

Clinical Relevance of HER-2/neu Expression in Germ-cell Testicular Tumors

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Abstract. *Background: Amplification and/or overexpression of HER-2/neu are associated with poor clinical outcome in several epithelial tumors. However, the exact prognostic role of HER-2/neu expression in testicular germ-cell tumors is equivocal. Patients and Methods: Since teratomas are relatively chemoresistant tumors, we evaluated the HER-2/neu alterations of 59 primary testicular teratomas and mixed germ-cell tumors containing teratomatous components using the standardized immunohistochemical method (IHC) (HercepTest) and Fluorescence in Situ Hybridization (FISH). Results: HER-2/neu overexpression was detected in 14 (24%) out of 59 specimens. With IHC, teratomatous and choriocarcinoma components showed significantly higher HER-2/neu expression compared to other histological subtypes of GCTs ($p=0.0095$). A statistically significant correlation ($p=0.0004$) can be established between HER-2/neu status and clinical stage of the disease. Similarly, a significant correlation was observed between HER-2/neu overexpression and clinical outcome ($p=0.0077$). None of the specimens had definite HER-2/neu gene amplification. Conclusion: Our results suggest that HER-2/neu overexpression is associated with an adverse clinical outcome and has a prognostic role in testicular germ-cell tumors. Further studies are needed to evaluate the exact background of HER-2/neu overexpression in germ-cell tumors and the role of anti-HER-2/neu antibodies in the treatment regimens for this malignancy.*

In Western countries, testicular germ-cell tumors (GCTs) account for up to 60% of all malignancies diagnosed in male patients between 20 and 40 years of age (1). GCT is one of the most sensitive tumors to cisplatin-based chemotherapy, since in disseminated cases a remission rate of 80% can be

obtained (2). However, up to 30% of patients with advanced tumor will not achieve a durable remission after initial treatment. Patients who experience disease progression during cisplatin-based chemotherapy or experience relapse after salvage treatment achieve long-term survival rates of less than 5% (3). In contrast to other histological subtypes, due to intrinsic chemoresistance, mature teratomas and choriocarcinomas are unresponsive to chemotherapy. In the former case primary surgical intervention has great importance. The fact that mature teratoma can be found in 30-40% of radiologically detected residual lesions after chemotherapy for non-seminomatous germ-cell tumors (NSGCTs) (4) renders salvage therapy necessary.

Improvement of the chemosensitivity of GCTs is dependent on a better understanding of their mechanisms of chemoresistance. The molecular as well as clinical differences that segregate curable from incurable disease are not defined. Identification of specific gene abnormalities or protein expressions that may be targeted by novel therapies appears the potentially most rewarding approach.

In recent years the importance of growth factors and growth factor receptors has been recognized in the pathogenesis of human neoplasms (5). Epidermal Growth Factor Receptor (EGFR) is one of the most widely investigated cell surface receptors. Although several subtypes of EGFR are known, only EGFR-2 (HER-2/neu, c-erbB-2) has major clinical relevance. EGFR-2 is overexpressed in the cell membrane in 25-30% of human breast cancers. HER-2/neu overexpression in breast cancer tissue samples correlates well with worse clinical outcome, shorter overall survival (6) and predicts relative chemoresistance to standard chemotherapy (7). Treatment of patients with breast cancers overexpressing HER-2/neu with trastuzumab (monoclonal antibody synthesized against the extracellular domain of HER-2/neu protein) results in a remission rate of 19-34% (8).

An increased expression of HER-2/neu has been demonstrated in several human tumors including cancers of the gastrointestinal tract (9), liver (10), lung (11), ovary (12), uterus (13), bladder (14) and prostate (15).

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Key Words: HER-2/neu, FISH, germ-cell testicular tumors.

In testicular GCTs, the HER-2/neu status, its prognostic role and possible therapeutic implications are still under investigation. Only a few papers have concentrated on this field. In unselected cases Shuin *et al.* (16) found high levels of EGFR expression in immature teratomas compared to other histological subtypes of GCTs. Hechelhammer *et al.* (17) found EGFR expression in the majority of teratomatous components of mixed GCTs. Our previous results revealed that HER-2/neu protein overexpression occurs in 25% of GCTs containing teratomatous components. The overexpression was restricted to the more differentiated histotypes, such as teratomatous or choriocarcinomatous components (18).

These facts encouraged us to determine the HER-2/neu expression in a larger series of pure teratomas and mixed GCTs containing teratomatous components. We used IHC and FISH to compare the protein overexpression and HER-2/neu gene amplification in primary testicular tumors. We assessed the association between the HER-2/neu status, clinical outcome and patients' clinical parameters.

Patients and Methods

Patients. Specimens were obtained by the semicastration of 59 patients with testicular tumor between the years 1992 and 2002. Only pure teratomas and mixed germ-cell tumors containing teratomatous components were enrolled in this study. Prior to surgery the patients received neither radio-, nor chemotherapy. The tumors were histopathologically classified according to WHO criteria (19). For clinical staging, physical examination, serum markers of the beta subunit of human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP), chest X-ray and computerized tomography (CT) scans were routinely used. All patients were staged according to UICC classification (20). Chemotherapy was performed according to Institutional protocol (21). Clinical response was measured according to generally used criteria in testis cancer (22). The clinical and pathological characteristics of patients are detailed in Table I. The median age of patients at orchiectomy was 32 years. Median follow-up after castration was 57 months. The overall 5-year survival was 91.5%.

IHC detection of HER-2/neu protein. Specimens were fixed in 4% buffered formalin. After paraffin embedding, 4-µm sections were cut. Heat-induced epitope retrieval was performed in a water bath (95°C, 50 minutes) and automated IHC staining with the DAKO HercepTest™ was performed on an automated immunostaining system (DAKO Autostainer) in strict accordance with the manufacturer's instructions. Negative and positive controls consisted of control cell lines included in the DAKO Kit (cell lines MDA-231, MDA-175 and SK-BR-3). To prove the reproducibility of IHC-staining, four slides of each specimen were examined.

Each IHC case was analyzed by 3 independent pathologists (J.T., L.M., M.B.). The IHC-stained slides were interpreted and scored on the scale of 0 to 3+, according to the FDA-approved guidelines for HercepTest™ (23); 2+ and 3+ reactions were assessed as positive.

Table I. *Clinical and pathological characteristics of the 59 patients.*

	No. of cases (%)	
Number of patients	59	(100%)
Mean age 32 years (range 16-60)		
5-year survival 91.5%		
Median follow-up 57 months (range 2-137)		
Histology of the primary tumor		
T	12	(20%)
T+S	5	(8%)
T+EC	26	(45%)
T+CC	1	(2%)
T+S+EC	6	(10%)
T+EC+YS	2	(3%)
T+EC+CC	7	(12%)
Clinical stage		
Early stage (I,IIA)	23	(39%)
Late stage (IIB,IIC,III)	36	(61%)
Therapy after semicastration		
Wait and see	3	(5%)
RLA	5	(8%)
Chemotherapy	36	(61%)
Chemotherapy+salvage surgery	1	(2%)
RLA+chemotherapy	13	(22%)
Chemotherapy+radiotherapy	1	(2%)
Clinical outcome		
No Evidence of Disease	8	(14%)
Complete Response	39	(66%)
Partial Response	4	(7%)
Stable Disease	1	(2%)
Progressive Disease	2	(3%)
Died	5	(8%)

T, teratoma; S, seminoma; EC, embryonal carcinoma; CC, choriocarcinoma; YS, yolk sac tumor; RLA; retroperitoneal lymphadenectomy.

Determination of HER-2/neu gene amplification by FISH. FISH was performed according to the Ventana HER-2/neu FISH protocol as an automated procedure for use on the Ventana BenchMark™ system. In brief, tumor blocks were cut in 1-µm sections. The sections were dewaxed by adding Xylene (100%, 3x2 min) and dehydrated in ethanol (100%, 2x2 min). After air drying, the sections were digested with protease (37°C, 40 min). Following rinsing (SCC, 3x2 min), the slides were dehydrated in a graded series of ethanol (95% and 100%, 1 min each) and were denatured (90°C, 10 min). Hybridization was performed with fluorescent (FITC)-labeled probes for HER-2/neu gene (Ventana INFORM 780-2840 Probe) according to the manufacturer's recommendation. Slides were counterstained by 4,6-diamidino-2-phenylindole dihydrochloride hydrate and visualized using a fluorescent microscope with FITC/DAPI dual band filter.

Table II. HER-2/neu status by immunohistochemistry.

HER-2/neu overexpression	No. of cases	
0	27	(46%)
+	18	(30%)
++	10	(17%)
+++	4	(7%)
Total	59	

At least 40 well-defined, representative, intact nuclei from each of two separate tumor areas were scored. FISH evaluation was performed only on tissue sections with uniform hybridization; overlapping nuclei were not evaluated.

HER-2/neu status was scored as follows: a. no amplification (up to 4 specific signals/nucleus). b. borderline amplification (4.1-5 specific signals/nucleus). c. definite amplification (more than 5 signals/nucleus).

Statistical methods. We analyzed the relationship between the HER-2/neu status and histological subtype, clinical stage and clinical outcome. Categorical variables were compared by the two-tailed Fisher's exact probability test. A probability ≤ 0.05 was considered to present statistical significance. The GraphPad Prism 2.01 (GraphPad Software Inc.) was used for calculations.

Results

IHC and its clinicopathological correlations. HER-2/neu overexpression was detected in 14 (24%) out of 59 specimens (Table II). Table III shows the relationship between HER-2/neu overexpression and histology of tumors (Figures 1, 2). Two out of 12 pure teratomas showed HER-2/neu positivity. Teratomatous and choriocarcinoma components showed significantly higher HER-2/neu expression compared to other histological subtypes of GCTs ($p=0.0095$).

The distribution of patients according to the stage of tumors and the HER-2/neu IHC status is shown in Table IV. All of the HER-2/neu overexpressing tumors were in advanced stage. A statistically significant correlation ($p=0.0004$) can be established between HER-2/neu IHC status and stage of the disease. Similarly, a significant correlation was found between HER-2/neu overexpression and clinical outcome ($p=0.0077$, Table V). In contrast to HER-2/neu-negative patients, only 56% of HER-2/neu-positive patients showed no evidence of tumor, or achieved complete response. Forty-four percent of the HER-2/neu-positive group lives with measurable tumor burden or died of disease.

FISH. None of the 59 specimens showed definite gene amplification. Borderline amplification (4.1 and 4.2) was found in 2 embryonal carcinoma (EC) components of mixed

Table III. Correlation between HER-2/neu immunostaining and histology.

Histological tumor type	Number of tumor components	HER-2/neu overexpression		% of positive cases	<i>P</i>
		-	+		
Seminoma	11	11	0	0%	0.0095
Embryonal carcinoma	41	37	4	10%	
Yolk sac tumor	2	2	0	0%	
Teratoma	59	46	12	20%	
Choriocarcinoma	9	4	5	56%	

Table IV. Correlation between HER-2/neu immunostaining and stage of tumor.

Clinical stage	No of cases	HER-2/neu overexpression		% of positive cases	<i>P</i>
		-	+		
Early stage (I, II/A)	23	23	0	0%	0.0004
Late stage (II/B-C, III)	36	22	14	39%	

Table V. Correlation between HER-2/neu overexpression and clinical outcome.

Clinical outcome	HER-2/neu overexpression				<i>P</i>
	-		+		
NED, CR	40	(89%)	8	(56%)	0.0077
AWD	3	(7%)	3	(22%)	
DOD	2	(4%)	3	(22%)	
Total	45	(100%)	14	(100%)	

NED: no evidence of disease; CR: complete response; AWD: alive with disease; DOD: dead as a result of disease.

GCTs (Figure 3). These cases, however, showed no HER-2/neu overexpression with IHC.

Discussion

The HER-2/neu oncogene and protein have been extensively investigated as a prognostic factor and more recently as a predictor of response to therapy in various human tumors. Anti-HER-2/neu therapy with trastuzumab, a targeted antineoplastic monoclonal antibody, has been shown to improve outcome for women with HER-2/neu

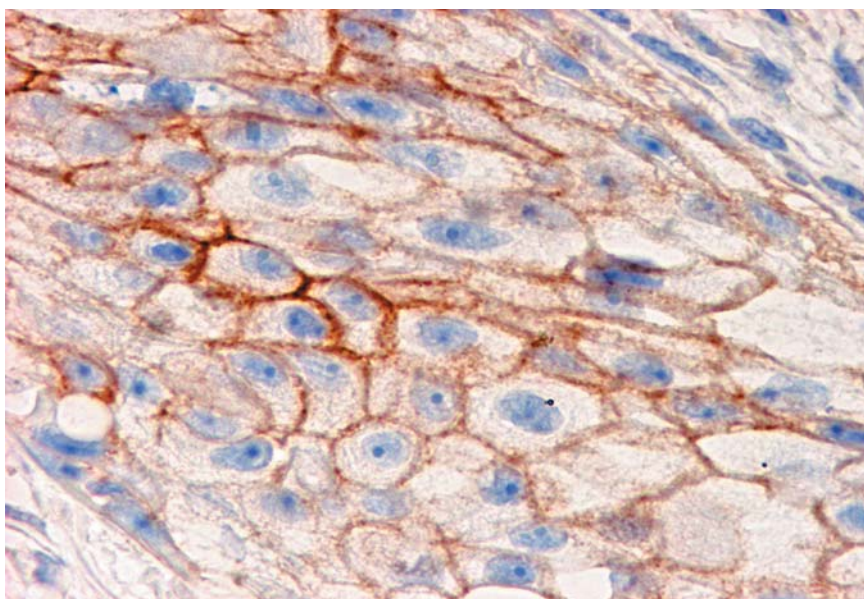


Figure 1. Pure mature teratoma. An intense staining of the entire membrane is observed in about 50% of the epithelial cells (3+ HER-2 positivity). (HercepTest™ x200)

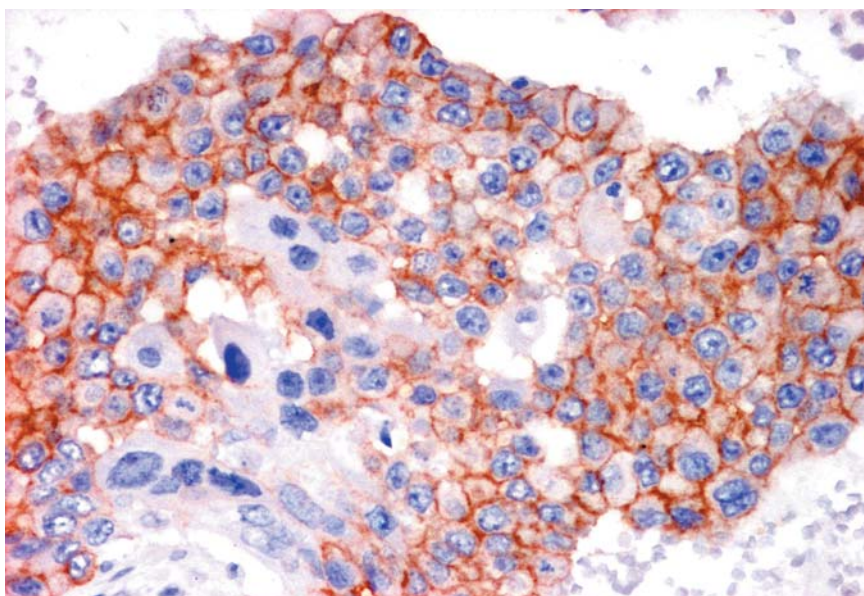


Figure 2. Mixed germ cell tumor, choriocarcinoma component. An intense staining of the entire membrane is observed in more than 50% of the cytotrophoblasts (3+ HER-2 positivity). (HercepTest™ x100)

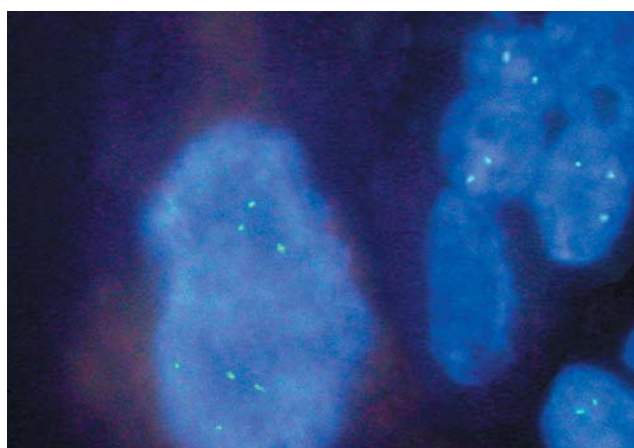


Figure 3. Embryonal carcinoma. Borderline amplification (3 to 7 signals/nucleus) with fluorescence in situ hybridization is probably due to polyploidy or high mitotic rate. Slide was stained with fluorescent (FITC)-labeled probes for HER-2/neu gene and with DAPI for DNA staining. (x600).

overexpressing metastatic breast cancer. This exemplary therapy would be beneficial for patients suffering from malignancies other than breast carcinoma.

The number of studies on the topic of the HER-2/neu status of GCTs is small and its prognostic role is equivocal. There is only one reported case of a chemoresistant GCT responding to trastuzumab treatment (24).

Because teratomas are chemoresistant tumors and previous studies showed that pure teratomas and teratomatous components of mixed GCTs overexpress HER-2/neu in a significant number of unselected cases, we examined a large series of NSGCTs containing teratomatous components.

To compare the HER-2/neu gene amplification and protein overexpression, we used standardized detection systems. The concordance between the results of these methods is 80-95% in breast cancer tissues (25). Although HER-2/neu was overexpressed in 14 (24%) out of 59 primary GCTs, we found only borderline amplification without protein overexpression in two EC components of mixed GCTs. Aneuploidy, polyploidy and high mitotic rate can explain this result. None of the tumor samples showed definite gene amplification. This finding partly correlates with the result of Soule *et al.* (26) who investigated 96 GCTs (primary orchiectomy specimens, post chemotherapy retroperitoneal lymph node resections, late relapse, primary mediastinal tumors). Twenty-two of these 96 GCTs overexpressed the HER-2/neu protein measured by IHC and only four of them showed HER-2/neu gene amplification by FISH. No gene amplification was demonstrated in primary testicular tumors. The discrepancy between the results of FISH and IHC suggests that mechanisms other than gene amplification may play a role in protein overexpression. Investigation into a correlation between mRNA expression and protein overexpression could confirm this hypothesis.

We found significantly higher HER-2/neu overexpression in the better differentiated teratomatous and choriocarcinoma components of GCTs than the less differentiated histological subtypes ($p=0.0095$). This is in concordance with the results of Shuin *et al.* (16), who examined the expression of 15 different protooncogenes (including *c-erbB-1* and *c-erbB-2*) in 26 primary GCTs (17 seminomas, 6 embryonal carcinomas, 3 immature teratomas). Three pure immature teratoma specimens showed high levels of *c-erbB-1* and *c-erbB-2* expression, whereas other types of GCTs showed negative or minimum levels of these protooncogenes with Northern blot analyses. Hechelhammer *et al.* (17) observed EGFR reactivity in 71% of the epithelial compartments of teratoma. In their study, EGFR staining was consistently detected in syncytiotrophoblastic cells. Moroni *et al.* (27) studied 24 testicular GCTs. Staining for cell membrane EGFR-1 was detected immunohistochemically in the 16 hCG-positive components of 18 NSGCTs. HER-2/neu expression was detected in 25% of EGFR-1-positive NSGCTs.

GCTs are derived from totipotential germ cells. Seminoma evolves from a common neoplastic precursor lesion, while EC arises from seminoma. The other differentiated forms of GCT, representing somatic (teratomatous) and trophoblastic differentiation, most commonly arise from EC. This differentiation recapitulates embryogenesis. The proliferation and differentiation control of epithelial cells is partly regulated by EGFRs. Therefore, EGFR-mediated cell activation may play a pivotal role in GCT development, differentiation and progression (27). Overexpression of HER-2/neu in the differentiated tumors, such as choriocarcinoma, or teratoma possibly correlates with a more metastasizing potential and/or with a poor response rate to chemotherapy.

The prognostic role of HER-2/neu overexpression is well demonstrated in breast cancer and in other epithelial tumors, including ovarian, gastric, lung and urinary bladder carcinomas as well. Extensive investigations have not been carried out so far to determine the correlation between HER-2/neu overexpression and clinical outcome of testicular GCTs. The present work provides evidence that HER-2/neu overexpression correlates with the stage ($p=0.0004$) and clinical outcome ($p=0.0077$) of GCTs containing teratomatous components. All of the HER-2/neu-positive cases were in advanced stage and 44% of these patients died, or did not achieve CR. Therefore HER-2/neu overexpression has a prognostic role in this set of tumors. Because of the low mortality rate, we could not define a correlation between HER-2/neu overexpression and overall survival. Five HER-2/neu-negative patients showed progression and two of them died. This suggests that HER-2/neu overexpression is not an absolute marker of chemoresistance.

Further investigations are needed to determine the background and exact role of HER-2/neu overexpression in GCTs. The abovementioned results should encourage us to prove the effectiveness of HER-2/neu-targeted pharmaceutical agents, through *in vitro* (cell-line) and *in vivo* (xenograft) experiments. Patients with advanced stage, progressive, HER-2/neu-positive tumors, which are resistant to conventional therapy, should be enrolled in phase II clinical trials to verify the therapeutic effect of trastuzumab in GCTs.

Acknowledgements

This study was supported by the grant 311/2003 ETT Ministry of Health, Social and Family Affairs and by the National Consortium of Oncology, Hungary (NFKP-48/2001).

References

- 1 Pottern ML, Brown M and Devesa SS: Epidemiology and pathogenesis of testicular cancer. *In*: Testicular and Penile Cancer. (Ernststoff MS, Heaney JA, Peschel RE eds). Oxford, Blackwell Science, 1998, pp 113-136.
- 2 Einhorn LH: Treatment of testicular cancer: a new and improved model. *J Clin Oncol* 11: 1177-1181, 1990.

- 3 Beyer J, Kramar A, Mandanas R, Linkesch W, Greinix A, Droz JP, Pico JL, Diehl A, Bokemeyer C, Schmoll HJ, Nichols CR, Einhorn LH and Siegert W: High-dose chemotherapy as salvage treatment in germ cell tumors: a multivariate analysis of prognostic variables. *J Clin Oncol* 14: 2638-2645, 1996.
- 4 Fizazi K, Tjulandin S, Salvioni R, Germa-Lluch JR, Bouzy J, Ragan D, Bokemeyer C, Gerl A, Flechon A, de Bono JS, Stenning S, Horwich A, Pont J, Albers P, De Giorgi U, Bower M, Bulanov A, Pizzocaro G, Aparicio J, Nichols CR, Theodore C, Hartmann JT, Schmoll HJ, Kaye SB, Culine S, Droz JP and Mahe C: Viable malignant cells after primary chemotherapy for disseminated nonseminomatous germ cell tumors: prognostic factors and role of postsurgery chemotherapy--results from an international study group. *J Clin Oncol* 19: 2647-2657, 2001.
- 5 Salomon DS, Brandt R, Ciardiello F and Normanno N: Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Hematol Oncol* 19: 183-232, 1995.
- 6 Menard S, Balsari A, Casalini P, Tagliabue E, Campiglio M, Bufalino R and Cascinelli N: HER-2-positive breast carcinomas as a particular subset with peculiar clinical behaviors. *Clin Cancer Res* 8: 520-525, 2002.
- 7 Allred DC, Harvey JM, Berardo M and Clark GM: Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 11: 155-168, 1998.
- 8 Vogel CL, Cobleigh M, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S and Stewart SJ: Superior outcomes with herceptin (trastuzumab) (H) in fluorescence *in situ* hybridization (FISH)-selected patients (abstract). *Proc Am Soc Clin Oncol* 20: 335a, 2001.
- 9 Ross JS and McKenna BJ: The HER-2/neu oncogene in tumors of the gastrointestinal tract. *Cancer Invest* 19: 554-568, 2001.
- 10 Ukita Y, Kato M and Terada T: Gene amplification and mRNA and protein overexpression of c-erbB-2 (HER-2/neu) in human intrahepatic cholangiocarcinoma as detected by fluorescence *in situ* hybridization and immunohistochemistry. *J Hepatol* 36: 780-785, 2002.
- 11 Nakamura H, Saji H, Ogata A, Hosaka M, Hagiwara M, Kawasaki N and Kato H: Correlation between encoded protein overexpression and copy number of the HER2 gene with survival in non-small cell lung cancer. *Int J Cancer* 103: 61-66, 2003.
- 12 Bookman MA, Darcy KM, Clarke-Pearson D, Boothby RA and Horowitz IR: Evaluation of monoclonal humanized anti-HER2 antibody, trastuzumab, in patients with recurrent or refractory ovarian or primary peritoneal carcinoma with overexpression of HER2: a phase II trial of the Gynecologic Oncology Group. *J Clin Oncol* 21: 283-290, 2003.
- 13 Santin AD, Bellone S, Gokden M, Palmieri M, Dunn D, Agha J, Roman JJ, Hutchins L, Pecorelli S, O'Brien T, Cannon MJ and Parham GP: Overexpression of HER-2/neu in uterine serous papillary cancer. *Clin Cancer Res* 8: 1271-1279, 2002.
- 14 Latif Z, Watters AD, Dunn I, Grigor KM, Underwood MA and Bartlett JM: HER2/neu overexpression in the development of muscle-invasive transitional cell carcinoma of the bladder. *Br J Cancer* 89: 1305-1309, 2003.
- 15 Fossa A, Lilleby W, Fossa SD, Gaudernack G, Torlakovic G and Berner A: Independent prognostic significance of HER-2 oncoprotein expression in pN0 prostate cancer undergoing curative radiotherapy. *Int J Cancer* 99: 100-105, 2002.
- 16 Shuin T, Misaki H, Kubota Y, Yao M and Hosaka M: Differential expression of protooncogenes in human germ cell tumors of the testis. *Cancer* 73: 1721-1727, 1994.
- 17 Hechelhammer L, Storkel S, Odermatt B, Heitz PU and Jochum W: Epidermal growth factor receptor is a marker for syncytiotrophoblastic cells in testicular germ cell tumors. *Virchows Arch* 443: 28-31, 2003.
- 18 Mandoky L, Geczi L, Bodrogi I, Toth J and Bak M: Expression of HER-2/neu in testicular tumors. *Anticancer Res* 23: 3447-3451, 2003.
- 19 Mostofi FK, Sesterhenn IA and Davis CJ: World health organisation international histological classification of germ-cell tumours of the testis. *In: Germ-cell Tumours*. (Jones WG, Ward MA and Anderson CK, eds). Oxford, Pergamon, 1986, pp 1-32.
- 20 Sobin LH and Wittekind C: UICC TNM Classification of Malignant Tumours. New York, Wiley-Liss. 1997, pp 174-179.
- 21 Geczi L, Gomez F, Horvath Z, Bak M, Kisbenedek L and Bodrogi I: Three-year results of the first educational and early detection program for testicular cancer in Hungary. *Oncology* 60: 228-234, 2001.
- 22 Flechon A, Rivorie M, Biron P and Droz JP: Importance of surgery as salvage treatment after high dose chemotherapy failure in germ cell tumors. *J Urol* 165: 1920-1926, 2001.
- 23 Perez EA, Roche PC, Jenkins RB, Reynolds CA, Halling KC, Ingle JN and Wold LE: HER2 testing in patients with breast cancer: poor correlation between weak positivity by immunohistochemistry and gene amplification by fluorescence *in situ* hybridization. *Mayo Clin Proc* 77: 148-154, 2002.
- 24 Kollmannsberger C, Pressler H, Mayer F, Kanz L and Bokemeyer C: Cisplatin-refractory, HER/neu-expressing germ-cell cancer: induction of remission by the monoclonal antibody Trastuzumab. *Ann Oncol* 10: 1393-1394, 1999.
- 25 Jacobs TW, Gown AM, Yaziji H, Barnes MJ and Schnitt SJ: Comparison of fluorescence *in situ* hybridization and immunohistochemistry for the evaluation of HER-2/neu in breast cancer. *J Clin Oncol* 17: 1974-1982, 1999.
- 26 Soule S, Baldrige L, Kirkpatrick K, Cheng L, Gilbert JL, Smith LR, Thurston VC, Vance GH, Einhorn L and Miller K: HER-2/neu expression in germ cell tumours. *J Clin Pathol* 55: 656-658, 2002.
- 27 Moroni M, Veronese S, Schiavo R, Carminati O, Sorensen B, Gambacorta M and Siena S: Epidermal growth factor receptor expression and activation in nonseminomatous germ cell tumors. *Clin Cancer Res* 7: 2770-2775, 2001.

Received February 4, 2004

Accepted April 8, 2004