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HER-2 Immunohistochemical Expression in Bone Sarcomas: A New Hope for Osteosarcoma Patients

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Abstract

BACKGROUND: Osteosarcoma and chondrosarcoma, remain the most common primary bone tumours. Questions have been raised about the prognostic influence of HER-2 in bone sarcomas, but so far the results have been debatable. The her-2 expression is possibly a predictor of chemotherapy response.

AIM: In this study, we investigated the extent of HER-2 expression in bone sarcomas, and attempted to correlate it with pertinent variables that will help to provide better treatment options, especially for metastatic ones.

MATERIAL AND METHODS: Fifty-two cases of bone sarcomas (32 osteosarcoma cases and 20 chondrosarcoma ones) were studied for HER-2 immunohistochemical expression then correlation with all available clinicopathologic features was done.

RESULTS: Most of the osteosarcoma cases exhibited membranous staining (78.1%). Strong staining was observed (score 3+) in 34.4%; while 21.9% showed moderate staining (score 2+); and 21.9% displayed weak staining (score 1+), on the other hand, no staining was detected in 7 out of 32 cases (21.9%) (score 0). As regards chondrosarcoma, the absence of staining in all examined cases was noted. Immunohistochemical HER-2 overexpression correlated significantly with osteosarcoma site with P value = 0.004, with variation relating HER-2 intensity score to the site of osteosarcoma (P = 0.051). A statistically significant negative correlation was detected between HER-2 expression and the presence of metastasis at time of diagnosis (P = 0.006), A significant correlation was also found regarding HER-2 score and presence of metastasis with P value = 0.046 as more than half of cases with no metastasis at diagnosis (17/28 cases, 60.7%) showed positive intensity score. A statistically significant correlation was detected between HER-2 expression and patients' age (P = 0.044). Also, HER-2 expression significantly correlated to histopathological detection of fibrous tissue, with P value = 0.033. Higher scores of HER-2 expression were associated with a significantly better differentiation (P = 0.038) since detection of wide areas of osteoid were associated with higher HER-2 scores.

CONCLUSION: Further research would still be needed to delineate HER-2 role being a new hope for therapeutic targeting in bone sarcoma patients, mainly osteosarcoma in contrast to chondrosarcoma that didn't express HER-2 at all.

Introduction

Osteosarcoma and chondrosarcoma, remain the most common primary bone tumours [1]. Osteosarcoma is a primary skeletal malignancy, signified by malignant spindle cells of mesenchymal origin with deposition of the immature osteoid matrix [2] [3]. Histologically, osteosarcoma subtypes can be separated into high and low-grades. Treatment of low-grade tumours involves surgery alone and bears a favourable prognosis. High-grade osteosarcoma should be regarded as micrometastatic at diagnosis and thereupon treated with both surgery and systemic chemotherapy [4].

Surgery and chemotherapy are relatively successful treatment modalities for localised disease, yet metastatic disease continues to be a challenging issue [5]. Nearly 15-20% of patients have evidence of metastases at diagnosis. Moreover, patients having the metastatic disease have a very poor prognosis, with approximately only 20-30% of them being long-term survivors, as compared to 65-70% of patients having localised disease [6]. The ability to stratify patients at diagnosis is imperative to identify prognostic factors, to select patients who might benefit from more intensive forms of therapy. Recognition of select malignant cellular features could make up the keystone of targeted therapy interferences [7].

Concerning chondrosarcoma, it may arise de novo and hence called primary, whereas those

developing on top of preexisting benign cartilaginous tumours are itemised as secondary. Chondrosarcomas being heterogeneous, can be categorised by anatomic location into central or peripheral. Besides conventional chondrosarcoma that exhibits hyaline cartilage differentiation, other types include dedifferentiated, mesenchymal, and clear cell ones [8].

Chondrosarcoma is typically considered to be resistant to conventional chemotherapy and radiotherapy [9]. Accordingly, surgical resection has been the primary form of treatment. Thus, identification of prognostic factors is of dire necessity, not only to predict patients' outcome but also to shape decisions as regards treatment. Novel therapeutic approaches have been appraised in an experimental study [10]. Human epidermal growth factor receptor 2 (HER-2) has been viewed as a protein of potential prognostic importance in addition to being a therapeutic target. Additionally, it is fundamentally involved in the pathogenesis of several human cancers. Through some pathways, it regulates cell growth, survival, cellular proliferation and differentiation [7] [11].

Questions have been raised about the prognostic influence of Her-2 in bone sarcomas, but so far the results have been debatable. Meanwhile, the HER-2 expression is possibly a predictor of chemotherapy response [12] [13]. In this study, we investigated the extent of HER-2 expression in bone sarcomas and attempted to correlate it with pertinent variables that will help to provide better treatment options, especially for metastatic ones.

Material and Methods

This retrospective study was conducted on 52 cases of bone sarcomas (32 osteosarcoma cases and 20 chondrosarcoma ones). Formalin-fixed paraffin-embedded tumour (FFPE) blocks of patients, diagnosed as osteosarcoma or chondrosarcoma were collected from Pathology Department, Kasr Alainy Hospital, Faculty of Medicine, Cairo University, after receiving approval from the institutional research ethics committee.

Four µm sections from each submitted tumour paraffin block were stained with hematoxylin and eosin, to assess pertinent histologic findings. Exclusion criteria for tumour blocks included scanty tumour tissue, poor fixation and overt necrosis. Serial sections (4 µm) were prepared and float-mounted on adhesive-coated glass slides for HER-2 staining. Primary antibodies included rabbit antihuman c-erbB2 (HER-2) oncoprotein antibody (DAKO, Dako Corporation, Carpinteria, CA, USA) at 1:200 dilution for HER-2. The DAKO Envision system (DAKO Envision labelled polymer, peroxidase) was used as the detection system for HER-2. For all cases, a negative control was the adjacent non-tumorous part of the specimen and the positive control was a known HER2/*neu*-overexpressing breast carcinoma.

Hematoxylin and eosin stained sections were evaluated by light microscopy along with Her-2 immunohistochemically stained slides were then scored. To determine the score of HER-2 expression, both the membrane and cytoplasmic staining patterns were estimated, and its intensity was scored on a scale of 0 to 3+. A reaction in > 20% of cells in the membrane (focal/linear) or cytoplasm, with an intensity score of 2 or greater, was considered positive, provided that the staining was limited to the tumour cells and did not represent background or artefact [14].

Clinical data were retrieved from patients' files, including the age, gender, site of a tumour, and metastasis presentation. Histopathologic features were assessed as regards type, grade, necrosis, and matrix differentiation. Her-2 immunohistochemical results were recorded. Findings were then tabulated.

Collected data was documented using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp., Armonk, NY, USA). Statistical analysis was done using descriptive statistics. Results were expressed as percentages, frequencies, and mean ± S.D. correlations were determined using the Chi-square $\chi 2$ test. A p value of less than 0.05 represented significance.

Results

Fifty two bone sarcoma cases were enrolled in this study. Median age was 40 years, with 27 (51.9%) males and 25 (48.1%) females. Patients ranged in age from 10 to 86 years, with a mean of 40.4 ± 19.888 years. The osteosarcoma series included 32 cases ranged from 10 to 86 years, with a mean of 32.38 ± 16.590 years, while chondrosarcoma cases included 20 cases with a range from 25 to 85 years and a mean of 53.25 ± 14.682 years.

All of the 52 cases were evaluated for HER-2 expression by immunohistochemical staining. Regarding cases of osteosarcoma, the vast majority of specimens exhibited membranous staining (either complete or not) (25 cases out of 32, 78.1%). A strong staining was observed in > 20% of cells in the membrane (focal/linear) or cytoplasm (score 3+, strongly positive cases) in 11 out of 32 cases (34.4%); while 7 cases out of 32 (21.9%) showed moderate staining (score 2+, equivocal or weakly positive); and 7 cases (21.9%) displayed weak staining in more than 20% of the tumor cells (score 1+, negative), on the

other hand, no staining was detected in 7 out of 32 cases (21.9%) (score 0, negative) (Figures 1, 2, and 3).

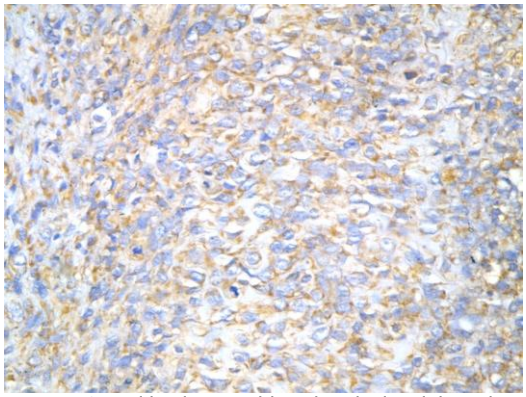

Figure 1: HER-2 positive immunohistochemical staining where the neoplastic cells of osteosarcoma showed staining in part of their membrane (low power)

As regards chondrosarcoma, the degree of immunohistochemical expression of HER-2 was similar in all cases given the absence of staining in all of the 20 examined cases (Figure 4).

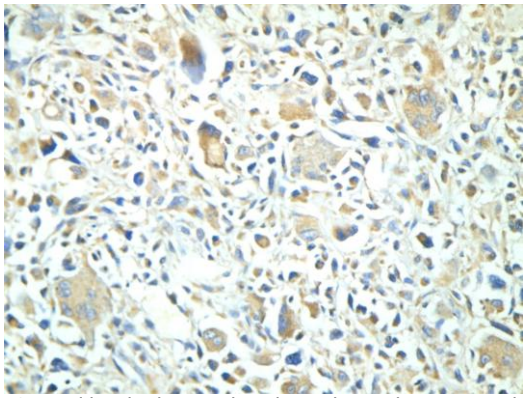

Figure 2: positive both cytoplasmic and membranous staining of HER-2 in a case of osteosarcoma (high power)

Clinicopathologic characteristics of cases and the results of HER-2 immunostaining are listed in Tables 1, 2, and 3.

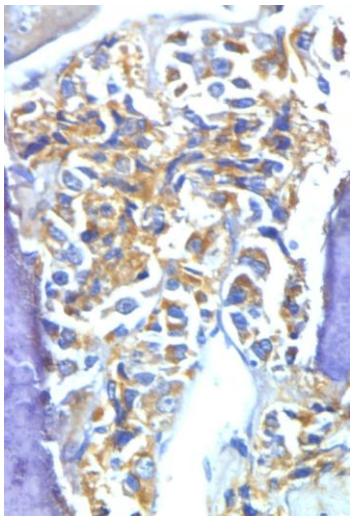

Figure 3: cytoplasmic staining of HER-2 without membrane staining in osteosarcoma case (high power)

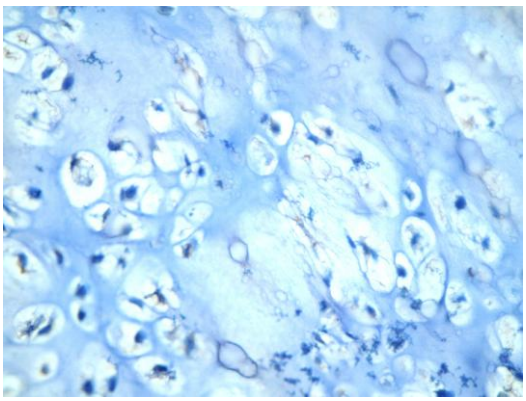

Figure 4: Chondrosarcoma with negative her-2 staining (high power)

Immunohistochemical HER-2 overexpression correlated significantly with osteosarcoma site with P value = 0.004, where 43.7% of the cases showed positive membranous staining, located in an extremity. Also, variation was found relating HER-2 intensity score to the site of osteosarcoma, and this difference nearly reached statistical significance, with P = 0.051.

Table 1: HER-2 immunohistochemical expression in correlation to osteosarcoma patients' clinicopathologic characteristics

		HER- 2 membranous staining			
		No membranous staining	Positive for any membranous staining	Total	P value
Gender	Female	1 (3.1%)	12 (37.5%)	13 (40.6%)	0.108
	Male	6 (18.8%)	13 (40.6%)	19 (59.4%)	
Site	Axial	3 (9.4%)	11 (34.4%)	14 (43.8%)	0.004
	Extremity	4 (12.5%)	14 (43.7%)	18 (56.2%)	
Presence of metastasis at the time of diagnosis	No metastasis	4 (12.5%)	24 (75.0%)	28 (87.5%)	0.006
	+ve for metastasis	3 (9.4%)	1 (3.1%)	4 (12.5%)	
Age group	Less than 20 yr	0 (0.0%)	10 (31.3%)	10 (31.3%)	0.044
	20 yr or more	7 (21.9%)	15 (46.9%)	22 (68.8%)	
Grade	High	7 (21.9%)	19 (59.4%)	26 (81.3%)	0.150
	Low	0 (0.0%)	6 (18.8%)	6 (18.8%)	
Presence of necrosis	Absent	2 (6.3%)	12 (37.5%)	14 (43.8%)	0.360
	Present	5 (15.6%)	13 (40.6%)	18 (56.3%)	
Presence of cartilage	Absent	5 (15.6%)	14 (43.8%)	19 (59.4%)	0.463
	Present	2 (6.3%)	11 (34.4%)	13 (40.6%)	
Presence of fibrous tissue	Absent	6 (18.8%)	10 (31.3%)	16 (50.0%)	0.033
	Present	1 (3.1%)	15 (46.9%)	16 (50.0%)	
Osteoid tissue detection	Focal	7 (21.9%)	19 (59.4%)	26 (81.3%)	0.150
	Wide	0 (0.0%)	6 (18.8%)	6 (18.8%)	

A statistically significant negative correlation was detected between HER-2 immunohistochemical expression and the presence of metastasis at the time of diagnosis (P = 0.006), as 85.7%, (24/28 cases) with the absence of metastatic deposits at the time of diagnosis showed HER-2 membranous staining. A significant correlation was also found regarding HER-2 score and presence of metastasis with P value = 0.046 as more than half of cases with no metastasis at diagnosis (17/28 cases, 60.7%) showed positive intensity score.

A statistically significant correlation was detected between levels of HER-2 immunohistochemical expression and patients' age (P

= 0.044) where HER-2 was expressed more in the younger age group (<20 years) in which all cases (10/10) showed membranous staining.

Table 2: Intensity scoring for HER-2 expression in correlation to clinicopathologic data of osteosarcoma cases

		HER-2 score				Total	P value
		0	1+	2+	3+		
Gender	Female	1 (3.1%)	3 (9.4%)	4 (12.5%)	5 (15.6%)	13 (40.6%)	0.403
	Male	6 (18.8%)	4 (12.5%)	3 (9.4%)	6 (18.8%)	19 (59.4%)	
Site	Axial	3 (9.4%)	3 (9.4%)	2 (6.3%)	6 (18.7%)	14 (43.7%)	0.051
	Extremity	4 (12.5%)	4 (12.5%)	5 (15.6%)	5 (15.6%)	18 (56.3%)	
Presence of metastasis at time of diagnosis	No metastasis	4 (12.5)%	7 (21.9%)	7 (21.9%)	10 (31.3%)	28 (87.5%)	0.046
	+ve for metastasis	3 (9.4%)	0 (0.0%)	0 (0.0%)	1 (3.1%)	4 (12.5%)	
Age group	Less than 20 yrs	0 (0.0%)	3 (9.4%)	2 (6.3%)	5 (15.6%)	10 (31.3%)	0.197
	20 yrs or more	7 (21.9%)	4 (12.5%)	5 (15.6%)	6 (18.8%)	22 (68.8%)	
Grade	High	7 (21.9%)	4 (12.5%)	5 (15.6%)	10 (31.3%)	26 (81.3%)	0.145
	Low	0 (0.0%)	3 (9.4%)	2 (6.3%)	1 (3.1%)	6 (18.8%)	
Presence of necrosis	Absent	2 (6.3%)	3 (9.4%)	4 (12.5%)	5 (15.6%)	14 (43.8%)	0.758
	Present	5 (15.6%)	4 (12.5%)	3 (9.4%)	6 (18.8%)	18 (56.3%)	
Presence of cartilage	Absent	5 (15.6%)	4 (12.5%)	4 (12.5%)	6 (18.8%)	19 (59.4%)	0.906
	Present	2 (6.3%)	3 (9.4%)	3 (9.4%)	5 (15.6%)	13 (40.6%)	
Presence of Fibrous tissue	Absent	6 (18.8%)	3 (9.4%)	3 (9.4%)	4 (12.5%)	16 (50.0%)	0.197
	present	1 (3.1%)	4 (12.5%)	4 (12.5%)	7 (21.9%)	16 (50.0%)	
Osteoid tissue detection	Focal	7 (21.9%)	4 (12.5%)	7 (21.9%)	8 (25.0%)	26 (81.3%)	0.038
	Wide	0 (0.0%)	3 (9.4%)	0 (0.0%)	3 (9.4%)	6 (18.8%)	

HER-2 immunohistochemical expression significantly correlated to histopathological detection of fibrous tissue, with P value = 0.033. Osteosarcoma cases showing fibrosis expressed HER-2 more frequently than those without a fibrous tissue.

Table 3: HER-2 immunohistochemical expression in correlation to chondrosarcoma patients' clinicopathologic characteristics

		HER-2 membranous staining			
		No membranous staining at all		Positive for membranous staining	
		Count	%	Count	%
Age group	Less than 52 yrs	10	50.0	0	0
	52 yrs or more	10	50.0	0	0
Gender	Female	12	60.0	0	0
	Male	8	40.0	0	0
Site	Axial	10	50.0	0	0
	Extremity	10	50.0	0	0
Biopsy	Excision	13	65.0	0	0
	Incision	7	35.0	0	0
Grade	I	16	80.0	0	0
	II	4	20.0	0	0
Cartilage	Focal	6	30.0	0	0
	Wide	14	70.0	0	0
Myxoid	No present	12	60.0	0	0
	Present	8	40.0	0	0
Necrosis	No	19	95.0	0	0
	Wide areas	1	5.0	0	0
Histological type	Chondrosarcoma, conventional	14	70.0	0	0
	Mesenchymal, Chondrosarcoma	1	5.0	0	0
	Myxoid Chondrosarcoma	5	25.0	0	0

Higher scores of HER-2 expression were associated with a significantly better differentiation (P = 0.038) since detection of wide areas of osteoid were associated with higher HER-2 scores.

There were no statistically significant differences in the other clinicopathologic features as patient gender, tumour grade, the presence of necrotic foci or cartilaginous areas between osteosarcomas cases expressing or lacking HER-2.

Overexpression of HER-2 has not noted in any of the chondrosarcomas studied cases with similar absence of expression in all cases regardless of diverse clinicopathologic characters.

Discussion

Bone tumours', mainly osteosarcoma and chondrosarcoma prognostic factors at diagnosis other than clinical stage have not been identified, and this is the only method that determines prognosis and therapy of patients. Therefore, there is a critical need for feasible prognostic and possibly therapeutic methods.

HER-2 has been applied as being a promising marker for targeted biological therapy. The development of trastuzumab, a humanised monoclonal antibody that binds specifically to HER's-2 prompted an opening to make use of this targeted therapy [15]. Thus, clinically the demand for HER-2 assessment is rapidly rising.

The HER-2 immunohistochemical expression has been detected in tissues derived from all three germ layers. Its expression has been observed in fetal tissue as well. In embryos, the expression of HER-2 was observed in the placenta, genitourinary tract epithelium, gastrointestinal tissue, pulmonary tract and the adrenal medulla. In contrast, HER-2 was not detected in liver, nervous tissue including brain, striated and smooth muscle, endothelium or fibroblasts of the embryo [16]. Adult tissue expression levels are lower than those in the early stages of development. These expression patterns ascertain the prospect for the involvement of HER-2 in a range of human neoplasms. The HER-2 expression has been recognised in some tumour types mainly breast, gastric, oesophageal, pancreatic, and many others. Even though there are agreement about antibodies and tyrosine kinase inhibitors targeting HER-2 in the breast, gastric and oesophagal adenocarcinomas, still there are other evident prospects in other tumour types [17].

In this study, we examined HER-2 expression in the commonest primary malignant bone tumours namely osteo and chondrosarcomas, immunohistochemically, as being a practical and cost-effective method available in most laboratories. Accordingly, immunohistochemistry is the most widespread method of HER-2 assessment.

In the current study, HER-2 has been identified as an important possible prognostic, therapeutic factor in osteosarcomas. First, we illustrated the immunohistochemical expression of HER-2 in osteosarcomas, where 78.1% of the studied cases (25/32) exhibited membranous staining (whether complete or not). Our results were higher than those of Gorlick et al., [18] who demonstrated somewhat high levels of HER-2 expression in 42.6% of their cases and Japanese investigators [19] who reported HER-2 overexpression in 42% of their patients. Moreover, Ma et al., [20] assured that 60.3% of their samples were HER-2 positive (ranging from low- to high-positivity). Additionally, Ebb et al., [21]

reported that around half of their cases showed positive HER-2 membranous staining. This high expression incidence was greater than what was previously demonstrated by Thomas et al., [22] and Kilpatrick [23] who both reported lack of membranous HER-2 immunoreactivity in their samples.

Moreover, Zhou et al., [24] revealed that the vast majority of their cases exhibited immunopositivity with focal to diffuse cytoplasmic reactivity yet without any membranous staining. This inconsistency could be explained by geographic and genetic variability between patients, sample size, differences in immunohistochemical processing (either decalcified or not and the duration of fixation), and variability in scoring methods with different thresholds or tumour heterogeneity [25]. Furthermore, the results are also contingent on the antibody used where Press et al. evaluated a panel of 28 antibodies (7 polyclonal and 21 monoclonal antibodies) on 187 cases previously shown to be HER-2 positive and demonstrated that the ability to detect HER-2 positive cells varied vastly from 6% to 82% [26].

In this research work, overexpression of HER-2 correlated significantly with the presence of osteosarcoma in an extremity rather than an axial site with P value = 0.004, also variation was noted relating the HER-2 intensity score to the osteosarcoma site, and this difference nearly reached statistical significance, with P value = 0.051. Also, a significant correlation was detected between the levels of HER-2 immunohistochemical expression and the patients' age (P = 0.044) where it was expressed more in the younger age group. Besides, a significant negative correlation was detected between HER-2 membranous immunohistochemical expression and absence of metastasis at the time of diagnosis (P = 0.006). This significant correlation was also attained as regards HER-2 score and the presence of metastasis with P value = 0.046, as more than half of cases which failed to show metastasis at diagnosis (60.7%, 17/28 cases) revealed positive intensity score. These findings support the belief that HER-2 level of expression may have a role in the early development of osteosarcoma, and it may act as a marker of good prognostic significance as it is down-regulated in tumors with metastatic deposits at the time of diagnosis. This is in agreement with the conclusions drawn by some previous investigators as Akatsuka and his colleagues [27], who demonstrated low levels of expression of HER-2 in metastatic lesions relative to the primary tumour. The majority of the earlier research studies demonstrated the poor prognostic effect of HER-2 overexpression mainly in the presence of metastasis as proclaimed by Gorlick et al., Ma et al., Onda et al., and Li et al., [18] [19] [20] [28] as they showed overexpression of HER-2 with poor prognostic factors mainly the presence of metastasis.

In this work, we reported that osteosarcomas with considerable fibrosis expressed HER-2 more frequently than osteosarcomas without, achieving a statistically significant relationship with a P value of 0.033. Also, higher scores of HER-2 immunohistochemical expression were associated with significantly better differentiation (P = 0.038) since detection of wide areas of osteoid were associated with higher HER-2 scores. Similar patterns of expression were also observed in the study by Zhou et al., [24] where they reported cytoplasmic staining more in undifferentiated spindle cells, while both membranous and cytoplasmic staining was detected in differentiated, malignant-appearing chondroblastic foci.

Regarding chondrosarcoma, the level of immunohistochemical expression of HER-2 was similar in all cases as we didn't find membranous staining in any of the examined cases. Our findings were just identical to those reported by Nelson et al. and Park et al., [17] [29] who couldn't detect immunohistochemical staining of HER-2 in their chondrosarcoma cases. Therefore, it is unlikely that targeted therapy against HER-2 overexpression could be used effectively in the treatment of such miserable patients also experiencing well-known resistance to most chemotherapeutic regimens.

In conclusion, Her-2 has expressed in bone sarcomas specifically osteosarcoma, and it is probably related to tumour prognosis and outcome. It may act as a marker of good prognostic significance as it is down-regulated in tumours with metastatic deposits at time of diagnosis.

Further clinicopathologic research work would still be needed to delineate its role being a new hope for therapeutic targeting in bone sarcoma patients, mainly osteosarcoma in contrast to chondrosarcoma that didn't express HER-2 at all.

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