On preoperative systemic treatment of muscle-invasive bladder cancer

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CHAPTER 6

A serendipitous preoperative trial of combined ipilimumab plus nivolumab For localized prostate cancer

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ABSTRACT

INTRODUCTION

Encouraging results have been observed by treating castration-resistant metastatic prostate cancer (PCa) patients with combined ipilimumab plus nivolumab. In other malignancies, pre-operative treatment with immune checkpoint inhibitors (ICIs) in the localized setting is associated with excellent responses.

In the NABUCCO trial, *n*=24 locally advanced muscle-invasive bladder cancer patients were treated with ipilimumab plus nivolumab, followed by radical surgery. This trial offered the unique opportunity to investigate the effect of these agents in PCa incidentally found at cystoprostatectomy.

PATIENTS AND METHODS

NABUCCO patients were evaluated for the presence of PCa and histopathological features of response to ICIs. Findings were compared to PCa incidentally found in a control cohort of bladder cancer patients. Representative PCa tissue sections from NABUCCO and control patients were stained for CD3, CD8 and CD45.

RESULTS

PCa was observed in 9/16 (56%) of eligible NABUCCO patients, compared with 48/121 (40%) in the control cohort. No histopathological features of response to ipilimumab plus nivolumab were observed. The number of $CD8^+$-cells, $CD3^+$-cells and $CD45^+$-cells in the PCa area was not statistically different between NABUCCO and control patients.

CONCLUSION

Taking several limitations of this retrospective study into consideration, we found no evidence for a major effect of ipilimumab plus nivolumab on incidental localized PCa.

CLINICAL PRACTICE POINTS

- Incidental prostate cancer was found in cystoprostatectomy specimens in 9 out of 16 patients treated in the NABUCCO trial.
- This was not statistically different from incidental prostate cancer observed after cystoprostatectomy in a control cohort.
- Based on histopathological analysis and immunohistochemistry, we found no evidence for response to ipilimumab and nivolumab in incidental prostate cancer in the NABUCCO trial.

INTRODUCTION

Immune checkpoint inhibition (ICI) has demonstrated impressive results in multiple malignancies[1-3]. However, the first trials with ICI monotherapy in prostate cancer (PCa) showed only limited clinical benefit[4,5]. Recently, more encouraging results in PCa were observed by combining nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) in the CheckMate-650 trial, showing an overall response rate of 25.0% in chemotherapy-naïve metastatic castration-resistant PCa patients[6].

In other malignancies, excellent results have been observed by treating patients with combined ICI in the localized, pre-operative setting[7,8]. This is also observed in cancer types where response to ICI is rare in the metastatic setting, such as microsatellite-stable colon cancer[9].

In the NABUCCO trial, locally advanced muscle-invasive bladder cancer (MIBC) patients were treated with ipilimumab plus nivolumab, followed by radical surgery[10]. As part of the surgical template in male patients, the prostate is removed (cystoprostatectomy). This presents a unique opportunity to investigate the effect of combined ipilimumab plus nivolumab on (incidental) PCa in the localized, pre-operative setting.

MATERIALS AND METHODS

NABUCCO STUDY DESIGN

NABUCCO is a prospective, single-arm trial testing the feasibility of pre-operative ipilimumab 3 mg/kg (day 1), ipilimumab 3 + nivolumab 1 mg/kg (day 22), and nivolumab 3 mg/kg (day 43) followed by resection with appropriate lymph node dissection in stage III (cT3-4aN0M0 and cT1-4aN1-3M0) resectable MIBC patients (*n*=24). For more information on the NABUCCO trial, refer to *Van Dijk et al., Nat Med, 2020*[10] and ClinicalTrials.gov:NCT03387761.

CONTROL COHORT PATIENT SELECTION

Patients that underwent radical surgery for bladder cancer in the Netherlands Cancer Institute from 2016 – 2019 were included in the control cohorts. Exclusion criteria were female sex, prior diagnosis of PCa, prostate-sparing surgery, previous systemic anticancer therapy except neo-adjuvant chemotherapy for MIBC, or previous radiotherapy in the pelvic area. Clinical data for eligible patients was collected from an institutional database in accordance with national and institutional ethical guidelines and approved under IRBdm20-092.

IMMUNOHISTOCHEMISTRY AND IMMUNE CELL QUANTIFICATION

Immunohistochemistry of formalin-fixed paraffin embedded tumor samples was performed on a BenchMark Ultra autostainer or Discovery Ultra autostainer (beta-2 Microglobulin). Briefly, paraffin

sections were cut at 3µm and deparaffinised. Antibodies used were CD3 clone SP7 (1/100 dilution, Spring/ITK), CD8 clone C8/144B (1/200 dilution, DAKO/Agilent), CD45 Clone 2B11PD7/26 (Ventana Medical Systems) and PSA, a polyclonal antibody (1/8000 dilution, DAKO). Bound antibody was detected using the OptiView and Ultraview DAB Detection Kits (Ventana Medical Systems). Slides were counterstained with Hematoxylin and Bluing Reagent (Ventana Medical Systems) and uploaded in SlideScore (https://www.slidescore.com/). Up to five randomly selected fields (0.8mm^2, magnification 20x) were generated in the PCa tumor area for each slide. Cells were counted manually by an experienced uropathologist (MvM) together with JvD. PCa tumors with a very small tumor focus (<0.8mm^2) were considered as '0' positive cells. The reported cell count for each patient is the mean for each of the five quantitated fields. For details concerning the multiplex immunofluorescent images (DAPI, PanCK, CD3, CD8, CD20, CD68 and FoxP3), see *Van Dijk et al., Nat Med, 2020*[10].

STATISTICAL ANALYSIS

All graphs and statistics were generated in GraphPad Prism 7.0.3. Illustrations were created in Adobe Illustrator CS6 (v. 16.0.3).

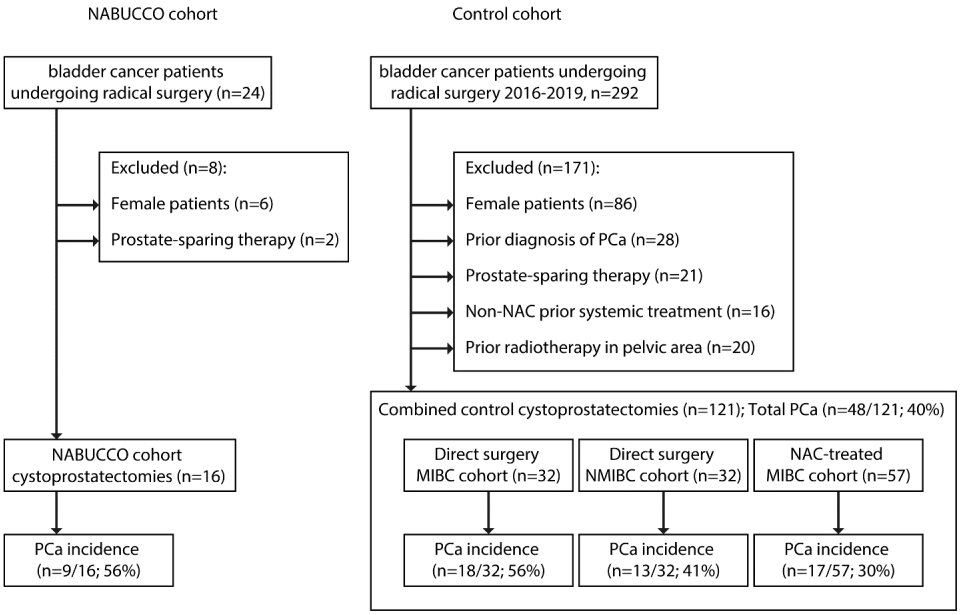

Figure 1 | Inclusion of patients. Left: All NABUCCO patients that underwent a cystectomy were included for analysis. Female patients and patients with prostate-sparing surgery were excluded. Right: All patients that underwent a cystectomy (2016 – 2019) were included for analysis. For the control cohort, we excluded female patients, patients with a prior diagnosis of PCa or prostate-sparing surgery, patients that were treated with systemic therapy before surgery other than platinum-based neo-adjuvant chemotherapy for MIBC, and patients that received prior radiotherapy in the pelvic area. Patients with prior local treatment for NMIBC were included. The remaining patients were subdivided over three cohorts: NMIBC patients, MIBC patients treated with neo-adjuvant platinum-based chemotherapy, and MIBC patients treated with a direct cystectomy.

RESULTS

In total, 24 stage III MIBC patients were treated with ipilimumab plus nivolumab in the NABUCCO trial[10]. As part of the eligibility criteria, only patients without prior diagnosis of PCa or with low-risk PCa were included. The presence of PCa was not actively excluded by prostate biopsies during screening, though prostate biopsies were obtained in patients undergoing prostate-sparing surgery. 16/24 patients underwent a cystoprostatectomy and were eligible for histological examination (Table 1; Figure 1, left). In 9/16 (56%) patients, incidental PCa was found. The majority of cases were low-grade PCa with a Gleason Score (Gleason) 3+3=6 in 7/9 cases and ≤pT2 in 8/9 cases (Table 1). Two patients were diagnosed with a clinically significant Gleason 4+4=8 PCa, including one patient with PCa metastases in multiple pelvic lymph nodes (Table 1).

We reassessed all prostate tissue sections from the NABUCCO cohort and compared these to the MIBC tissue sections. As described previously, response of MIBC to ipilimumab plus nivolumab was characterized by fields of fibrosis and necrosis, infiltration of immune cells, and formation of tertiary lymphoid structures (Figure 2A)[10]. 5/16 patients had a complete pathological response of MIBC (31%, ypT0N0) and 8/16 showed major pathological downstaging of MIBC (50%, <ypT2N0), often exhibiting a prominent immune infiltrate in the tumor bed[10]. No correlation was found between the response of MIBC to ipilimumab plus nivolumab and the occurrence of PCa. In addition, there was no fibrosis, necrosis or other histopathological features of response to ICI in any of the nine PCa specimens (Figure 2B).

Without a full pre-treatment diagnostic work-up for PCa, it is possible that we underestimate the response to ipilimumab plus nivolumab in PCa. To this end, we retrospectively collected data from control patients treated with a radical cystoprostatectomy in our hospital from 2016 – 2019 to compare the incidence and characteristics of PCa to our findings in the NABUCCO cohort (Figure 1, right). Baseline data was collected for three cohorts: MIBC patients treated by direct cystoprostatectomy (without prior systemic treatment, n=32), for three cohorts: MIBC patients treated by direct cystoprostatectomy (without prior systemic treatment, n=32), MIBC patients treated with neo-adjuvant platinum-based chemotherapy followed by cystoprostatectomy (n=57) and non-muscle-invasive bladder cancer (NMIBC) patients (n=32) (Table 1; Figure 1, right). Incidental PCa was found in 48 of the 121 patients in the control cohorts, which was not significantly different from the NABUCCO cohort (40% versus 56%; p=0.28; Table 1). This was also comparable to previously published cohorts[11,12]. Gleason 3+3=6 was found in 33/48 (69%) cases in the combined control cohorts versus 7/9 (78%) cases in the NABUCCO cohort (p=0.71). pT-stage for PCa was ≤pT2 in 44/48 (89%) cases in the other cohorts versus 8/9 (89%) cases in the NABUCCO cohort (p=1.00; Table 1; Figure 2C). In summary, when comparing PCa in the NABUCCO cohort against a control cohort, no significant differences in terms of incidence, Gleason score or disease stage were found. This suggests that we are not underestimating response to ipilimumab plus nivolumab in PCa in NABUCCO patients.

Table 1 | Patient characteristics in urothelial cancer cohorts

	NABUCCO patients	Direct surgery MIBC patients	NAC-treated MIBC patients	NMIBC patients	Statistics
Number of patients eligible	16	32	57	32	
Mean age in years (std)[a]	69.4 (6.8)	70.7 (9.3)	65.3 (9.0)	67.2 (9.4)	p=0.38[b] p≥0.26[c]
Mean PSA at baseline in µg/l (std)	3.40 (3.45)	3.14 (3.30)	2.28 (3.04)	2.48 (2.04)	p=0.30[d] p≥0.42[e]
number of patients with incidental prostate cancer (% of eligible patients)	9 (56)	18 (56)	17 (30)	13 (41)	p=0.28[f] p=1.00[g]
Gleason score (% of PCa)					p=0.71[h] p=0.67[i]
3+3=6	7 (78)	11 (61)	12 (71)	10 (77)	
3+4=7	0 (0)	4 (22)	5 (29)	3 (23)	
4+3=7	0 (0)	3 (16)	0 (0)	0 (0)	
4+4=8	2 (22)	0 (0)	0 (0)	0 (0)	
T-stage PCa (% of PCa)					p=1.00[j] p=0.64[k]
pT1	0 (0)	0 (0)	0 (0)	0 (0)	
pT2	8 (89)	14 (78)	17 (100)	13 (100)	
pT3a	0 (0)	3 (22)	0 (0)	0 (0)	
pT3b	1 (11)	1 (6)	0 (0)	0 (0)	
N-stage for PCa					
pN0	8 (89)	18 (100)	17 (100)	13 (100)	
pN1	1 (11)	0 (0)	0 (0)	0 (0)	
Pre-treatment clinical TNM stage for bladder cancer (% of eligible patients)					
<cT2N0	0 (0)	0 (0)	0 (0)	32 (0)	
cT2N0	0 (0)	21 (65)	4 (7)	0 (0)	
cT2N+	0 (0)	1 (3)	7 (12)	0 (0)	
cT3N0	7 (44)	8 (25)	21 (37)	0 (0)	
cT3N+	5 (31)	2 (6)	12 (21)	0 (0)	
cT4aN0	4 (25)	0 (0)	6 (11)	0 (0)	
cT4aN+	0 (0)	0 (0)	7 (12)	0 (0)	

[a] Mean age at time of cystectomy for all eligible patients;
[b] two-tailed t-test, mean age in NABUCCO versus all control cohorts combined;
[c] 1-way ANOVA followed by Dunnet's multiple comparisons test, mean age for NABUCCO cohort versus all control cohorts separately;
[d] two-tailed t-test, mean PSA in NABUCCO versus all control cohorts combined;
[e] 1-way ANOVA followed by Dunnet's multiple comparisons test, mean age for NABUCCO cohort versus all control cohorts separately;
[f] Fisher's exact test, comparing incidence of PCa in NABUCCO versus all control cohorts combined;
[g] Fisher's exact test, comparing incidence of PCa in NABUCCO versus MIBC cohort treated with direct cystectomy;
[h] Fisher's exact test, comparing GS ≤3+3=6 versus >3+3=6 in NABUCCO versus all control cohorts combined;
[i] Fisher's exact, comparing GS ≤3+3=6 versus >3+3=6 in NABUCCO versus MIBC cohort treated with direct cystectomy;
[j] Fisher's exact test, comparing ≤T2 versus >T2 in NABUCCO versus all control cohorts combined;
[k] Fisher's exact, comparing ≤T2 versus >T2 in NABUCCO versus MIBC cohort treated with direct cystectomy;
(N)MIBC = (Non-)Muscle-invasive bladder cancer; NAC = Neo-adjuvant chemotherapy; PSA = Prostate-specific antigen; PCa = Prostate cancer

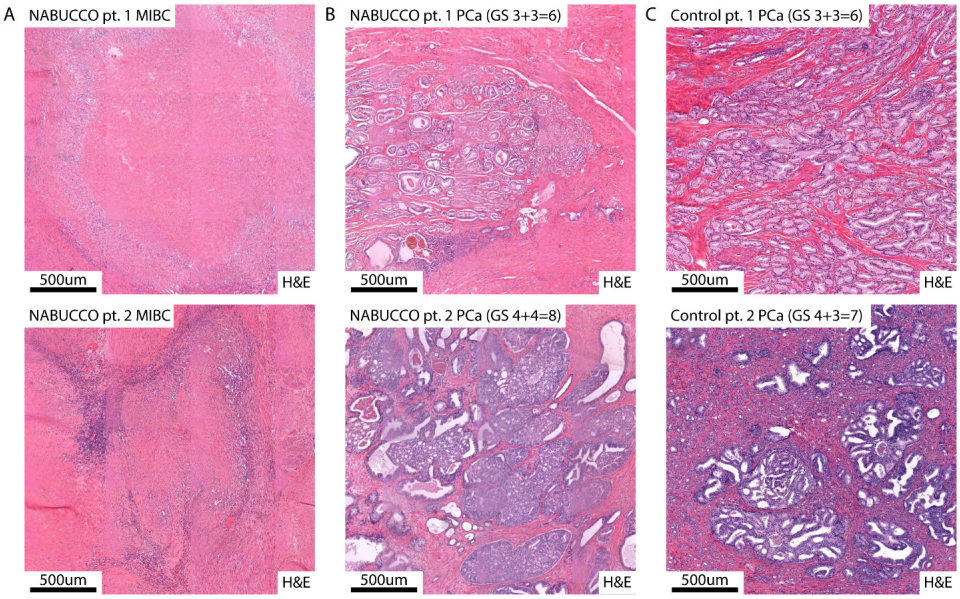

Figure 2 | Histological features of bladder cancer and prostate cancer observed in NABUCCO compared to patients treated with a direct cystectomy without prior neo-adjuvant treatment. A, H&E stainings of representative slides of the MIBC tumor bed in two NABUCCO patients showing extensive necrosis and fibrosis, surrounded by immune infiltrate (top panel: pt#1; ypT0N0, bottom panel, pt#2; ypT3bN0). **B,** H&E stainings of representative slides of PCa in the same NABUCCO patients as in (A), showing a PCa focus with vital tumor cells without any histopathological features of response (top panel: pt#1, GS3+3=6. pT2a; bottom panel: pt#2, GS4+4=8, pT3b). **C,** H&E stainings of representative slides of PCa in two MIBC patients without neo-adjuvant treatment showing a PCa focus with vital tumor cells (top panel: pt#1, GS3+3=6, pT2a; bottom panel: pt#2, GS4+3=7, pT3a).

Despite the absence of histological features related to response in PCa in NABUCCO patients, it is still possible that treatment with ICI results in the recruitment of effector T-cells into the PCa microenvironment, as described previously for pre-operative treatment with sipuleucel-T[13]. We assessed infiltrating immune cells in the prostate tumor microenvironment as a result of treatment with ipilimumab plus nivolumab versus a control cohort. To exclude potential effects from neo-adjuvant chemotherapy or previous treatment for NMIBC, we compared NABUCCO patients to MIBC patients who underwent cystoprostatectomy without any neo-adjuvant systemic treatment. Representative PCa tissue sections were stained for CD3, CD8 and CD45. We found no statistical difference in cells per mm^2 in the PCa tumor area in control patients and NABUCCO patients for any of the immune cell markers (CD3: 110 versus 124 cells per mm^2 p=0.80; CD8: 25 versus 15 cells per mm^2 p=0.25; CD45: 74 versus 80 cells per mm^2 p=0.85, Figure 3). Based on these data, we found no evidence for recruitment of effector T-cells in the PCa microenvironment as a result of treatment with ipilimumab plus nivolumab.

DISCUSSION

Treatment with combined ICI has shown impressive results in multiple malignancies. Superior results have been observed in the localized, pre-operative setting, as demonstrated for MIBC in the NABUCCO trial[10]. Given the encouraging results with ipilimumab plus nivolumab in metastatic PCa, we expected to find a similar response in localized PCa. Surprisingly, the PCa incidence, pT-stage, Gleason score and infiltration of immune cells was comparable between the NABUCCO cohort and the control cohorts. Thus, in sharp contrast to the high activity against MIBC in this cohort, we found no evidence that ipilimumab plus nivolumab induces a meaningful anti-cancer immune response in localized PCa.

There are several potential explanations why ICI would not be effective in localized PCa. The Checkmate-650 trial observed a higher response rate in metastatic PCa patients with a higher tumor mutational burden[6]. On average, patients with locoregional PCa have a lower tumor mutational burden compared to patients with metastatic PCa[14]. 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 MIBC invasion of the prostate showed an impressive response in the bladder and in MIBC invading the prostate (Figure 4A and 4B), whereas no histopathological signs of response were observed in the concurrent primary Gleason 4+4=8 PCa or in three PCa lymph node metastases (Figure 4B and 4C).

This retrospective study has several limitations. As a pre-treatment PCa diagnosis was not actively investigated in patients who were planned for cystoprostatectomy, there may have been patients having a PCa with a complete pathological response to ipilimumab plus nivolumab. In addition, PCa found in our analysis was mostly low-risk disease and would generally not have required treatment. In conclusion, in contrast to other cancer types, we find no evidence for a major effect of ipilimumab plus nivolumab on incidental, localized PCa.

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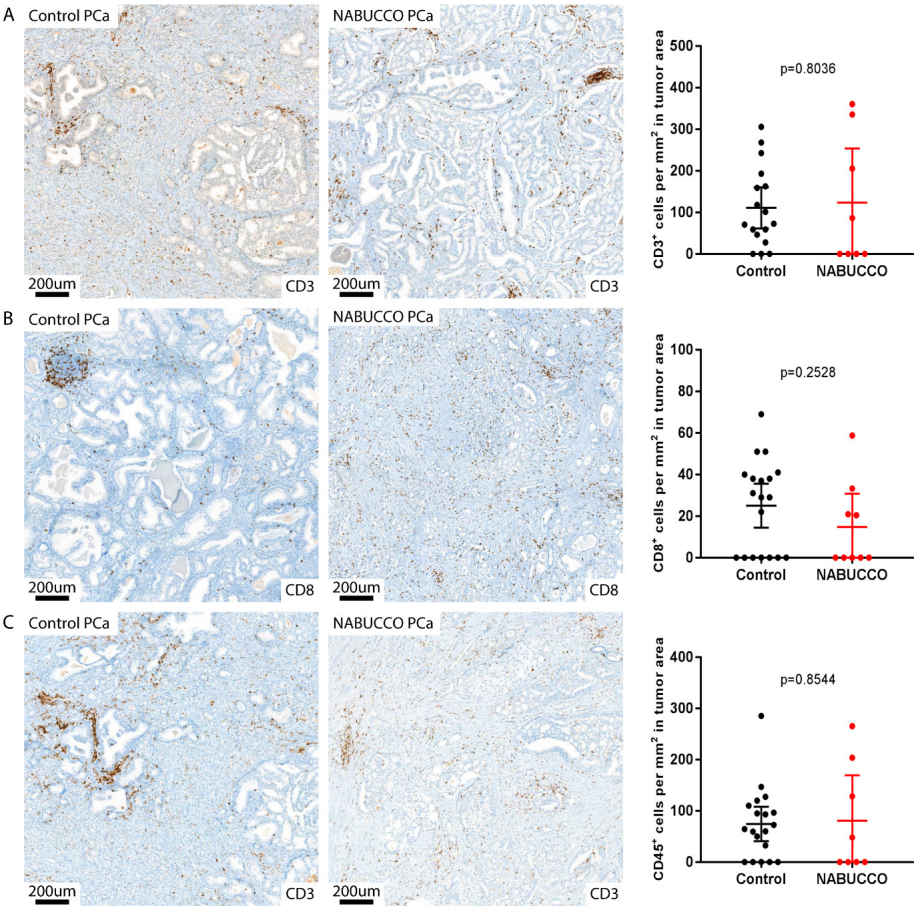

Figure 3 | Expression of CD3, CD8 and CD45 in prostate cancer in NABUCCO cohort and control cohort treated with direct cystectomy. Representative images of CD3 (**A**), CD8 (**B**) and CD45 (**C**) IHC for PCa in a MIBC patient without neo-adjuvant treatment (left) and PCa in a NABUCCO-patient (middle). Graphs (right) show the number of positive cells per mm^2 in the PCa tumor area per cohort per cell surface marker. P-value is given for unpaired t-test, error bars show 95% CI.

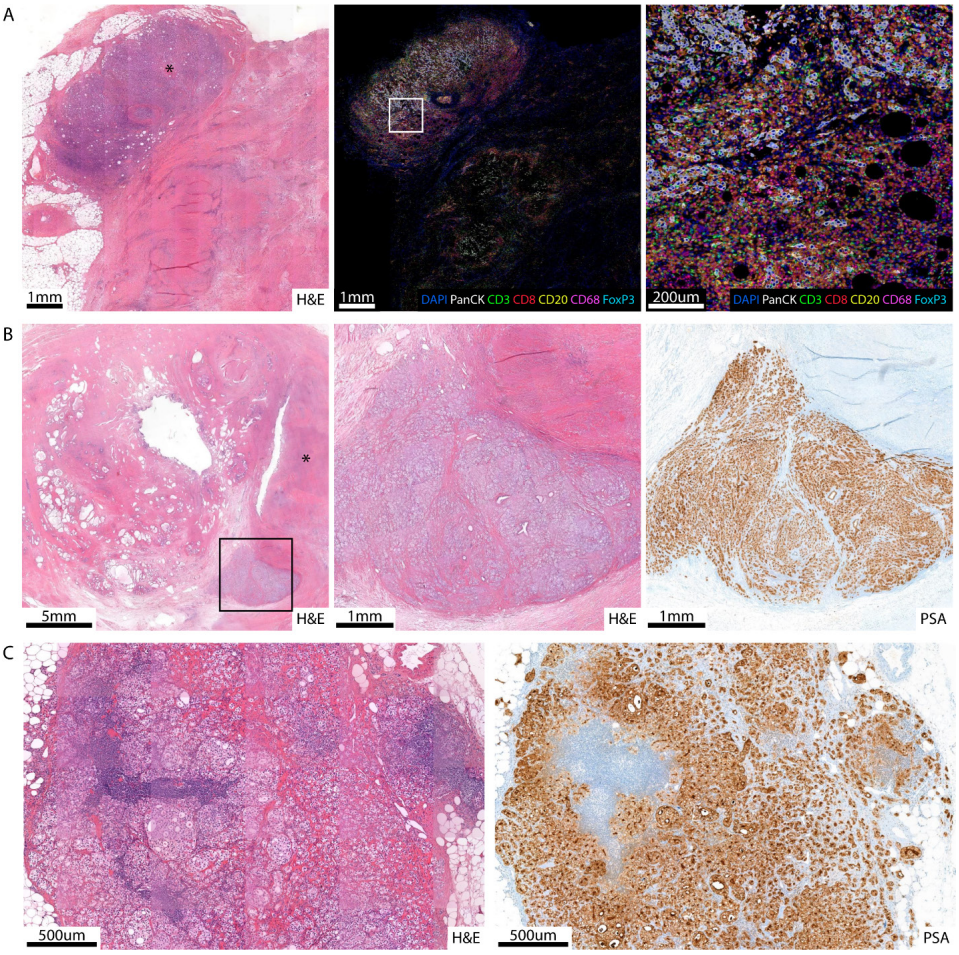

Figure 4 | NABUCCO patient with pT3bN1 incidental PCa. A, Left panel, H&E staining: bladder tumor area with central field of necrosis, indicative of response to ipilimumab plus nivolumab. In the topleft area in the panel, a vital tumor remnant is visible, marked by an asterisk (*). Middle panel, multiplex immunofluorescent staining: Comparable tumor area as visible in the left panel. The white square marks the area that is visible in the right panel. Right panel, multiplex immunofluorescent staining: Close-up shows individual tumor cells (PanCK^{+}), surrounded by T-cells (CD3^{+}CD8$^{+/-}$), B-cells (CD20^{+}) and macrophages (CD68^{+}). **B,** Left panel, H&E staining: Prostate with field of necrotic bladder tumor on the right, marked by an asterisk (*).The black square marks the area that is visible in the middle and right panel. Middle and right panel: Close-up shows field of PCa (middle: H&E, right: PSA). **C,** PCa cells in local lymph node (left: H&E, right: PSA).

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