Association of an immune gene signature with pathologic response and outcome after neoadjuvant pembrolizumab, compared to neoadjuvant chemotherapy, in muscle-invasive bladder cancer

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BACKGROUND

- The PURE-01 study (NCT02736266) evaluated the use of pembrolizumab before radical cystectomy (RC) in muscle-invasive bladder cancer (MIBC).
- Preliminary biomarker results suggested that there were opportunities for discriminating those patients who might be treated with a single-agent immunotherapy approach instead of standard-of-care therapy, namely cisplatin-based neoadjuvant chemotherapy (NAC) in cisplatin-fit patients or immediate RC for cisplatin-ineligible patients.[1,2]
- The ability to accurately select those patients who are most likely to benefit from a neoadjuvant single-agent checkpoint inhibitor instead of NAC, or both in combination, will be important to delineate future treatment strategies.

STUDY OBJECTIVE

To evaluate the ability of immunotherapy-related biomarkers to predict pathological complete response (CR: ypT0N0) and relapse-free survival (RFS) in pembrolizumab versus NAC-treated patients presenting with comparable clinical features.

METHODOLOGY

Patient Populations

Specimen collection and sample processing for PURE-01 were conducted using Decipher® (Decipher Biosciences Laboratory, San Diego, CA, USA), a clinical-grade whole-transcriptome assay.[3,4] Data generation for the NAC cohort has been described.[3] Cohort details are provided in **Table 1**.

Molecular Subtyping and Gene Expression Signatures

The TCGA and Consensus classifiers were downloaded from GitHub.[5] The Genomic Subtypes Classification (GSC) subtypes were assigned by first identifying neuroendocrine (NE)-like patients[6] and then classifying the remaining tumors using the Seiler 2017 model.[3] The Immune190 signature score calculations have been previously described.[4,7] The interferon gamma (IFN-γ), interferon alpha (IFN-α) and inflammatory response signatures are part of the hallmark gene sets (MSigDB).[8]

Statistical Analysis

The primary endpoint of the study was pathological complete response (CR: ypT0N0). Partial response (PR: ypTa/is/1N0) and recurrence-free survival (RFS) were secondary endpoints. All outcome data was censored at 24 months of follow-up. Univariable and multivariable logistic regression analyses were used to evaluate the association of pre-specified clinical factors (cT-stage, gender, age, smoking status) and immune signature scores with pathological CR. Analyses were performed in R v3.4.1 (R Foundation, Vienna, Austria).

Table 1. Clinical characteristics of the PURE-01 and NAC cohorts

Variables		PURE-01 n (%)	NAC n (%)
Total		84	140
Age	Median (IQR)	68 (62-74)	62 (54-70)
Sex	Male		
	Female	12 (14)	37 (26)
Cisplatin-ineligible*		8 (9.5)	0
Smoking Status	Non smoker	23 (23)	0
	Current smoker	22 (26)	0
	Former smoker	39 (46)	0
	Unavailable	0 (0)	140 (100)
Histology	Pure UC	61 (72.6)	n.a.
	UC + variant histology	23 (27.4)	n.a.
Clinical T-stage	cT2	40 (47.6)	65 (46)
	cT3	43 (51)	50 (36)
	cT4	1 (1)	25 (18)
Pathological T-stage	pT0	31 (37)	55 (39.3)
	pTa/pTis	13 (15.5)	8 (5.7)
	pT1	3 (3.6)	8 (5.7)
	pT2	10 (12)	26 (18.6)
	pT3	21 (25)	30 (21.4)
	pT4	1 (1.2)	12 (8.6)
	Unavailable	5 (6)	1 (0.7)
Number of nodes removed	Median (IQR)	27 (21-34)	20 (13-27)
	No	62 (74)	99 (71)
	Yes	13 (15.5)	16 (11.4)
pN+	Unavailable	9 (11)	25 (18)

Abbreviations: IQR: interquartile range; n.a.: not available; NAC: neoadjuvant chemotherapy.
*according to Galsky criteria for the PURE-01 cohort; based on investigator decision in the NAC cohort.

RESULTS

Molecular subtypes are not associated with pathological response to pembrolizumab

Molecular subtyping was used to classify the PURE-01 cohort into basal squamous (Ba/Sq, n=26), luminal non specified (LumNS, n=14), luminal papillary (LumP, n=16), luminal unstable (LumU, n=19), Stroma-rich (n=6), and NE-like (n=3) subtypes according to the Consensus model, with similar patterns for the TCGA and GSC models (**Figure 1**).

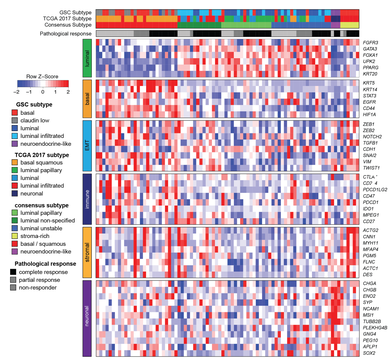

Figure 1: Molecular subtyping and biological characterization of the PURE-01 cohort (n=84) using selected MIBC marker genes. The Consensus, TCGA and GSC are indicated in the covariate tracks. A covariate for pathological response defined as complete (ypT0N0), partial (ypT$_{a/is/1}$N0) or non-responder (≥ypT2) is also provided.

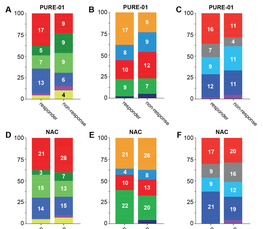

Figure 2: Pathological response rates according to molecular subtype for the PURE-01 cohort (n=84): (A) Consensus, (B) TCGA, and (C) GSC and the NAC cohort (n=126): (D) Consensus, (E) TCGA, and (F) GSC, respectively. Response is dichotomized as complete (ypT0N0) plus partial (ypT$_{a/is/1}$N0) response versus non-response (≥ypT2) on radical cystectomy. The number of cases for each subtype are indicated in white text.

The rate of major responses was not significantly associated (p>0.2 for all tests) with molecular subtypes in either the PURE-01 or NAC cohorts (**Figure 2**). However, the LumU tumors from the Consensus model had the highest response rate of 68.4%. In PURE-01, the Ba/Sq (Consensus) and Basal squamous (TCGA) tumors had favorable major response rates of 65.4% for both the Consensus and TCGA, and 7/11 (63.6%) of GSC Claudin-low tumors showed a complete or major response. In the NAC cohort, there was no association between subtype and pathological response, which was consistent with the original NAC study.[3]

Immune molecular subtypes are associated with favorable RFS

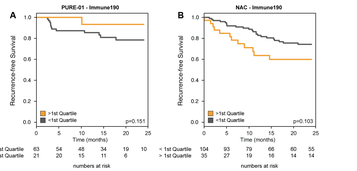

Figure 4: Kaplan-Meier curves for the association of the immune190 signature with RFS in (A) PURE-01 and (B) NAC cohorts.

In PURE-01, patients with basal-type tumors or higher immune activity obtained more major pathological responses (CR+PR). We next explored the RFS endpoint as a function of molecular subtype in both PURE-01 and NAC (**Figure 5**). Here, we observed NE-like tumors had the worst outcome in both groups (33% and 0% 2-year RFS for PURE-01 and NAC, respectively), whereas Claudin-low tumors had the best RFS outcome with pembrolizumab (no recurrences within 2-y) compared to NAC. The remaining subtypes had RFS ranging from 53-89% for the Consensus model, 75-86% in TCGA, and 74-92% in the GSC (**Figure 5**).

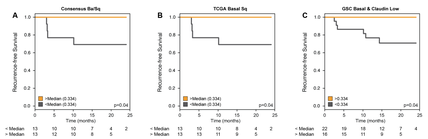

Figure 6: RFS in Basal-type tumors across the (A) Consensus classifier, (B) TCGA and (C) GSC classifiers, split according to the median values of Immune190 signature.

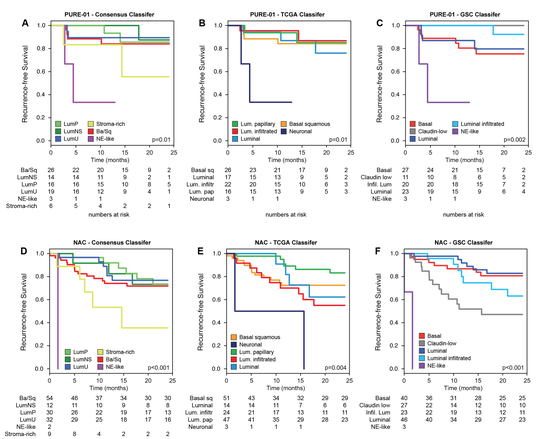

Figure 5: RFS by molecular subtype. For PURE-01 (A) Consensus, (B) TCGA and (C) GSC. For NAC (D) Consensus, (E) TCGA and (F) GSC. The number of events for each subtype is shown in brackets. Log-rank p-values and the number of patients at risk are indicated. *1 patient had missing RFS information in NAC cohort.

Immune signatures are highly associated with pathological response to pembrolizumab but not to NAC

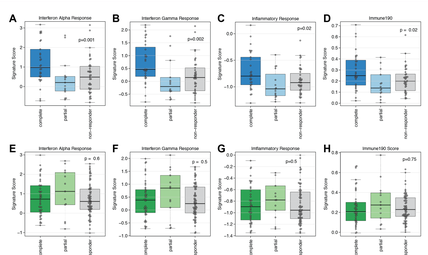

Figure 3: Association of immune signatures with pathological response (complete: ypT0N0; partial: ypT$_{a/is/1}$N0; non-response: ≥ypT2). For PURE-01 (A) Interferon alpha response, (B) Interferon gamma response, (C) Inflammatory response and (D) Immune190 signature score. For NAC (E) Interferon alpha response, (F) Interferon gamma response, (G) Inflammatory response and (H) Immune190 signature score.

Three immune-associated signatures showed similar distributions to Immune190 across the molecular subtypes in both cohorts and were significantly associated with pathological response in PURE-01 (**Figure 3A-D**). Significance was retained on multivariable analyses for all four signatures, by adjusting for sex and cT-stage (**Table 2**). On univariable analyses, higher TMB was not associated with a better response (defined as CR; p=0.06). None of the four immune signatures were significant for pathological response in the NAC cohort (**Figure 3E-H**).

Table 2. Univariable and multivariable logistic regression analyses on the association with ypT0N0 in the PURE-01 cohort.

Signature	Variable Name	Unadjusted OR (95% CI)	p-value*	Adjusted OR (95% CI)	p-value*
Immune190	Sex (Male)	1.13 (0.32-4.57)	0.9	1.64 (0.41-7.84)	0.51
	cT-stage (2 vs 3-4)	0.29 (0.1-0.74)	0.01	0.3 (0.11-0.79)	0.02
	Signature (continuous)	1.53 (1.1-2.2)	0.01	1.51 (1.09-2.17)	0.02
IFNγ	Sex (Male)	1.13 (0.32-4.57)	0.9	1.81 (0.43-9.25)	0.44
	cT-stage (2 vs 3-4)	0.29 (0.1-0.74)	0.01	0.3 (0.1-0.79)	0.02
	Signature (continuous)	1.1 (1.04-1.18)	0.003	1.11 (1.04-1.19)	0.004
IFNα	Sex (Male)	1.13 (0.32-4.57)	0.9	1.74 (0.42-8.68)	0.47
	cT-stage (2 vs 3-4)	0.29 (0.1-0.74)	0.01	0.29 (0.1-0.76)	0.02
	Signature (continuous)	1.07 (1.02-1.13)	0.004	1.07 (1.02-1.13)	0.006
Inflammatory	Sex (Male)	1.13 (0.32-4.57)	0.9	1.74 (0.42-8.65)	0.47
	cT-stage (2 vs 3-4)	0.29 (0.1-0.74)	0.01	0.3 (0.11-0.8)	0.02
	Signature (continuous)	1.23 (1.06-1.46)	0.009	1.23 (1.05-1.46)	0.01

Abbreviations: CI: confidence interval; F: female; M: male; OR: odds ratio.
*2-sided Wald test p-value.

CONCLUSIONS

- Transcriptome profiling revealed that tumors with higher levels of pre-existing immune infiltration had a favorable clinical response to neoadjuvant pembrolizumab, but not to platinum-based NAC.
- Pending the results of the ongoing randomized studies, this is a first step towards the incorporation of selected molecular subtypes or immune signatures into the next clinical trials to help guide patient selection for immune checkpoint blockade.

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