Abstract

**Background:** An improved risk assessment of patients with bladder cancer (BC) is important to optimize clinical management.

**Objective:** To identify whether immune cell subpopulations and cancer cell-intrinsic features are associated with outcome and response to first-line chemotherapy in BC.

**Design, setting, and participants:** Primary tumor tissue from 785 patients with BC (stage Ta-T4b) were stained using multiplex immunofluorescence (CD3, CD8, FOXP3, CD20, CD68, CD163, and MHC-I) and immunohistochemistry (pancytokeratin, CK5/6, GATA3, programmed death 1 [PD-1], and programmed death ligand 1 [PD-L1]). A digital image analysis quantified staining results within the carcinoma cell and stromal part of the tumor.

**Outcome measurements and statistical analysis:** Primary endpoints were progression-free survival, recurrence-free survival, and response to first-line chemotherapy. Optimal cutoff values for investigated markers were estimated using maximally selected rank statistics and receiver operating characteristic for each primary endpoint. Time-to-event analyses were performed using Cox regression analyses.

**Results and limitations:** Several immune subpopulations were independently associated with clinical outcomes. Especially, high PD-1 and PD-L1 expression was independently associated with an increased risk of recurrence and progression in non-muscle-invasive tumors, but with a lower risk of recurrence in muscle-invasive tumors. Furthermore, we observed a lower likelihood of response to first-line chemotherapy in patients with basal differentiation features. Finally, a model combining clinical risk factors with our most evident prognosticator improved prediction accuracy compared with clinical risk factors alone for progression in non-muscle-invasive BC and recurrence in...
1. Introduction

Bladder cancer (BC) is a highly molecularly heterogeneous disease [1], and the phenotype of each cancer cell is influenced by a multitude of cancer cell-intrinsic and cell-extrinsic features, which may drive disease development and treatment resistance [2].

BC is characterized by a high tumor mutational burden (TMB), and a higher TMB has been associated with an increased T-cell influx in different cancer types [3,4]. However, tumors can escape immune surveillance through alterations in neoantigen processing or presentation, or through upregulation of programmed death ligand 1 (PD-L1) [5]. High PD-L1 expression has been associated with a poor prognosis in both non–muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC) [6–9]. Recent transcriptomic profiling of BC has provided additional insights, revealing that immune infiltration is greatly associated with the different molecular subtypes: high immune infiltration has been associated with class 2b in NMIBC and the basal/squamous (Ba/Sq) subtype in MIBC [10,11].

The prognostic value of immune system biomarkers in BC has been addressed in several studies [12,13]. A high level of cytotoxic T lymphocyte (CTL) infiltration has generally been associated with a favorable prognosis in BC [7,14,15]; however, it has also been associated with an increased risk of recurrence in NMIBC [16]. High infiltration of macrophages and regulatory T cells (Tregs) have primarily been linked to tumor progression [17,18], but high levels of Tregs have also been associated with a positive prognosis in BC [19]. Consequently, larger studies are needed to obtain consistent reliable results in order to determine optimal predictors of tumor recurrence, progression, and treatment response. Here, we present multiplex immunofluorescence (mIF) and immunohistochemical analyses of tumors from 785 patients with BC. We use a digital image analysis to investigate the spatial dynamics of cancer cell-intrinsic and cell-extrinsic features during disease development and their impact on clinical outcomes.

2. Patients and methods

2.1. Patient details and samples

Biological specimens (n = 785) from transurethral resection of bladder tumor or cystectomy collected between 1992 and 2014 were retrieved from 12 Danish hospitals. Tissue samples were placed on tissue microarrays (TMAs). Further details regarding samples, procedures, and clinical follow-up are listed in the studies of Lindskrog et al [11], Taber et al [20], and Jensen et al [21], and in the Supplementary material. Informed written consent to take part in future research projects was obtained from all patients, and the study was approved by the National Committee on Health Research Ethics (#1706291 and #1300174).

2.2. Endpoints

Recurrence-free survival and progression-free survival were measured in months from the time of primary surgery to the event or end of follow-up. Evaluation of first-line chemotherapy response was based on pre- and post-treatment imaging according to RECIST 1.1. Survival data were updated in June 2020.

2.3. Immunofluorescence, immunohistochemistry, and imaging

Detailed information on antibodies and staining protocols used in this study is described in Supplementary Table 1 and the Supplementary material. We utilized a tyramide signal amplification strategy for mIF detection of panel 1 (CD3, CD8, and FOXP3) and panel 2 (CD20, CD68, CD163, and HLA-A, HLA-B, HLA-C), and standard bright-field immunohistochemistry for the detection of pancytokeratin, programmed death 1 (PD-1), PD-L1, GATA3, and CK5/6, as described previously [20,22].

2.4. Automated quantification of scanned images

A digital image analysis was carried out using the Visiopharm image analysis software version 2018.9.5.5952 (Visiopharm A/S, Hørsholm, Denmark), as described in the Supplementary material. Identification and quantification of specific cell types were based on the colocalization of selected markers or positive brown staining in near proximity to a nucleus, as shown in Figure 1. MHC-I staining was quantified using an H score (1 × [% area low intensity] + 2 × [% area moderate intensity] + 3 × [% area high intensity]).

2.5. RNA-derived mutational load

Total RNA-seq data were available for a subset of NMIBC (n = 135) and MIBC (n = 32) patients. The RNA-based mutational calling has been described previously [11,20].
2.6. Quantification and statistical analysis

Statistical comparisons between groups were performed using Fisher’s exact test, Kruskal-Wallis test, or Wilcoxon rank sum test. The log-rank test was used to compare survival curves. Maximally selected rank statistics and receiver operating characteristic (ROC) statistics were used to estimate the optimal cutoff for time-to-event endpoints and treatment response, respectively. The prognostic potential of individual markers was analyzed by univariate and multivariable Cox regression analyses. For multivariable testing, all clinicopathological parameters significant in the univariate analysis were included. We performed ROC and time-dependent ROC statistics, and compared areas under the curve (AUCs) using DeLong test and inverse probability of censoring weighting, respectively. Significance levels were adjusted for multiple testing using the Bonferroni method ($p_{adjust}^*$). All $p$ values below 0.05 were considered significant. Data analysis was performed using R version 3.6.1 (RStudio Team, 2019).
**Fig. 2 – Immune infiltration associated with stage, grade and cell-intrinsic features.** Only patients with available cell counts for all immune cell types and > 200 cells counted within the specific area of interest were included in this analysis. (A) Overall immune cell fraction compared to tumor stage (B) Total immune cell fraction for the carcinoma cell region of interest (CROI) compared to tumor grade in non-muscle invasive bladder cancer (NMIBC). (C) Overall immune cell fraction compared to tumor grade in non-muscle invasive bladder cancer (NMIBC). (D-E) CROI MHC-I and PD-L1 cell expressions association with immune cell infiltration in NMIBC and muscle invasive bladder cancer (MIBC). (F) RNA-derived tumor mutation burden (TMB) according to the total immune cell infiltration in NMIBC. (G) Distribution of CK5/6 and GATA3 positive cells within the CROI stratified by the most dominant staining pattern and tumor stage. (H) Comparison between the differentiation features and consensus gene expression subtypes in NMIBC (n = 135) and MIBC (n = 32). (I-J) Differentiation features and immune cell infiltration in NMIBC and MIBC. For boxplots, the centerline represents the median, box hinges represent the first and third quartiles, whiskers represent ±1.5 * interquartile range (IQR) and points represent outliers. P values were calculated using the Kruskal-Wallis test or the Wilcoxon rank sum test for continuous variables and Fisher’s exact test for categorical variables.
3. Results

3.1. Clinicopathological data and staining results

A total of 785 patients were included in this retrospective study: 220 with NMIBC (pTa-T1), 441 with localized MIBC (pT2-T4a), and 124 with advanced BC (T4b, N+, or M+). Summarized clinical and histopathological information is available in Supplementary Table 2. An overview of the included cohort, study design, and representative staining results is shown in Figure 1.

3.2. Overall immune infiltration association with histopathological and cancer cell-intrinsic features

We first investigated the overall degree of immune cell infiltration and cancer cell-intrinsic markers in 541 patients with available staining results from both immune panels. Overall, immune infiltration increased significantly with tumor stage (both regions, \( p < 0.001 \); carcinoma cell region, CROI, \( p < 0.001 \); Fig. 2A and 2B) and grade (NMIBC only, \( p = 0.025 \); Fig. 2C, and Supplementary Fig. 1A and 1B).

For NMIBC and MIBC, overall high (>median) immune infiltration was associated with high MHC-I and PD-L1 carcinoma cell expression (MHC-I: \( p < 0.001 \); PD-L1: \( p < 0.001 \); Fig. 2D and 2E, and Supplementary Fig. 2A–H). Furthermore, RNA-seq–derived TMB data were available for a subset of NMIBC (\( n = 135 \)) cases, and we found that tumors with high immune infiltration within the carcinoma cell part had a significantly higher TMB (\( p = 0.018 \); Fig. 2F, and Supplementary Fig. 2I and 2J).

We also investigated differentiation features in NMIBC and MIBC defined by the most dominant GKS/6 and GATA3 staining patterns (Fig. 2G and the Supplementary material). NMIBC cases were almost exclusively luminal (72%) or double positive (24%), whereas 50% of MIBC cases were luminal, 17% basal, 16% double positive, and 16% double negative. As described in the study of Lindskrog et al [11], the double positive differentiation feature in NMIBC was associated with transcriptomic UROMOL 21 class 3 (Fig. 2H). Furthermore, we observed a partial overlap between the four differentiation features in MIBC and the consensus molecular subtypes (74% luminal/double positive tumors were classified as luminal papillary or luminal unstable, and 80% of basal tumors were classified as Ba/Sq) [10]. We observed no significant difference in overall immune cell infiltration across differentiation features (NMIBC: \( p = 0.26 \); MIBC: \( p = 0.21 \), Fig. 2I and 2J, and Supplementary Fig. 2K–N).

3.3. Specific immune subsets among different stages and differentiation features

Next, we explored immune cell subpopulations across tumor stage and differentiation features (Fig. 3A). Generally,
we observed that Ta and T1 tumors were characterized by lower infiltration of the different subsets of immune cells and low PD-L1, PD-1, and MHC expression compared with higher-stage disease (Fig. 3B). Only infiltration with CTLs were found to be significantly higher in Ta tumors than in higher-disease stages.

In NMIBC, the two major differentiation features (luminal and double positive) showed similar immune composition and higher-disease stages.

Fig. 4 – Immune subsets and cell-intrinsic features predict clinical outcomes in NMIBC. (A) Forest plot based on univariate Cox regression analyses for recurrence (black) and progression (blue) in NMIBC. The different immune cell subsets and immune evasion markers are stratified by optimized cut-offs (Supplementary Table 3-4). Dots represent hazard ratios (HR) and lines the corresponding 95% confidence intervals (95% CI). Multivariable Cox regression analyses were performed separately for each variable together with the European Association of Urology (EAU) risk score and the variable BCG treatment either before or after the analyzed tumor. P values were calculated by the Wald test. The multivariable p values (p.adjust) were corrected by the Bonferroni correction (p.adjust*). Luminal = CK5/6-GATA3+, Double positive = CK5/6+GATA3+. (B-C) Time dependent receiver operating characteristic (ROC) curves censored at 24 months for different immune cell subsets and immune evasion markers are stratified by optimized cut-offs (Supplementary Table 3-4). Dots represent hazard ratios (HR) and lines the corresponding 95% confidence intervals (95% CI). Multivariable Cox regression analyses were performed separately for each variable together with the European Association of Urology (EAU) risk score and the variable BCG treatment either before or after the analyzed tumor. P values were calculated by the Wald test. The multivariable p values (p.adjust) were corrected by the Bonferroni correction (p.adjust*). Luminal = CK5/6-GATA3+, Double positive = CK5/6+GATA3+.
Fig. 5 – Immune subsets and cell-intrinsic features predict clinical outcomes in MIBC. (A) Forest plot based on univariate Cox regression analyses for recurrence (black) and logistic regression for response to first-line chemotherapy (blue) in muscle invasive bladder cancer (MIBC). Different immune cell subsets and cell-intrinsic features are stratified by optimized cut-offs (Supplementary Table 5-6). Dots represent hazard ratios (HR) for recurrence and odds ratios (OR) for response. The corresponding lines are 95% confidence intervals (95% CI). Multivariable Cox regression analysis was performed separately for each variable with the clinicopathological factors significant in the univariate analysis and the date of the FFPE material (90’s/00’s/10’s).

(B) Time dependent receiver operating characteristic (ROC) curves censored at 24 months for different Cox regression models predicting recurrence (n=307). The two strongest predictors in the multivariable analysis (Fig.5A) are included. P values are calculated by the Wald test for Cox regression and the Students test for logistic regression. The multivariable analyses were performed separately for each variable with the clinicopathological factors significant in the univariate analysis and the date of the FFPE material (90’s/00’s/10’s).

(C) ROC curves for different logistic regression models predicting response to first-line chemotherapy (n=152). The strongest predictor in the multivariable analysis (Fig.5A) is included together with the differentiation marker variable (Basal/Not Basal). P values are calculated by the DeLong test and test if the AUC changes significantly when an additional variable is included in the model. *p<0.05, **p<0.01, *NS=not significant (>0.05); yr inc. = years increment; M/F = males/females; aBC = advanced bladder cancer; C-index = concordance index; PS = performance status.
3.4. Prognostic role of immune subtypes in NMIBC and MIBC

For a subset of MIBC patients, we have previously reported that immune infiltrated (high infiltration into the CROI) and immune excluded (immune cells restricted to the SROI) tumors were associated with a better response to first-line chemotherapy than immune desert (few immune cells present in both regions) tumors [20]. No natural clustering into these immune subtypes was observed in either NMIBC or MIBC in this study (Supplementary Fig. 4A and 4B). Immune subtypes were not prognostic in NMIBC (Supplementary Fig. 4C and 4D). However, recurrence-free survival was significantly improved in MIBC patients with infiltrated tumors compared with the other subtypes (p = 0.019; Supplementary Fig. 4E).

We also identified subtypes based on hierarchical clustering of immune cell subsets and immune evasion markers. The resulting clusters had no prognostic value in NMIBC (p = 0.24–0.93; Supplementary Fig. 5). However, for MIBC, Kaplan-Meier survival analysis indicated improved survival for patients with clusters 2 and 4, enriched with immune cells and high PD-L1 expression (p = 0.003; Supplementary Fig. 6A–C). Furthermore, we observed the lowest response rate to first-line chemotherapy in cluster 3, characterized by tumors with low immune infiltration (overall difference, p = 0.3; Supplementary Fig. 6D).

3.5. Immune subpopulations and cell-intrinsic features predicting clinical outcomes in NMIBC

First, we assessed whether the overall immune cell infiltration could predict clinical outcomes for patients with NMIBC. However, overall immune infiltration had no significant prognostic value in NMIBC (Fig. 4A, and Supplementary Tables 3 and 4). We then asked whether the different subsets of immune cells and cell-intrinsic features have prognostic value in NMIBC. For that, we estimated optimal cutoff values for predicting recurrence and progression (Supplementary Tables 3 and 4), and performed a univariate and a multivariable Cox regression analysis for each candidate biomarker. For multivariable testing, we adjusted for the currently applied risk assessment tool by the European Association of Urology (EAU) and BCG treatment either before or after the analyzed tumor (Fig. 4A).

High infiltration of the different subsets of T lymphocytes (T helper cells, CTLs, and Tregs) was independently associated with a low risk of recurrence compared with low infiltration. This was most evident for CTLs (CROI: p = 0.015; SROI: p = 0.0007; Fig. 4A, and Supplementary Tables 3 and 4). High infiltration of CTLs within the carcinoma cell region was also associated with a low risk of progression (CROI: p = 0.036; SROI: p = 0.28).

The strongest independent predictor of recurrence was high PD-1 expression within the stromal region (CROI: p = 0.004; SROI: p = 0.0001); high expression of PD-L1 within the carcinoma cell area was likewise associated with an increased risk of recurrence (CROI: p = 0.0081; SROI: p = 0.029). We also observed that high expression of PD-1 and PD-L1 was independently associated with a higher risk of progression (PD-1: CROI: p = 0.004; SROI: p = 0.012; PD-L1: CROI: p = 0.0002; SROI: p = 0.23).

We also studied the prognostic value of differentiation features and found that luminal tumors (GATA3’CK5/C6K) had a lower risk of progression than double positive tumors (GATA3’CK5/C6K; p = 0.004; Fig. 4A).

To test whether the EAU risk score combined with our most promising predictors of recurrence and progression could improve the prediction compared with EAU risk score alone, we performed time-dependent ROC analyses. Neither stromal PD-1 expression nor stromal CTL infiltration improved the prediction accuracy for recurrence (PD1: p = 0.22; CTLs: p = 0.26; Fig. 4B and Supplementary Fig. 7). For progression, addition of PD-1 or PD-L1 expression in the carcinoma cell region improved the prediction accuracy (PD-1: p = 0.020; PD-L1: p = 0.021; Fig. 4C and Supplementary Fig. 7) compared with the EAU risk score alone. However, a model containing both PD-1 and PD-L1 did not significantly improve the prediction further (p = 0.21; Fig. 4C and Supplementary Fig. 7).

3.6. Immune subpopulations and cell-intrinsic features predicting clinical outcomes in MIBC

In a similar manner as for NMIBC, we analyzed the clinical value of the overall immune cell infiltration, immune cell subsets, and cancer cell-intrinsic features in MIBC (Supplementary Tables 5 and 6). Here, we assessed whether these have any prognostic or predictive value independent of clinicopathological factors (Fig. 5A). We also adjusted for sample age, as we observed a significant difference in the overall degree of immune cell infiltration in MIBC in relation to the age of the formalin-fixed paraffin-embedded (FFPE) material (p < 0.001; Supplementary Fig. 8).

We observed that high overall immune cell infiltration within the carcinoma cell region was independently associated with a lower risk of recurrence than low infiltration (CROI: p = 0.038 SROI: p = 0.6; Supplementary Table 5 and 6), whereas high stromal immune cell infiltration was independently associated with a better response to first-line chemotherapy (CROI: p = 0.77; SROI: p = 0.044).

A high proportion of T helper cells was independently associated with a lower risk of recurrence (CROI: p = 0.0098; SROI: p = 0.018). High infiltration of CTLs within the carcinoma cell region was also associated with a low risk of recurrence (CROI: p = 0.015; SROI: p = 0.17). On the contrary, high infiltration of stromal M2 was independently associated with an increased risk of recurrence (CROI: p = 0.013; SROI: p = 0.005). Interestingly, we observed that high stromal infiltration of different immune cell subsets (T helper cells, CTLs, M1, and M2) was associated with a better response to first-line chemotherapy (p = 0.024–0.0096).
PD-1 expression in both regions showed a strong association with clinical outcome; high expression was associated with a lower risk of recurrence compared with low expression (CROI: \( p \text{adjust}^* = 0.0015; \) SROI: \( p \text{adjust}^* = 0.0021; \) Fig. 5A, and Supplementary Tables 5 and 6). As previously described for this cohort [20], high PD-1 expression was associated with a high probability of chemotherapy response (CROI: \( p \text{adjust}^* = 0.022; \) SROI: \( p \text{adjust}^* = 0.0096). High expression of PD-L1 was also associated with a lower risk of recurrence (CROI: \( p \text{adjust}^* = 0.0011; \) SROI: \( p \text{adjust}^* = 0.002). Importantly, these results hold true when disease-specific survival is selected as an endpoint for both PD-1 (CROI: \( p \text{adjust}^* = 0.019; \) SROI: \( p \text{adjust}^* = 0.046) and PD-L1 (CROI: \( p \text{adjust}^* < 0.001; \) SROI: \( p \text{adjust}^* = 0.068; \) Supplementary Fig. 9).

In accordance with our previous study assessing gene expression consensus subtypes [20], we observed that basal tumors have a poor response to first-line chemotherapy compared with the other differentiation features (\( p \text{adjust} = 0.0075; \) Fig. 5A).

An analysis of the two strongest independent predictors of recurrence, PD-1 and PD-L1 expression within the carcinoma cell region, showed that high PD-L1 expression improved the predictive accuracy compared with N-stage alone (PD-L1: \( p = 0.0023; \) PD-1: \( p = 0.26; \) Fig. 5B and Supplementary Fig. 10). Inclusion of both variables in the model did not provide additional value (\( p = 0.082). Finally, we assessed whether basal differentiation compared with the strongest immune marker for predicting response to first-line chemotherapy, PD-1 expression in the carcinoma cell region, could improve the AUC compared with clinicopathological factors alone. Both markers improved the AUC, but not significantly (PD-1 CROI: \( p = 0.21; \) basal: \( p = 0.19; \) Fig. 5C). It should be noted that the ROC analyses are based on fewer patients than the odds ratios (Fig. 5A) because of the need of a complete overlap between all variables in the ROC analysis (152 vs 163 patients).

4. Discussion

Here, we present a large-scale study exploring the clinical impact of the immune contexture and cancer cell-intrinsic features in BC. Consistent with previous findings, we observed that immune infiltration in BC is highly stage dependent [23]. We observed a significant increase in immune cell infiltration within the carcinoma cell region across stages. One explanation for the migration of immune cells into the tumor core concurrently with infiltration depth could be the much higher TMB found in MIBC than in NMIBC [24,25], resulting in a higher neoantigen burden, ultimately making MIBC more immunogenic.

In NMIBC, a high proportion of the different T-cell subsets (T helper cells, CTLs, and Tregs) were independently associated with a low risk of recurrence, consistent with previous reports [12], whereas only high CTL infiltration within the carcinoma cell region was independently associated with a low risk of progression. For MIBC, high infiltration with T helper cells in both regions and CTLs within the carcinoma cell region were independent prognostic markers of recurrence. Tregs favor a protumorigenic immune response and have been associated with a poor prognosis in several cancer types [26]. This paradoxical prognostic effect of Tregs observed in NMIBC has been described by others [19,27,28], and may be explained by a Treg-mediated downregulation of matrix metalloproteinase 2 [27]. Taken together, our data might suggest that infiltration of T-cell subsets have a greater prognostic potential in NMIBC than in MIBC. Recent studies have focused on exhausted and dysfunctional T cells in cancer, which may explain why T cells are not as effective in later stages [29]. Interactions with immunosuppressive cells, such as M2 macrophages and Tregs, are considered important factors mediating T-cell dysfunction [29]. In our study, high stromal M2 infiltration was independently associated with a high risk of recurrence in MIBC, in line with previous findings [12,30,31].

Another known mechanism of T cell dysfunction is PD-1/PD-L1 interactions [29]. High expression of PD-L1 and its counterpart PD-1 is generally considered to be an indicator of a poor prognosis in both NMIBC and MIBC [6,32,33], but positive correlations with prognosis have also been reported [14,28]. This discrepancy can partly be attributed to the large variability observed between antibodies (especially PD-L1) and nonstandardized cutoff values [34,35]. In this study, high PD-1 and PD-L1 expression was associated with an increased risk of progression and recurrence in NMIBC. In MIBC, we observed an opposite trend, where high expression of PD-1 and PD-L1 correlated with a lower risk of recurrence. It seems counterintuitive that high PD-1 and PD-L1 expression is associated with a favorable prognosis in MIBC, and the biology underlying this needs to be explored in future studies.

Accumulating evidence suggests that the composition of the immune landscape in tumors may contribute to the cytotoxic effects of chemotherapy [36,37]. In BC, the ratio of CD8+ to FOXP3+ has been linked to a neoadjuvant chemotherapy response in a small study of 41 patients [38]. In our study, we observed that high stromal immune infiltration of both T helper cells, CTLs, and macrophages (M1 and M2) was associated with a better response to first-line chemotherapy. We also observed that basal tumors are less likely to respond to first-line chemotherapy as recently reported [20,39]. Others have reported that luminal tumors and immune-infiltrative basal tumors respond better to neoadjuvant chemotherapy in MIBC [40]. In our cohort, basal tumors were characterized by high infiltration of CTLs within the carcinoma cell part, and also a high level of immunosuppressive PD-L1-positive cells. Thus, T-cell dysfunction in our advanced cohort may explain the discrepancy. Additional studies are needed to fully establish the predictive value of the basal differentiation feature.

Multiple studies have investigated the immune landscape in BC, however using mainly single-marker immunohistochemistry and analog quantification, or not including spatial organization of immune cells. Our study is limited by the retrospective study design, use of TMAs, and potential batch-to-batch effect. Finally, it should be noted that our approach does not identify the cell types that expressed PD-1 and PD-L1.
5. Conclusions

Our results highlight several cancer cell-intrinsic and cell-extrinsic features associated with clinical outcomes in BC, and importantly we show that our most promising markers (PD-1 and PD-L1) combined with clinical risk factors improved the prediction accuracy compared with clinical factors alone. If validated by additional studies, this could potentially aid future clinical management.

Author contributions: Lars Dyrskjøt had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Taber, Prip, Steiniche, Dyrskjøt.

Acquisition of data: Taber, Prip, Agerhåæk, Jensen, Steiniche.

Analysis and interpretation of data: Taber, Prip, Lamy, Dyrskjøt.

Drafting of the manuscript: Taber, Prip, Dyrskjøt.

Critical revision of the manuscript for important intellectual content: Taber, Prip, Lamy, Agerhåæk, Jensen, Steiniche, Dyrskjøt.

Statistical analysis: Taber, Prip, Lamy.

Obtaining funding: Taber, Prip, Dyrskjøt.

Administrative, technical, or material support: Taber, Prip, Steiniche, Dyrskjøt.

Supervision: Dyrskjøt.

Other: None.

Financial disclosures: Lars Dyrskjøt certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Lars Dyrskjøt has sponsored research agreements with C2i, AstraZeneca, Natera, Photocure, and Ferrin; has an advisory/consulting role at Ferrin; has sponsored research agreements with Medac, Photocure ASA, Cepheid, and Ferrin.

Funding/Support and role of the sponsor: This work was funded by the Novo Nordisk Foundation (NNF17OC0024464), Independent Research Fund Denmark (7016-003659b), Aarhus University, the Danish Cancer Biobank, KV Fondev, Toyota-Fonden (Denmark), and Beckett-Fonden.

Acknowledgments: We thank Jeanette Børger Georgsen and Kristina Lynstlund Lauridsen for technical assistance regarding the proteomic analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euo.2022.01.008.

References


