ANNALES
UNIVERSITATIS MARIE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LVIII, N 1, 21 SECTIO D 2003

Pulmonary Department, Medical University of Lublin

PAWEŁ KRAWCZYK, SYLWIA CHOCHOLSKA,
JANUSZ MILANOWSKI

# *Anti-HER therapeutic agents in the treatment of non-small-cell lung cancer*

## HER GENE FAMILY

HER-receptor family consists of four related genes, designed as *HER1-HER4*. These genes encode four structurally similar transmembrane proteins (EGFR, HER2, ErB-3, and ErB-4) with tyrosine kinase activity that dimerise on binding with a number of ligands, including epidermal growth factor (EGF) and transforming growth factor alpha (TGFα). There is strong laboratory and clinical evidence to implicate members of this family, especially *HER1* and *HER2*, to carcinogenesis in a variety of epithelial tissues. Intracellular signalling initiated by homo- or heterodimers of members of this family leads to stimulation of cellular proliferation. Cells transformed by *HER* genes also demonstrate increased invasive and metastatic capacity.

Amplification of the *HER2* gene (*c-erbB-2*/neu) was one of the first genetic changes associated with the pathogenesis of human cancers. Overexpression of the *HER2* gene encoding a 185-kD transmembrane glycoprotein was found to contribute to oncogenic transformation, tumorigenesis and metastatic potential in breast cancer, for instance. Several studies found a correlation between overexpression of *HER2* and a shorter disease-free and overall survival in women affected by the disease. Therefore, being also associated with potentially more aggressive tumours, *HER2* overexpression detected in 25–30% of human breast cancers is considered as a negative prognostic factor (5,6,11).

Overexpression of HER2/neu protein is observed in 20–66% of resected non-small-cell lung cancer (NSCLC) tumours and was shown to predict poor patient outcome in multiple series (1). Experiments with NSCLC cell lines show that HER2 overexpression increases chemoresistance, invasiveness and metastatic potential of the cells. Positive HER2 expression is most often seen in adenocarcinomas in comparison with squamous-cell carcinomas or large-cell carcinomas, and is rarely seen in small-cell lung cancer (SCLC) (9).

Mature epidermal growth factor receptor (EGFR) encoded by the *HER1* gene is a 170-kD transmembrane glycoprotein with four functional domains: an extracellular ligand-binding domain, a transmembrane domain, the catalytic protein kinase domain, and the COOH-terminal regulatory domain. Many of the ligands that bind to EGFR are expressed in malignant tissues. The expression of EGFR and its ligand – TGFα within tumours is consistent with an autocrine or paracrine mechanism of growth stimulation (12).

*HER1* alterations seem to play an equally important or even greater role than overexpression of *HER2* in the development of lung cancer. EGFR overexpression and/or amplification were reported in many cases of head and neck cancer. Gene amplification, however, could only account for a fraction of these cases. Additional mechanisms like promoter

alterations and intragenic mutations are candidate mechanisms that contribute to EGFR overexpression. Shintani et al. reported two consistent intragenic *HER1* alterations in malignant human oral keratinocytes: a truncated form of *EGFR* and tumour-associated polymorphism at position 2073 generating a unique *BsrI* restriction site (10,12). Gebhardt et al. examined the influence of a highly polymorphic CA dinucleotide repeat in the *HER1* gene on transcription. They demonstrated that *HER1* transcription is inhibited by approximately 80% in alleles with 21 CA repeats. Transcription of *HER1* starts at multiple initiation sites within the GC-rich promoter that lacks a TATA or CAAT box. It was shown that the first intron of *HER1*, as of several other genes, has an important regulatory function. Moreover, a polymorphic simple sequence repeated (SSR) with 14–21 CA dinucleotides may play a crucial role in inhibition or activation of cell proliferation (8).

Overexpression of EGFR and HER2 is one of the earliest and the most consistent abnormalities in bronchial epithelium of risk smokers (2,7). It is present at the stage of basal cell hyperplasia and persists through squamous metaplasia, dysplasia and carcinoma *in situ* (7). Moreover, the impact of EGFR and HER2 co-expression on patients' survival may be additive (2).

## EVALUATION AND INTERPRETATION OF TUMOUR *HER* EXPRESSION

The most common methods evaluating *HER2* status, in breast cancer patients, for instance, are immunhistochemical (IHC) assay and fluorescence *in situ* hybridisation (FISH) assays. The correlation between IHC-detected protein overexpression and FISH-detected gene amplification is generally strong. IHC may be performed using different commercial polyclonal or monoclonal antibodies. The most popular is DAKO HerceptTest with rabbit antihuman HER2 polyclonal antibody. The intensity of membrane staining is evaluated according to the following manufacturers' criteria: scores 0 and 1+ are considered as normal (negative for overexpression) and scores 2+ and 3+ are considered as positive for *HER2* overexpression. *HER2* status may be also determined using the flow cytometry method (6,11).

Another method detecting *HER2* expression is evaluation of serum HER2 extracellular domain (ECD) level. HER2 ECD is secreted into circulation and its concentration can be determined in the serum of patients with metastatic cancers. It was suggested that serum HER2 ECD level measurement is clinically useful in breast cancer cases for detecting early recurrence or metastasis and for predicting patients' response to hormonal therapy (6).

Evaluation of EGFR overexpression is more difficult than *HER2* status determination. To assess EGFR status, the expression of *HER1* mRNA is examined by Northern blot analysis. Western blot may be used to detect cellular levels of EGFR protein. IHC assay applying monoclonal antibody produced by Upstate Biotechnology is useful for EGFR immunodetection. Finally, detection of point mutation in *HER1* and of additional *BsrI* polymorphic site in this gene as well as determination of CA repeat number in intron 1 of *HER1* by PCR technique are currently being explored (8,12).

EGFR and *HER2* status assessment as well as detection of gene amplification or mutation may be important methods for patients' selection for anti-cancer therapy using anti-HER agents. The humanized anti-*HER2* monoclonal antibody trastuzumab (Herceptin) demonstrated activity in clinical trials in women afflicted with metastatic breast cancer overexpressing *HER2*. *HER2*-non-overexpressing subjects are not responsive to anti-*HER2* therapy (6,11). The efficacy of therapy needs precise interpretation of tumour *HER2* expression, which is accessible in breast and oral cancer and difficult in lung cancer. In non-small-cell lung cancer, the therapy may be reserved only for patients with diagnosis of malignant tissue presence in biopsy specimens or for subjects after surgical tumour resection.

## HER-RECEPTOR FAMILY TARGETING

Herceptin is the first anti-*HER* drug to be approved for treatment of cancer. Trastuzumab caused $G_1$ cell cycle arrest and growth inhibition in cells expressing *HER2*. Response rates to the antibody given as a single agent in women with *HER2* overexpressing breast tumour ranged from 12 to 27%. Clinical trials evaluating the combination of trastuzumab with traditional chemotherapeutic agents potentially enhanced antitumour activity. In phase II trial in women with refractory metastatic breast cancer, the use of anti-*HER2* antibody in combination with cisplatin resulted in higher response rates than previously reported for cis-platin alone. In phase III trial patients, who received trastuzumab in combination with doxorubicn plus cyclophosphamide or single-agent paclitaxel, significantly longer time of progression, higher overall response rate and longer median overall survival compared with patients who had received chemotherapy alone were observed. At present, the herceptin is recommended for metastatic breast cancer with overexpression of HER2. Such therapy is generally well tolerated. Serious haematological toxicity and neuropathy are infrequent but the cardiac complications are more common side effects (5,6,11).

Preclinical data suggest a role for trastuzumab in the treatment of non-small-cell lung cancer. On phase II, trials show that trastuzumab can be added to standard chemotherapy in the treatment of patients with advanced NSCLC without additional toxicity, and with promising efficacy. Using HercepTest, Hirsh et al. and Bunn et al. showed that only 25% of NSCLC show 2+ or greater *HER2* expression and less than 10% tumours have 3+ overexpression. As determined by FISH analysis, the high degree of *HER2* gene amplification is lower in NSCLC than in breast tumours. The authors concluded that trastuzumab alone and in combination with chemotherapeutic agents should be tested in NSCLC patients and that *HER2* status should be assessed by both IHC and FISH methods (3,9). Cox et al. obtained similar results showing that herceptin may target only a small proportion of NSCLC tumours and be of limited clinical value in the disease, particularly in adjuvant setting (4).

A variety of new approaches to target HER2 are evaluated in clinical trials. 2C4 is a novel monclonal antibody that interferes with ligand-dependent signalling mediated by HER2-containing dimer. This antibody binds to a different epitope than herceptin. It is active in several *HER2*-non-overexpressing models that are non-responsive to herceptin. Antibodies against HER2 can also be used to target cytotoxic molecules, e.g. radioisotopes, biological toxins and chemotherapeutic agents to the cancer cells (5).

Analogously to the therapeutic approaches to target *HER2*, new drugs are developed to target EGFR. OSI-774 (erlotinib, Tarceva), a small molecule quinazolie, is a potent, selective inhibitor of EGFR tyrosine kinase. In preclincial studies, it demonstrated >70% inhibition of EGFR autophosphorylation and led to marked growth inhibition of HN5 and A432 xenografts.. In phase II clinical studies, objective responses were observed in patients with NSCLC, head and neck cancer as well as ovarian cancer. In phase II trails, in NSCLC patient, additive effect of Tarceva is observed in combination with cis-paltin, doxorubicin and gemcitabine. Tarceva results in a objective response rate of 12.3% in patients with EGFR overexpression NSCLC after failure of platinum-based chemotherapy. More than 60% of NSCLC are characterized by overexpression of EGFR and EGFR status is evaluated at scores of 2+ or 3+. Erlotinib at a daily *per os* dose of 150 mg is well tolerated but the most common side effects are acneiform dermatitis in 50% of patients and grade 1-2 diarrhoea in 32% of subjects. The mean survival after Tarceva therapy was 37 weeks and 48% patients survive more than one year (5,13).

Other anti-EGFR drugs in an advanced stage of clinical testing include humanized monoclonal antibody – Cetuximab and small molecule ZD1839 (Iressa). In addition, small molecules, which inhibit multiple members of HER family (pan-HER kinase inhibitors), are also studied in order to carefully evaluate their efficacy and safety in clinical trials (5). It is also possible that the optimum therapy in some patients may involve a combination of two or more of these agents.

## REFERENCES

1. Azzoli C. G. et al.: Trastuzumab in the treatment of non-small cell lung cancer. Seminars in Oncology, 29, 59, 2002.
2. Brabender J. et al.: Epidermal growth factor receptor and HER2-neu n RNA expression in no-small cell lung cancer is correlated with survival. Clin. Cancer Res., 7, 1850, 2001.
3. Bunn P. A. et al.: Expression of Her-2/neu in human lung cancer cell lines by immunohistochemistry and fluorescence in situ hybridisation and its relationship to *in vitro* cytotoxicity by trastuzumab and chemotherapeutic agents. Clin. Cancer Res., 7, 3239, 2001.
4. Cox G. et al.: Herceptest: HER2 expression and gene amplification in non-small cell lung cancer. Int. J. Cancer, 92, 480, 2001.
5. Dinghra K.: Her-receptor family targeting: new therapeutic options. II European Spring Oncology Conference. Latest Advances on Anticancer Drug in Clinical Development, 12-15.06.2002, Marbella, Malaga (Spain).
6. Esteva F. J. et al.: Phase II study of weekly docetaxel and trastuzumab for patients with HER-2 – overexpressing metastatic breast cancer. J. Clin. Oncol., 20, 1800, 2002.
7. Franklin W. A. et al.: Epidermal growth factor receptor family in lung cancer and premalignancy. Seminars in Oncology, 29, 3, 2002.
8. Gebhardt F. et al.: Modulation of epidermal growth factor receptor gene transcription by polymorphic dinucleotide repeat in intron 1. J. Biol. Chem., 19, 13176, 1999.
9. Hirsch F. R. et al.: HER2/neu expression in malignant lung tumors. Seminars in Oncology, 29, 51, 2002.
10. Reiter J. L. et al.: Correspondence re: S. Shintani et al., Itragenic mutations analysis of the human epidermal growth factor receptor (EGFR) gene in malignant human oral keratinocytes. Cancer Res., 60, 766, 2000.
11. Seidman A. D. et al.: Weekly trastuzumab and paclitaxel therapy for metastatic breast cancer with analysis of efficacy by HER2 immunophenotype and gene amplification. J. Clin. Oncol., 19, 2587, 2001.
12. Shintani S. et al.: Intragenic mutations analysis of the human epidermal growth factor receptor (EGFR) gene in malignant human oral keratinocytes. Cancer Res., 59, 4142, 1999.
13. Talent European Investigators Workshop. 19-20.03.2002, Budapest (Hungary).

## SUMMARY

*HER* gene family (*HER1-HER4*) encodes structurally similar transmembrane proteins (EGFR, HER2, ErB-3, and ErB-4) with tyrosine kinase activity. Dimerised on binding with a number of ligands, including epidermal growth factor (EGF) and transforming growth factor alpha (TGFα), these proteins stimulate epithelial cell proliferation. HER2 and EGFR overexpression is detected in the cells of many tumours, mainly in breast, lung and oral cancer and may be connected with *HER2* gene amplification or point mutations as well as with the presence of overactive polymorphic forms of *HER1* gene. The first medication of a proved efficacy in breast cancer treatment was trastuzumab (Herceptin) – monoclonal antibody against HER2 protein. Trastuzumab was effective only in the case of patients with high *HER2* expression evaluated by immunohistochemical methods and with gene amplification ascertained by fluorescence *in situ* hybridisation assays. In non-small-cell lung cancer (NSCLC), HER2 overexpression was detected only in a few cases. Therefore, trastuzumab treatment seems to be problematic in NSCLC patients. A small molecule quinazolie (erlotinib, Tarceva) is a promising therapeutic agent selectively blocking EGFR. Phase III Tarceva clinical trail in NSCLC patients showed that their survival is prolonged and that the medication acts together with other chemotherapeutic agents like cis-paltin and gemcitabine.

Leki blokujące białka rodziny *HER* w leczeniu niedrobnokomórkowego raka płuc

Rodzina genów *HER* (*HER1 – HER4*) koduje strukturalnie podobne białka o aktywności kinaz tyrozynowych (EGFR, HER2, ErB-3, and ErB-4). Białka te, łącząc się z szeregiem ligandów, np. nabłonkowym czynnikiem wzrostu (EGF) i (TGFα), ulegają dimeryzacji, powodując proliferację komórek nabłonkowych. Wzmożona ekspresja białek HER2 i EGFR stwierdzana jest w komórkach wielu nowotworów, przede wszystkim w raku sutka, płuc oraz jamy ustnej i może wiązać się z amplifikacją genu *HER2* lub punktowymi mutacjami i występowaniem nadreaktywnych form polimorficznych genu *HER1*. Pierwszym lekiem, którego skuteczność została udowodniona w leczeniu raka sutka, był trastuzumab (Herceptin) – przeciwciało monoklonalne blokujące białko HER2. Trastuzumab działał wyłącznie u pacjentek, u których metodami immunohistochemicznymi stwierdzano wysoką ekspresję *HER2*, a techniką fluorescencyjnej hybrydyzacji *in situ* – amplifikację genu. W niedrobnokomórkowym raku płuca wzmożona ekspresja *HER2* była stwierdzana w nielicznych przypadkach i dlatego zasadność stosowania trastuzumabu wydaje się problematyczna. Duże nadzieje wiążą się natomiast z małą cząsteczką kinazoliny o nazwie erlotinib (Tarceva), która selektywnie blokuje białko EGFR. Badania kliniczne nad Tarcevą w niedrobnokomórkowym raku płuca weszły w III fazę i wskazują na przedłużenie czasu życia chorych oraz synergistyczne działanie leku z chemioterapeutykami, takimi jak cisplatyna i gemcytabina.