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# Target RNA expression by tumor molecular subtypes in patients with advanced urothelial carcinoma or muscle invasive bladder cancer: exploratory analyses from JAVELIN Bladder 100 and the Tempus database

M. Eckstein,<sup>1</sup> N. Klümper,<sup>2</sup> P. Grivas,<sup>3</sup> E. Grande,<sup>4</sup> J. Brägelmann,<sup>5</sup> J. Hoffman,<sup>6</sup> J. Mazur,<sup>7</sup> O. Bogatyrova,<sup>7</sup> V. Grünwald<sup>8</sup>

<sup>1</sup>University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; <sup>2</sup>University Hospital Bonn, Bonn, Germany; <sup>3</sup>University of Washington, Fred Hutchinson Cancer Center, Seattle, WA, USA; <sup>4</sup>Department of Medical Oncology, MD Anderson Cancer Center, Madrid, Spain; <sup>5</sup>University of Cologne and University Hospital Cologne, Cologne, Germany; <sup>6</sup>EMD Serono Research & Development Institute, Inc., Billerica, MA, USA, an affiliate of Merck KGaA; <sup>7</sup>Merck Healthcare KGaA, Darmstadt, Germany; <sup>8</sup>University Hospital Essen, Essen, Germany

## CONCLUSIONS

- We report exploratory analyses of RNA expression for actionable molecular targets by molecular subtype in patients with advanced urothelial carcinoma (UC) or muscle-invasive bladder cancer (MIBC) from the JAVELIN Bladder 100 phase 3 trial and Tempus real-world database
- In both the clinical trial and real-world cohorts:
  - NECTIN4, HER2, HER3, and TROP2 RNA expression was heterogeneous across molecular UC subtypes and was highest in luminal subtypes
  - EGFR RNA expression was highest in the basal/squamous (Ba/Sq) subtype, which was the most frequent subtype in the Tempus cohort
  - A strong correlation was observed for RNA expression of NECTIN4 vs HER2, HER3, and TROP2
- Studies to further assess the activity of novel therapies, relevant targets, and biomarkers in consensus molecular subtypes or histological subtypes may be warranted to individualize treatment and optimize clinical outcomes in patients with advanced UC

## PLAIN LANGUAGE SUMMARY

- Previously, researchers found that urothelial cancers can be sorted into groups using levels of different molecules. These groups are called molecular subtypes
- Nectin-4, HER2, HER3, EGFR, and TROP2 are proteins that are often found in urothelial cancer cells
  - These proteins are targets for different treatments that are being studied in people with urothelial cancer
- In this study, researchers looked at levels of signals that tell cells to make these proteins, called messenger RNA, in different molecular subtypes of urothelial cancer
- Researchers found that levels of messenger RNA for Nectin-4, HER2, HER3, EGFR, and TROP2 differed between the molecular subtypes. They also found that levels of messenger RNA for Nectin-4, HER2, HER3, and TROP2 within the same cancer were often similar
- These results may help with designing new studies to find out which treatments might work best in people with urothelial cancer based on the features of their cancer

## BACKGROUND

- Molecular targets of investigational or novel therapies for patients with UC include:
  - Nectin-4: a transmembrane protein involved in cell adhesion that is expressed at moderate to high levels in the majority of UC tumors<sup>1</sup>
  - HER2: a receptor tyrosine kinase involved in cell proliferation, survival, and mobility that is overexpressed in a subset of UC tumors and has been associated with poor prognosis<sup>2,3</sup>
  - HER3: a receptor tyrosine kinase involved in resistance to therapies targeting other HER receptors<sup>4</sup>
  - EGFR: a receptor tyrosine kinase involved in cell proliferation and survival that is overexpressed in a subset of UC tumors<sup>5,6</sup>

- TROP2: a transmembrane glycoprotein that is expressed at high levels in the majority of UC tumors<sup>7</sup>
- UC or BC tumors can be classified into 6 consensus molecular subtypes: Ba/Sq, stroma-rich (SR), luminal unstable, luminal papillary, luminal nonspecified, and neuroendocrine (NE)-like<sup>8</sup>
- Previous studies have reported that protein expression levels of molecular targets are heterogeneous across molecular subtypes of UC/BC<sup>2,3,5,7</sup>
- Limited data are available regarding RNA expression levels of molecular targets

- In the JAVELIN Bladder 100 trial, avelumab first-line (1L) maintenance + best supportive care (BSC) significantly prolonged overall survival and progression-free survival vs BSC alone in patients with advanced UC that had not progressed with 1L platinum-based chemotherapy<sup>9,10</sup>
- Here, we report exploratory analyses of RNA expression of actionable molecular targets, including NECTIN4, HER2, HER3, EGFR, and TROP2, within consensus molecular subtypes in patients with advanced UC from the JAVELIN Bladder 100 phase 3 trial and in patients with MIBC or advanced UC from the Tempus real-world database

## METHODS

- JAVELIN Bladder 100 (NCT02603432) was an international, randomized, phase 3 trial that enrolled patients with unresectable locally advanced or metastatic UC who were progression free after 4-6 cycles of 1L platinum-based chemotherapy<sup>9,10</sup>
- Patients were randomized 1:1 to receive avelumab + BSC or BSC alone until disease progression, unacceptable toxicity, or patient withdrawal
- The primary endpoint was overall survival, measured from randomization
- Tempus is a national database of deidentified patient data from US clinical practice<sup>11</sup>
- Only data from patients with advanced UC or MIBC (recorded in the Tempus database as disease stage II-IV, or T2-4, N1-3, or M1) were analyzed

- For the current analysis, whole-transcriptome profiles in available tumor samples were generated using RNA sequencing
- Transcript levels were quantified using Personalis ACE Technology (JAVELIN)<sup>12</sup> or kallisto version 0.44 (Tempus)
- Positive status for RNA expression of NECTIN4, HER2, HER3, EGFR, and TROP2 was defined using median expression of the selected, clinically relevant tumor-associated antigens (TAAs) for bladder cancer: PVRL4, ERBB2, ERBB3, EGFR, TACSTD2, CD276, AXL, PTK7, CD44, and CDH6
- For the majority of TAAs, the prevalence of TAA-high tumors was consistent with published literature<sup>13-18</sup>

## RESULTS

- The analysis population included biomarker-assessable patients from the JAVELIN Bladder 100 trial (n=560) and Tempus database (n=419)
  - In the JAVELIN Bladder 100 cohort, all patients had advanced UC
  - In the Tempus cohort, patients had either MIBC or advanced UC
- In the JAVELIN Bladder 100 cohort, the most frequent subtype was luminal (46.4%), followed by SR (36.1%) (Figure 1)
- In the Tempus cohort, the most frequent subtype was Ba/Sq (35.1%), followed by luminal (33.7%) (Figure 1)
- In both cohorts, positive RNA expression of TROP2, NECTIN4, and EGFR, based on previously reported thresholds, was observed in all subtypes at different frequencies (Figure 1)
  - Positive HER2 and HER3 RNA expression was observed most frequently in luminal subtypes
- In both the JAVELIN Bladder 100 and Tempus cohorts, NECTIN4, HER2, HER3, and TROP2 RNA expression was highest in luminal subtypes, followed by SR and Ba/Sq, and lowest in the NE-like subtype (Figure 2 and Table 1)
  - EGFR RNA expression was highest in Ba/Sq, followed by luminal and SR, and lowest in the NE-like subtype
- In both cohorts, a strong correlation (R>0.5) was observed for RNA expression of NECTIN4 vs HER2, HER3, and TROP2 (Figure 3)

Figure 1. Expression of actionable molecular targets by molecular subtype

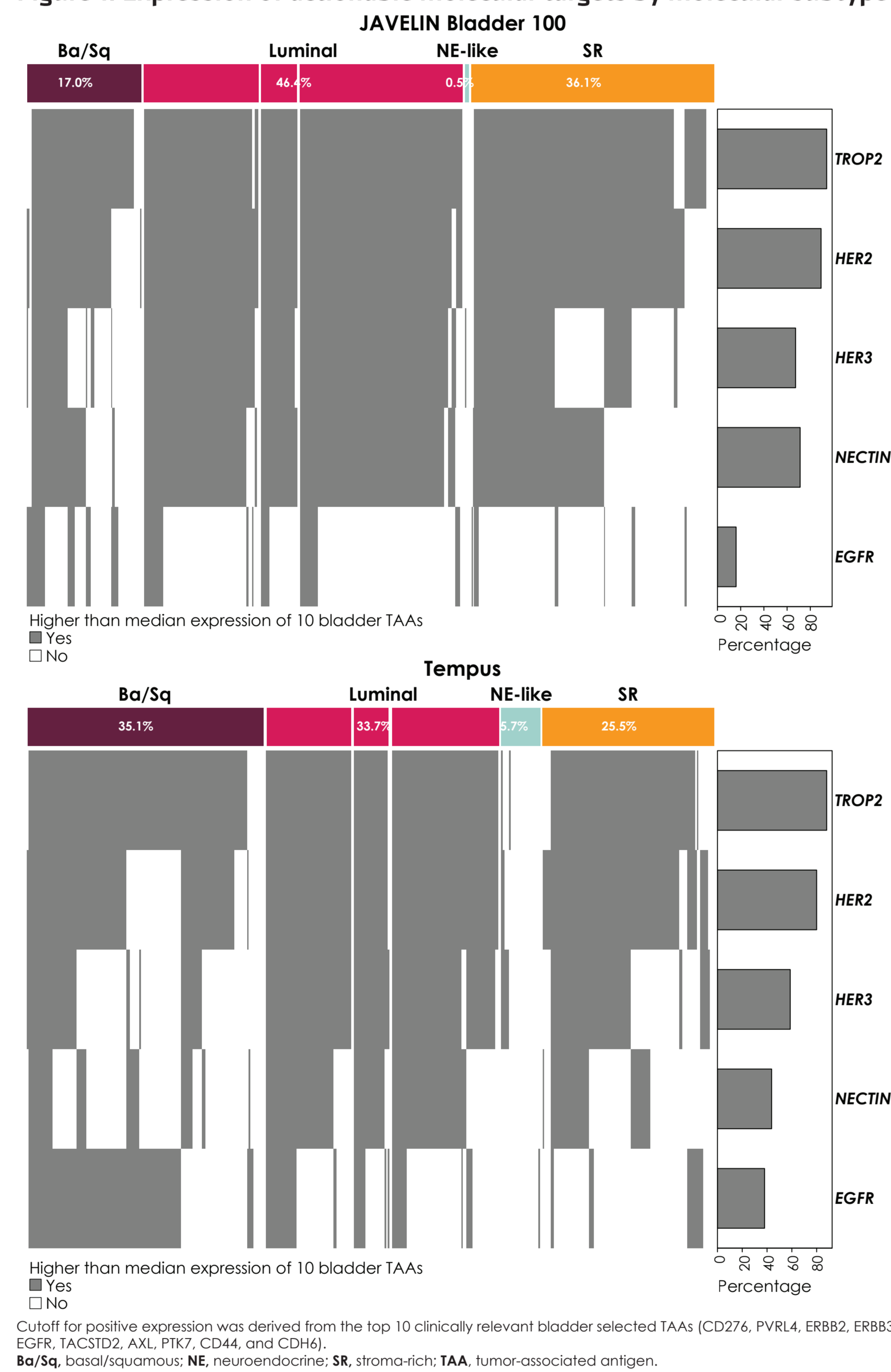


Figure 2. Molecular target expression in subgroups defined by molecular subtype

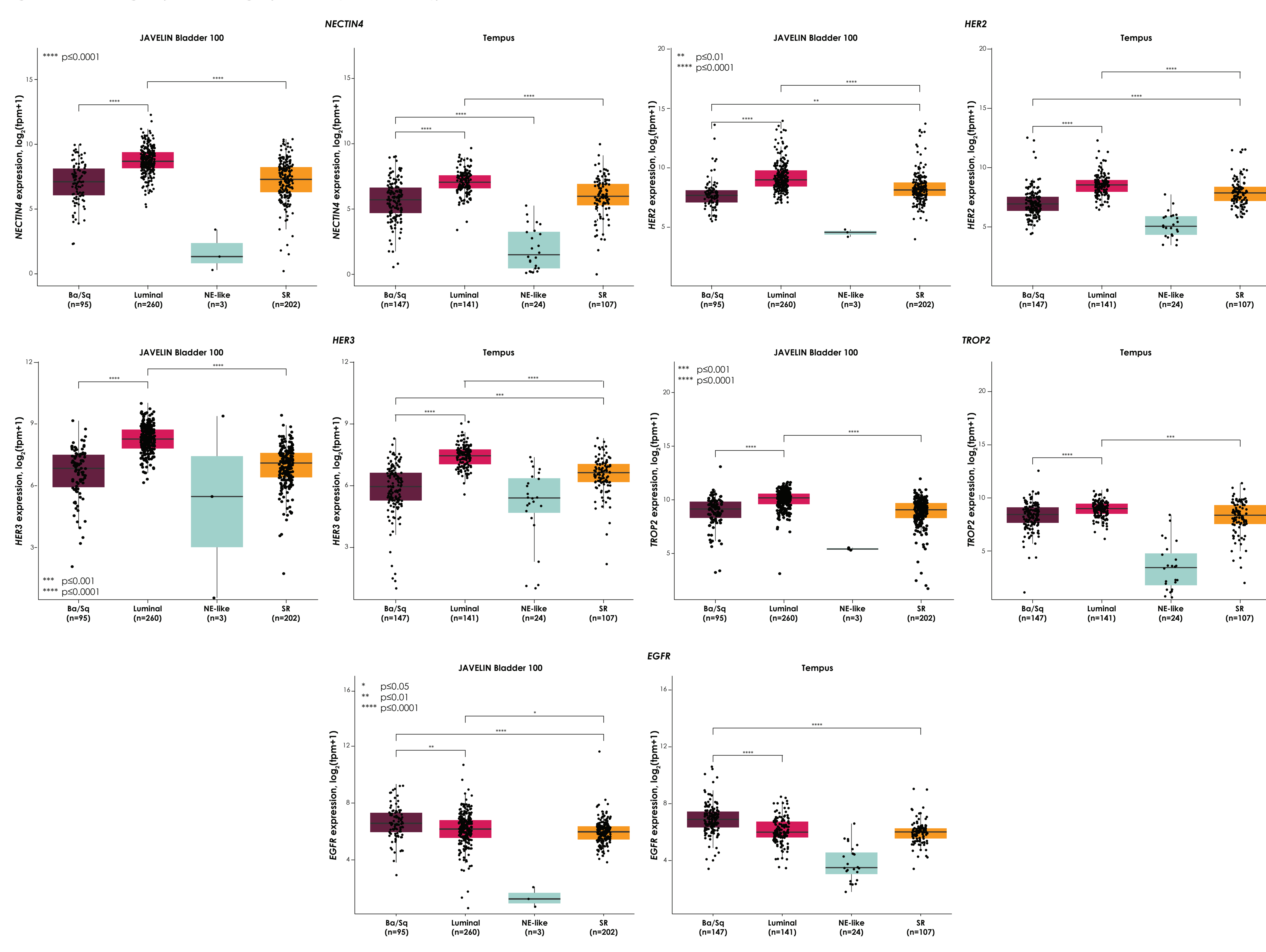


Table 1. Relative molecular target RNA expression levels in subgroups defined by molecular subtype

Molecular subtype	JAVELIN Bladder 100 (n=560)						Tempus (n=419)					
	n (%)	Log <sub>2</sub> fold change in tpm vs luminal					n (%)	Log <sub>2</sub> fold change in tpm vs luminal				
		NECTIN4	HER2	HER3	TROP2	EGFR		NECTIN4	HER2	HER3	TROP2	EGFR
Ba/Sq	95 (17.0)	-1.584	-1.353	-1.431	-1.038	0.342	147 (35.1)	-1.362	-1.610	-1.511	-0.557	0.912
Luminal*	260 (46.4)	0	0	0	0	0	141 (33.7)	0	0	0	0	0
NE-like	3 (0.5)	-8.079	-4.560	-2.821	-4.798	-5.180	24 (5.7)	-6.160	-3.526	-2.075	-5.695	-2.607
SR	202 (36.1)	-1.401	-0.888	-1.170	-1.109	-0.178	107 (25.5)	-1.088	-0.676	-0.826	-0.624	0.016

Ba/Sq, basal/squamous; NE, neuroendocrine; SR, stroma-rich; tpm, transcripts per million.

\*Reference.

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Correspondence: Markus Eckstein, markus.eckstein@uk-erlangen.de



Figure 3. Correlation of molecular target expression

