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Original Research Article

Study of her2/neu and ki-67 expression in invasive urothelial carcinoma of bladder and their correlation with grade and stage

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ABSTRACT

Background: Urinary bladder cancer is one of the costliest diseases, which requires lifelong surveillance due to its rapid aggressive clinical course. Here, HER2/neu and Ki-67 expression, is studied, as important prognostic marker with therapeutic implications in invasive urothelial bladder carcinoma (IUBC).
Objective: To study expression of HER2/neu and Ki-67 in histologically diagnosed cases of IUBC and their clinicopathological correlation.
Materials and Methods: An observational, cross-sectional, study was performed in Pathology Department, NRS Medical College, Kolkata, from February 2020 to July 2021. Tissue sections, after proper processing, were stained in H&E. A total of 75 IUBC cases were histopathologically diagnosed with grade and stage. HER2/neu and Ki-67 expression by immunohistochemistry was studied in them; which was compared with clinicopathological variables like age, sex, risk factors, histological type, grade, stage, etc. Statistical analysis software EPI INFO (TM) 7.2.2.2 was used, $p<0.05$ was taken to be statistically significant.
Results: Among 75 cases of IUBC, 60% were high grade and 72% were of lower (pT1) stage. HER2/neu was positive in 52% cases; significantly associated with males, haematuria, smoking, necrosis, high grade ($p<0.00001$) and higher stage (pT2/3/4) ($p=0.0005$). Ki-67 index was high in 56% cases; significantly associated with >50 years age group, haematuria, smoking, necrosis, LVI, high grade ($p<0.00001$) and higher stage ($p<0.0001$). Positive HER2/neu expression was also significantly associated with high Ki-67 index ($p<0.00001$).
Conclusion: Positive HER2/neu expression and high Ki-67 index is significantly associated with high grade and stage of tumours. So, these patients may be considered for targeted therapy using Trastuzumab in addition to conventional therapy.

1. Introduction

Urinary bladder cancer is the tenth most common cancer worldwide. With global incidence of 573,278 new cases in 2020;[1] 330,380 new cases in 2012;[2] it has shown an increasing trend over the years. It is 3-4 times more common in males than in females.[3] In India, 21,096 new cases and 11,154 deaths due to bladder cancers were reported in 2020.[1] According to recent reports of the National Cancer Registry Programme, the overall incidence of bladder cancer in India is 2.25% (per 100,000 annually), 3.67% in males and 0.83% in females.[4]

Infiltrating urothelial carcinoma is the most common malignancy of the urinary tract. The defining histological criterion is invasion beyond basement membrane:[3] beyond:

1. Lamina propria (pT1)
2. Muscularis propria (pT2)

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pT1 tumours are treated with Transurethral resection of bladder tumours (TURBT) ± intravesical BCG immunotherapy. pT2 or higher stages are treated with radical cystectomy ± preoperative chemotherapy. So, depth of invasion is very important criterion for determining stage and guiding treatment.[5]

50-70% of superficial urothelial carcinomas may recur and 15-25% progress to a higher stage, grade or metastatic disease.[6] Hence, bladder cancer is considered as one of the costliest diseases, which requires lifelong surveillance due to its rapid aggressive clinical course. So, early diagnosis and institution of molecular targeted therapies help to prevent recurrence, metastasis and improve patient survival.

ERBB2, a proto-oncogene located on 17q12, encodes HER2/neu, a member of the human epidermal growth factor receptor family.[7] Overexpression of HER2/neu in high grade and stage of urothelial tumors, aside from having prognostic significance in muscle invasive bladder carcinoma;[3,8] may have therapeutic implications- addition of target therapy using humanized monoclonal antibody (trastuzumab) to conventional therapy may prolong 5-year survival. Encouraging preclinical results have been achieved with trastuzumab conjugated with cytotoxic agent DM1 in HER2/neu positive tumours.[3,9]

Ki-67, a cell-cycle related nuclear protein, is encoded by MK167 gene and is expressed by proliferating cells.[10] Ki-67 is increased in high grade carcinoma (due to biological aggressiveness) with or without muscle invasion. Thus, Ki-67 index helps in diagnosis, guiding therapy; is an important prognostic molecular marker in Non-muscle invasive bladder cancer.[3,8]

Accurate prognosis with any single factor is difficult. Hence, combination of histology and immunohistochemical expression of HER2/neu and Ki-67 are needed for ease of diagnosis, prognostic prediction and therapy.

Though plenty research work has been done regarding HER2/neu and Ki-67, few effective studies have been done concentrating on invasive urothelial bladder carcinoma (IUBC) in the Indian subcontinent, especially in West Bengal.

Thus, our study intends to correlate HER2/neu and Ki-67 expression in IUBC with its grade, stage and other clinicopathological parameters like age, sex, histological type, necrosis, etc.

2. Materials and Methods

2.1. Sample selection

An institutional based observational, cross-sectional, study was conducted in the Pathology Department in collaboration with Urology Department, NRS Medical College and Hospital, Kolkata, West Bengal from February 2020 to July 2021. The work was approved by the Institutional Ethical Committee (No/NMC/448) and informed consent was obtained from the study population.

2.2. Inclusion criteria

The diagnosed cases of IUBC in TURBT samples, radical cystectomy specimens and cold cup biopsies.

2.3. Exclusion criteria

1. Non-neoplastic bladder lesions (inflammation, etc.).
2. Bladder carcinomas other than urothelial carcinoma.
3. Non- invasive urothelial bladder carcinoma.
4. Non-representative samples (extensive necrosis, etc.)

Hence, a total of 75 cases were enrolled.

Census method of sampling was used. Data was collected using a pre-designed, pre-tested semi-structured schedule on dependent variables like HER2/neu and Ki-67 expression and independent variables like clinical-pathological profile including age, sex, histological type, grade, stage, etc. Data was collected by observations, record review and laboratory techniques including histopathology and immunohistochemistry.

2.4. Histopathology

All tissue samples were collected in 10% buffered formalin and processed for routine histopathological examination. Five micrometers thick sections from formalin fixed, paraffin embedded blocks were cut and stained with hematoxylin and eosin for histopathological diagnosis of IUBC type, tumor grade [WHO/ISUP classification 2016], tumor stage (pT) [American Joint Committee on Cancer, 2010], necrosis, lympho-vascular invasion (LVI) and perineural invasion (PNI).

2.5. Immunohistochemistry (IHC)

For IHC staining, 3 μm thick sections from formalin fixed paraffin embedded tissues were taken on poly L Lysine coated slides. IHC was done using HER2/neu and Ki-67 antibody (rabbit monoclonal antibodies), peroxide block and the steps mentioned in the kit were followed. Negative control was achieved by omitting primary antibody in both cases.

2.6. Scoring of HER2/neu IHC

Interpretation of HER2/neu was done according to ASCO/CAP guidelines.[11,12] A known case of HER2/neu positive breast carcinoma was used as positive control. Positivity was assessed as brown colour, cell membrane staining of malignant cells. Score 0/Negative: No staining observed OR incomplete, faint/barely perceptible membrane staining within ≤ 10% of invasive tumour cells. Score 1+/negative: Incomplete, faint/barely perceptible membrane staining in > 10% of invasive tumour cells.

Score 2+/equivocal: Circumferential membrane staining that is incomplete and/or weak/moderate in >10% of invasive tumour cells OR complete, intense circumferential membrane staining in ≤ 10% of invasive tumour cells. Score 3+/positive: Complete, intense circumferential membrane staining in >10% of invasive tumour cells. For the purpose of our study, score 2+ and score 3+ were considered positive.

2.7. Scoring of Ki-67 IHC

The samples were analysed following recommendations from the International Ki-67 in Breast Carcinoma Working Group [13], as in other bladder carcinoma studies [14]; whereby positive Ki-67 staining was defined as only positive nuclear staining (dark brown colour) counting of at least 1000 nuclei at high power (40x objective). Chronic tonsillitis tissue was used as positive control of Ki-67.

The Ki-67 score (Ki-67 index/ proliferation index) was expressed as the percentage of positively staining nuclei among the total number of nuclei in the area scored. For proper grouping of results, IUBC cases were categorised into: Cases with low Ki-67 score: ≤20% positive nuclei and Cases with high Ki-67 score: >20% positive nuclei.

2.8. Statistical analysis

Statistical analysis was performed with help of Epi Info (TM) 7.2.2.2 EPI INFO, a trademark for the Centers for Disease Control and Prevention (CDC).

Descriptive statistical analysis was performed to calculate the means with corresponding standard deviations (S.D.). Test of proportion was used to find Standard Normal Deviate (Z) to compare different proportions. Chi-square (χ^2) test was performed to find the association between different study variables. t-test was used to compare two means. $p<0.05$ was taken to be statistically significant.

3. Results

3.1. Clinicopathological findings

Patient demography details, clinical presentation, risk factors and radiological findings are listed in Table 1.

Histopathological parameters like necrosis, LVI and PNI were all present in high grade tumours. These and more parameters are listed in Table 2.

3.2. HER2/neu expression and its correlation with clinicopathological parameters:

Out of 75 cases, HER2/neu was positive in 39 cases (52%).

Positive HER2/neu expression was significantly associated with squamous, glandular differentiation, and micropapillary variant of IUBC; necrosis, high grade ($p<0.00001$) and higher stage (pT2/pT3/pT4) ($p=0.0005$) [Table 2]; also, with males, haematuria and smoking.

3.3. Ki-67 index and its correlation with clinicopathological parameters

Out of 75 cases, high Ki-67 proliferation index was seen in 42 cases (56%).

High Ki-67 index was significantly associated with all histological types of IUBC except the usual type, necrosis, LVI, high grade ($p<0.00001$) and higher stage ($p<0.0001$) [Table 3]; also, with patients aged >50 years, haematuria, smoking.

Table 1: Patient demography details, clinical presentation, risk factors and radiological findings. (n=75)

Parameters	Number of patients	Percentage (%)
Age		
• ≤50 years	21	28
• >50 years	54	72
Mean age: 57.01±12.97 years		
Median age: 55 years		
Range: 25-84 years		
Sex		
• Male	63	84
• Female	12	16
M:F ratio =5.25:1		
Clinical presentation		
• Haematuria (microscopic/gross)	69	92
• Irritative urinary symptoms	24	32
• Lower abdominal pain	9	12
Risk Factors		
• Smoking	30	40
• Arsenic exposure	6	8
• Painter (paint- aryl amines)	3	4
• None	36	48
Specimen type		
• TURBT	63	84
• Radical cystectomy	6	8
• Cold cup biopsy	6	8
Location		
• Lateral wall	45	60
• Posterior wall	12	16
• Trigone	9	12
• Bladder neck	6	8
• Dome	3	4

3.4. Correlation between HER2/neu expression and Ki-67 proliferation index

Positive HER2/neu expression was significantly associated with high Ki-67 index ($p<0.00001$) such that 84.6% of HER2/neu positive cases showed high Ki-67 index. [Table 4]

Table 2: Association between HER2/neu status and different histopathological parameters of patients. (n=75)

Parameters	No. of cases	%	Status of Her2/neu		p value
			Negative (%)	Positive (%)	
Histological type					0.0042
-Usual type	50	66.67	29 (58)	21 (42)	
-Squamous differentiation	12	16	3 (25)	9 (75)	
-Glandular differentiation	6	8	0 (0)	6 (100)	
-Sarcomatoid variant	3	4	3 (100)	0 (0)	
-Micropapillary variant	3	4	0 (0)	3 (100)	
-Clear cell variant	1	1.33	1 (100)	0 (0)	
Necrosis					0.0062
-Present	24	32	6 (25)	18 (75)	
-Absent	51	68	30 (58.8)	21 (41.2)	
LVI					0.08
-Present	12	16	3 (25)	9 (75)	
-Absent	63	84	33 (52.4)	30 (47.6)	
PNI					0.08
-Present	3	4	0 (0)	3 (100)	
-Absent	72	96	36 (50)	36 (50)	
Histological grade					**<**0.00001
-Low grade	30	40	24 (80)	6 (20)	
-High grade	45	60	12 (26.7)	33 (73.3)	
Tumor stage					0.0005
-pT1	54	72	33 (61.1)	21(38.9)	
-pT2	12	16	0 (0)	12 (100)	
-pT3	6	8	3 (50)	3 (50)	
-pT4	3	4	0 (0)	3 (100)	

Table 3: Association between Ki-67 score and different histopathological parameters of patients. (n=75)

Parameters	Ki-67 score (proliferation index)		p value
	Low (%)	High (%)	
Histological type			0.00001
-Usual type	33 (66)	17 (34)	
-Squamous differentiation	0 (0)	12 (100)	
-Glandular differentiation	0 (0)	6 (100)	
-Sarcomatoid variant	0 (0)	3 (100)	
-Micropapillary variant	0(0)	3 (100)	
-Clear cell variant	0 (0)	1 (100)	
Necrosis			**<**0.00001
-Present	0 (0)	24 (100)	
-Absent	33 (64.7)	18 (35.3)	
LVI			
-Present	0 (0)	12 (100)	0.0004
-Absent	33 (52.4)	30 (47.6)	
PNI			
-Present	0 (0)	3 (100)	0.17
-Absent	33 (45.8)	39 (54.2)	
Histological grade			<0.00001
-Low grade	27 (90)	3 (10)	
-High grade	6 (13.3)	39 (86.7)	
Tumor stage			
-pT1	33 (61.1)	21 (38.9)	
-pT2	0 (0)	12 (100)	<0.0001
-pT3	0 (0)	6 (100)	
-pT4	0 (0)	3 (100)	

Table 4: Association between HER2/neu status and Ki-67 score of patients. (n=75)

Status of HER2/neu	Ki-67 score (proliferation index)		Total
	High (>20%)	Low (≤ 20%)	
Negative	9	27	36
Row%	25	75	100
Column %	21.4	81.8	48
Positive	33	6	39
Row%	84.6	15.4	100
Column %	78.6	18.2	52
Total	42	33	75
Row%	56	44	100
Column %	100	100	100

p value < 0 00001

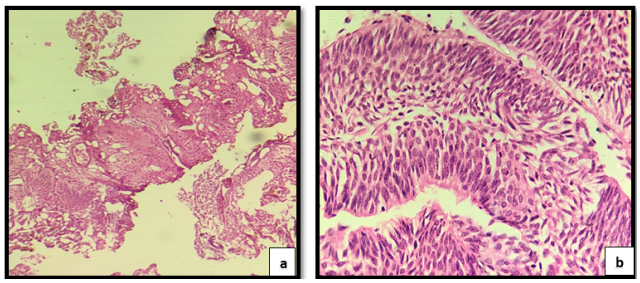

Fig. 1: a: Lamina propria invasive urothelial bladder carcinoma (IUBC)- pT1. (H&E;100X); **b:** Low grade IUBC (H&E; 400X).

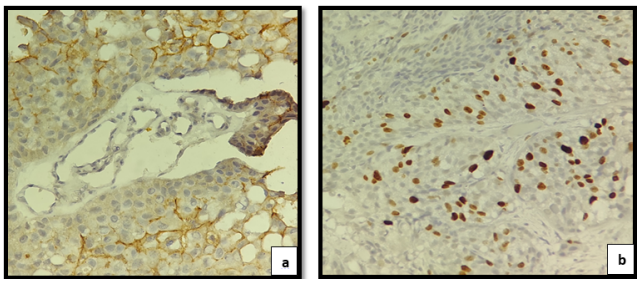

Fig. 2: Low grade IUBC. (400x), **a:** IHC staining: shows HER2/neu: 1+ score (NEGATIVE); **b:** IHC staining: shows low Ki-67 index (≤20%)

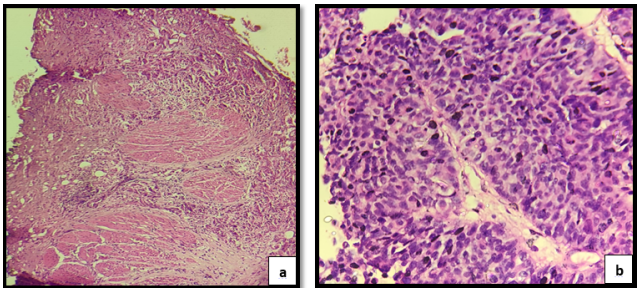

Fig. 3: a: Muscularis propria IUBC-pT2. (H&E; 100x); **b:** High grade IUBC (H&E; 400x)

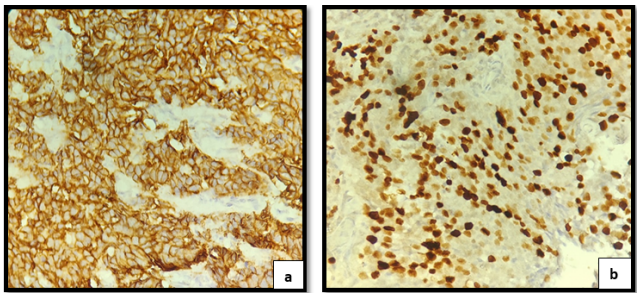

Fig. 4: High grade IUBC (400x); **a:** IHC staining: shows HER2/neu 3+ score (POSITIVE); **b:** IHC staining: shows high Ki-67 index (>20%)

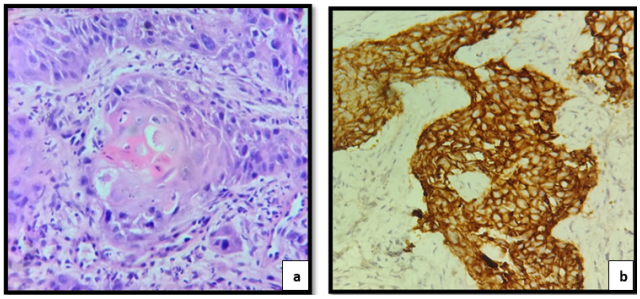

Fig. 5: a: Squamous differentiation of IUBC. (H&E- 400x); **b:** IHC staining of squamous differentiation of IUBC:HER2/ neu 3+ score (POSITIVE). (400x)

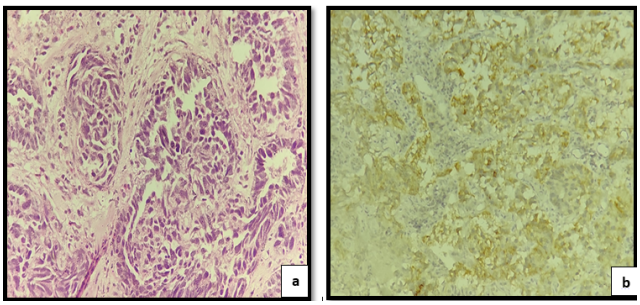

Fig. 6: a: Glandular differentiation of IUBC. (H&E-400x); **b:** IHC staining of glandular differentiation of IUBC: HER2/neu 2+ score (POSITIVE). (400x)

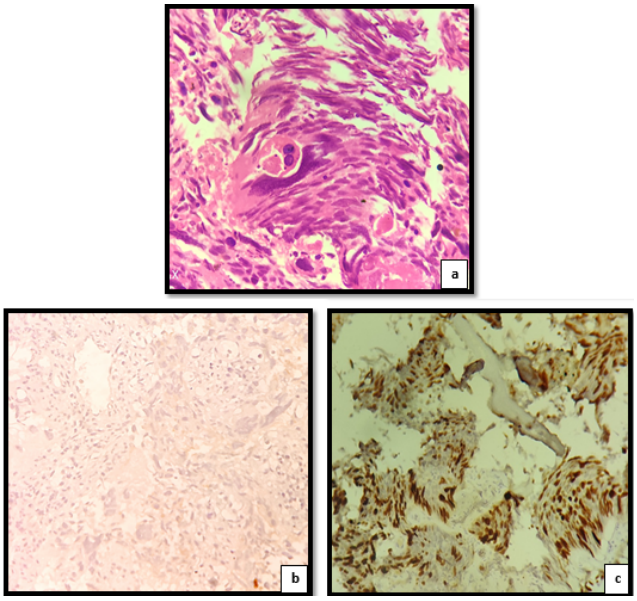

Fig. 7: a: Sarcomatoid variant of IUBC. (H&E-400x); **b:** IHC staining of sarcomatoid variant of IUBC: HER2/neu 0 score (NEGATIVE). (400x); **c:** IHC staining of sarcomatoid variant of IUBC: high Ki-67 index (>20%). (400x)

4. Discussion

Urinary bladder carcinoma is a common multistage progressive malignancy ranking 10^{th} in worldwide cancer incidence and responsible for significant mortality and morbidity. In the past few decades, many studies have explored the prognostic value of various biomarkers involved in the molecular pathogenesis of bladder cancer.

Agarwal et al. found the mean age of patient to be 55.9 years (range of 21-83 years), most common age group being 40-60 years;[15] similar to our study. There was male predominance with M:F ratio of 7.2:1 (as compared to our study with 5.25:1).

Nedjadi et al. found statistically insignificant association between age, sex and HER2/neu expression;[16] in contrast to our study, where HER2/neu expression was more common in males. Wang et al. reported Ki-67 had no gender preference,[17] like our study.

Thakur et al. found that most common clinical symptom of invasive urothelial carcinoma was haematuria (90%), followed by urgency, nocturia and dysuria;[14] consistent with our study.

Agarwal, et al found that majority of patients (68%) had history of smoking;[15] similar to our study. Asmi et al. studied 75 cases, out of which majority (64%) were TURBT samples;[4] like our study.

The most common anatomical site of tumour was found to be lateral wall of bladder-64.8% in Agarwal et al.[15] and 37% in Stephenson et al.[18] similar to our study (60%).

In our study, the most common histological type was the usual type (66.6%) of IUBC, followed by squamous differentiation (16%). Similarly, Kim et al. reported squamous differentiation as the most common (40%) type of divergent differentiation.[19] Our study, also found significant association between positive HER2/neu expression and squamous differentiation, glandular differentiation, and micropapillary variant of IUBC, quite similar to Ching et al.[20] All histological types of IUBC, except the usual type, showed high Ki-67 index with significant association in our research.

Kumar M et al. reported majority (90%) of cases with LVI to have high Ki-67 index; and no significant association between LVI and HER2/neu expression; PNI had insignificant association with both markers;[21] consistent with our study. Our study also reported significant association between necrosis and both markers; 75% of cases with necrosis showed positive HER2/neu expression and 100% of cases with necrosis showed high Ki-67 index.

Agarwal et al. had 63.5% high grade and 32.4 low grade invasive cases,[15] quite alike our study. Thakur et al. had majority (67%) pT1 tumours and 32.9% higher stage tumours,[14] like this study.

In our study, 52% cases showed positive HER2/neu expression, which was significantly associated with high grade ($p<0.00001$) and higher stage (pT2/pT3/pT4) ($p=0.0005$); such that 80% of low grade tumours showed negative HER2/neu expression and 73.3% of high grade tumours showed positive HER2/neu expression; 61.1% of pT1 tumours showed negative HER2/neu expression and 85.7% of higher stages showed positive HER2/neu expression. This is supported by Nedjadi et al., Kumar M et al., and Asmi et al.[4,16,21] in contrast to Kumar S et al. where no correlation was found between HER2/neu expression and tumour grade and stage.[22]

In our study, 56% of 75 IUBC cases, showed high Ki-67 score, which was significantly associated with high grade ($p<0.00001$) and high tumour stage ($p<0.0001$) such that 86.7% of high grade tumours showed high Ki-67 index and 90% of low grade tumours showed low Ki67 index; 61.1% of pT1 showed low Ki-67 index and 100% of higher stages showed high Ki-67 index. This is supported by Stepan et al., Wang et al., Kumar M et al. and Thakur et al.[12,14,17,21]

Finally, we aimed to determine whether the expression of HER2/neu is associated with a more aggressive behaviour by its correlation with Ki-67 index and found 84.6% HER2/neu positive cases showed high Ki-67 index with significant association ($p<0.0001$); 66.6% of high grade tumours showed co-expression (positive HER2/neu and high Ki-67 index); consistent with Kumar M et al, where co-expression of both markers correlated well with tumour grade and muscle invasion.[21] Thus, Ki-67 and Her2/neu co-expression was superior to a single marker expression in predicting tumour aggression, progression and prognosis.

5. Conclusion

In conclusion, 52% and 56% of IUBC, showed positive HER2/neu expression and high Ki-67 index respectively. Both positive HER2/neu expression and high Ki-67 index was significantly associated with higher grade and higher stage (pT); and most of HER/neu positive cases (84.6%) had high Ki-67 index. Thus, HER2/neu positive bladder cancers, when given target therapy with trastuzumab in addition to conventional therapy, may give better response and improved survival.

6. Conflict of Interest

None.

7. Source of Funding

None.

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