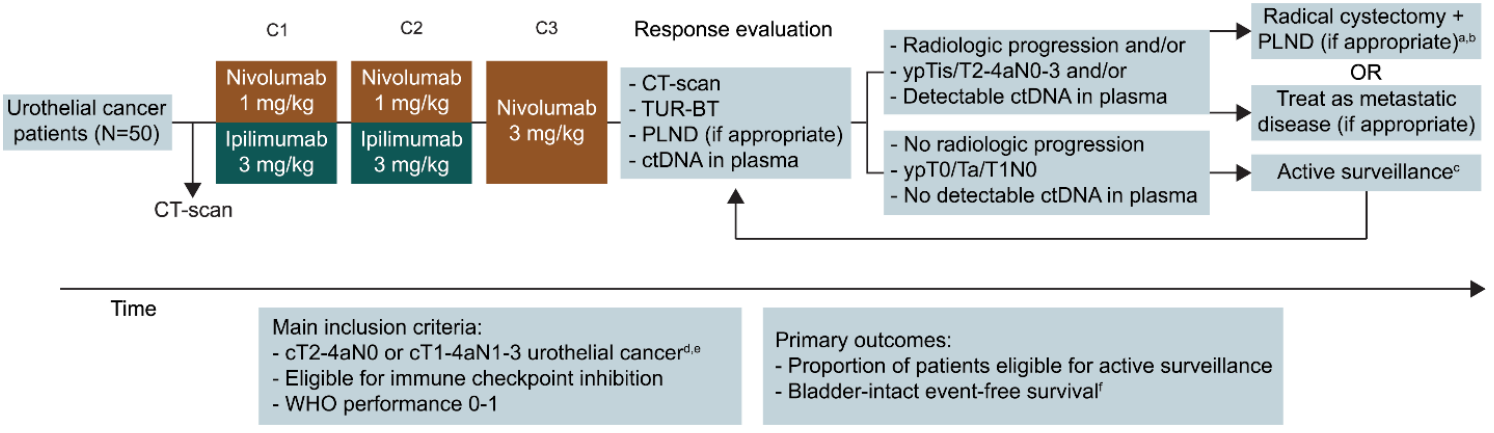

Figure 2 | Graphic overview of the Night's Dream Trial. Eligible patients receive ipilimumab and nivolumab as described for cohort 2A in the NABUCCO trial, followed by a response evaluation. Based on the outcome, patients will be followed with active surveillance or treated with radical cystectomy with a pelvic lymph node dissection if appropriate. Depending on the on the response evaluation outcome, patients will be treated with BCG or as metastatic disease instead. a) Alternatively, eligible patients can be treated with chemoradiation; b) Alternatively, eligible patients with CIS can be treated with BCG; c) Active surveillance will include regular cystoscopies, CT-scans and ctDNA in plasma; d) A maximum of 50% of cT2N0 patients will be permitted; e) Patients with lymph node metastasis at baseline will be treated with a PLND during response evaluation in addition to TUR-BT. *TUR-BT: Transurethral resection of the bladder tumor, ctDNA: circulating tumor DNA, PLND: Pelvic lymph node dissection*

CTDNA IN URINE TO PREDICT PATHOLOGICAL RESPONSE IN THE BLADDER

In contrast to ctDNA in plasma, ctDNA in urine was not predictive for pathological response or PFS. When looking at the data in detail, a number of observations can be made. Primarily, when looking at the graph in Chapter 5, Extended Data Figure 6a, we observe that a number of patients with a pathological complete response still show detectable ctDNA in urine prior to surgery. This could be explained by residual non-vital tumor that is being degraded and is still actively or passively shedding cell-free DNA. In addition, ctDNA is also detected prior to surgery in patients with residual non-muscle-invasive disease (Chapter 5, Extended Data Figure 6). Finally, we have one example of patient with a complete pathological response in the bladder and a single lymph node metastasis that showed rapid disease progression after treatment. This patient showed clearance of ctDNA in the urine prior to surgery, but did show increasing levels of ctDNA in plasma over the course of treatment. This exemplifies that urine possibly reflects the bladder micro-environment but does not reflect the systemic presence of ctDNA which is probably more relevant in terms of disease progression.

RECENT DEVELOPMENTS FOR BLADDER-SPARING TREATMENT IN MUSCLE-INVASIVE BLADDER CANCER

Radical cystectomy is a surgical procedure with significant morbidity and mortality[29]. In NABUCCO, three patients refused radical surgery, one of whom was treated with transurethral resection of the bladder tumor and a lymph node dissection. Two patients refused surgery altogether and are currently in follow-up. None of these patients did show any signs of disease recurrence.

Another recent trial (HCRN GU16-257) investigated the feasibility of cisplatin plus gemcitabine plus nivolumab in cT2-4N0M0 urothelial cancer patients without standard consolidating treatment[30]. After initial treatment and clinical restaging, 33 patients (43%) achieved a clinical complete response and opted to forego direct radical cystectomy. Nine of these patients developed a local recurrence and were treated with a salvage radical cystectomy. Of these 33 patients, two developed distant metastases during follow-up[30]. Given these encouraging results and the high rate of pathological complete response after treatment with ipilimumab plus nivolumab in NABUCCO, we would argue that foregoing consolidating treatment could also be feasible in a subset of patients treated with ipilimumab and nivolumab.

NIGHT'S DREAM TRIAL: A PHASE 2 TRIAL OF COMBINED NIVOLUMAB PLUS IPILIMUMAB FOLLOWED BY ACTIVE SURVEILLANCE FOR LOCALIZED OR LOCALLY ADVANCED UROTHELIAL CANCER

As part of the discussion of this thesis, I would like to propose a phase 2 trial to assess the feasibility of treating patients with combined ipilimumab (3 mg/kg) plus nivolumab as is described in NABUCCO cohort 2A, followed by clinical restaging based on radiological imaging, pathological assessment of the bladder tumor by TUR-BT and measuring ctDNA in plasma. The predictive value of ctDNA in plasma prior to surgery might aid to properly select patients that could safely forego

or postpone radical cystectomy. Patients with no signs of radiologic progression, no evidence of muscle-invasive disease (or carcinoma *in situ*) after transurethral resection of the bladder tumor and no detectable ctDNA in plasma after treatment with ipilimumab and nivolumab will not receive any consolidating treatment but instead will be regularly monitored with radiological imaging, cystoscopy and ctDNA measurements. Patients with evidence of residual muscle-invasive disease (or carcinoma *in situ*) after transurethral resection, detectable ctDNA in plasma and/or radiologic progression will be treated with radical cystectomy including removal of the locoregional lymph nodes. Patients with ypTisN0 will be counseled for treatment with BCG or radical cystectomy accordingly. Alternatively, patients will be treated with chemoradiation treatment if eligible. Patients with local recurrence during active surveillance will be treated accordingly with a re-TUR-BT, BCG, radical cystectomy (with PLND) or chemoradiation treatment. Patients with lymph node positive disease will be eligible for the study as we have observed complete pathological responses in NABUCCO in these patients. However, these patients will be treated with a pelvic lymph node dissection in addition to the transurethral resection of the bladder tumor. Patients with cT2N0 tumors will also be eligible for this study as these patients have an *a priori* lower chance of disease recurrence after treatment and will benefit from a bladder-sparing treatment. However, this patient category will be limited to have a sufficient number of patients with locally advanced disease participating. Patients with upper tract tumors will not be eligible in this trial.

Overall, this trial will include patients with cT2-4aN0M0 or cT1-4aN1-3M0 BC. All patients should be eligible to receive ipilimumab and nivolumab and should have a WHO performance score of 0-1. Primary outcome will be proportion of patients eligible for active surveillance meaning no signs of radiologic progression, no evidence of muscle-invasive disease (or carcinoma *in situ*) after transurethral resection of the bladder tumor, no vital lymph node metastasis in case of a PLND and no detectable ctDNA in plasma. Bladder-intact event-free survival for active surveillance-eligible patients will be monitored as a co-primary endpoint as recommended[31]. Events are defined as muscle-invasive recurrence in the bladder or in the distal ureter, nodal or distant recurrence, death related to disease progression or related to treatment and/or radical cystectomy. Secondary outcomes will include immune-related toxicity, changes in ctDNA as measured in plasma during treatment with, rate of non-muscle invasive recurrence, PFS and OS for the population as a whole and pathological complete response (ypT0Nx) and residual non-muscle-invasive disease (ypTa/Tis/T1Nx) for those patients undergoing radical cystectomy, Forty-seven percent of patients In NABUCCO cohort 1 and 2A combined had no residual muscle-invasive disease or carcinoma *in situ* after radical cystectomy. In addition, a total of 6 patients (16%) showed disease progression during follow-up in NABUCCO 1 and 2A combined. Taking these numbers into account, we will enroll a total of 50 patients in this trial, including a maximum of 50% (n=25) patients with cT2N0 tumors. We assume a pathological response rate (ypT0/Ta/T1N0) of around 50% (n=25). Survival outcomes for this population will be compared to results from NABUCCO cohort 1 and 2A and from the INDIBLADE study.

CHAPTER 6 – A SERENDIPITOUS PRE-OPERATIVE TRIAL OF COMBINED IPILIMUMAB PLUS NIVOLUMAB FOR LOCALIZED PROSTATE CANCER

In chapter 6, we examined the incidence and characteristics of prostate cancer in sixteen patients treated with ipilimumab and nivolumab followed by radical cystoprostatectomy in NABUCCO cohort 1. In addition, we compared the findings from NABUCCO to a control cohort consisting of 121 patients treated with muscle-invasive BC that were treated with neoadjuvant chemotherapy followed by radical cystoprostatectomy or treated with a direct radical cystoprostatectomy. Surprisingly, the incidence of prostate cancer, pT-stage, Gleason score and infiltration of immune cells were all comparable between the NABUCCO cohort and the control cohort. In conclusion, we found no evidence that ipilimumab plus nivolumab induces a meaningful anti-cancer immune response in localized prostate cancer.

It is currently not well understood why localized prostate cancer does not respond well to immunotherapy. This study does provide some insights into this phenomenon. Firstly, we observe no correlation between (lack of) response in muscle-invasive BC and the incidence of prostate cancer, supporting the idea that the patient immune system is still intact and not suppressed due to the presence of prostate cancer. In addition, the prostate tumor microenvironment could potentially disrupt the function of ICI antibody molecules or prevent access to relevant immune cells. However, one particular patient in the NABUCCO cohort with invasion of muscle-invasive BC into the prostate showed an impressive response in the bladder and also in part of the bladder tumor that was invading the prostate (Chapter 6, Figure 4). In contrast, no histopathological signs of treatment response were observed in the concurrent prostate tumor or in three prostate cancer lymph node metastases (Chapter 6, Figure 4). This suggests that the prostate micro-environment is not necessarily detrimental for the effect of ICI.

Tumor mutational burden might also explain why prostate cancer does not respond well to treatment with ICI. It has been found that the average tumor mutational burden is lower in prostate cancer compared to tumor types that are more prone to respond to treatment with ICI, such as melanoma, non-small cell lung cancer and BC[32]. In addition, a correlation between response rate and tumor mutational burden in metastatic prostate cancer patients treated with ipilimumab plus nivolumab in the Checkmate 650 trial[33]. Finally, it has been observed that patients with localized prostate cancer have a lower tumor mutational burden compared to patients with metastatic prostate cancer[34].

As the diagnosis of prostate cancer was not actively investigated in patients who were planned for cystoprostatectomy, no adequate pre-treatment prostate cancer information is available. This excludes the possibility to properly compare pre- and post-treatment prostate cancer characteristics, which could be considered necessary to properly evaluate the treatment effect of

ipilimumab and nivolumab on localized prostate cancer. Potentially, there may have been patients with prostate cancer with a partial or complete response after treatment with ipilimumab and nivolumab that was no longer detectable at the time of radical cystoprostatectomy. However, we observed that the incidence and grade of prostate cancer was not statistically different between the different cohorts. Of note, the incidence of prostate cancer was numerically slightly higher in the patients treated with ipilimumab and nivolumab, and this cohort also included the single patient with prostate cancer-related lymph node metastases.

In conclusion, there is only limited evidence for the efficacy of ICI in prostate cancer, which is in sharp contrast other cancer types. In our study we also did not find any evidence for a relevant effect of ipilimumab plus nivolumab on incidental, localized prostate cancer. More research is required to find out if there are other immune-related (combination) treatments that will improve the treatment of prostate cancer.

CLOSING WORDS

For many years, cisplatin-based chemotherapy followed by radical surgery was the only option for patients with locally advanced BC and provided only a marginal benefit. In the last years, a plethora of new treatment options and combinations thereof have become available including ICI and enfortumab vedotin. In addition, with improved efficacy of neoadjuvant treatment strategies, bladder-sparing treatments are currently becoming a valid alternative treatment for an increasing number of patients.

As we conclude this exciting chapter of research, let us not only celebrate the advancements made but also acknowledge the ongoing journey ahead. With continued dedication and collaboration, we can pave the way for a future where bladder cancer treatment is not just about managing the disease but about achieving enduring remission and improved quality of life for all those affected.

REFERENCES

1. Challita-Eid, P.M., *et al.* Enfortumab Vedotin Antibody-Drug Conjugate Targeting Nectin-4 Is a Highly Potent Therapeutic Agent in Multiple Preclinical Cancer Models. *Cancer Res* 76, 3003-3013 (2016).
2. Powles, T., *et al.* Enfortumab Vedotin in Previously Treated Advanced Urothelial Carcinoma. *N Engl J Med* 384, 1125-1135 (2021).
3. Powles, T.B., *et al.* LBA6 EV-302/KEYNOTE-A39: Open-label, randomized phase III study of enfortumab vedotin in combination with pembrolizumab (EV+P) vs chemotherapy (Chemo) in previously untreated locally advanced metastatic urothelial carcinoma (la/mUC). *Annals of Oncology* 34, S1340 (2023).
4. Sridhar, S., *et al.* 2365MO Study EV-103 cohort L: Perioperative treatment w/ enfortumab vedotin (EV) monotherapy in cisplatin (cis)-ineligible patients (pts) w/ muscle invasive bladder cancer (MIBC). *Annals of Oncology* 34, S1203 (2023).
5. Necchi, A., *et al.* Phase 3 KEYNOTE-905/EV-303: Perioperative pembrolizumab (pembro) or pembro + enfortumab vedotin (EV) for muscle-invasive bladder cancer (MIBC). *Journal of Clinical Oncology* 41, TPS585-TPS585 (2023).
6. Hoimes, C.J., *et al.* KEYNOTE-B15/EV-304: Randomized phase 3 study of perioperative enfortumab vedotin plus pembrolizumab versus chemotherapy in cisplatin-eligible patients with muscle-invasive bladder cancer (MIBC). *Journal of Clinical Oncology* 39, TPS4587-TPS4587 (2021).
7. Cancer Genome Atlas Research, N. Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature* 507, 315-322 (2014).
8. Robertson, A.G., *et al.* Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. *Cell* 171, 540-556 e525 (2017).
9. Bajorin, D.F., *et al.* Adjuvant Nivolumab versus Placebo in Muscle-Invasive Urothelial Carcinoma. *N Engl J Med* 384, 2102-2114 (2021).
10. van der Heijden, M.S., *et al.* Nivolumab plus Gemcitabine-Cisplatin in Advanced Urothelial Carcinoma. *N Engl J Med* 389, 1778-1789 (2023).
11. Powles, T., *et al.* Pembrolizumab alone or combined with chemotherapy versus chemotherapy as first-line therapy for advanced urothelial carcinoma (KEYNOTE-361): a randomised, open-label, phase 3 trial. *Lancet Oncol* 22, 931-945 (2021).
12. Galsky, M.D., *et al.* Atezolizumab with or without chemotherapy in metastatic urothelial cancer (IMvigor130): a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* 395, 1547-1557 (2020).
13. Ascierto, P.A., *et al.* Ipilimumab 10 mg/kg versus ipilimumab 3 mg/kg in patients with unresectable or metastatic melanoma: a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol* 18, 611-622 (2017).
14. Wolchok, J.D., *et al.* Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. *Lancet Oncol* 11, 155-164 (2010).
15. Lebbe, C., *et al.* Evaluation of Two Dosing Regimens for Nivolumab in Combination With Ipilimumab in Patients With Advanced Melanoma: Results From the Phase IIIb/IV CheckMate 511 Trial. *J Clin Oncol* 37, 867-875 (2019).
16. Rozeman, E.A., *et al.* Identification of the optimal combination dosing schedule of neoadjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma (OpACIN-neo): a multicentre, phase 2, randomised, controlled trial. *Lancet Oncol* 20, 948-960 (2019).
17. Janjigian, Y.Y., *et al.* CheckMate-032 Study: Efficacy and Safety of Nivolumab and Nivolumab Plus Ipilimumab in Patients With Metastatic Esophagogastric Cancer. *J Clin Oncol* 36, 2836-2844 (2018).

18. Antonia, S.J., *et al.* Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial. *Lancet Oncol* 17, 883-895 (2016).
19. Hammers, H.J., *et al.* Safety and Efficacy of Nivolumab in Combination With Ipilimumab in Metastatic Renal Cell Carcinoma: The CheckMate 016 Study. *J Clin Oncol* 35, 3851-3858 (2017).
20. Sharma, P., *et al.* Nivolumab Alone and With Ipilimumab in Previously Treated Metastatic Urothelial Carcinoma: CheckMate 032 Nivolumab 1 mg/kg Plus Ipilimumab 3 mg/kg Expansion Cohort Results. *J Clin Oncol* 37, 1608-1616 (2019).
21. Yau, T., *et al.* Efficacy and Safety of Nivolumab Plus Ipilimumab in Patients With Advanced Hepatocellular Carcinoma Previously Treated With Sorafenib: The CheckMate 040 Randomized Clinical Trial. *JAMA Oncol* 6, e204564 (2020).
22. Wolchok, J.D., *et al.* Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. *N Engl J Med* 377, 1345-1356 (2017).
23. Einerhand, S.M.H., *et al.* Survival after neoadjuvant/induction combination immunotherapy vs combination platinum-based chemotherapy for locally advanced (Stage III) urothelial cancer. *Int J Cancer* (2022).
24. Pfister, C., *et al.* Randomized Phase III Trial of Dose-dense Methotrexate, Vinblastine, Doxorubicin, and Cisplatin, or Gemcitabine and Cisplatin as Perioperative Chemotherapy for Patients with Muscle-invasive Bladder Cancer. Analysis of the GETUG/AFU V05 VESPER Trial Secondary Endpoints: Chemotherapy Toxicity and Pathological Responses. *Eur Urol* 79, 214-221 (2021).
25. Zargar, H., *et al.* Neoadjuvant Dose Dense MVAC versus Gemcitabine and Cisplatin in Patients with cT3-4aN0M0 Bladder Cancer Treated with Radical Cystectomy. *J Urol* 199, 1452-1458 (2018).
26. Flaig, T.W., *et al.* Study EV-103: Neoadjuvant treatment with enfortumab vedotin monotherapy in cisplatin-ineligible patients (pts) with muscle invasive bladder cancer (MIBC): Updated results for Cohort H. *Journal of Clinical Oncology* 41, 4595-4595 (2023).
27. Kamat, A.M., *et al.* Definitions, End Points, and Clinical Trial Designs for Bladder Cancer: Recommendations From the Society for Immunotherapy of Cancer and the International Bladder Cancer Group. *J Clin Oncol* 41, 5437-5447 (2023).
28. Lindskrog, S.V., *et al.* Circulating Tumor DNA Analysis in Advanced Urothelial Carcinoma: Insights from Biological Analysis and Extended Clinical Follow-up. *Clin Cancer Res* 29, 4797-4807 (2023).
29. Stimson, C.J., *et al.* Early and late perioperative outcomes following radical cystectomy: 90-day readmissions, morbidity and mortality in a contemporary series. *J Urol* 184, 1296-1300 (2010).
30. Galsky, M.D., *et al.* Gemcitabine and cisplatin plus nivolumab as organ-sparing treatment for muscle-invasive bladder cancer: a phase 2 trial. *Nat Med* 29, 2825-2834 (2023).
31. Balar, A.V., *et al.* Pembrolizumab monotherapy for the treatment of high-risk non-muscle-invasive bladder cancer unresponsive to BCG (KEYNOTE-057): an open-label, single-arm, multicentre, phase 2 study. *Lancet Oncol* 22, 919-930 (2021).
32. Chalmers, Z.R., *et al.* Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med* 9, 34 (2017).
33. Sharma, P., *et al.* Nivolumab Plus Ipilimumab for Metastatic Castration-Resistant Prostate Cancer: Preliminary Analysis of Patients in the CheckMate 650 Trial. *Cancer Cell* 38, 489-499 e483 (2020).
34. Ryan, M.J. & Bose, R. Genomic Alteration Burden in Advanced Prostate Cancer and Therapeutic Implications. *Front Oncol* 9, 1287 (2019).