

## *ESMO Research Fellowship Report*

Title: Pharmacogenetic study of Fc Gamma Receptor and HER2 genes in relation to treatment of breast cancer

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Home Institute: St. Chiara University Hospital - Pisa - Italy

Host Institute: Northern Institute for Cancer Research - Newcastle University - UK

### **Background**

Pharmacogenetics, generally referred to as the study of genetic variation that gives rise to differing response to drugs, is becoming more relevant in the diagnosis, treatment, and recovery of cancer patients. The problem faced when treating cancer is the outstanding variability in treatment outcome. Unpredictability among a population of patients who are affected with the same malignancy can show varying associated toxicities in drug treatment ranging from zero effect through lethal doses. However, using pharmacogenetics there are promising advancements in the development of effective agents which will enable 'personalized cancer chemotherapy' to become routine for the clinical practice. This individualization is most advanced in the field of breast cancer.

Breast malignancies are the leading cause of cancer deaths amongst women in the United Kingdom, accounting for 18% of all cancers, although death rates from the disease are decreasing.

Approximately 20% of breast cancers over-express a receptor called HER2, which is the target for the monoclonal antibody therapy trastuzumab (Herceptin®). Trastuzumab is currently in use to treat patients with high risk or recurrent HER2 positive breast cancer.

Many mechanisms of action have been proposed for trastuzumab (inhibition of the PI3K pathway, immune-mediated response, induction of HER2 down-regulation, inhibition of angiogenesis etc).

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) has been demonstrated to play a role in the immune-mediated activity of trastuzumab.

Fc Gamma Receptors are the receptors for the Fc region of IgG; they are present in most cells of the immune system and link the humoral to the cellular response and play key-role in ADCC.

Single nucleotide polymorphisms (SNPs) have been identified in the HER2, Fc Gamma Receptor and related genes, which may have implications in HER2 expression and the mechanism of action of trastuzumab.

### **Project**

We proposed to take blood samples from 750 appropriately consented patients for genotype analysis of these SNPs (to include 150 HER2-positive patients with late-stage/ metastatic breast cancer to be recruited for additional Fc Gamma Receptor genotyping, plus 600 patients for HER2 genotyping who have previously had their breast cancer HER2 receptor tested and who are under follow up for either early- or late-stage breast cancer). These patients are followed at the Northern Centre for Cancer Treatment in Newcastle. We are also sought permission from patients to use pathology samples previously taken from their breast cancer in order to analyse if there is a difference between host cell and cancer cell genotype. In order to be included in the study, patients must have had their tumour tested for HER2 expression. We are using these genotype data to identify SNP frequencies and to analyse their influence on HER2 expression. In addition, for those patients receiving trastuzumab (in the presence or absence of chemotherapy) we are measuring levels of trastuzumab and biomarkers (circulating HER2 extracellular domain - ECD) in their blood sample. For such patients, treatment outcome (objective response to therapy, time-to-progression, overall survival, side effects) will be compared with laboratory results.

### **Methods**

Genotyping of SNPs in peripheral nucleated blood cells is performed by Fluorogenic 5' nuclease assay using the endogenous 5' nuclease activity of AmpliTaq Gold DNA polymerase.

The following functional SNPs have been evaluated so far:

- HER2-I655V (residue in transmembrane domain of HER2)
- HER2-A1170P (residue in the carboxyl-terminal regulatory domain of HER2)
- FCGR3A-V158F (residue in the extracellular domain of Fc Gamma Receptor IIIA, which directly interacts with the lower hinge region of IgG1)
- FCGR2A-H131R (residue located in the second extracellular Ig-like domain of Fc Gamma Receptor IIA)

Immunological methods are being developed and validated to measure trastuzumab and biomarkers in plasma.

In particular, in order to measure trastuzumab levels in plasma samples, a home-made cell-based ELISA assay was developed, using SKBR3 cells (a HER2 over-expressing cell-line) as capture antigen.

Briefly, about  $10^5$  cells per well are seeded in a 96-wells plate, after coating with Poly-D-Lysine (to improve cell adherence). In order to obtain the same number of cells per well and reduce inter-assay variability, single-use aliquots of cells were prepared and pre-stored at  $-80^{\circ}\text{C}$ .

After fixing cells with formalin and blocking plate, to prevent non-specific bindings, the plate is incubated with the primary antibody (trastuzumab).

After wash, the plate is incubated with the secondary antibody (HRP-conjugated Anti-human Goat IgG).

Finally, after another wash, the substrate is added and colour development is observed and measured by the spectrophotometer.

## **Results**

166 patients have been recruited in the study so far. All of them are evaluable with respect to germ-line genotype.

64 of them had HER2-positive tumours and 102 had HER2-negative tumours.

Among the HER2-positive, there were 50 early stage and 14 advanced stage patients.

The vast majority of HER2-positive patients received herceptin, as adjuvant (41) or palliative treatment (12).

We also managed to collect a total of 32 plasma samples

For each of the 4 SNPs we tested, it was possible to distinguish rare allele homozygous, heterozygous and major allele homozygous patients.

102 patients have been genotyped for the two HER2 SNPs so far.

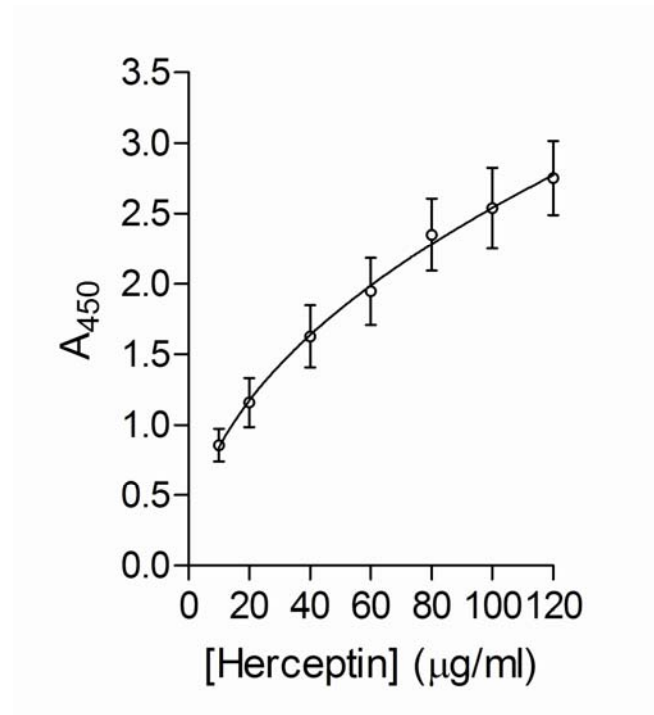
The genotypes were in Hardy-Weinberg equilibrium and the frequencies of the alleles were very much comparable with literature data in a Caucasian population (our population was almost entirely Caucasian).

The recruitment is currently ongoing.

So far there doesn't seem to be any correlation between both the HER2 SNPs and HER2 expression in primary tumours, although there might be a trend for the SNP in the intracellular carboxyl-terminal tail (HER2-A1170P): minor allele (Proline) carriers seem more likely to have a HER2-positive tumour compared to homozygous Alanine patients.

With regard to the cell-based ELISA method for trastuzumab, all the standard validation experiments have been successfully completed (including including

determination of the lower limit of quantification, intra- and inter-assay variability, variability among different batches of SKBR3 cells, freeze and thaw stability, long and short term storage stability) and a methodology paper is currently in preparation. This is the average standard curve for all the validation assays; the “goodness of fit” was quite high ( $R^2 = 0.9972$ )



The determination of trastuzumab levels in patients is ongoing. Different ELISA commercial assays are being evaluated in order to measure HER2 - ECD in plasma.

### **Future steps in the project**

The recruitment is currently ongoing and the study will be extended to other centres in the Northern Cancer Network.

The role of ADCC in trastuzumab therapy will be further evaluated, by considering other possibly relevant SNPs and a haplotype analysis, as well as evaluating other possible factors in FFPE tumour blocks.

I also wish to study more in depth ADCC by planning laboratory cytotoxicity assays.

Other possible mechanisms of action of trastuzumab will also be considered.

Finally, this pharmacogenetic research will be extended to other medical treatments of breast cancer, such as chemotherapy, hormone therapy or new biological targeted agents.

### **Personal considerations**

During my fellowship I had the opportunity to broaden my experience this is a partial list of activities:

- To attend Oncology Clinics at Northern Centre for Cancer Treatment (NCCT) in Newcastle, and take part in Clinical Trial Unit meetings;
- To learn some fundamental laboratory techniques, such as DNA extraction and genotyping, ELISA assays, Western Blotting assays, basic cell culture techniques;
- To learn how a research laboratory group works, also attending periodic laboratory meetings;
- To attend Northern Institute for Cancer Research (NICR) seminars, research fora and translational research meetings, which involved me in a very stimulating research environment

As my experience has been overall very positive, I applied for a temporary post as Clinical Research Associate in the Institute.

My application has been accepted, so for the next three years I will be working here, on the main topic of "Breast Cancer Pharmacogenetics", and I will have the opportunity of achieving a PhD degree as well.

### **Acknowledgements**

I'd like to thank ESMO for giving me this exciting opportunity, Prof Hilary Calvert, Prof Alan Boddy, Dr Mark Verrill and Prof Ruth Plummer, who are supervising me, Dr David Jamieson, who introduced me to the laboratory techniques, Dr Jo Lee, all Pharmacology staff at NICR and all clinical staff at NCCT.