SYSTEMATIC LITERATURE REVIEW AND TESTING OF HER2 STATUS IN UROTHELIAL CARCINOMA (UC)

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Introduction

- HER2 expression has been extensively studied in breast and gastric cancers; however, the prevalence of HER2 protein expression and gene amplification (encoded by *ERBB2*) and their clinical relevance in urothelial cancer (UC) has not been well defined[1]
- Testing for HER2 expression in UC is not part of current routine practice, and there is no standardized method for testing, adding to the uncertainty around the role of HER2 in UC
- Recent clinical trials suggest an emerging role for HER2-targeted therapy in locally advanced and metastatic urothelial cancer (LA/mUC) with HER2 overexpression[2–4]
- An understanding of the prevalence of HER2 protein expression and gene amplification in UC is needed, given these potential new treatment opportunities

Objectives

1. To report updated findings of a systematic literature review (SLR) of HER2 status in UC
2. To report findings of new, optimized UC-specific laboratory methods to evaluate HER2 status by immunohistochemistry (IHC) and *in situ* hybridization (ISH)

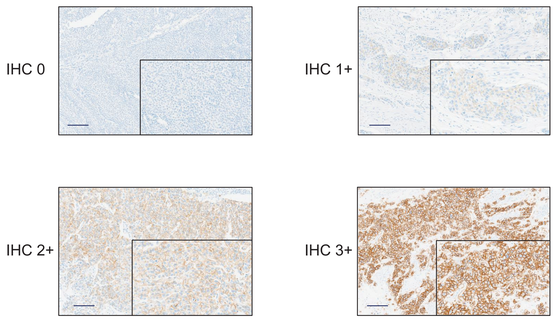
Methods

Systematic Literature Review of HER2 Status in Urothelial Carcinoma

- The SLR was conducted in accordance with Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines[1,5]
- MEDLINE and EMBASE, in addition to abstracts presented at select oncology congresses, were searched to identify English-language studies reporting HER2 protein expression or gene amplification data for UC tumor samples
- Key objectives of the SLR were to determine:
  - Prevalence of HER2 expression in UC
  - Methods used for assessing HER2 expression and gene amplification
- Definitions used for the SLR:
  - HER2-positive (HER2+): IHC score of 3+ or IHC 2+ and ISH+/fluorescence in situ hybridization (FISH)+
  - HER2-low: IHC 2+ and ISH/FISH-negative or IHC 1+
  - HER2-zero (previously known as HER2-negative): IHC 0
- The updated results extend the original publication timeline[1] (January 2000 to October 2021) to include studies published from October 2021 to July 2022
- Weighted averages from studies that presented data allowing for categorization of HER2 status as HER2+, HER2-low, or HER2-zero using the above predefined criteria were used to calculate prevalence

Figure 1. HER2 Protein Expression in Urothelial Cancer

Representative images of UC scored with the HER2 UC algorithm to determine HER2 protein levels (IHC scores 0–3). Scale bars, 100µM.



Proprietary HER2 UC Assays

- Commercially sourced, formalin-fixed, paraffin-embedded surgical resections of primary UC were evaluated by trained readers for:
  - HER2 protein expression using the VENTANA HER2/neu (4B5) Rabbit Monoclonal Primary Antibody IHC assay (**Figure 1**)
  - HER2 gene amplification using the VENTANA HER2 Dual ISH DNA Probe Cocktail that quantitatively detects both ERBB2 and its residing chromosome, chromosome 17 (Chr17), using a two-color chromogenic stain
- HER2 IHC staining in UC was scored using a modified version of the scoring algorithm used for gastric cancer (**Table 1**)
  - A modified gastric cancer algorithm was used because HER2 staining patterns in UC are more consistent with those observed in gastric cancer than in breast cancer, given that tumor cell membranes in urothelial and gastric cancers contain more incomplete lateral and/or basolateral HER2 staining, and stain intensities may be more heterogeneously expressed, as compared with breast cancer
  - HER2 positivity in UC, however, is more localized to cell membrane over cytoplasm as compared with gastric cancer
- HER2 gene amplification was defined by a HER2/Chr17 ratio ≥2.0
- Further details of assay methods are available via the QR code

Table 1. Scoring Criteria for HER2 (4B5) Staining Intensity of Tumor Cells in UC Tissue

Staining Pattern	HER2 Score		HER2 Expression Assessment	HER2 Clinical Status
No reactivity, or membranous reactivity at any staining intensity in <10% of tumor cells	0		Negative	IHC-Zero
Weak membranous reactivity in ≥10% of tumor cells; reactivity occurs in only part of the cell membrane	1+		Positive	HER2-Low
Moderate, complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells	2+ (Reflex to ISH)	ISH Non-amplified	Positive	HER2-Low
		ISH Amplified		Positive/ overexpression
Strong, complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells	3+		Positive	Positive/ overexpression

Results

Updated Systematic Literature Review Findings

- A total of 98 unique studies were included (50 LA/mUC, 32 earlier stage UC, 16 mixed population/unspecified)
- A variety of criteria were used to define HER2 expression, and most studies defined their own criteria (n=46) or did not mention criteria in the study methods (n=36)
- Multiple assays using IHC, ISH/FISH, and next generation sequencing (NGS) with different assay conditions, scoring criteria, and cut-off values were used
- Of the studies including sufficient data to categorize HER2+ status using our predefined criteria, HER2+ prevalence ranged from 6.7% to 37.5% (weighted average, 12.3%; 95% CI, 7.8% to 16.8%; 6 studies, N=971 patients)[3,6-10] in LA/mUC
- HER2-low prevalence ranged from 13.3% to 75.0% (weighted average, 47.9%; 95% CI, 16.7% to 79.0%; 4 studies, N=275 patients) in LA/mUC[6,8-10]
- Only one small study (N=25 patients) categorized HER2 status for earlier stage UC[11]

Proprietary HER2 UC Assay Results

- Of 362 UC samples evaluated (**Table 2**), 57 were HER2+ (15.7%; 95% CI, 12.4%–19.9%), 103 were HER2-low (28.5%; 95% CI, 24.1%–33.3%), and 202 were HER2-zero (55.8%; 95% CI, 50.7%–60.8%)
- Collectively, 160 (44.2%; 95% CI, 39.2%–49.3%) UC samples had a HER2-positive/overexpression or HER2-low clinical status (**Table 3**)
- The HER2 gene was amplified in 51 samples (14.1%), of which 37 (72.5%) were stage III or IV muscle-invasive UC

Table 2. HER2 Status by UC Stage[a]: Assay Results

		N (row %)		
Stage	N	HER2+	HER2-low	HER2-zero
Stage I	7	1 (14)	0	6 (86)
Stage II	133	17 (13)	46 (35)	70 (53)
Stage III	192	37 (19)	50 (26)	102 (55)
Stage IV	30	2 (7)	7 (23)	21 (70)
All	362	57 (16)	103 (28)	202 (56)

a Stage information as supplied by the tissue vendor. Staging may have been based on clinical information or on tissue samples different from those included in the current study

Table 3. Summary of HER2 Status

HER2 Status	N	Percentage of samples (95% CI)
HER2+ and HER2-low	160	44.2% (39.2%–49.3%)
HER2+/overexpression	57	15.7% (12.4%–19.9%)
HER2-low	103	28.5% (24.1%–33.3%)
HER2-zero	202	55.8% (50.7%–60.8%)

Conclusions

- The systemic literature review revealed substantial heterogeneity of HER2 status in UC and highlighted a lack of standardized methods for defining and assessing HER2 status
- In our large study using optimized UC-specific laboratory methods, 44.2% of UC samples were found to be HER2+ or HER2-low, and HER2 status distribution was consistent with that previously reported for patients with LA/mUC
- These findings call for optimizing and standardizing UC-specific testing methods to support further investigations of the clinical role of HER2 alterations and of HER2-targeted therapy in UC
- Study limitation: Commercially sourced samples, as used for the HER2 IHC and ISH assays, have an unknown degree of preselection, and the impact of specimen handling, such as fixation time, is unknown

References

1. Scherrer E, et al. Front Oncol. 2022;12:1011885.
2. Patelli G, et al. Cancer Treat Rev. 2022;104:102351.
3. Sheng X, et al. Clin Cancer Res. 2021;27(1):43-51.
4. D'Angelo A, et al. Cancer Chemother Pharmacol. 2022;90(3):191-205.
5. Page MJ, et al. BMJ. 2021;372:n71.
6. Fleischmann A, et al. Eur Urol. 2011;60(2):350-357.
7. Oudard S, et al. Eur J Cancer. 2015;51(1):45-54.
8. Banerji U, et al. Lancet Oncol. 2019;20(8):1124-1135.
9. Li S, et al. J Clin Oncol. 2022;40(6_suppl):524.
10. Zhou L, et al. J Clin Oncol. 2022;40(6_suppl):515.
11. Latif Z,et al. Br J Cancer. 2003;89(7):1305-1309.

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