1 Final publishable summary report

1.1 Executive summary

The RESPONSIFY project has the aim to find genome based biomarkers to predict response and resistance to anti-HER2 as well as anti-angiogenic therapies in breast cancer. These investigations should lead to new molecular diagnostic approaches. The identification of novel modulators of response through whole-genome screening approaches using cell lines and fresh frozen material was the basis for marker validations, which were performed in a large tumour tissue bank from clinical trials. The projects focused on tissue-derived predictive factors for anti-HER2 directed therapy. In addition, serum markers for response to anti-angiogenic therapy were evaluated. As main research areas, immunological changes as well as mutations in tumour tissue were studied. The project partners have developed an mRNA based immune predictive test ("ImmunoPredict"), which monitors changes in the immunological status in breast cancer tissue. This test will be used in future clinical trials as concomitant biomarker. In addition, tumour infiltrating lymphocytes as a surrogate for an active intratumoural immune environment were found to be predictive for response to anti-HER2 as well as chemotherapy and were linked to good prognosis. Standardized approaches for TIL evaluation were developed. *PIK3CA* mutations are a marker for reduced response to double anti-HER2 treatment in neoadjuvant clinical trials of the German Breast Group.

The RESPONSIFY results were confirmed in other clinical trials and a meta-analysis integrating the data generated by other groups was performed to increase acceptance of *PIK3CA* as an additional diagnostic marker. Based on the results of RESPONSIFY, tumour infiltrating lymphocytes as well as *PIK3CA* mutation analysis have been included in the German breast cancer guidelines. Additional biomarkers derived from screening approaches of RESPONSIFY have been transferred to a RT-PCR based test platform and show first promising results which merit further validation. The integrated health economic project demonstrated that biomarker-based stratification of patients can improve economics efficiency. Within the RESPONSIFY project, a user-friendly tool has been developed that will help clinicians to integrate health economic analyses in future biomarker strategies.

For clinical trial management, the web-based electronic data capturing system MedCODES® has been developed to capture biomarker as well as other data from clinical trials. This enables the conduction of clinical registration trials with integrated central biomarker testing. Based on the results of RESPONSIFY, we plan to integrate the MedCODES® system and the FFPE based "ImmunoPredict" assay in future clinical trial as final validation step for patient stratification to better define the treatment population for therapy approaches.

1.2 A 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 (personalized medicine) through the use of molecular diagnostic approaches is one of the most demanding challenges facing cancer medicine today. Despite this consensus, there are only a few biomarker tests established in the market and significant barriers exist for the transfer of innovation from research to the commercial market in the European diagnostic healthcare enterprises. Moreover, to implement in clinical practice the use of high throughput technologies for biomarker research, the critical block to high potential candidates is the access to a sufficient number of biological specimens, along with annotated high quality clinical outcome data to help overcome the hurdle of distinguishing genes and proteins with true biological and hence biomarker potential from false positives. Quality biomarker candidate discovery can only come from stringently characterized specimens which have been handled in a standardized way from specimen acquisition, quality control, processing, handling and storage as well as the coordinated efforts of experts in the appropriate fields of biology, therapeutics and analysis of multiple levels of high dimensional genomic information.

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 and one management partner.

To reach the overall aim, RESPONSIFY has performed large-scale screening studies to identify new markers and – in parallel – has extensively validated markers based on pre-specified 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 realisation 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 (Markov model) as a basis for evaluating and comparing different testing strategies for various biomarkers in breast cancer

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 approaches 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 within 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 relevant for response to anti-HER2 therapy as well as carboplatin-based chemotherapy.

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 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 performed with defined targeted treatment approaches to compare the treatment-related molecular changes in tumour tissue. These trials are important to investigate molecular changes in tumour tissue 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, which have been investigated several neoadjuvant clinical trials. 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 investigated in clinical validation cohorts.

Both biomarker identification approaches were accompanied by product development activities with the aim of a diagnostic IVD test. Those activities included the successful transfer of biomarkers identified and selected in the screening studies to a diagnostic PCR platform followed by technical feasibility studies with instruments and reagents, selection of suppliers, pre-analytical studies, as well as establishment of a manufacturing and quality control concept for the new assay under ISO13485 conditions. Moreover proto-type PCR assays were successfully manufactured and quality controlled for the validation studies in GeparQuattro and GeparSixto.

These investigations and development activities have resulted in a new mRNA based-biomarker test, the “ImmunoPredict” assay, as well as a standardized approach for evaluation of tumour-infiltrating lymphocytes (TIL) in histological slides. The standardized TIL assessment has already been implemented in the ongoing GeparOcto trial. It is planned 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 filed a patent application to get IPR protection for “ImmunoPredict”, have evaluated the market and have established a commercialization plan. Morover, we 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.

1.3 Description of the main S&T results/foregrounds

1.3.1 Overview and structure:

The scientific and technological 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

1.1: genomic screening approaches using cell lines (WP2)
1.2: genomic screening approaches using tissue samples (WP3)
1.3: realization of clinical window studies to evaluate molecular changes associated with short-term therapy (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

2.1: histopathological markers such as tumour-infiltrating lymphocytes (WP3 and 5)
2.2: evaluation of mRNA markers including immune markers (WP3 and WP5)
2.3: mRNA markers derived from screening approaches and window studies (interaction between WPs 2, 3 and 5)
2.4: DNA based markers (mutation analyses) (WP1, WP3, WP5)
2.5: 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

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

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

3.1 Setup of the infrastructure for tissue-distribution, quality control and standardization of methods (WP1)

3.2 Development of a software platform for integration of biomaterial management into clinical trials (WP6)

3.3 Health economical analyses (WP7)

3.4 Continuous assessment of IP issues as well as commercial and medical options for development of IVD tests (WP8)

1.3.2 Description of work performed and results

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

1.3.2.1.1 Genomic screening approaches using cell lines (WP2)

As part of the RESPONSIFY consortium, we have performed genome-wide loss-of-function (shRNA) and gain-of-function (transposon) screens to identify novel genes that determine resistance and/or sensitivity to HER2 inhibition in a panel of breast cancer cell lines. Using these approaches we have found several novel modulators of response to HER2 in each cell line treated with either lapatinib or afatinib. Comparing the targets among different cell lines, allowed us to identify common modulators that could represent potential therapeutic targets. In particular, from the transposon screens we obtained a list of targets that could be further validated and characterized in functional cell culture studies. Two of these genes were, then, transferred to WP5 for additional validations in clinical cohorts. Interestingly, there was an overlap of markers derived from the transposon screens with markers from proteomics analysis performed on samples collected from patients in a clinical window trial using trastuzumab.

1.3.2.1.2 Genomic screening approaches using tissue samples (WP3)

In parallel to the cell line based evaluations, tissue samples from clinical trials were evaluated by proteomics, DNA sequencing and transcriptomics. Markers derived from these investigations were transferred to other work packages for further validation.

1.3.2.1.3 Conduction of clinical window studies to evaluate molecular changes associated with short-term therapy (WP1 and 3)

A total of four presurgical window studies have been performed within RESPONSIFY that have included both antibody-based therapies, such as trastuzumab and pertuzumab, and tyrosine kinase inhibitors such as afatinib. Understanding the mechanisms of resistance to these compounds is essential to be able to develop alternative strategies for tumour treatment.

Two window studies were conducted in a multicentric setting (GeparSepto and DAFNE). The first window-of-opportunity study was implemented into the larger GeparSepto study and some patients were randomly assigned to receive for a short period of time one of three anti-HER2 treatments (trastuzumab, pertuzumab or the combination of both) before they were entering the main GeparSepto trial. In the DAFNE trial the window of opportunity was an

integrated part of the study and all patients received the anti-HER2 treatment before receiving chemotherapy. In both studies the patients were biopsied to retrieve tumour material for further investigations at the end of the window-of-opportunity before they started chemotherapy as neoadjuvant treatment. Surgery was performed after the end of a 24 weeks chemotherapy.
In addition, two monocentric window-of-opportunity studies were conducted in which patients received an anti-HER2 treatment for a short period of time before surgery. Tumour samples before and after therapy were collected. The molecular changes linked to short-time targeted therapy were evaluated by proteomics as well as gene expression profiling. Interestingly, there was an overlap of markers derived from the transposon screens (WP2) with markers from proteomic studies of window trial samples (WP3), and one of these markers was selected for further validation in WP5.

1.3.2.2 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

1.3.2.2.1 Tumour-infiltrating lymphocytes as histopathological markers of therapy response (WP3 and WP5)

In this part of the project, we have investigated tumour-infiltrating lymphocytes (TILs) in different clinical study cohorts (including FinHer, GeparQuattro, GeparQuinto, GeparSixto and GeparSepto). We have shown that TILs are associated with an increased response to chemotherapy plus anti-HER2 treatment, and with improved survival. In our evaluations, TILs seemed to be particularly relevant in patients treated with trastuzumab (FinHer trial) as well as patients receiving a platinum-based chemotherapy (GeparSixto study). Validation studies are currently ongoing outside of the project. These results led to the integration tumour-infiltrating lymphocytes into the German breast cancer guidelines (www.ago.online.de).
For analytical standardization for TILs a guideline review article has been published and a first international ring trial has been performed. Based on the results of this ring trial, we have developed a standardized evaluation software for assessment of TILs that will be validated in future studies.

1.3.2.2.2 Markers on the mRNA level including immune markers (WP5)

In parallel to the investigation of TILs, we have evaluated a set of immunologically relevant mRNAs, including immune checkpoint markers that are currently under investigation as new therapeutic targets. These checkpoint markers were transferred to the validation studies based on the results in the FinHer cohort. In addition, three mRNA markers from the screening studies were transferred to the validation work packages, these markers were evaluated in the GeparQuattro cohort.

1.3.2.2.3 Markers on the mRNA level derived from screening approaches and window studies (WP 5)

A total of three additional markers were evaluated in more than 200 samples from the GeparQuattro cohort. Two of these markers were selected from the transposon screen in WP2. The third marker was derived from tissue-based screening investigations in WP3.

1.3.2.2.4 Serum markers to investigate response to anti-angiogenic therapy WP3)

In this part of the project, we searched for predictive tests for efficacy of Bevacizumab, a humanised antibody directed towards vascular endothelial growth factor (VEGF) with a modest effect in unselected breast cancer patients. We analysed two isoforms of VEGF; VEGF-A and –C in serum samples from patients included in the GeparQuinto trial. VEGF-A could function as a predictive test in the group of hormone receptor positive breast cancer while VEGF-C had a predictive value in triple negative breast cancer when pCR at surgery was set as end-point. However, the addition of Bev did not improve survival and consequently VEGF-A and –C had no impact on survival.

We also performed a pilot study of circulating microRNAs associated with resistance to Bev. In summary, 18 microRNAs were identified out of which 4 were involved in regulation of VEGF-A. As the VEGF´s not were associated to prognosis and Bev not is expected to have a major role in breast cancer treatment, the RESPONSIFY consortium decided not to continue the development of diagnostic tests in this area.

1.3.2.2.5 PIK3CA mutations and response to anti-HER2 therapy

In the RESPONSIFY project *PIK3CA* mutations were described as marker of resistance to anti-HER2 therapy based on evaluations of ~600 tumour samples. Within the GeparSixto (double anti-HER2 treatment) study we prospectively evaluated the *PIK3CA* mutations by Sanger Sequencing. In two retrospective cohorts from the GeparQuinto and GeparQuattro study with (mono-anti-HER2 therapy either trastuzumab or lapatinib) *PIK3CA* was assessed as well. This is one of the most common mutations, altered in about 20-25% of HER2-positive breast cancer. For the first time it could be demonstrated that tumours harbouring a *PIK3CA* mutation have a significantly lower pathological complete response rate, especially when the double blockade was given. The predictive effect of *PIK3CA* mutations seemed particularly relevant in hormone-receptor positive tumours. A similar trend was found in the DAFNE study in 65 tumours.
Other groups have shown similar results overall and by conducting a metaanalysis with almost 1000 patients these results could be confirmed, in particular for the group of hormone-receptor positive patients. Based in the results of RESPONSIFY, the analysis of *PIK3CA* mutations was included in the German breast cancer guidelines (www.ago.online.de).

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

One of the objectives of RESPONSIFY was the transfer of biomarkers identified by the screening studies to the diagnostic platforms of the SMEs commercializing the final test. Therefore, Sividon designed and successfully transferred immune system-related biomarkers as well as biomarkers identified in the screenings studies to their diagnostic PCR platform. A product development process was started including technical feasibility studies with instruments and reagents, selection of suppliers, pre-analytical studies, as well as establishment of a manufacturing and quality control concept for the new assay under ISO13485 conditions.

Moreover proto-type PCR assays were successfully manufactured and quality controlled for the validation studies in GeparQuattro and GeparSixto. These product development activities form the technical basis for the implementation of the ImmunPredict assay in to future clinical trials for monitoring of the immune system in breast cancer as well as for the final development of an IVD test.

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

1.3.2.4.1 Infrastructure for tissue-sampling, quality control and standardization (WP1)

At beginning of the project the backbone and logistics have been built and strengthened. We identified the possible cohorts with the appropriate biomaterial and set up standard operating procedures how these biomaterials could further be prepared and distributed from the central biobank from the German Breast Group. In parallel the biomaterial collection in various neoadjuvant studies was continued. In four neoadjuvant trials we implemented a window-of-opportunity study, where the patients receive a targeted therapy before they entered the main study or received surgery. These studies were an additional source for fresh frozen material. In total, more than 2000 nucleic acid isolations from more than 1100 tissue samples have been successfully performed in RESPONSIFY for the evaluation of DNA- and RNA-based biomarkers.

1.3.2.4.2 Software platform for clinical trial management (WP6)

A standardized **software for EDC** (electronic data capturing) and trial management was developed to support the integration of biomarker testing into the clinical trial environment, in particular for registration trials. In this EDC software additional functions have been implemented including access for central pathology to enter the results of the central assessment of HER2, ER, PR, Ki67, TILs and other predefined markers. In addition, a biomaterial tracking system has been integrated to allow the documentation of samples received, remuneration tracking and sample processing tracking, The system allows the import of lists of samples as well as individual data entry. For reporting of clinical data, the possibility to enter, assess and manage serious adverse events (SAEs) has been integrated into the system. The quality of the data in electronic SAEs as opposed to paper SAEs is

significantly improved and reconciliation with non-serious side effects can be easier performed.
Other modules built into the system include the Case Report Form Builder, which allows CRFs to be built fully in the EDC environment. Being template-based it enables standardized and rapid design and creation of new CRFs. The Monitoring Role for Issue Tracking allows monitors to track site, center, documentation and safety issues. The Monitoring Support Role enables monitors to follow up on queries on site and to see the data field history. The PET module enables data entry and external review, again within the EDC environment. The MedCODES platform is expected to be validated in March 2015 according to FDA CFR Part 11. This will enable us to conduct registration trials within the academic environment on a high level.

1.3.2.4.3 Health economical analyses (WP7)

Health economic evaluation is an integral element of the RESPONSIFY approach because predictive testing has the potential to save resources without affecting patient outcomes or quality of care. On the other hand, such favourable impact cannot be taken for granted. Health economic modelling can identify the most cost- effective approaches to the use of predictive tests. The health economics work package has been integrated into RESPONSIFY to assess under what circumstances biomarkers developed during the project may be cost-effective in combination with specific therapies.

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The health economics work package addressed five main tasks:

1. The first task included the construction of three core decision-analytic models as a basis for performing cost-effectiveness analyses of diagnostic and therapeutic strategies in the field of breast cancer. These cover the adjuvant, the neo-adjuvant and the metastatic settings, respectively. The core models have been used in the assessment of existing biomarkers and new biomarkers identified by the project consortium. Specifically, an adjuvant model for HER2-negative, estrogen receptor (ER)-positive breast cancer patients was developed and there was a first application to a real decision problem (Endopredict model). A neo-adjuvant model for HER2-positive breast cancer patients was constructed in accordance with the study-design of the GeparQuattro trial. A metastatic model for HER2-positve breast cancer patients was developed and will form a core model for future health economic studies in the metastatic setting.
2. Core input parameters and decision problems were defined. The decision problems to be studied in WP7 (e.g. testing and treatment strategies to be compared, relevant perspectives, countries for which the analyses were to be performed) and required model parameters were defined in close cooperation with the consortium partners. In particular, current state-of-the-art testing regimens and treatment schedules were defined for various settings. For this purpose, a health economic interest group was established. The information deriving from these meetings was linked with collected external data (Task 7.3.) and inserted into the various core models as a prerequisite of further analysis of the health economics of RESPONSIFY-generated biomarkers (Task 7.4)
3. Model inputs from external sources included mainly health state utilities (quality of life weights) and unit costs:

a. Health state utilities: we performed a comprehensive search for published literature containing information on utilities of breast cancer patients in different disease states;
b. Unit costs: information on resource use and unit costs was collected for the Germany and UK health care systems, for various treatment settings. Cost information was extracted for surgery, diagnostics, consultations, medical examinations, chemotherapy, endocrine and targeted therapy, side effects of chemotherapy, follow-up care, recurrence, metastasis, and last 4 weeks of life / death.

4. In the fourth task, several health economic evaluations were performed or prepared, including evaluations of new biomarkers identified or further evaluated by the RESPONSIFY consortium.

   In this first adjuvant model, it was assessed whether it is cost-effective to use a consortium member-generated test (EndoPredict®, Sividon Diagnostics) in addition to common clinical guidelines to support decision making in patients with estrogen receptor-positive, HER-2-negative breast cancer. The results from this analysis were integrated into the commercialization plans of an SME (WP8) within RESPONSIFY. In the neo-adjuvant setting, the cost-effectiveness of VEGF-C level determination to guide the use of bevacizumab in triple-negative breast cancer patients was determined. A further adjuvant model was developped to determine the costs and effects of TIL level determination for predicting trastuzumab use (FinHER study) among HER-2-pos breast cancer patients. This model will provide the basis of a future model assessing the cost-effectiveness of using the *PIK3CA* marker to guide the administration of trastuzumab and pertuzumab as a HER-2 double-blockade. The ongoing GeparSixto (NCT01426880) and GeparSepto (NCT01583426) trials will be used to measure treatment effects in the relevant groups of breast cancer patients.

5. A tool has been constructed to provide interested, non-health economist users with approximate insights into the health economic performance characteristics and potentially possible price of new biomarkers predicting response to a therapy of interest. The tool covers the adjuvant, neoadjuvant and metastatic treatment settings. Users can either make use of pre-set, trial-based clinical data valid for specific groups of breast cancer patients receiving specific treatments, or can make their own assumptions regarding patient pathways (e.g. proportion of patients expected to develop metastasis, time to metastasis and average survival once in this state). They can select a health system perspective or a societal perspective of cost assessment, and different discount rates. Key parameters affecting the results are the prevalence of the biomarker of interest, the performance characteristics of the test of interest, the treatment effect of the therapy of interest, and the costs of the test and therapy of interest. For example, higher sensitivity and specificity of the test as well as better clinical effectiveness of the therapy of interest will improve cost-effectiveness. The key strength of the tool is its adaptability to various settings, scenarios and countries. This leads to the main limitation of the tool which, due to its unspecific nature, will generate general trends rather than precise cost-effectiveness estimates. The quality of the results generated will depend on the quality of the model inputs available to the user, except where pre-set inputs can sensibly be used.

   A search for available biomarker tests and tests in development has also been conducted and key characteristics were extracted from the literature and accessible

websites (such as methods and test used, targeted patient populations, test accuracy and test costs).

1.3.2.4.4 IP issues as well as options for development of IVD tests (WP8)

The inclusion of diagnostic SME partners with expertise and know-how in development of molecular biomarker test was central for transfer of the validated biomarker assays to a routine diagnostic platform to support the commercialization and dissemination of the results of the RESPONSIFY project. During RESPONSIFY, one patent application was filed, on additional licensing agreement between the partners was finalized and one patent application is currently under evaluation. There has been a formal assessment of market potential and economic risks of diagnostic tests based on RESPONSIFY results and a commercialization plan was established. A first PCR-based test assay, the "ImmunoPredict" test has been selected for further development as a standardized way to monitor the immune status in the tumour tissue in the context of clinical trials.

1.4 The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

1.4.1 Overview

The overall aim of this project is to develop standardized FFPE-based predictive IVD tests for anti-HER2 and anti-angiogenic therapy in breast cancer to improve risk to benefit ratio for women diagnosed with cancer as well as cost to benefit ratio.

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

1. **ImmunoPredict molecular assay:** "ImmunoPredict" is a standardized PCR assay to be used by molecular pathology labs to assess a molecular immune signature in formalin-fixed, paraffin-embedded (FFPE) routine tumour tissue.
   We plan to implement the "ImmunoPredict" assay into future clinical trials to monitor the immune system in breast cancer. This could be particularly relevant for trials of immune checkpoint inhibitors. Protocols for those clinical trials are currently under preparation at the German Breast Group.

2. **Additional biomarker approaches:** In addition to the "ImmunoPredict" assay, further genes identified in the molecular screens are currently under investigation.
   These biomarkers will allow us to better understand the molecular mechanisms of resistance to therapy in breast cancer and to identify novel potential targets for the development of more specific treatments.

3. **Standardized test for tumour-associated lymphocytes:** The second biomarker test is a standardized approach to evaluate tumour-infiltrating lymphocytes in breast cancer using standardized image analysis software. We have published a guideline article for standardization of TIL evaluation during histopathological assessment and conducted a first ring trial. In the final phase of RESPONSIFY, a software prototype has been

developed for standardized evaluation of TILs. Both approaches ("ImmunoPredict" as well as the TIL assay) are based on the main result of RESPONSIFY that the immune system is critical for response to chemotherapy as well as anti-HER2 therapy. Tumour-infiltrating lymphocytes have been included in the German AGO guidelines for evaluation of breast cancer samples.

4. **Serum markers for response to anti-angiogenic therapy:** 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 anymore in the therapy of breast cancer. Therefore it was decided to discontinue further development of this diagnostic approach.

5. ***PIK3CA* mutations and anti-HER2 therapy:** One additional major focus of RESPONSIFY was the evaluation of *PIK3CA* mutations in breast cancer. 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. *PIK3CA* mutation analysis has also been included in the German AGO guidelines for evaluation of breast cancer samples.

6. **Health economic evaluation** of predictive testing has the potential to save resources without affecting the quality of care. By assessing health economic characteristics of new biomarkers, generation of an evidence base has begun to sustain efficient decision making on novel test-treatment combinations in breast cancer management.

1.4.2 Potential impact including socio-economic impact and wider societal implications

1.4.2.1 Rationale

Breast cancer is the main research focus of RESPONSIFY because it is the most common cancer in women worldwide with more than 1.1 million women newly diagnosed annually, accounting for 14% of all female cancer deaths. In Europe, approximately 463,000 women show new cases of breast cancer each year. 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 out- comes and minimize unnecessary toxicity. To date, the response rate to breast cancer therapy is approximately 20-40 %. The RESPONSIFY researchers aim to increase this rate considerably by the development of validated biomarker approaches.

RESPONSIFY's main goal is the development **of new** standardized biomarker tests, which can indicate whether and how a specific treatment affects an individual patient. This approach to a more **"personalized medicine"** will facilitate response prediction and select patients to a specific treatment from non-responders.

1.4.2.2 Impact for cancer research and clinical management of breast cancer patients

The RESPONSIFY project contributed to get breast cancer therapy more tailored to individual patients. Translated into clinical practice, this will help physicians to **make decisions and avoid ineffective treatments.**

RESPONSIFY investigated the interaction of tumour cells with the immune system. We were able to show that tumours with a high immune infiltrate have an increased response to neoadjuvant chemotherapy, and this finding can be validated by immunological gene signatures. This approach is particularly relevant considering the new clinical trials on immunotherapy that investigate immune check-point inhibitors in breast cancer. RESPONSIFY has evaluated the molecular targets of these inhibitors, which are linked to therapy response. In addition, RESPONSIFY evaluated the role of mutations for the therapy of breast cancer. In particular mutations of the *PIK3CA* gene are relevant for resistance to combined anti-HER2 therapies.

With help of novel standardized biomarker tests, **physicians could evaluate the prognosis and determine the treatment strategy early on.** By this approach, it will be possible to start treatment already before surgery, in a so called neo-adjuvant therapy, which can help to reduce the tumour burden before surgery and to evaluate response. The advantage of neoadjuvant therapy is that the effective response of the therapy on the tumour is immediately visible.

The European society will benefit from the knowledge gained as RESPONSIFY resulted in and presented in a variety of publications **new insights into cancer patient management** through improved molecular diagnostics and, therewith, the possibility of avoiding ineffective therapies and/or improvement of therapeutic concepts by selection of effective drugs.

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, who currently have only very limited therapeutic options.

RESPONSIFY **SME partners** will use the predictive tests discovered in the project in their prospective clinical trials in future. Through this way, the tests will be used by several hundred of key investigators worldwide.

1.4.2.3 RESPONSIFY dissemination activities

Due to the broad impact, RESPONSIFY's international dissemination and networking activities targeted various interest groups:

- the general public with focus on target groups
- the scientific and medical communities
- stakeholders from healthcare and insurances

The RESPOINSIFY consortium has established a broad strategy of public information including the creation of a public website, a promotion flyer, and finally presentation of the project and of results at public and scientific events and conferences in order to improve the awareness of the population on the problems of breast cancer treatment and the way to find solutions within the European Union.
A List of publications is presented at the RESPONSIFY website www.RESPONSIFY-FP7.eu.

The results of RESPONSIFY, in particular the data on the relevance of immune markers, have been summarized in a review article entitled 'The evaluation of tumour-infiltrating lymphocytes (TILs) in breast cancer: Recommendations by an international TILs Working Group 2014' that was published in Annals of Oncology 26: 259-271 2015 in 2014. This **review article** is also relevant for the dissemination of diagnostic tests, because it contains a **standardized guideline** for the evaluation of tumour-infiltrating lymphocytes that can be used by pathologists all over the world to integrate this parameter into standardized diagnostic testing.

Results were disseminated by presentations during national and international conferences, by publications in international peer-reviewed bio-medical journals. Several publications are currently under preparation. 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. For example, at the San Antonio Breast Cancer meeting 2013, three abstracts with RESPONSIFY results were selected for oral presentation. Additional oral presentations with RESPONSIFY results were presented at the ASCO 2014 in Chicago, the ESMO 2014 in Madrid and the EBCC conference 2014 in Glasgow, as well as other scientific conferences. Project's interdisciplinary workshops have been used to discuss results and options for intellectual property protection between the various experts of the consortium and invited external guest speakers. RESPONSIFY results will be presented in the upcoming scientific Gordon Conference on "Cancer Genetics and Epigenetics", which will be held in Italy in April 2015.

For standardization of evaluation of tumour-infiltrating lymphocytes, an international **working group** consisting of more than 40 pathologist and clinicians has been formed.

For the general public three **press releases** have been released for RESPONSIFY, one at the time of the start of the project, one with the results of the presentations at SABCS 2013, and one at the end of the project with the overall results and the announcement of the successful finalization of the project. The project was included in the Parliaments Magazine of the European Commission. Dr. Denkert has given a lecture for the public audience at Maxim-Gorki Theatre in Berlin in December 2012 including RESPONSIFY results.

Furthermore, the project is presented on the website of the European Commission in the EC news room area.

The **RESPONSIFY website** gives a general overview on the research field and lists all relevant publications of the project partners.

1.4.2.4 Strategies for the exploitation - management of intellectual property

The continuous evaluation of IP and exploitation options have been integrated in the RESPONSIFY project. Specific sessions for the SME partners were included in the project meetings and there was a separate work package for exploitation and IP issues. One patent application has been filed and a second patent application is currently under evaluation. In addition, one licensing agreement between partners has been finalized to obtain freedom-to-operate for the “ImmunoPredict” assay.

Based on these discussions with different experts and stakeholder groups a consolidated market analysis was done and a commercialization plan for the RESPONSIFY products was generated, which will **strengthen the position of the RESPONSIFY SME partners** on the molecular diagnostic market in oncology.

1.5 The address of the project public website, if applicable as well as relevant contact details

Public website address: http://www.responsify-fp7.eu

Contact details:

For all enquiries concerning RESPONSIFY, please contact the project coordinators

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