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HER2/neu Expression: A Predictor for Differentiation and Survival in Children With Wilms Tumor

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Abstract Wilms tumor is a mixed embroynal neoplasm of the kidney . HER2 is an onco-protein. Its over-expression could be implicated in the development of many tumors. The clinico-demographic and pathological data of 28 Wilms tumor patients were , reviewed. The tissue samples were examined by light Microscopy then immunohistochemical staining for HER2/neu expression. Additional 28 normal surrounding renal tissue specimens were included. There was significant differences between HER2/neu positive and HER2/neu negative Wilms tumors in relation to stage, histological phase and epithelial differentiation ($P>0.05$ for all). The overall survival advantage was noticed if Wilms tumor was at early stages (I and II) (Log-rank=13.23 and P>0.001), homologous epithelial differentiation (Log-rank=6.01 and $P=0.04$), as well as HER2/neu positive tumors (Log-rank=6.14 and $P=0.013$). A statistical significant trend toward a longer recurrence free survival was, noticed if Wilms tumor was at early stages (Log-rank=21.22, $P>0.0000$) and if HER2/neu positive (Log-rank=8.53, $P=0.004$). HER2/neu expression in Wilms tumor could be a marker for epithelial and homologous differentiation and its expression could be a good predictor for overall survival and longer recurrence free survival.

Keywords Her2/neu · Oncoprotein survival · Wilms tumor · Differentiation

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Introduction

Wilms tumor is a complex mixed embroynal neoplasm of the kidney [1]. It is the most common renal tumor of the childhood and accounts for about 6% of all childhood cancer [2]. In Egypt, 44 cases were recorded in recent Cancer Pathology Registry (CPR), 2003–2004 (0.45 % of total malignancy) [3]. The biologic behavior of Wilms tumor is difficult to predict on the basis of histopathologic findings alone [4].

The mean age at diagnosis is 3.5 years. The most common feature at presentation is an abdominal mass. Other signs and symptoms include abdominal pain, hypertension, fever, hematuria and anemia [5]. National Wilms Tumor Study Group (NWTS) recommends an initial radical operation followed by adjuvant chemotherapy with or without radiotherapy.The International Society of Pediatric Oncology (SIOP) emphasizes the efficacy of pre-nephrectomy treatment [2]. With the advent of multimodal therapy, the prognosis of Wilms tumor is good. The overall survival rate is about 90% [6].

HER2 (human epidermal growth factor receptor 2), is an onco-protein, [7]. Activation of this onco-protein, triggers intracellular signaling events, crucial for cell growth, proliferation, differentiation and survival [8].

An over-expression of c-erbB-2 onco-protein cause over-expression of the receptor and could be implicated in the

development of many types of tumors like breast, ovary, salivary glands, GIT, prostate, lung, liver, kidney and bladder cancer [9] to increase the metastatic potential and to promote chemoresistance [10].

Some independent clinical and biological predictors for Wilms tumor have been identified for predicting survival specially stage and anaplasia [11] Currently increased attention has been focused on new biological parameters for malignancy using molecular markers in a risk stratification strategy has been proposed, [9] The objectives of the current study were to investigate HER2/neu expression in Wilms tumor and its impact on the prognosis reflected on survival.

Materials and Methods

The clinico-demographic and pathological data of the studied 28 Wilms tumor patients were retrospectively, retrieved and reviewed from the registries and files of the Pediatrics Hemato-Oncology Unite in Minoufiya University hospital and Oncology Unit in Suez Canal University hospital as well as the Medical Statistics Department of the National Cancer Institute, Cairo University for the Wilms tumor cases diagnosed, managed and followed up during the period from January 1999 to September 2006. They were analyzed for the following clinical characteristics; age at diagnosis, gender, initial symptoms, location, size, stage, survival and recurrence. All patients were managed according to the NWTS protocol [2]. An overall survival was defined as the time from initial diagnosis to death or last follows up [12]. The Recurrence free survival was defined as length of time after treatment during which no cancer is found [13].

We examined 28 cases of Wilms tumor that were retrieved from the pathology departments' archives. 28 normal surrounding renal tissue specimens were included.

The hematoxylin and eosin stained slides were reviewed by two pathologists to determine the histological phase , predominant cell type ,blastemal pattern arrangement, epithelial as well as mesenchymal differentiation and anaplasia according to Perlman et al., [11]. Teratoid variant of Wilms tumor is a tumor that demonstrated greater than 50% of their volume occupied by heterologous differentiation [14].

HER2/neu IHC staining was performed on formalin fixed, paraffin embedded material that were sectioned at 5 ųm thickness and placed onto positive charged slides. HER2/neu IHC staining was performed using the Universal Dakocytomation Labelled stretavidin-Biotin® 2 system, Horseradish Peroxidase (LSAB®2 System, HRP Kit, Code No. k0679). The antibody used in this study was concentrated, polyclonal rabbit anti-human c-erbB-2 oncoprotein (HER2/neu) (DAKO Herceptest). (Code No. A0485).

Negative controls were obtained by omitting the primary antibody. Positive HER2/neu breast duct carcinoma NOS cases were served as a positive control in each run.

HER2/neu immunoreactivity was evaluated by semi-quantitave scoring (SQS) using a light microscope according to Jimenez et al., [15] (Table 1) by two pathologists in separate settings. Un-intentional bias was prevented by coding patient tissue samples.

The data were collected, tabulated and processed on an IBM PC compatible computer using SPSS version 11. X^2 test for comparison was used. Univariate survival analyses were made and survival curves were drown according to Kaplan-Meier method. Log — rank test was performed. Tests were considered significant when P values were ≤ 0.05 and highly significant when P value≤ 0.01 [16].

Results

The demographic data of the studied 28 cases of Wilms tumor were as follow; the age at diagnosis of the studied group ranged from 1.5 to 10 years with mean ± SD of 5.3± 2.47 years and median of 5 years. M/F ratio was 1:1. Seventeen patients (60.7%) were presented by abdominal mass, 10(35.7%) with abdominal pain, 7(25%) with hypertension, 6 (21.4%) presented with hematuria and 3 cases (10.7%) with fever. None of studied patients was syndromatic or had any genitourinary abnormality. Eight patients (28.6%) were stage I, 9 (32.1%) stage II, 7 (25%) stage III, one (3.6%) stage IV and three (10.7%) were stage V. The size of Wilms tumors ranged from 2.5–25 cm with 8.27±5.23. Four tumors (14.3%) were multi-centric.

Table 1 Score for HER2/neu over expression

Score	Assessment	Staining pattern
O	Negative	No staining or membrane staining is observed in >10% of tumor cells.
1+	Negative	Faint or barely perceptible membrane staining is detected <10% of tumor cells; the cells are only stained in part of their membrane
2+	Weak to moderate positive	Weak to moderate complete membrane staining is observed in< 10% of tumor cells
3+	Strong positive	Strong complete membrane staining is Observed in <10% of tumor cells.

Among the studied patients; 6 (21.4%) died of disease (DOD) and 7 (25%) had recurrent tumor during the period of the study.

Among 28 cases, 18 (64.3%) showed a triphasic pattern, 6 (21.4%) biphasic pattern. Four cases (14.3%) showed a monophasic pattern composed of blastemal cells. According to the predominant cell type; one tumor (3.6%) showed predominance of mesenchymal element, 14 (50%) showed blastemal and 13 (46.4%) showed epithelial. Blastemal element displayed in 27 cases; 14 showed serpentine pattern and 13 showed diffuse architecture. Twenty three cases had epithelial elements, 3 showed hetrologous differentiation (squamous and mucous secreting cells "teratoid variant") and 20 showed homologous differentiation Twenty cases showed mesenchymal elements, one case showed hetrologous differentiation and 19 cases showed homologous differentiation ,.Anaplasia was detected in 4 cases (14.3%) 3 cases with focal and one diffuse (Table 2).

In normal kidney specimens, HER2/neu showed positive immunoreactivity in the cell membrane of endothelial cells and renal tubules, whereas the glomeruli were not entirely stained (Table 3) (Fig. 1 A).

In Wilms tumors, HER2/neu immunostaining was diffuse and the findings tended to vary widely. Among the 27 specimens with blastemal cells, only 3 (11.1%) showed positivity. Fifteen (65.2%) of 23 cases with epithelial differentiation showed positive HER2/neu immunoreactivity. Only 6 (30%) of 20 cases with mesenchymal differentiation showed HER2/neu positivity (Table 3) (Fig. 1 B and C).

No significant differences were found between HER2/neu positive and negative cases regarding age at diagnosis, gender, size of the tumor, centricity, predominant cell type, blastemal architectural pattern, mesenchymal differentiation or anaplasia as well as living state or recurrence ($P>0.05$). The HER2/neu positive cases were statistically significantly

Table 2 Characteristic of Wilms tumor studied cases

Case	Histological phase	Predominant cell type	Blastemal Pattern	Epithelial differentiation	Mesenchymal differentiation	Anaplasia	Stage	State
1	Triphasic	Blastemal	Serpentine	Homologous	Homologous	No	I	Living
2	Triphasic	Blastemal	Diffuse	Homologous	Homologous	No	III	Living
3	Triphasic	Blastemal	Serpentine	Homologous	Homologous	Focal	II	Dead
4	Triphasic	Epithelial	Serpentine	Homologous	Homologous	No	V	Living
5	Triphasic	Epithelial	Diffuse	Homologous	Homologous	No	V	Living
6	Triphasic	Blastemal	Diffuse	Homologous	Homologous	No	II	Living
7	Biphasic	Mesenchymal	Diffuse	No	Homologous	Focal	IV	Living
8	Monophasic	Blastemal	Diffuse	No	No	No	III	Dead
9	Biphasic	Epithelial	No	Hetrologus	Hetrologous	No	III	Living
10	Triphasic	Epithelial	Diffuse	Homologous	Homologous	Diffuse	V	Dead
11	Triphasic	Blastemal	Serpentine	Hetrologus	Homologous	No	II	Living
12	Monophasic	Blastemal	Diffuse	No	No	No	III	Living
13	Biphasic	Blastemal	Diffuse	Hetrologus	No	No	II	Living
14	Monophasic	Blastemal	Diffuse	No	No	No	III	Living
15	Monophasic	Blastemal	Serpentine	No	No	No	I	Dead
16	Biphasic	Epithelial	Serpentine	Homologous	No	No	III	Living
17	Triphasic	Epithelial	Serpentine	Homologous	Homologous	No	I	Living
18	Triphasic	Blastemal	Serpentine	Homologous	Homologous	Focal	III	Dead
19	Triphasic	Epithelial	Serpentine	Homologous	Homologous	No	I	Living
20	Triphasic	Epithelial	Serpentine	Homologous	Homologous	No	II	Living
21	Triphasic	Epithelial	Diffuse	Homologous	Homologous	No	I	Living
22	Biphasic	Epithelial	Serpentine	Homologous	No	No	II	Living
23	Triphasic	Epithelial	Diffuse	Homologous	Homologous	No	I	Living
24	Biphasic	Epithelial	Diffuse	Homologous	No	No	I	Living
25	Triphasic	Blastemal	Diffuse	Homologous	Homologous	No	III	Dead
26	Triphasic	Blastemal	Serpentine	Homologous	Homologous	No	II	Living
27	Triphasic	Epithelial	Serpentine	Homologous	Homologous	No	I	Living
28	Triphasic	Epithelial	Serpentine	Homologous	Homologous	No	II	Living

Table 3 HER2/neu expression in the studied cases

	HER2/neu expression in normal renal tissue			*HER2/neu expression in Wilms tumor studied cases*		
Cases	Glomeruli	Tubules	Endothelium	Blastemal	Epithelial	Mesenchymal
1	0	1	1	0	1	0
2	0	1	1	0	1	0
3	0	3	2	0	1	0
4	0	3	1	0	2	0
5	0	2	2	0	1	0
6	0	2	1	0	1	1
7	0	2	1	0	No	1
8	0	2	1	0	No	No
9	0	2	1	No	0	0
10	0	2	1	0	1	0
11	0	2	1	0	2	0
12	0	1	1	0	No	No
13	0	2	1	0	2	No
14	0	2	1	0	No	No
15	0	2	1	1	No	No
16	0	2	1	0	1	No
17	0	3	1	1	3	2
18	0	2	1	1	3	1
19	0	2	1	1	2	2
20	0	2	2	1	3	2
21	0	3	2	1	3	3
22	0	2	2	2	3	No
23	0	3	1	1	3	1
24	0	2	1	1	2	No
25	0	2	1	1	2	2
26	0	2	1	3	3	2
27	0	2	1	2	3	1
28	0	2	1	1	3	0

higher in early stages (I&II) than other stages, in triphasic tumors than bi-or mono-phasic and in homologous differentiated tumors than none or heterologously differentiated (Table 4), (Fig. 1 B, C and D).

No significant relationship was found between the overall survival or recurrence free survival and demo-clinico-pathologic data, like age at diagnosis, gender, site, histological phase, predominant cell type, blastemal pattern, mesenchymal differentiation, anaplasia as well as the whole tumor differentiation. A statistical significant trend toward the overall survival advantage was noticed if Wilms tumor was in early stage (I and II) (Log-rank=13.23 and P< 0.001), ,homologous epithelial differentiation (Log-rank= 6.01 and P=0.04), as well as HER2/neu positive tumors (Log-rank=6.14 and P=0.013) (Table 4) ,(Fig. 2).

A statistical significant trend toward a longer recurrence free survival was, however, noticed if Wilms tumor was unicentric (Log-rank=7.68 and P<0.006), in early stage (Log-rank=21.22 and P>0.0000) and HER2/neu positive (Log-rank=8.53 and P =0.004) (Fig. 3).

Discussion

Wilms tumor arises from pluripotent embryonic kidney precursor cells [17].

Studying 28 children of Wilms tumor treated according to NWTS in multicenters,(Egypt) ,the results revealed that the age at presentation ranged from 1.5 to 10 years with mean ± SD of 5.3±2.47 years and the median age was 5 years. The M/F ratio was 1:1. This finding is consistent that mentioned in the literatures [2, 11]. The most common symptom among our patients was abdominal mass in 17 children (60.7%) followed by abdominal pain in 10 patients (35.7%) then hypertension in 7 (25%) while 6 patients (21.4%) had hematuria and 3 cases (10.7%) presented with

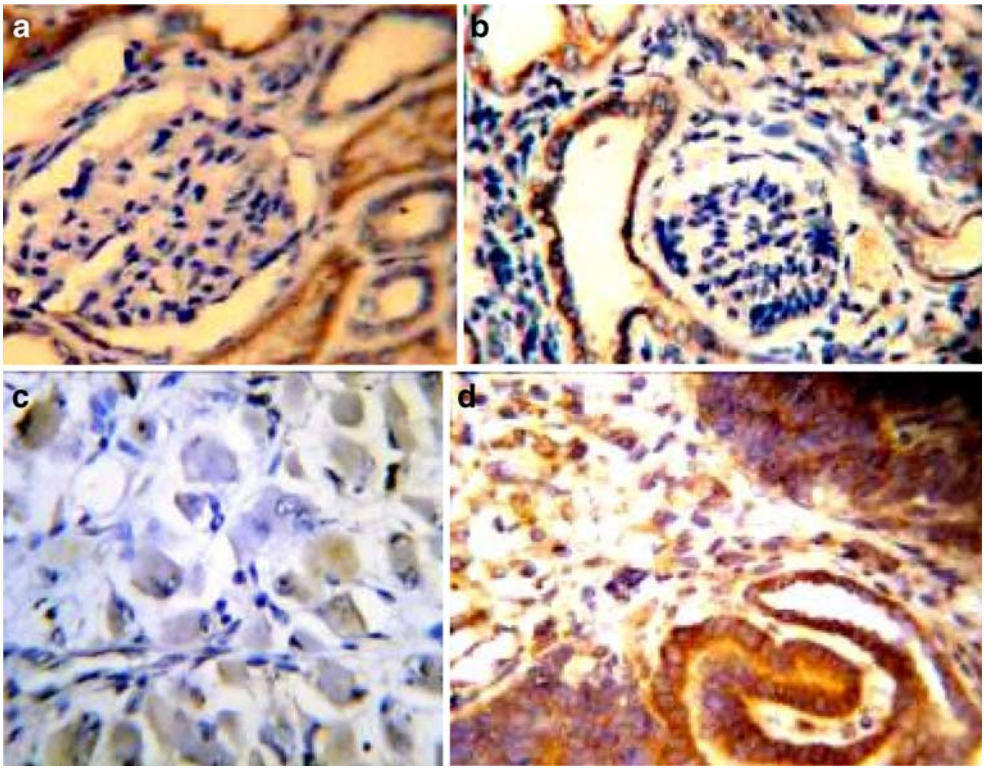

Fig. 1 (**A**): Normal kidney showed strong (score 3) membranous immunoreactivity in tubular epithelium only. (**B**): Classic Wilms tumor showed strong (score 3) membranous immunoreactivity in well formed tubules and negative (score 0) staining in a primitive glomerulus. (**C**): Teratoid variant of Wilms tumor showing negative HER2/neu immunostaining in skeletal muscle. (**D**): Classic Wilms tumor displayed strong (score 3) membranous immunoreactivity in tubular epithelium and negative (score 0) in mesenchymal component

fever. Our findings are in agreement with those published in literatures [1, 2].

The presence of EGFR peptides and their receptors has been found in normal developing kidney[18] and is required for tubulogenesis [19].Wilms tumor is classified as a primitive, multilineage malignancy of embryonic renal precursors that shares histological features with the developing normal kidney [17].

In the current study, we detected HER2/neu to be expressed in normal kidney tissue specimens, it showed positive immunoreactivity in the cell membranes of endothelial cells and renal tubules whereas the glomeruli were not entirely stained (Table 3 and Fig. 1 A), suggesting that this protein is a normal membrane constituent of epithelial renal tissue but not of glomeruli. This result is consistent with Salem et al., [7]. We can thus propose that

Table 4 Relation between HER2/neu over expression and some clinico-pathological characteristics of Wilms tumors

Parameters	HER2/neu		Total	Test of significance	P value
	− Ve No (%) 13 (100)	+ Ve No (%) 15 (100)	No (%) 28 (100)		
Stage					
I	2 (15.4)	6 (40.0)	8 (100)	X^2=9.59	0.048*
II	2 (15.4)	7 (46.7)	9 (100)		
III	6 (46.2)	1 (6.7)	7 (100)		
IV	1 (7.7)	-	1 (100)		
V	2 (15.3)	1 (6.7)	3 (100)		
Histologic phase:					
Triphasic	6 (46.2)	12 (80)	18 (100)	X^2=5.89	0.05*
Biphasic	3 (23.1)	3 (20)	6 (100)		
Monophasic	4 (30.7)	-	4 (100)		
Epithelial differentiation:					
No	5 (38.5)	-	5 (100)	X^2=7.03	0.03*
Homologous	7 (53.8)	13 (86.7)	20 (100)		
Hetrologous	1 (7.7)	2 (13.3)	3 (100)		

*: significant

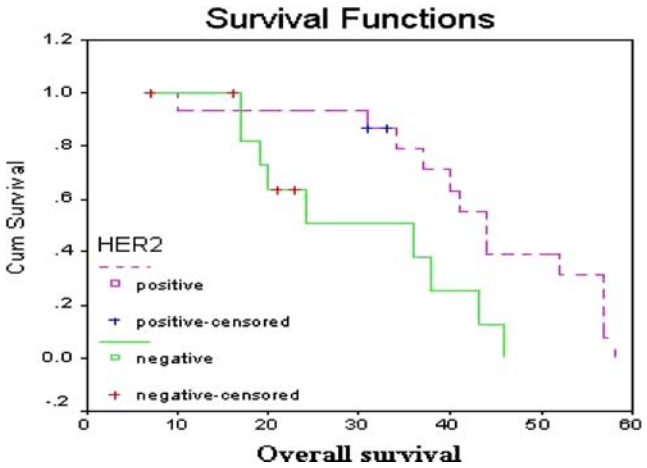

Fig. 2 The overall survival analysis in patients with Wilms tumors by HER2/neu over expression

HER2/neu could be one of the chemical directors of ureteric bud differentiation (tubulogenes) during normal nephrogenesis. This proposal is in agreement with Salem et al., and Rivera & Haber [7, 17].

In Wilms tumor samples, the staining patterns appear to differ regarding each component. The HER2/neu protein expression in immunoreactive epithelial cells was 65.2 % and was 30 % in mesenchymal cells, whereas the blastemal component stained in only 11.1 % (Table 3 and Fig. 1 B and C). This study included 3 cases of teratoid variant of Wilms tumor; one case had both squamous component and skeletal muscle (Fig. 1 D) and did not showed HER2/neu positivity, the second had squamous cells and the third had mucous secreting epithelium and both of them displayed moderate positive immunoreactivity for HER2/neu.

The strong positive HER2/neu staining according to SQS was in epithelial element 9/23 (39.1%) followed by mesenchymal component, 1/20 (5%) whereas in the blastemal component only 1/27 (3.7%) revealing that the epithelial component expressed higher percentage of positivity and higher SQS. Similar findings have also been confirmed in Wilms tumor [7], uterine carcinosarcoma [20] and biphasic synovial sarcoma [21].

Akin to Potti et al., [8], in development of Wilms tumor, high level of HER2 expression contribute to epithelial differentiation and this is in agreement with our results of Wilms tumor samples.

Wilms' tumor is a curable disease in the majority of affected children. More than 90% of patients survive 4 years after diagnosis[5, 6, 22].

Several factors were considered in the prognosis of Wilms tumor; with early stage and absence of anaplasia being the most important predictors for survival [2, 11, 23, 24].

In the last decade there has been great interest in the HER2/neu proto-oncogene concerning tumor biology [25].

An over-expression of this onco-protein has been fond to correlate with a poor prognosis in a variety of malignant tumors, such as breast and ovarian cancers [9] and to increase the metastatic potential and to promote chemo resistance [10]. Other studies revealed its over-expression to be associated with histological differentiation and a favorable prognosis in both thyroid carcinoma and synovial sarcoma [22, 26, 27].

Considering the clinico-pathological characteristics of the studied Wilms tumor patients, none of age at diagnosis, gender, size of the tumor, centricity, predominant cell type, blastemal architectural pattern, mesenchymal differentiation or anaplasia as well as living state and recurrence showed statistical significant difference between HER2/neu positive and negative tumors (P>0.05).

The HER2/neu positive tumors were significantly higher in early stages (I&II) than advanced stages, in triphasic tumors than bi-or mono-phasic and in homologous differentiated tumors than heterologous differentiated (Table 4) concluding that HER2 over-expression favors presentation as a localized homogonously differentiated Wilms tumor thus it could have a good prognostic impact.

Studying the association between HER2/neu expression and survival, ; overall survival advantage was noticed if Wilms tumor was at early stages (I and II) (Log-rank= 13.23 and P<0.001), homologous epithelial differentiation (Log-rank=6.01 and P=0.04), as well as HER2/neu positive tumors (Log-rank=6.14 and P=0.013) (Fig. 2).

A statistical significant trend toward a longer recurrence free survival was noticed if Wilms tumor was unicentric (Log-rank=7.68 and P>0.006) in early stages (Log-rank= 21.22 and P>0.0000) and HER2/neu positive (Log-rank= 8.53 and P=0.004) (Fig. 3).

Nuclear anaplasia in Wilms tumor is associated with an adverse outcome [2]. Anaplasia was detected in 4 out of the studied 28 patients (14.3%), 3 was focal and only one

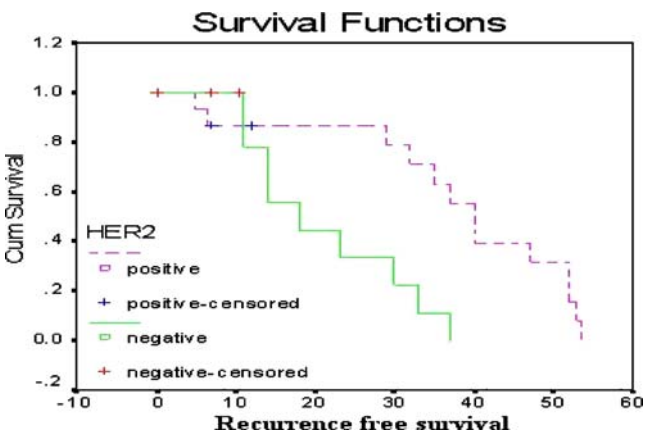

Fig. 3 Recurrence free survival analysis in patients with Wilms tumors by HER2/neu over expression

diffuse. Absence of impact of anaplasia on survival among our patients can be explained by small number of cases.

Staging of Wilms tumor is one of the well established independent clinical and biological predictors that have been identified for predicting survival [2, 11]. Our results of longer overall and recurrence free survivals for patients in early stages are in agreement with this.

The good impact of HER2 expression on overall and recurrence free survival for our Wilms tumor patients; are in line with Ghanem et al., [4] who concluded that HER2 may not play an important role in the aggressive behavior of Wilms tumor and with Salem et al., [7] who stated that HER2/neu overexpression might be associated with a good prognosis in Wilms tumor; however; it is difficult to conclude due to the small number of cases under their study. Yokoi et al., [25],suggested that erbB-2 in an in vivo model might serve as a therapeutic target.

However, there is still a lack of general consensus regarding the possible prognostic impact of this marker in Wilms tumor and still not clear whether HER2/neu may act with other clinic-pathological parameters to influence either the response to chemotherapy or clinical outcome.

In conclusion, the present study suggested that HER2/neu expression in Wilms tumor could be a golden marker for epithelial homologous differentiation pathways. These findings may therefore help to explain the development of Wilms tumor as a primitive, multi-lineage malignancy of embryonic renal precursors from the standpoint of histological differentiation. HER2/neu expression in Wilms tumor could be a good predictor for overall survival advantage and longer recurrence free survival.

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