1 Publishable summary

1.1 Summary description of project context and objectives

Breast cancer is the most common cancer in women with more than 1.1 million women newly diagnosed annually, accounting for 14% of all female cancer deaths. While the disease is curable in early stages, about 50% of patients present with stage II or III tumours. Today almost all women are candidates for different systemic therapies (endocrine-, trastuzumab-, chemotherapy), so that suitable and validated predictive assays are urgently needed to optimise clinical outcomes and minimize unnecessary toxicity. There is a broad consensus that individualisation of therapy through the use of molecular diagnostic approaches is one of the most demanding challenges facing cancer medicine.

The RESPONSIFY project has the overall goal of identifying standardized, clinically implementable predictive biomarkers, that can be used to better select breast cancer patients for chemotherapy and anti-HER2 treatment. The RESPONSIFY consortium consists of 11 partners from 6 European countries, including 4 research and development focussed SMEs. To reach the overall aim, RESPONSIFY has performed large-scale screening approaches to identify new markers and – in parallel – has extensively validated markers based on prespecified hypotheses.

The following objectives were addressed within RESPONSIFY:

1. Identification and discovery of predictive biomarkers using novel and established genome-wide based techniques
2. Identification of genes modulating HER2-inhibitor sensitivity by whole genome based-screening including functional validations
3. Transfer of novel and established molecular markers to a diagnostic platform to take forward for further validation and functional characterization
4. Validation of candidate biomarker assays in large clinical trial cohorts and conduction of window-of-opportunity trials for focussed evaluation of therapeutic agents
5. Integration of the biomarkers into a formal development process for CE-marked IVD (in vitro diagnostics) tests, including a commercialization and dissemination plan
6. Set up of a commercially available web-based centralised database for clinical & biomarker data management within clinical trials.
7. To establish a core health economic model as a basis for evaluating and comparing costs and effects of different test-treatment strategies
8. To further improve and develop a functional research infrastructure including tissue collection, SOPs, central sample management and integrated bioinformatics.
9. IPR protection of results by patent applications and negotiation of licensing agreements between partners to commercialize a diagnostic test.

In order to achieve these aims, the RESPONSIFY project has been structured into two parallel approaches:

In large screening projects we have utilized novel genomic technologies to identify new genes involved in resistance to anti-Her2 therapy. In sequencing approaches, we have looked for gene alterations that could be linked to therapy resistance. Markers found in these approaches have been further validated in the project.

In parallel to these screening approaches, we have performed hypotheses-based evaluations that were based on previous results of the partners. A major focus of RESPONSIFY was on the detailed evaluation of immunological alterations in breast cancer. We have validated previous results that an immune activation in tumour tissue is predictive for increased response to therapy. Furthermore, we have shown for the first time that the immune system might be particularly relevant for response to anti-HER2 therapy as well as carboplatin-based chemotherapy. Moreover, biomarkers from both approaches were transferred to a diagnostic PCR platform and product development activities to establish a diagnostic test have been performed.

1.2 Description of the work performed so far and main results

During the first part of RESPONSIFY one major task was the functional identification of biological markers modulating HER2 inhibitors based on whole genome screening assays using human cell-lines. In parallel the identification of biomarker candidates for anti-HER2 therapy by novel genome based technologies has been performed in tumour samples from clinical cohorts. Within the RESPONSIFY project, four “window-of-opportunity” trials have been conducted with defined targeted treatment approaches to compare the molecular changes induced by therapy in tumour tissue. These trials are important to investigate molecular changes associated with response and resistance to therapy. Candidate biomarkers from the literature and from previous projects have been evaluated with a strong focus on tumour-infiltrating lymphocytes and immune mRNA markers. In addition we have evaluated mutations in tumour tissue, in particular mutations of the *PIK3CA* gene as a predictive factor for anti-HER2 therapy.

During the second part of RESPONSIFY the main focus was on the validation of markers based on results from part 1 of the project. We have further validated *PIK3CA* mutations as well as tumour-infiltrating lymphocytes in additional clinical cohorts. Based on results from period 1, immune checkpoint inhibitor expression was evaluated at the mRNA level in two clinical cohorts. Three additional markers from the screening investigations that have been shown to be involved in HER2 response and resistance have been studied in clinical validation cohorts. These investigations have resulted in a new mRNA based-biomarker test, the “ImmunoPredict” assay, as well as a standardized approach for evaluation of tumour-infiltrating lymphocytes (TILs) in histological slides. The standardized TIL assessment has already been implemented in the ongoing GeparOcto trial. We plan to integrate the “ImmunoPredict” assay in further clinical trials of the German Breast Group, in particular in those trials that investigate immunomodulatory agents such as immune checkpoint inhibitors.

In parallel to the molecular investigations, we have performed health economical analyses and developed a user-friendly evaluation tool that can be used to approximate the impact of new biomarker tests and stratified therapy approaches from a health economical point of view.

The results of the RESPONSIFY project can be generally divided into several areas:

1: Screening studies to identify new molecular markers of resistance and therapy response

- Genomic screening approaches using cell lines (WP2)
- Genomic screening approaches using tissue samples (WP3)
- Clinical window-of-opportunity studies to evaluate molecular changes associate with short-term biological treatment (WP1 and 3)

2: Hypothesis-based investigations of new markers as well as validation studies of existing markers from previous projects of the partners and from the screening approaches

- Histopathological markers such as tumour-infiltrating lymphocytes (WP5)
- Evaluation of mRNA markers including immune markers (WP5)
- mRNA markers derived from screening approaches and window studies (interaction between WPs 2, 3 and 5)
- Serum markers to investigate response to anti-angiogenic therapy (WP3)

3: Transfer of biomarkers to a diagnostic platform, manufacturing of proto-type test for hit validation, and development of a diagnostic IVD test

- Transfer of markers identified and selected in WP2 and WP3 to a diagnostic platform (WP4)
- Manufacturing of proto-type tests for hit validation in WP5 (WP4)
- Product development process (WP4, WP8)

4: Development of new tools for management of clinical trials, IVD test implementation, establishment of a commercialization plan as well as health economical studies

- Setup of the infrastructure for tissue-distribution, quality control and standardization of methods (WP1)
- Development of a web based electronic data capturing system for integration of data and biomaterial management for clinical trials (WP6)
- Health economical analyses (WP7)
- Continuous assessment of IP issues as well as options for development of IVD tests (WP8)

1.3 The expected final results and their potential impact and use

1.3.1 Screening studies to identify new molecular markers of resistance and therapy response

The genome-wide screens have resulted in a list of targets that could be further validated in functional cell culture studies. Interestingly, there was an overlap of these markers with markers from proteomic analyses of clinical window trial samples. Three markers were selected for further validation in clinical cohorts.

1.3.2 Hypothesis-based investigations of new markers as well as validation studies of existing markers from previous projects of the partners and from the screening approaches

We have shown that TILs as well as immune mRNAs are predictive for increased response to chemotherapy, in particular in patients treated with trastuzumab or platinum-based chemotherapy. Predictors of response to anti-angiogenic therapy were identified in serum samples. *PIK3CA* mutations were described as predictors of anti-HER2 response.

1.3.3 Transfer of biomarkers to a diagnostic platform, manufacturing of proto-type test for hit validation, and development of a diagnostic IVD test

Biomarkers were successfully transferred to a diagnostic PCR platform. Proto-type PCR assays were successfully manufactured. These results are the basis for the planned implementation of the ImmunoPredict assay in further clinical trials of the German Breast Group.

1.3.4 Development of new tools for management of clinical trials, IVD test implementation as well as health economical studies

Standardized software for trial management was developed to support the integration of biomarker test into clinical trials. In the health economic project three core models were constructed and user-friendly tool was developed to assess health economic characteristics of existing and novel biomarkers. During RESPONSIFY, one patent application was filed, one licensing agreement between the partners was finalized and one patent application is currently under evaluation.

The results of RESPONSIFY have already been published in 10 publications at the time of the project end, additional manuscripts have been submitted and will be published in the future. RESPONSIFY results have been presented as oral presentations at several major international conferences.

The potential impact and use of the results of the RESPONSIFY project

As a result of RESPONSIFY, several standardized diagnostic approaches have been established for further development and integration in clinical trials and diagnostic practice:

1. We plan to implement the "ImmunoPredict" assay into future clinical trials for monitoring the immune system in breast cancer.
2. In addition we have developed a standardized approach to evaluate tumour-infiltrating lymphocytes in breast cancer. We have published a guideline article, conducted a first ring trial and developed a software prototype.
3. For anti-angiogenic therapy, RESPONSIFY has identified serum markers that are linked to response in the neoadjuvant setting. However, these markers were not related to prognosis, and anti-angiogenic therapy is not expected to play a major role in the treatment of breast cancer. Therefore it was decided not to further develop this diagnostic approach.
4. We showed that PIK3CA mutated tumours have a reduced response to anti-HER2 therapy. After additional validation studies, this opens the door for diagnostic tests based on Sanger sequencing and targeted exome sequencing for detection of PIK3CA mutations in breast cancer.

The impact of these results will be a better stratification of breast cancer patients for therapy. For upcoming trials of immunotherapy, we have prepared standardized molecular assays that could be further developed in to companion diagnostics. This will help to improve therapy for breast cancer patients, and it might be particularly relevant for patients with triple-negative breast cancer, for which therapeutic options are currently very limited.

RESPONSIFY Website: www.RESPONSIFY-FP7.eu