Oncogramme Responses of Breast Tumour Cells Treated with Herceptin Correlate with HER2/C-ERB B2 Pathological Status

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Abstract. *Background: Among targeted therapies, Herceptin is a monoclonal antibody successfully used on patients with breast cancer expressing Human Epidermal Growth Factor Receptor-2 (HER2 receptors). Oncogramme is a method developed to predict anticancer activity of molecules and thus individualize chemotherapeutic strategies. Before this ex vivo test enters clinical validation, it was desirable to correlate breast cancer cell responses to Herceptin observed through Oncogramme with HER2 expression by these cells. Materials and Methods: Breast tumour fragments were dissociated and obtained cells were cultured in defined medium. After Herceptin treatment, cytotoxicity was detected by cell death analysis, and responses compared to tumour HER2 status were determined by pathologists. Results: Cell responses to increasing doses of Herceptin obtained with Oncogramme were in correlation with HER2 expression. Conclusion: Comparison between Herceptin responses obtained with Oncogramme and HER2 status of breast tumour cells confirmed that Oncogramme is a reliable method for prediction of patient cell sensitivity to anticancer drugs.*

Oncogramme is an individualized tumour response test developed for predicting tumour responses to breast cancer treatments (1). This test is performed according to specific processes developed for clinical diagnosis. First, the tumour dissociation procedure is optimized to obtain both high cell viability and high rate of culture success. Then cells are cultured in a chemically defined medium (OncoMiD for Breast) whose formulation was intended to (i) limit phenotypic variations resulting from batch-to-batch variations in serum, and (ii) allow tumour cells to survive and proliferate while limiting fibroblastic invasion. A previous study has already demonstrated the feasibility of Oncogramme on non-selected breast tumours, using relevant chemotherapies (1). This technique is currently being evaluated through a phase I clinical trial.

Breast tumours overexpressing Human Epidermal Growth Factor Receptor-2 (HER2, also termed C-ERB B2) display an aggressive cancer progression phenotype (2). Treatment with trastuzumab (Herceptin™ ; Genentech/Roche, South San Francisco, CA, USA), a humanized anti-HER2 antibody, has been shown to extend survival in patients with HER2-overexpressing cancer (3), and it has become the standard of care in patients with increased HER2 (4, 5).

The aim of this study, as a preclinical evaluation, was to compare Oncogramme responses to increasing doses of Herceptin with the HER2 pathological status of tested tumours.

Materials and Methods

Collection of breast tissue. Ten cancerous breast tissues were obtained from fresh surgical specimens (Clinique du Colombier, Limoges, France) with fully informed consent of the patients. Tumours were selected by pathologists and fragments were transmitted to Oncomedics only when it did not prevent complete pathologic diagnosis. Pathologists provided HER2 status within one month after surgery. Tissues were collected in transport medium (OncoMid-Via for breast; Oncomedics, Limoges, France) and kept at 4˚C for a maximum of 48 hours before dissociation (1).

Primary culture. Cell dissociation was performed with OncoMid-Diss kit for breast (Oncomedics). Cell viability was determined by a Trypan blue exclusion assay (Sigma, Saint-Quentin Fallavier, France), and cells (2×10^6 cells per flask) were seeded in 75 cm^2 dishes (Nunc, Langenselbold, Germany). Cells were cultured in OncoMiD for breast chemically defined medium (Oncomedics) in a humidified incubator (Binder CS 150, Tuttlingen, Germany) at 37˚C, under an atmosphere of 95% air 5% CO_2.

Oncogramme. Ten to 14 days after tissue dissociation, cells were seeded in 8-well Lab-Tek slides (Nunc, Langenselbold, Germany) (2×10^4 cells/well) and maintained for three days in culture with increasing doses (0 for control, 1, 100 and 200 μg) of Herceptin (kind gift of Institut Bergonié, Bordeaux, France). Viable and dead

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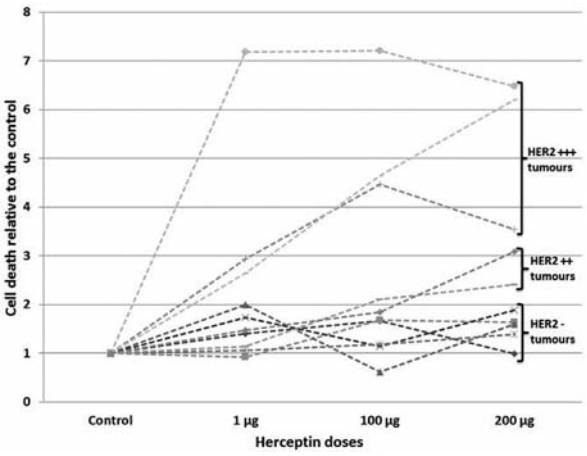

Figure 1. *Cell death of breast tumour cells treated with increasing doses of Herceptin.*

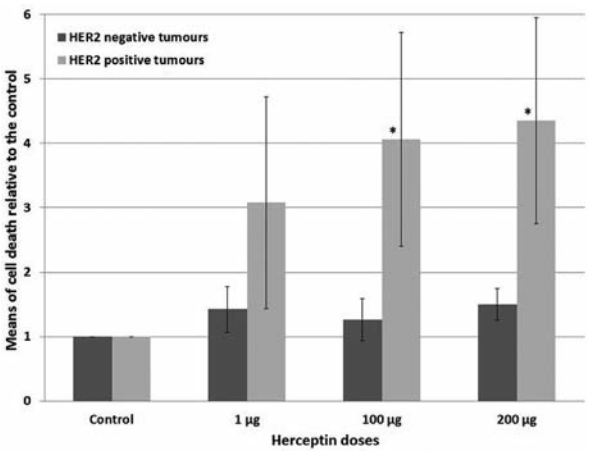

Figure 2. *Means of HER2-positive and -negative tumour cell death with increasing doses of Herceptin (*symbol indicates a p-value <0.05).*

cells were labelled using Live/Dead Viability/Cytotoxicity kit for mammalian cells (Molecular probes, Leiden, The Netherlands). Cells were observed through fluorescence microscopy using NIS-Element BR 3.1 software (Nikon, Champigny sur Marne, France). After cell counting, the percentage of dead cells was determined and the cell death ratio was calculated in reference to values obtained for the control (set at 1). Results are displayed as the mean±SEM. Statistical analysis was performed using Tukey test and InStat 3 software version 3.10 (GraphPad Software, San Diego, CA, USA). A p-value <0.05 was considered as statistically significant.

Results

Cell death analyses after Herceptin treatment. Ten Oncogrammes were obtained with various response profiles (Figure 1). For HER2-negative tumours (n=5), no significant difference between control (untreated cells) and Herceptin-treated cells means was observed (p>0.05), while HER2-positive cells (n=5) exhibited a significantly increased cell death mean compared to the control when treated with Herceptin at 100 and 200 µg (Figure 2). Indeed, for HER2-positive tumours, cell death means for control, 100 and 200 µg Herceptin-treated cells were respectively 1 *vs*. 4.06±1.66 (p<0.05) and 4.34±1.6 (p<0.05).

Discussion

Cell death observed through the Oncogramme technique on breast tumour cells was significantly increased in HER2-positive cells in the presence of Herceptin, while no statistical differences for HER2-negative cancer cells were observed. These results, thus, show that Oncogramme can determine the *ex vivo* response of tumour cells to Herceptin and confirm that the test could be useful to predict clinical responses of patients to known anticancer drugs. Final validation must now pass through a phase I clinical assay (6, 7). Oncogramme may also be used to investigate new drug candidates. Promising therapeutic approaches, such as monoclonal antibody-targeted therapies, could be evaluated by this assay, and companion diagnostics and mechanisms subsequently identified.

Conflict of interests

CL is a founder and CEO of Oncomedics, and has an equity position in the company. SG is CSO of Oncomedics and has an equity position in the company. CBMP is an employee at Oncomedics. VF and AG declare no conflict of interest.

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