

EphA2 expression across molecular and histological subtypes in muscle-invasive bladder cancer and its association with Nectin-4 and HER2

▶ LB012

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BACKGROUND

- ▶ Despite aggressive multimodal therapy, muscle-invasive bladder cancer (MIBC) is associated with poor prognosis¹
 - MIBC can be classified into 6 consensus molecular subtypes: luminal papillary (LumP), luminal nonspecified (LumNS), luminal unstable (LumU), stroma-rich, basal/squamous (Ba/Sq), and neuroendocrine (NE)-like²
 - MIBC may also be classified by histological subtype³

THERAPEUTIC TARGETS IN MIBC

- ▶ Nectin-4- and HER2-targeting agents have shown benefit in patients with bladder cancer^{4,5}
 - Squamous differentiated histology is associated with lower levels of Nectin-4 expression and decreased efficacy with Nectin-4-targeted therapy⁶
- ▶ Targeting erythropoietin-producing hepatocellular receptor A2 (EphA2) may also be an effective treatment approach for patients with bladder cancer⁷
 - EphA2 is a member of the receptor tyrosine kinase family and is highly expressed in a variety of solid tumors^{7,8}
 - Clinical trial data for patients with variant and divergent histologies are limited,^{9,10,11} including a lack of information on EphA2 expression across MIBC subtypes⁷
 - Preliminary anti-tumor activity with nuzefatide pevedotin (nuzefa; formerly BT5528), an EphA2-targeting Bicycle[®] Drug Conjugate, has been shown in patients with advanced urothelial carcinoma, including EphA2-positive patients¹²
 - Nuzefa has high specificity for EphA2 ($K_D=1.9$ nM), demonstrating binding to this single protein from 5500+ surface protein targets in a Retrogenix membrane array¹³

OBJECTIVE

- ▶ Here, we report exploratory analyses of Nectin-4, EphA2, and HER2 expression and correlation to molecular and histological subtypes in MIBC to identify patients who may benefit from targeted therapy

METHODS

- ▶ Tumor samples were grouped by consensus molecular (based on transcriptome signature) and histologic subtype
- ▶ Membrane protein expression of EphA2, Nectin-4, and HER2, plus RNA expression, were quantified by IHC and whole-transcriptome sequencing, respectively
- ▶ For membranous EphA2 and Nectin-4 expression, the difference in H-score (H-diff) was assessed via the Kruskal-Wallis test
- ▶ HER2 status was assessed using the gastric algorithm; HER2 positive was defined as 1+/2+/3+ and negative defined as 0
- ▶ Nectin-4 membrane was assessed using H-score; Nectin-4 positive was defined as H-score ≥ 150 and negative/low defined as H-score < 150
- ▶ RNA transcript levels were quantified using Kallisto V0.44
- ▶ A tumor proportion score (TPS) >1 was used to define EphA2 positive; negative defined as TPS ≤ 1
- ▶ EphA2 staining was performed using rabbit monoclonal anti-EphA2 antibody (Cell Signaling Technology 6997); scoring was performed by a pathologist/medical examiner

ACKNOWLEDGMENTS

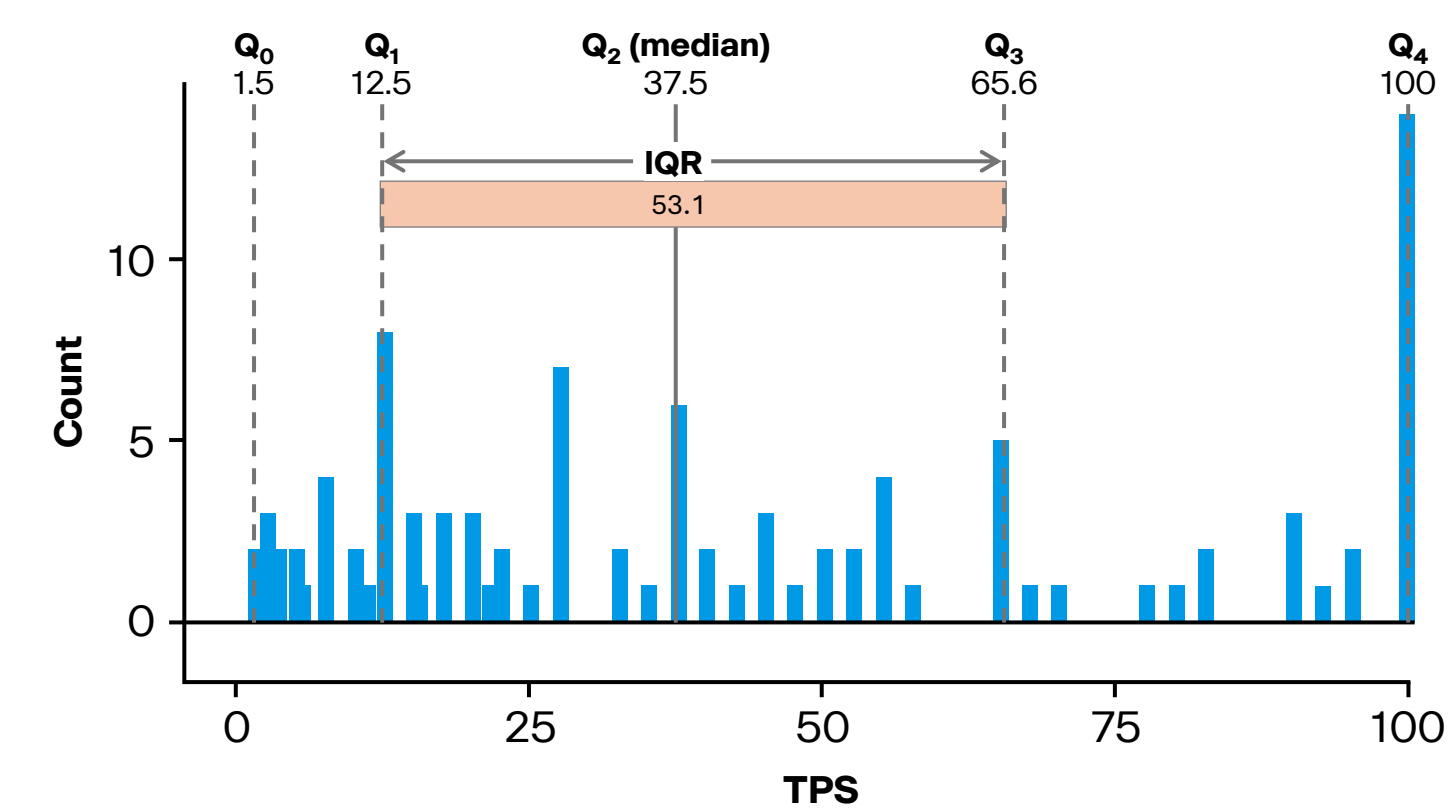
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RESULTS

Membranous EphA2 (TPS >1) was expressed in:

- ▶ 36% of 285 MIBC cases defined by histological subtype (Figure 1, Table 1)
- ▶ 34% of 234 MIBC cases defined by molecular subtype (Table 1)

FIGURE 1. DISTRIBUTION OF EPHA2-POSITIVE TUMOR SAMPLES BY TPS>1 (N=104) GROUPED BY HISTOLOGY



EphA2, erythropoietin-producing hepatocellular receptor A2; IQR, interquartile range; MIBC, muscle-invasive bladder cancer; TPS, tumor proportion score.

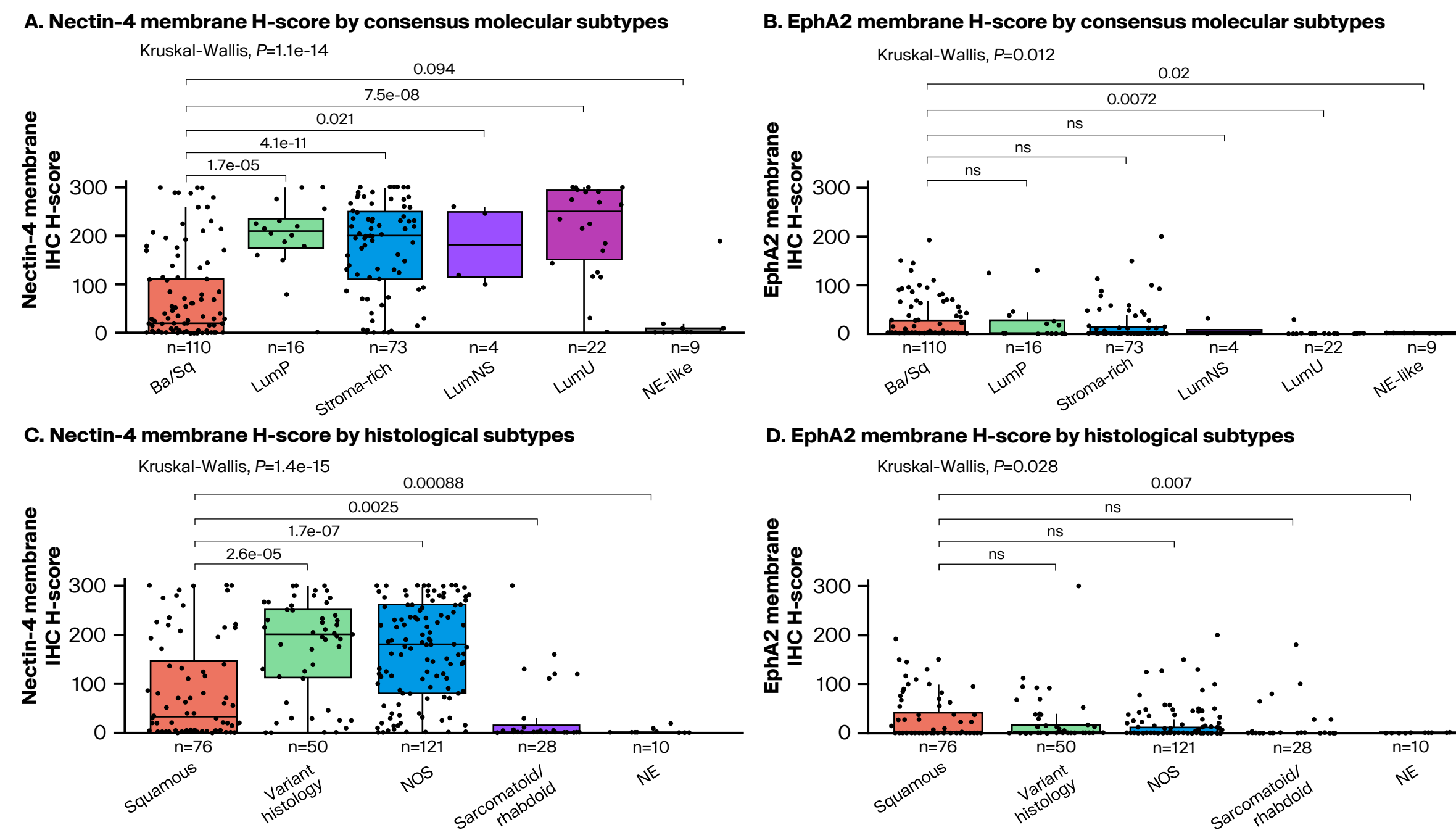
TABLE 1. EPHA2 EXPRESSION IN MIBC MOLECULAR AND HISTOLOGICAL SUBTYPES

| Consensus molecular subtype | Molecular | | | Histological | | | |
|-----------------------------|-----------|------------|-------------------------|------------------------|---------|------------|-------------------------|
| | Total N | % of total | EphA2 membrane TPS>1, % | Histology variant type | Total N | % of total | EphA2 membrane TPS>1, % |
| All | 234 | 100% | 34% | All | 285 | 100% | 36% |
| Ba/Sq | 110 | 47% | 40% | Squamous | 76 | 27% | 46% |
| LumP | 16 | 7% | 50% | Variant histology | 50 | 17% | 38% |
| Stroma-rich | 73 | 31% | 36% | NOS | 121 | 42% | 36% |
| LumNS | 4 | 2% | 25% | Sarcomatoid/rhabdoid | 28 | 10% | 25% |
| LumU | 22 | 9% | 5% | NE | 10 | 4% | 0% |
| NE-like | 9 | 4% | 0% | | | | |

Ba/Sq, basal/squamous; EphA2, erythropoietin-producing hepatocellular receptor A2; LumNS, luminal non-specified; LumP, luminal papillary; LumU, luminal unstable; MIBC, muscle-invasive bladder cancer; NE, neuroendocrine; NOS, urothelial carcinoma-not otherwise specified; TPS, tumor proportion score.

- ▶ The highest proportion of patients with tumors expressing EphA2 were seen in:
 - LumP (50%), Ba/Sq (40%), and stroma-rich (36%) molecular subtypes (Table 1)
 - Squamous (46%), variant histology (38%), and urothelial carcinoma-not otherwise specified (NOS; 36%) histological subtypes (Table 1)
- ▶ The most common subtypes included:
 - **Molecular:** Ba/Sq (n=110/234; 47%) and stroma-rich (n=73/234; 31%) (Table 1)
 - **Histological:** NOS (n=121/285; 42%) and squamous (n=76/285; 27%) (Table 1)

FIGURE 2. COMPARISON OF PROTEIN EXPRESSION OF NECTIN-4 AND EPHA2 IN MIBC MOLECULAR AND HISTOLOGICAL SUBTYPES



Ba/Sq, basal/squamous; EphA2, erythropoietin-producing hepatocellular receptor A2; H-score, histochemical score; IHC, immunohistochemistry; LumNS, luminal non-specified; LumP, luminal papillary; LumU, luminal unstable; MIBC, muscle-invasive bladder cancer; NE, neuroendocrine; NOS, urothelial carcinoma-not otherwise specified; ns, not significant.

- ▶ Nectin-4 protein expression was relatively lower in the Ba/Sq and NE-like molecular subtypes (Figure 2A), and in the squamous, sarcomatoid/rhabdoid, and NE histological subtypes (Figure 2C) compared with other subtypes evaluated
- ▶ EphA2 protein expression was relatively higher in the Ba/Sq molecular subtype (Figure 2B) and in the squamous, histological subtype (Figure 2D) compared with other subtypes evaluated

TABLE 2. EPHA2, NECTIN-4, AND HER2 MEMBRANE EXPRESSION ACROSS MIBC HISTOLOGICAL SUBTYPES

| Group | Nectin-4 H-score result | HER2 | EphA2 TPS result | MIBC ^a (N=285) | Squamous (n=76) | Variant histology (n=50) | NOS (n=121) | Sarcomatoid/rhabdoid (n=28) | NE (n=10) |
|-----------------------------------|-------------------------|----------|------------------|---------------------------|-----------------|--------------------------|-------------|-----------------------------|-----------|
| Nectin-4 single target positivity | All | - | Positive | 37% | 46% | 38% | 36% | 25% | 0 |
| | Positive | - | All | 44% | 25% | 66% | 58% | 7% | 0 |
| | Positive | - | Positive | 17% | 13% | 22% | 22% | 0 | 0 |
| Nectin-4 vs. EphA2 | Negative/low | - | Positive | 20% | 33% | 16% | 14% | 25% | 0 |
| | Positive | - | Negative | 27% | 12% | 44% | 36% | 7% | 0 |
| | Negative/low | - | Negative | 37% | 42% | 18% | 28% | 68% | 100% |
| HER2 single target positivity | - | All | Positive | 37% | 46% | 38% | 36% | 25% | 0 |
| | - | Positive | All | 32% | 13% | 38% | 47% | 14% | 0 |
| | - | Negative | Positive | 24% | 40% | 18% | 19% | 21% | 0 |
| | - | Positive | Negative | 19% | 7% | 18% | 31% | 11% | 0 |
| HER2 vs. EphA2 | - | Negative | Negative | 45% | 47% | 44% | 34% | 64% | 100% |
| | - | Positive | Negative | 45% | 47% | 44% | 34% | 64% | 100% |

^aSamples with Nectin-4, HER2, and EphA2 IHC scores, and histological subtypes available.
EphA2, erythropoietin-producing hepatocellular receptor A2; H-score, histochemical score; HER2, human epidermal growth factor 2; IHC, immunohistochemistry; MIBC, muscle-invasive bladder cancer; NE, neuroendocrine; NOS, urothelial carcinoma-not otherwise specified; TPS, tumor proportion score.

- ▶ 33% of patients with squamous histological subtype had EphA2 positive disease (membrane TPS >1) and Nectin-4 negative/low disease (H-score <150) (Table 2)
- ▶ 32% of patients with Ba/Sq molecular subtype had EphA2 positive disease (membrane TPS >1) and Nectin-4 negative/low disease (H-score <150)
- ▶ 40% of patients with squamous histological subtype had EphA2 positive disease (membrane TPS >1) and HER2 negative disease (0) (Table 2)
- ▶ 35% of patients with Ba/Sq molecular subtype had EphA2 positive (membrane TPS >1) and HER2 negative disease (0)

ABBREVIATIONS

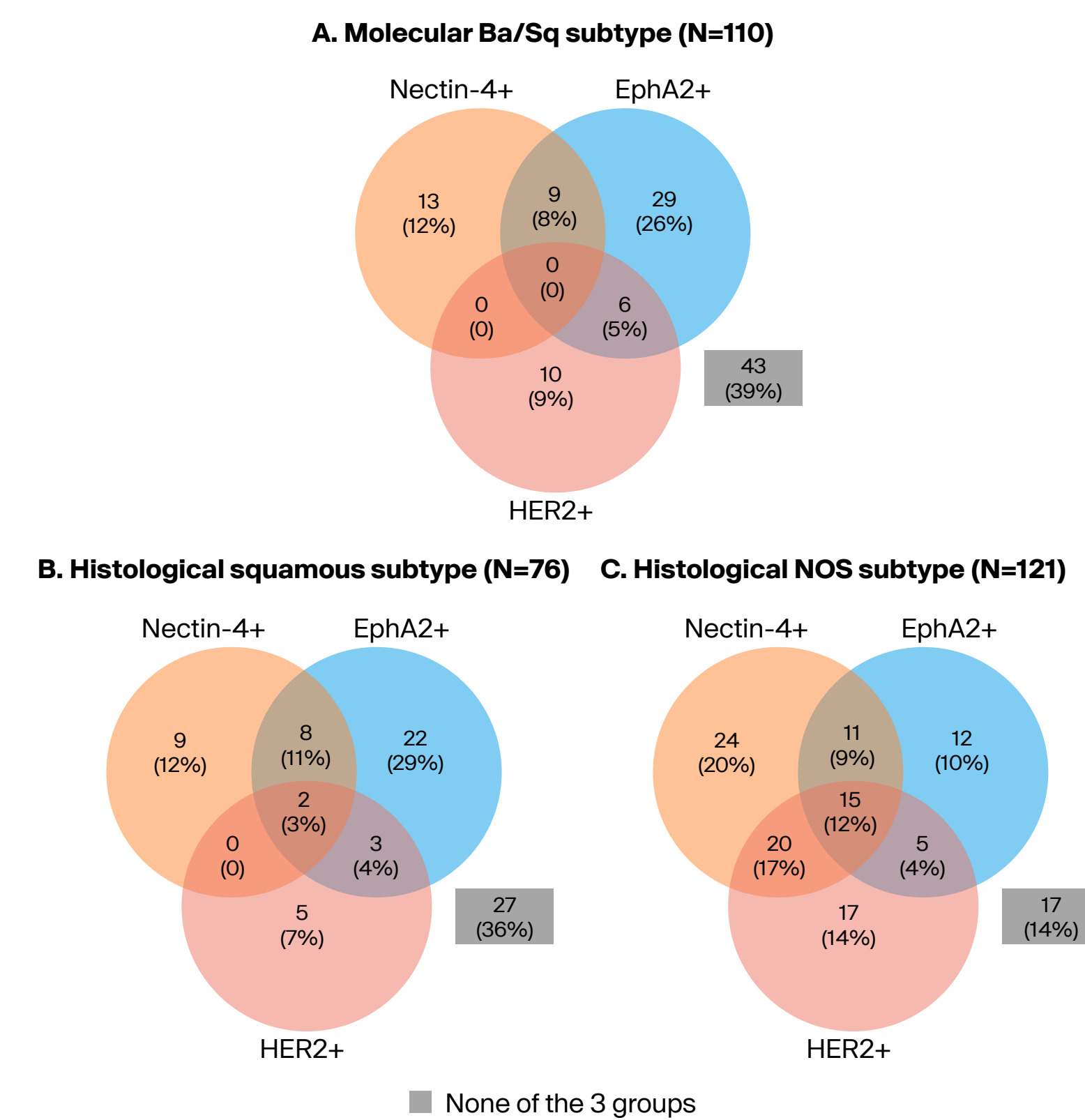
Ba/Sq, basal/squamous; EphA2, erythropoietin-producing hepatocellular receptor A2; H-score, histochemical score; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; LumNS, luminal non-specified; LumP, luminal papillary; LumU, luminal unstable; NE, neuroendocrine; MIBC, muscle-invasive bladder cancer; NOS, urothelial carcinoma-not otherwise specified; ns, not significant; RNA, ribonucleic acid; TPS, tumor proportion score.

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- ▶ There was no obvious correlation between *NECTIN4* ($R^2=0.02$) or *ERBB2* (HER2) ($R^2=0.028$) with *EPHA2* RNA expression in matched MIBC samples (n=241), Ba/Sq molecular subtype, or squamous histological subtype
- ▶ This was also the case in matched samples for *NECTIN4* with *EPHA2* in patients with Ba/Sq molecular ($R^2=0.011$) and squamous ($R^2=0.063$) histological subtypes

FIGURE 3. EPHA2, NECTIN-4, AND HER2 PROTEIN EXPRESSION IN MIBC MOLECULAR AND HISTOLOGICAL SUBTYPES



Ba/Sq, basal/squamous; EphA2, erythropoietin-producing hepatocellular receptor A2; HER2, human epidermal growth factor 2; MIBC, muscle-invasive bladder cancer; NOS, urothelial carcinoma-not otherwise specified.

- ▶ 26% and 29% of patients with Ba/Sq molecular subtype and squamous histological subtype were EphA2 positive (membrane TPS >1), Nectin-4 negative/low (membrane H <150) and HER2 negative (0) (Figure 3)

CONCLUSIONS

- ▶ These data indicate that patients with MIBC with a Ba/Sq molecular subtype and/or squamous histology who have limited treatment options may benefit from EphA2-targeted treatment, warranting further investigation of EphA2 as a novel therapeutic target in MIBC and other bladder cancers

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