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European Journal of Cancer 40 (2004) 924–925

European Journal of Cancer

www.ejconline.com

Editorial Comment

In relation to Anninga *et al.* Overexpression of the *HER*-2 oncogene does not play a role in high-grade osteosarcomas

1. HER-2 expression in osteosarcoma—triumph of hope over reality?

The history of medicine teaches us that doctors are as prey to the whim of fashion as shoppers in the high street. The current trend in oncology is for the use of molecularly targeted therapy, to the extent that some pundits predict that we will shortly cease to use cytotoxic chemotherapy, in spite of evidence to the contrary. It is inevitable that in this context we seek to find evidence for the presence of novel targets that might be amenable to treatment, in our disease of interest.

HER-2, a transmembrane receptor of the epidermal growth factor receptor (EGFR) superfamily, is associated with a metastatic phenotype in breast cancer and is also overexpressed in a proportion of patients with ovarian cancer. Trastuzumab (Herceptin®), a monoclonal antibody against the HER-2 receptor, is licensed for the treatment of metastatic breast cancer, either in combination with paclitaxel for patients who cannot be given an anthracycline or as monotherapy following prior treatment with anthracyclines and taxanes. It has been approved for the treatment of tumours that score 3+ for HER-2 expression, using internationally agreed criteria. In the light of this knowledge, reference laboratories were established to ensure that the diagnosis of HER-2 overexpression was made accurately, using a standard test—the DAKO HERCEPTEST® [1].

It is not surprising that reports of HER-2 positivity in osteosarcoma should also be viewed as a potential opportunity to use trastuzumab for the treatment of refractory patients [2]. However, we know that expression of a receptor tyrosine kinase by a tumour does not necessarily indicate that it is important for the proliferation or survival of those tumour cells. For example, in gastrointestinal stromal tumours the receptor tyrosine kinase KIT, receptor for stem-cell factor, is overexpressed, and strong membrane staining for CD117 is accepted as a standard diagnostic test in this disease [3], albeit not in isolation. The tyrosine kinase inhibitor imatinib is an extremely effective treatment for patients with advanced inoperable or metastatic gastrointestinal stromal tumours (GIST) [4]. KIT is usually constitutively activated by specific mutations in GIST [5]. However, while imatinib is effective in tumours with the commonest, exon 11, *KIT* mutation, if *KIT* is wild type the response rate is low, i.e. 10%, rather than 85% [6]. Those patients who do respond may well do so as a result of imatinib-sensitive activating mutations in the gene for platelet-derived growth factor receptor-α [7]. While KIT may be expressed by a variety of other tumours, such as small cell lung cancer, it is not mutated in these situations and to date evidence is lacking for antitumour activity with imatinib in common solid tumours [8].

Although a clear correlation has been established between response to trastuzumab and HER-2 expression in breast cancer, this has not been true for the tyrosine kinase inhibitor gefitinib and EGFR expression in lung cancer. Gefitinib produces a response rate of 10–15% in non-small cell lung cancer [9] but it has proved difficult to define which patients are likely to benefit. This does not appear to be predicted by the level of expression of EGFR using conventional immunohistochemical techniques, either in patients or in the laboratory [10]. The doses of gefitinib currently in use undoubtedly inhibit the EGFR-signalling pathway in surrogate tissues [11] and larger doses appear to produce more toxicity without increasing antitumour activity [12].

Returning to the case of HER-2 and osteosarcoma, the literature is rather inconsistent. The early study by Gorlick and colleagues used immunohistochemistry alone in a retrospective analysis of 53 patients [2]. HER-2 staining of 2+ or more was regarded as positive, which is questionable, but nevertheless membrane staining of 3+ and 4+ was observed in a proportion of cases. There was no confirmation of HER-2 expression via gene amplification using either FISH or RT-PCR. HER-2 expression did appear to correlate with clinical behaviour in that analysis. HER-2 overexpression via gene amplification has been claimed to be common by some [13] and to be very rare by others [14]. Some

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2004.02.001

laboratories have found that not only were they unable to demonstrate gene amplification using RT-PCR but also that HER-2 staining was predominantly cytoplasmic [15]. This has also been reported by others and suggests that the HER-2 staining is an artefact, since if functional, HER-2 should be present in the cell membrane.

The meticulous study reported by Anninga *et al.* [18] examined 33 osteosarcoma patients and failed to find any evidence of strong HER-2 membrane staining using the DAKO HERCEPTEST®, nor evidence of mRNA overexpression by RT-PCR or gene amplification using FISH. They demonstrate the importance of using all available positive and negative controls for the immunohistochemical findings. They used cell lines with known negative or positive HER-2 expression as the negative and positive controls for immunohistochemistry, something not done hitherto. In their study, even when moderate HER-2 expression was seen this was not associated with evidence of enhanced gene expression. They suggest that previous reports of gene amplification in the literature might be false positives if *HER-2* gene copy number was not linked to chromosome 17 centromere number, as recommended, since aneusomy of chromosome 17 is common in osteosarcoma. However, osteosarcomas have a complex karyotype and marked genetic instability. Amplifications and rearrangements affect a number of chromosomes, 8q, 17p and 20q being the commonest [16]. This being the case, low levels of *HER-2* gene amplification could genuinely be found, although not at the levels seen in breast cancer, where 25–100 copies of the gene per cell have been reported [17]. Anninga *et al.* [18] also suggest that the only study appearing to show convincing membrane staining for HER-2 [2] did so because a different antibody, 5B5, was used. However, in that report it states that the results with 5B5 were compared with the HERCEPTEST® and 29/33 samples scored similarly; hence this does not appear to be the sole explanation [2].

Taking all the data into account it does seem likely that HER-2 overexpression in osteosarcoma is indeed rare and is not associated with a fundamentally important signalling process that is amenable to therapeutic intervention. It is notable that there have still been no reports of positive clinical trials of trastuzumab in this disease. Only a clinical trial with meticulous prospective evaluation of HER-2 expression would answer this question finally, but the data suggest that such a study would never be justified.

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