PROJECT FINAL REPORT

Mutanome Engineered RNA Immuno-Therapy

Grant agreement no.:	601939
Project acronym:	MERIT
Project title:	Mutanome Engineered RNA Immuno-Therapy
Funding scheme:	FP7-CP-FP
Period covered:	01 June 2013 to 30 November 2017
Project coordinator name:	Prof. Dr. Ugur Sahin
Project coordinator:	BioNTech AG
Phone:	+49- 6131-9084-0
Fax:	+49- 6131-9084-390
E-mail:	ugur.sahin@biontech.de
Project website address:	http://merit-consortium.eu/

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF TERMS AND ABBREVIATIONS

BIO	BioNTech AG, Mainz, Germany
BioNTech IMFS GmbH	BioNTech Innovative Manufacturing Service GmbH
CIMT	Cancer Immunotherapy
CSP	Clinical Study Protocol
DLT	Dose-limiting toxicity
DP	Drug product
DSMB	Data and Safety Monitoring Board
ELISPOT	Enzyme linked immune spot assay
ER	Estrogen receptor
GMP	Good manufacturing practice
HER2	Human epidermal growth factor receptor
HMGB1	High-mobility group protein B1
IB	Investigator's Brochure
ICD	Immunogenic cell death
IFN	Interferon
IFNAR	Interferon-α/β receptor
IGR	Institute Gustave Roussy, Villejuif, France
IMP	Investigational medicinal product
IMPD	Investigational Medicinal Product Dossier
IVAC	Individualized Vaccines Against Cancer
MAP1LC3B/LC3B	Microtubule-associated proteins 1A/1B light chain 3B
MERIT	Mutanome Engineered RNA Immuno-Therapy
NGS	Next generation sequencing
PR	Progesterone receptor
RNA	Ribonucleic acid
RT qPCR	Real-time quantitative polymerase chain reaction
TAA	Tumor-associated antigen
TNBC	Triple-negative breast cancer
UU	Uppsala Universitet, Sweden
UZH	University Hospital Zurich, Switzerland
VUB	Vrije Universiteit Brussel, Belgium
WHO	World Health Organization
WP	Work package

1 FINAL PUBLISHABLE SUMMARY REPORT

1.1 Executive Summary

The **M**utanome **E**ngineered **R**NA **I**mmuno-**T**herapy (MERIT) project was funded by the European Commission under the 7th Framework Programme and ran from 2013 to 2017. A pioneering ribonucleic acid (RNA)-based immunotherapy approach to target individual tumor antigen signatures and create a biomarker-guided personalized treatment of cancer should be clinically translated and industrially validated. MERIT was implemented by a consortium of five European partners in academia and industry. The scientific-technological work program was structured into seven work packages (WPs), covering preclinical, clinical, project management, and dissemination activities.

A novel, highly personalized and on-demand manufactured RNA vaccine-based immunotherapy for patients with triple-negative breast cancer (TNBC) was investigated. The vaccine was tailored to the individual genomic profile of the tumour of each patient. The main objective of the project was to assess the clinical safety, feasibility and biological efficacy of the MERIT approach in a multi-center phase I/II trial. Until the end of the MERIT project funding, ten patients were vaccinated with RNA Warehouse vaccines coding for shared tumor-associated antigens (TAAs) The treatments were well-tolerated and a primary immune response analysis indicated that immune system activation was triggered by the RNA Warehouse vaccinations. The TNBC-MERIT trial will be continued after the funding period and the first vaccination with the highly individualized Mutanome RNAs, targeting patient-individual mutations, is planned for Q1 2018.

The project also included a broad scientific program: (i) The Development of a fast and accurate mutation caller (ConfIdent) as well as a fast and reliable sequencing read aligner (ComPass) have been finalized and a new pattern recognition semi-metric, Poisson-Binomial Radius (PBR) for mutational analysis was implemented. (ii) T-cell priming experiments providing evidence for development of T-cell memory upon $RNA_{(LIP)}$ immunization (iii) the capacity of Protamine-RNA nanoparticles (“PR particles”) of different sizes and surface charges to induce steerable immunomodulation were evaluated in mice and (iv) the optimal combination therapy for treatment of metastasized disease as well as several packaging systems to enhance the uptake and cell tropism of the injected mRNA have been determined.

The MERIT concept and clinical trial design have been presented to a broad audience on numerous conferences. Scientific publications compiling data of the MERIT project are planned after completion of the clinical trial. Please visit the project webpage at http://merit-consortium.eu/ for further information.

1.2 Summary of Project Context and Objectives

1.2.1 The Concept

Triple-negative breast cancer (TNBC) is an aggressive, molecularly heterogeneous cancer defined by a lack of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2) overexpression.[1] TNBC accounts for approximately 15-25% of all breast cancer patients, is more likely to affect younger women and is frequently associated with a progressed clinical stage at diagnosis. Due to the molecular heterogeneity and lack of common targetable molecular alterations, many targeted therapies (e.g., PARP-, EGFR-, anti-VEGF and multi-kinase inhibitors) fail to provide clinical benefit in a significant fraction of TNBC patients. The five-year survival rate is less than 80%. Recent studies report a high immunogenicity of TNBC tumors indicating that inducing an integrated anti-tumor immune response directed against both shared and mutated tumor-antigens on a single- patient basis is a particularly suitable approach to meet the high unmet medical need.

In the TNBC-MERIT trial, TNBC patients after surgery and chemotherapy are vaccinated with individualized as well as fully personalized cancer vaccines to stimulate the patient's immune system. T cells are activated against the cancer to specifically recognize tumor cells and destroy them.

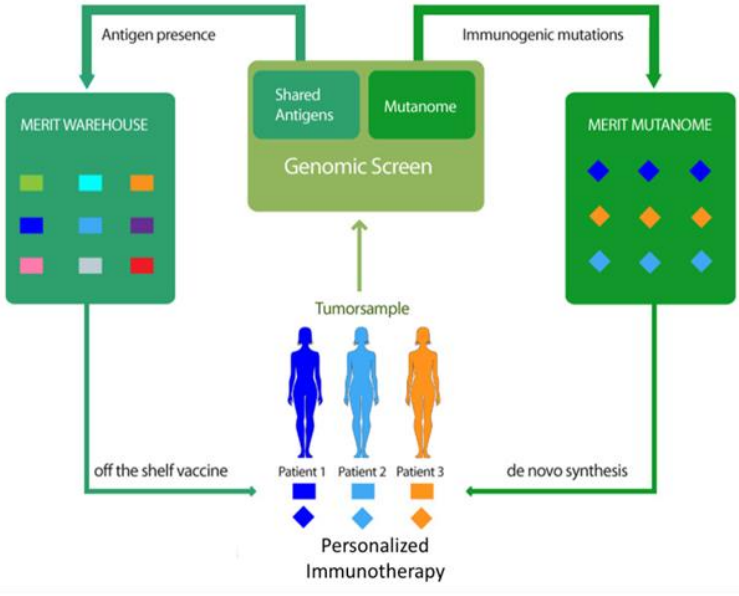

Figure 1: Concept of the TNBC-MERIT trial.

The TNBC-MERIT trial consists of a combination of two different concepts for treatments of patients, the Warehouse concept and the Mutanome concept. The Warehouse concept targets shared tumor-associated antigens (TAA) expressed in the respective patient´s tumor, whereas the Mutanome concept is a fully personalized cancer vaccine, targeting patient-specific non-synonymous mutations identified by NGS.

The TNBC-MERIT trial consists of two different concepts for treatments of TNBC patients, the Warehouse concept and the Mutanome concept (Figure 1). The treatments are given either

separately or consecutively. The Warehouse concept targets shared TNBC-specific antigens selected based on their immunogenic potential. The Mutanome concept is a fully personalized cancer vaccine, targeting patient-specific non-synonymous mutations identified by NGS. During the clinical trial patients receive an individualized combination of up to four RNAs from the off-the-shelf RNA Warehouse corresponding to their tumor antigen-expression profile in eight vaccination cycles (Figure 2). We have further established a Mutanome clinical grade process to identify tumor-specific immunogenic mutations for inclusion in the vaccine. In this multi-step process somatic mutations in the tumor of the patient are determined by next generation sequencing (NGS). Mutanome RNAs targeting the unique mutation signature of the individual patient are engineered and produced on demand and administered as tailored treatment during the clinical trial. Patients either receive eight vaccination cycles with Warehouse RNA (ARM1) or eight cycles of Warehouse treatment followed by eight additional vaccination cycles with fully personalized Mutanome RNA (ARM2). At the time of writing this report, the first patient had been recruited to ARM2, received Warehouse treatment, but not yet Mutanome treatment. The TNBC-MERIT trial will be continued after the end of the funding period and first vaccination with the fully personalized Mutanome RNAs is planned for Q1 2018.

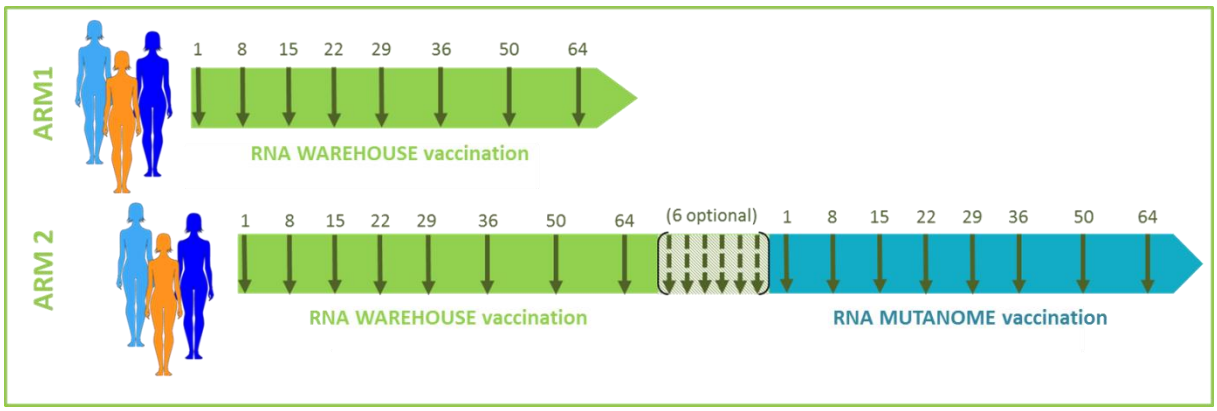

Figure 2: Vaccination schedule for treatment of patients included in ARM1 and ARM2.

Clinical study related procedures should be performed after the result of antigen expression is available for a given patient. Patients included in ARM1 receive eight RNA Warehouse vaccinations. Patients of ARM2 receive eight cycles of Warehouse RNA, followed by additional eight vaccinations with Mutanome RNA. Arrows indicate vaccinations and the day of each vaccination is indicated by the respective number.

An extensive analysis of immunological and molecular biomarkers is performed for each patient who completes vaccination. The vaccine-induced cellular immune response is determined by a robust and sensitive screening assay and by various aspects of immunogenic cell death and its correlation with T cell infiltrates are investigated. The obtained data are compiled in a database for efficient correlation analyses.

1.2.2 The Objectives

The main objective of the ongoing MERIT project is to assess the clinical safety, feasibility, and biological efficacy of the individualized as well as the fully personalized RNA cancer vaccines for treatment of TNBC patients in a multi-center phase I/II trial.

Further MERIT objectives are:

- to develop a computational medicine platform to allow for rapid identification of immunogenic shared and mutated antigens in TNBC patients.
- to set-up an RNA vaccine Warehouse of shared tumor antigens addressing > 95% of TNBC patients.
- to set-up a manufacturing process for timely on-demand manufacturing of personalized RNA vaccines targeting multiple tumor- specific mutations.
- to study associated biomarkers to identify molecular and immunological signatures that correlate with clinical events following treatment.
- to identify synergistic compounds and optimized protocols of MERIT vaccines.
- to establish a high-level European network for research on novel, personalized immunotherapy for treatment of TNBC patients.

To achieve these objectives, the MERIT project was divided into work packages (WPs) focusing on the preclinical part (WPs 1-3 and 6), the clinical part (WPs 4 and 5) and on general project management and dissemination (WP7).

1.2.3 The Project Phases and Tasks

Preclinical/translational project phase (WPs 1-3 and WP6)

The first aim of the preclinical/translational project phase was the preclinical development of new RNA-based immune-stimulatory compounds, including the preclinical proof-of-concept. The optimal immunotherapy strategy of RNA vaccines was investigated in a CT26 murine lung cancer model. The formulation of the vaccine was optimized with respect to delivery route, dose level and schedule in monotherapy, and in combination with chemotherapy for treatment of metastasized disease in a mouse model.

For the MERIT Warehouse approach, eight well-characterized antigens which are shared by a large fraction of TNBC patients and carry immunogenic T cell epitopes were selected. The selection was based on (i) differential expression profiling (tumor versus multiple healthy tissues), (ii) testing presentation of T cell epitopes by tumor cell lines and (iii) analysis of cellular localization and cell cycle dependent expression. The aim of WP1 was to manufacture the eight selected Warehouse RNAs. Manufacturing, testing and release were performed in compliance with good manufacturing practice (GMP) regulations by BioNTech Innovative Manufacturing Service GmbH (BioNTech IMFS GmbH, former EUFETS GmbH). Furthermore, a Real-time quantitative polymerase chain reaction (RT qPCR)-based diagnostic

assay was to be established and implemented for screening of the selected TAAs during the clinical trial by BIO.

For the MERIT Mutanome RNA approach, patient-specific mutations are identified from the patient's tumor sample. The identified mutations are ranked and the epitopes with the highest prioritization chosen to be manufactured by BioNTech IMFS GmbH. The fully personalized RNA vaccines as well as liposomes and diluent required for the preparation of the ready-to-use investigational medicinal product (IMP) are assembled to kits. Labeling and packaging of patient-individual kits is performed at BIO´s affiliated entity BioNTech RNA Pharmaceuticals GmbH.

The team at Uppsala Universitet (UU, Sweden) developed a novel statistical algorithm for mutation detection and analysis. To support the risk/benefit assessment of first-in-man testing BIO generated a comprehensive preclinical data set. The immunogenicity of mutation-based RNA vaccines was analyzed in a murine mammary carcinoma model. Vaccination was performed by systemic and repetitive application to simulate the treatment protocol in humans. It was shown that the established process of discovering tumor-specific mutations in other models also led to detection of immunogenic mutations with anti-tumoral effect in the mouse breast cancer model.

The competent authority was consulted for scientific advice before preparation and submission of the study documents. Subsequently, the Investigator's Brochure (IB), the Investigational Medicinal Product Dossier (IMPD), the Clinical Study Protocol (CSP), and the Informed Consent Form of the TNBC-MERIT trial were submitted to the regulatory agencies and ethics committees. The MERIT trail was approved in Germany, France, Belgium and Sweden and was entered to the world health organization (WHO) approved clinical trial registry (ClinicalTrials.gov).

<u>Clinical project phase (WP4 and WP5)</u>

The clinical project phase of the MERIT project is ongoing. Patients are enrolled with advanced stages of TNBC following surgery of the primary tumor. Archived tumor specimens obtained at therapeutic surgical intervention are used for gene expression profiling and vaccine design. Patients are eligible for enrolment if at least two MERIT RNA Warehouse antigens are expressed in their tumor. Patients with symptomatic brain metastases, known clinically relevant autoimmune disease or systemic immunosuppression are excluded.

The aim of the clinical project phase is to demonstrate feasibility of the novel personalized vaccine approach in patients with TNBC. This includes determination of safety, investigation of biological activity by immune monitoring, and first clinical benefits as determined by response of tumor lesions according to immune related response criteria. A dedicated data safety monitoring board (DSMB) was installed for continuous safety and feasibility assessment. For each patient who completed vaccination, extensive analysis of immunological and molecular biomarkers is performed. To detect and classify vaccine-

induced cellular immune response, immune monitoring is performed at the established centralized immune monitoring facility of BIO´s GxP Analytics Unit. Here, enzyme-linked immuno spot (ELISPOT) assays are employed as a robust and sensitive screening assay to determine the patient´s immune response by comparing antigen-specific T cells before and after the vaccination cycle. Moreover, markers for immunogenic cell death are investigated by the Institute Gustave Roussy (IGR, Villejuif, France). Those analyses cover the evaluation of the endoplasmic reticulum stress response pathway, activation of the autophagy machinery, and interferon (IFN) fingerprinting. Correlative studies between immunogenic cell death markers and T cell infiltrates are also ongoing.

General provisions (WP7)

As project coordinator, BIO was responsible for budget and timelines, ensuring overall quality, and coordinating interactions within the MERIT consortium and with the European Commission. Taking full responsibility for the monitoring the scientific progress, managing the clinical trial and the dissemination of results were tasks of the respective WP. For the latter purpose, a project specific website address was secured for reporting to the scientific community and to the public (Section 1.4.4.2).

1.2.4 The Partners

The MERIT project was led by BioNTech AG (BIO, project coordinator), a highly innovative biotechnology company dedicated to applying the best science for the benefit of patients. The collaboration of the BIO with academic partners and clinical research partners provided a unique opportunity for clinical translation of the innovative therapeutic concept and to achieve the industrial objectives of BIO within this project and even beyond. The international collaboration is mandatory to (i) recruit a clinical trial within reasonable time frame, (ii) to provide a whole range required competences and include world leading experienced partners, and to (iii) ensure further development and commercialisation. All clinical partners (Institute Gustave Roussy, IGR; Uppsala University, UU; Vrije Universiteit Brussel, VUB) are international experts in the field of TNBC treatment and have a proven capability to recruit in multi-center clinical trials and long-time experience of successfully execution of clinical trials.

The industrial partner (BIO), academic and clinical research partners (IGR, VUB, UU, and University Hospital Zurich, UZH) combined complementary knowledge and competences needed to translate this multi-disciplinary approach of individualized patient treatment and the highly specialized work modules (*e.g*. biomarker, immune monitoring, whole genome analyses and development of new compounds). All partners are renowned scientists and experts in their clinical and/or research fields and have demonstrated long-standing commitments to the development of immunotherapies and biomarker research.

Furthermore academic centers in Europe recruited patients and produced scientific data for the MERIT project. The personalized vaccines were produced at BIOs´s subsidiaries BioNTech IMFS GmbH and BioNTech RNA Pharmaceuticals GmbH.

1.3 Description of the Main Results

1.3.1 Overview

MERIT is an ongoing successful project involving numerous partners and generating interesting scientific results. The MERIT work packages are summarized below.

1.3.2 RNA Vaccine Production (WP1)

Objectives

The main objectives of WP1 were to manufacture GMP-grade MERIT Warehouse and Mutanome RNA for the clinical trial and to manufacture GMP-grade Kits containing all vaccine components for the clinical trial.

Summary of progress

Plasmid DNA templates were established at BIO containing codon-optimized versions of selected TNBC-associated antigens. RNA drug products were subsequently manufactured and tested in compliance with GMP regulations and each was accompanied by a Certificate of Analysis (CoA). Every RNA vaccine contained one RNA drug substance encoding the specific TAA. BioNTech IMFS GmbH successfully manufactured the eight RNAs for use in the MERIT clinical trial. The drug products were stored as independent batches and stability studies were conducted to determine their shelf-life. The material used for stability studies was produced under GMP conditions. A stability study was performed for each Warehouse RNA vaccine, including test of RNA content, identity, purity and potency. Subsequently, the preparation of the investigational medicinal product (IMP) kits was afterwards performed at BioNTech IMFS GmbH. A defined process covering every step from initiation of production to release of the final product was established according to GMP regulations. All required components were stored and all steps performed under defined and controlled conditions. Every step was precisely documented and underwent quality control procedures. Patient-specific kits composed of up to four RNA drug products (DPs) that were chosen according to the stratification of the patient´s tumor and kits containing diluent and excipient required for preparation of the ready-to-use IMP were produced following GMP guidelines.

1.3.3 Preclinical Studies and Regulatory Affairs (WP2)

Objectives

The main objectives of WP2 were i) to design a phase I/II clinical trial, ii) to obtain primary pharmacodynamics data in animal models supporting the MERIT clinical testing concept of anti-tumoral RNA vaccine, iii) to obtain scientific advice for the MERIT clinical trial by European regulatory authorities and iv) to prepare and submit the data package for the clinical trial application.

Summary of progress

To support the risk/benefit assessment of the first-in-man testing, BIO performed a number of preclinical studies. It was shown that the established process of discovering tumor-specific mutations in other models also lead to the detection of immunogenic mutations that have an anti-tumoral effect in a mouse breast cancer model. Regarding the Warehouse RNA vaccines targeting human cancer antigens, BIO showed a potential patient benefit in *in vivo* mouse models with immune responses and cytolytic activity of induced T cells on a transgenic human HLA-background.

BIO received positive feedback on the basic concept and scientific rationale of the MERIT approach at a Scientific Advice Meeting with members of the Paul-Ehrlich Institute (PEI, competent authority Germany). Following this meeting, the IB, the IMPD, and the CSP were prepared and submitted to the regulatory agency (PEI, Germany) and ethics committees (Mainz and Heidelberg) in November 2014. The CSP was developed by the Consortium as a whole and was discussed in its outlines during the MERIT Annual Meeting with the consortium and as a full document thereafter. The MERIT clinical study was approved in all participating countries and registered on the WHO approved registry ClinicalTrial.gov (https://clinicaltrials.gov/) with the following ClinicalTrials.gov Identifier: NCT02316457. The press release on completion of patient recruitment was postponed as the clinical study is ongoing.

1.3.4 Cancer Genomics (WP3)

Objectives

The main objectives of WP3 were i) to set up a targeted screening for the presence of shared tumor antigens in patient tumors, ii) to optimize a clinical screening process for genome-wide tumor somatic mutations and iii) to enhance sensitivity and specificity of mutation detection in clinical samples.

Summary of progress

BIO selected TAAs and established a diagnostic assay which was implemented for screening the TAAs based on RT qPCR during the clinical trial. In addition, BIO established the genome-wide screening process for TNBC in clinical use. RNA from over 50 TNBC samples was sequenced by NGS, demonstrating the successful application of the NGS platform and computational pipelines for TNBC samples. RNA and DNA from a variety of tumor types and sample formats were sequenced. In summary, the lab and computational platform ran successfully and were established for clinical use.

The Warehouse vaccine is composed of three individual antigens selected from the pre-manufactured RNA Warehouse, consisting of a total of eight different antigens. Additionally, the universal tumor-associated antigen p53 was included in the vaccine for every patient. At the time of writing this report, the expression profiles of 14 tumor samples (13 ARM1 and one ARM2 patient) have been analyzed. Ten patients have been enrolled in ARM1 and

antigens for vaccination have been selected according to a defined selection algorithm. An average of four to five warehouse antigens (ranging from two to seven) were identified per patient sample and a warehouse antigen was expressed in 56% (ranging from 20-100%) of samples. Analysis of the first 10 patients of ARM1 showed that the individualized concept is reasonable as every patient showed a different expression pattern. Different sets of antigens were selected for all patients so far.

The first ARM2 patient was recruited in Germany in October 2017. The patient´s tumor and blood samples were analyzed and NGS was used to identify expressed non-synonymous tumor-specific mutations. The selected targets were approved by a panel of expert and the DNA templates were produced and transferred to the GMP manufacturing facility. The two individual RNA constructs were successfully produced beginning of January 2018, sterility and analytic tests of release criteria are ongoing. The first treatment with the on-demand produced Mutanome vaccine is scheduled for Q1 2018.

In addition to work for the clinical trial, a novel statistical algorithm for mutation detection and analysis was developed by the team at Uppsala University (UU). This algorithm can be integrated into a mutational analysis software for further Mutanome studies. UU has also developed a somatic mutation caller (ConfIdent), characterized by a high platform portability, multi-processor capability, competitive execution speed and improved accuracy at detection of important classes of somatic mutations (InDels) over the available solutions.

1.3.5 Phase I/II Clinical Trial (WP4)

Objectives

The main objective of WP4 was to conduct a clinical phase I/II trial featuring a novel approach for a fully personalized vaccine in patients with TNBC.

Summary of progress

The TNBC-MERIT trial was initially registered at the EudraCT database and received its unique identifier 2014-002274-37. Each amendment of to the clinical trial application was updated the online information in the EudraCT database. Furthermore, the clinical trial was initially registered at ClinicalTrials.gov and received its unique identifier NCT02316457.

Until the end of the EU funding of the MERIT project, enrolment for both cohorts I and II of ARM1 was completed and all six patients have received all eight Warehouse vaccinations. Moreover, all three patients for cohort III of ARM1 are enrolled and already under treatment (see Figure 3). With the recommendation of the DSMB to open cohort III of ARM1, the recruitment for cohort I of ARM2 could be initiated. The first patient in ARM2 has already been recruited and started treatment with Warehouse RNA (see Figure 3). Three clinical sites (Mainz and Heidelberg in Germany as well as Uppsala in Sweden) have already actively enrolled patients and will continue their activities beyond the end of the EU funding. Moreover, the opening of additional clinical sites is planned.

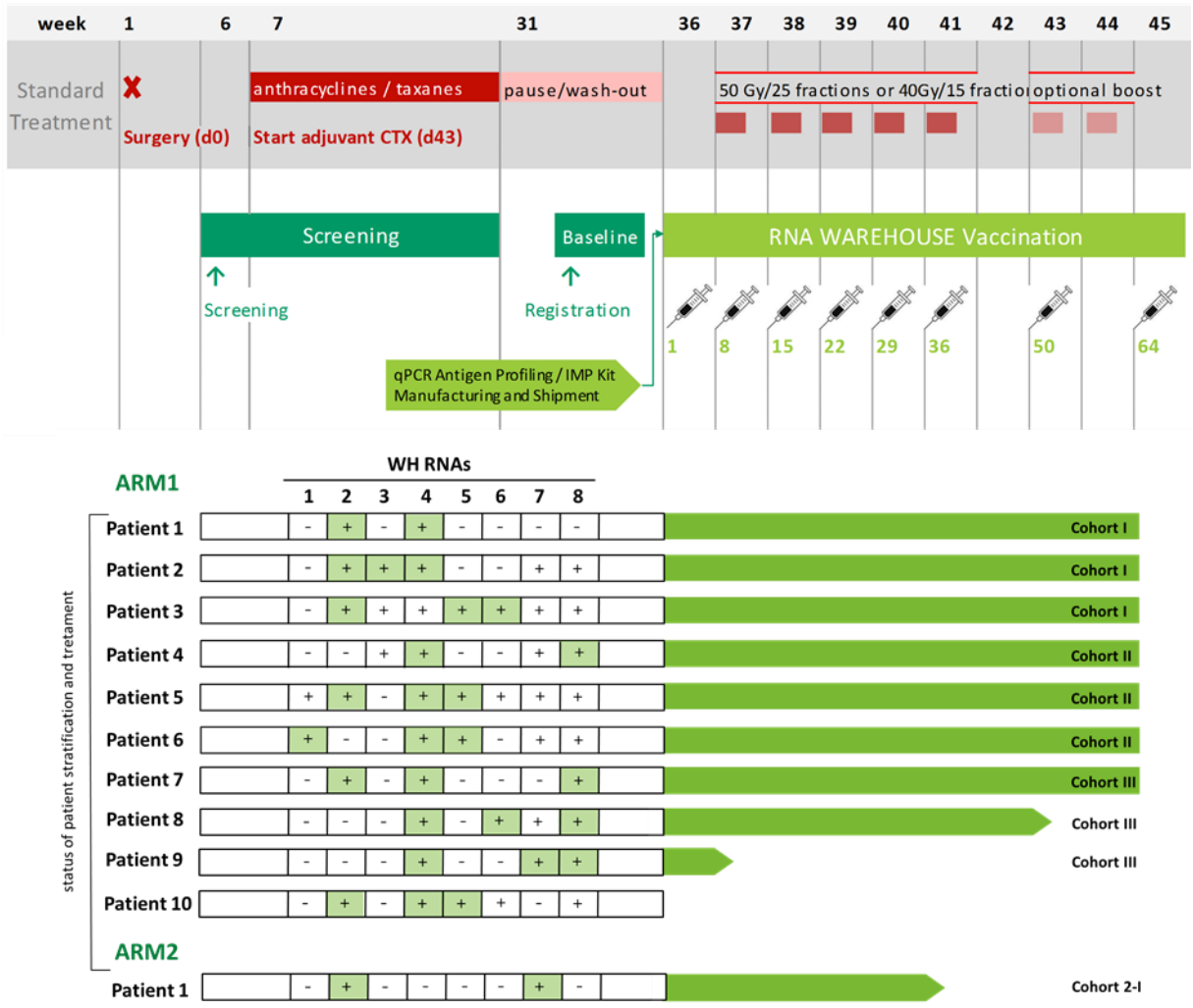

Figure 3: Status of patient stratification and vaccination.

At least two warehouse antigens were found per tumor in ten patients of ARM1 and one patient of ARM2 via the stratification procedure. Pluses indicate positive stratification for the respective antigen, minuses indicate negative stratification for the respective antigen. Antigens marked by a green square were selected for vaccination of the patient. Seven patients have already received all eight vaccinations with Warehouse RNA, while two are currently under treatment (indicated by the green bars). Syringes indicate single vaccinations. Red squares indicate radiotherapy fractions, which are given in parallel to RNA Warehouse vaccination.

1.3.6 Translational Biomarker Research (WP5)

Objectives

The main objectives of WP5 were to generally conduct translational biomarker research and to specifically analyze the relevance of immunogenic cell death biomarkers in the efficacy of the vaccine.

Summary of progress

Immune monitoring of each patient that completed vaccination was performed at the centralized immune monitoring facility of BioNTech´s GxP Analytics Unit. Since MERIT Warehouse vaccines were expected to lead to activation of antigen-specific cytotoxic T cells

(CTLs) and helper T cells, T-cell responses against the selected warehouse antigens were monitored specifically and individually for each patient and antigen. Here, ELISPOT assays were employed as a robust and sensitive screening assay to determine the patient´s immune response by comparing antigen-specific T cells before and after the vaccination cycle. In the case of one of the patients, the available material was insufficient for this approach, instead bulk PBMCs were tested by ELISPOT. After optimization of the biomarker program, samples from the first two dose cohorts of ARM1 were analyzed. Preliminary data of ELISPOT analysis revealed CD4- as well as CD8-mediated responses. The amplification of pre-existing immune responses against the analyzed tumor-associated antigens as well as *de novo* vaccine-induced responses were observed in several patients.

The team at Institute Gustave Roussy (IGR) investigated markers for immunogenic cell death in samples of different tumor types. The analysis of human breast cancer samples revealed that the absence of microtubule-associated proteins 1A/1B light chain 3B (MAP1LC3B/LC3B)-positive puncta in the cytoplasm or the loss of nuclear high-mobility group protein B1 (HMGB1) expression by malignant cells, are associated with poor prognostic features. Biomarker studies in melanoma support the idea of a mutual dialogue between the cell-intrinsic stress pathway and the immune effectors. In addition, preliminary data showed that, in esophageal cancer, the expression of the two studied markers (HMGB1 and LC3B) had no impact on the survival of patients, but follow-up studies will be performed.

Moreover, tissue sections as well as whole blood were collected from TNBC breast cancer patients enrolled in the TNBC-MERIT trial at various time points. With these samples the team at IGR will investigate various aspects of immunogenic cell death. Next to immunohistochemical detection of the tumor immune infiltrate (CD4, CD8, Foxp3), the expression level of mRNA related to interferon-α/β receptor (IFNAR) pathways and immunogenic cell death (ICD) will be determined by RT-qPCR.

1.3.7 Preclinical Optimization Studies (WP6)

Objectives

The main objectives of WP6 were i) to optimize the vaccine's immunogenicity via delivery route/dose/schedule/combined chemotherapy, ii) to augment vaccine induced T cell responses by recombinant RNA coding for immune stimulators and iii) to optimize the vaccine's efficacy via systemic immunomodulation using RNA based nanoparticles.

Summary of progress

BIO investigated the optimal immunotherapy strategy of RNA vaccines in a CT26 lung cancer mouse model. Here, intranodal immunization with naked RNA was compared to intravenous immunization with liposomal complexed RNA. Moreover, different immunization schedules (short term interval versus weekly immunization) were compared and the ability of RNA vaccines to induce memory T cell response was evaluated. Based on these investigations, an

optimized vaccination strategy for RNA vaccines was defined and successfully implemented in the clinical study protocol. Within the clinical trial, RNA complexed with liposomes is applied intravenously, once weekly for eight weeks. In addition UZH tested mRNA vaccination ($RNA_{(LIP)}$) one day after injection of Doxorubicine (classical chemotherapy for triple negative breast cancer patients) or Gemcitabine (potential chemotherapy for triple negative breast cancer patients). None of the two chemotherapeutic drugs affected the uptake of $RNA_{(LIP)}$ in vivo. However, the chemotherapies did not improve and eventually reduced the immune response triggered by the vaccine. Thus, it was recommended not to use chemotherapies in combination with $RNA_{(LIP)}$.

The team at Vrije Universiteit Brussel (VUB) analyzed the combination therapies of dendritic cells modifying proteins for the treatment of metastasized disease in a preclinical model. Here, various reagents were tested in several mouse tumor models for immune-therapeutic potential. Reagents were tested in mono- or combination therapies by injection into tumors or tumor draining lymph nodes. This work showed the feasibility of using mRNA encoding immunomodulating proteins to modulate the tumor microenvironment and has been published [2]. Furthermore several packaging systems have been evaluated to enhance the uptake and cell tropism of the injected mRNA.

The UZH team also investigated immunostimulatory RNA formulated in Protamine nanoparticles (“PR particles”) for cancer therapy. They found that systemic induction of interferon-alpha and anti-lung metastasis activities were most efficiently triggered by intravenous injections of neutral PR particles with an average diameter of 120 nm or less. This required an adequate formulation and a specific administration schedule. In addition, chemotherapeutic moieties were incorporated into RNA in order to generate immunochemotherapeutic RNA nanoparticles that may be used for debukling large tumors prior to ($RNA_{(LIP)}$) vaccination. These results have been published recently.[3]

.

1.3.8 Dissemination and Project Management (WP7)

Objectives

The objectives of WP7 were to i) install efficient management and administration of the project in accordance with legal and ethical requirements for projects in the European Union, ii) establish management structures and procedures for efficient communication between the consortium partners and iii) implement monitoring and controlling processes for timely identification of potential problems and initiation of corrective actions. Further objectives were iv) to develop a risk management and mitigation structure and v) to coordinate exploitation strategies and vi) to disseminate the project activities within the consortium and beyond. vii) Lastly, a networking and communication platform for

dissemination of project-related results to the scientific community and the general public as a fundamental aspect of translational research should be provided.

Summary of progress

The MERIT specific project office as central management platform was implemented at BIO during the setup phase. The MERIT website (http://merit-consortium.eu/) was setup in August 2013 with restricted access to the consortium partners. The website was finally available for the public in September 2013.

The internal status reports 1, 2 and 3 were posted on the MERIT website including an overall project update. All consortium partners have access to the password-protected area of the webpage that contains the MERIT internal status report for download.

The MERIT project was introduced and progress was presented by the MERIT consortium to experts in the field of oncology and immunology at the DNA Vaccine Conference 2014 in San Diego. In addition, abstracts were submitted and selected for presentation by an international panel of experts at the annual meeting of the American Association for Cancer Research in 2015 and 2016 in Philadelphia and New Orleans. Moreover, an editorial in the 'EU Research' journal was published in 2015. Abstracts were submitted and the MERIT concept was selected for presentation at the CIMT meetings in 2014, 2015, 2016 and 2017. CIMT is the largest European meeting with focus on cancer immunotherapy of the Association for Cancer Immunotherapy (CIMT) and thus constitutes an ideal platform for the MERIT consortium to present and discuss new findings generated within the project. In addition, the MERIT concept was presented at the ESMO Immuno-Oncology Congress in Geneva, Switzerland. The publication of MERIT project results in high impact peer review journals was postponed to the end of the trial.

1.4 Potential Impact and Main Dissemination Activities and Exploitation of Results

1.4.1 Impact for Life Science and Medical Use

TNBC is a molecularly heterogeneous and aggressive type of breast cancer. Due to the molecular heterogeneity and the lack of common targetable molecular alterations (ER, PR, HER2), many targeted therapies fail to provide clinical benefit in a significant fraction of TNBC patients. Recent studies report a high immunogenicity of TNBC tumors supporting the MERIT approach of inducing an integrated anti-tumoral immune response directed against shared and mutated tumor-antigens on a single- patient basis. This approach might be particularly suitable for treatment of patients with TNBC, where the five-year survival rate is less than 80%, so the unmet medical need is high.

Personalized medicine is currently under comprehensive investigating and it is turning into a technology that can be made accessible for patients.[4, 5] MERIT is, to our knowledge, the

first multi-center clinical trial where immune-therapeutics are actively tailored to the needs of individual patients. With the successful completion of the MERIT project, we gained information about the safety and feasibility profile and achieved the first indications of cellular response and therefore we believe that the MERIT consortium has advanced personalization of medicine to the next level. MERIT has significantly strengthened the direct cooperation between leading TNBC centers all over Europe with regard to patient care and translational research and given new hope for TNBC patients. The creation and implementation of such a network has the potential to move Europe to a global leading position in the field of next-generation personalized cancer medicine.

1.4.2 Impact on Industry

As stated within our business development plan BioNTech AG is outstandingly positioned in the landscape of venture-capital financed drug developing biotech companies within Europe. The first promising results of the Individualized Vaccines Against Cancer (IVAC) melanoma trial have highlighted this position worldwide. BioNTech entered into a strategic collaboration with Genentech Inc. in September 2016.

This collaboration with Genentech Inc. and with F-Hoffmann La Roche is of crucial importance, because it is a long-term collaboration and together with the financially strong partner it opens new opportunities for clinical development and commercialization of BioNTech's technology platform, worldwide, including in particular the countries of the European Union. Under the collaboration agreement BioNTech grants a co-exclusive license to Genentech and F-Hoffmann La Roche to its intellectual property for research, development and commercialization of pharmaceutical products in the field of neoepitope mRNA. This may include results created under the FP7-Project MERIT. BioNTech has reserved an exclusive license on its intellectual property in order to conduct the clinical trial under the FP7-Project MERIT and BioNTech maintains the possibility to conduct subsequent clinical trials in the relevant fields of breast cancer and melanoma. Further, BioNTech will manufacture the mRNA vaccines at its sites in Germany for all clinical trials within the collaboration and therefore further develop its technology and build up new manufacturing locations in Germany/Europe.

The first major achievement of this collaboration was the positive feedback from the FDA concerning our new Mutanome process during the pre-IND meeting in April 2017. BioNTech and Genentech have now commenced the first Mutanome trial in the United States of America. Due to the positive experiences of BioNTech with the European competent authorities of Germany, Sweden and Belgium with the MERIT project, further clinical trials are prepared for these countries, too. This will further improve the European position within the fast developing field of personalized cancer medicine.

1.4.3 Impact on Other Areas of Research

Synthetic and recombinant RNAs have the potential to become part of the most important vaccine technology platform in the 21st century. BioNTech has been acknowledged in Nature Medicine [6, 7] for their contribution to this field and the results of the first RNA based Mutanome trial in melanoma has been published in Nature in 2017.[8] The scientific and technological lead of RNA platforms and RNA vaccines will be further increased by MERIT. With the aim to master the regulatory, technical and clinical requirements associated with the paradigm shifts of a rationally designed individualized therapy, the MERIT consortium may contribute to contemporary medical and operational best practice.

1.4.4 Dissemination Activities

1.4.4.1 Management of Intellectual Property

Identifying commercializable DPs was a primary outcome of this project, as this is the only way for such therapeutic concepts to sustainably reach the patients. Thus, the MERIT consortium is committed to enable development of commercializable DPs by BIO. MERIT is driven by existing intellectual property and will generate novel intellectual property. Thus, a mutual definition of IP relationships was necessary for the MERIT project and was defined in the consortium agreement. The contracts guaranteed that the respective interests of the partners were respected. BIO will proceed with advanced clinical development of MERIT beyond the life-time of the project.

1.4.4.2 Exploitation of Project Results

The MERIT project team has strived to ensure a knowledge distribution and promotion of the MERIT concept and clinical trial to a broad audience. In summary, the project findings were disseminated in the form of scientific presentations and publications in form of posters and reports, which were sent regularly to the European Commission. First results of the scientific program have been published.[2,3] Moreover, information was made publicly available on the MERIT website (http://merit-consortium.eu/).

Logo and project templates

A clear and appealing logo was designed for the MERIT project to give the project an easily recognizable corporate identity (Figure 4). To establish MERIT as a brand and to promote corporate identity within the consortium, standard templates for documents and power point presentations were compiled.

Figure 4: Project logo.

Official project website

The MERIT website (http://merit-consortium.eu/) was launched on September 13th 2013 for internal communication via a restricted section of the website as well as for external communication with the scientific and public community.

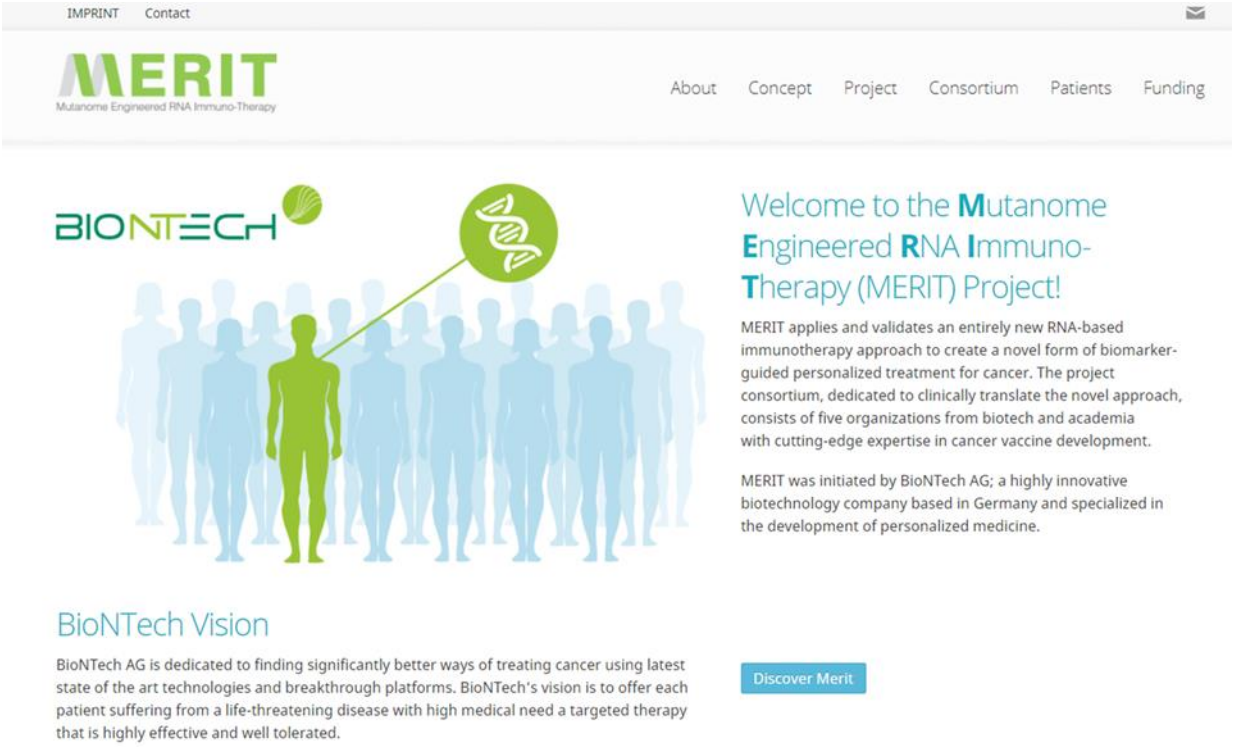

Figure 5: Home of the MERIT project website.

Consortium meetings

During the MERIT project funding period, one kick-off meeting and four consortium meetings were held (Table 1). The discussions during these meetings were important contributions to the MERIT concept.

Table 1: List of consortium meetings during the MERIT project funding period.

Meeting	Date	Location	Host
Kick-off-Meeting	26 June 2013	Mainz, Germany	Ugur Sahin, BioNTech AG
First Annual Meeting	10 October 2014	Zurich, Switzerland	Dr. Steve Pascolo, University Hospital of Zurich
Second Annual Meeting	20 October 2015	Brussel, Belgium	Kris Thielemans and Jacques de Grève, Vrije Universiteit Brussel
Third Annual Meeting	06 September 2016	Mainz, Germany	Ugur Sahin, BioNTech AG
Fourth Annual Meeting	12 September 2017	Uppsala, Sweden	Tobias Sjöblom and Henrik Lindman, Uppsala University

Conference presentations and publications

The MERIT project team aimed at presenting the scientific advances in the field of cancer genomics, bioinformatics, immunology, personalized immunotherapy and biomarker development on international conferences.

Thus, the MERIT concept was presented to the field of oncology and immunology at the CIMT Annual Meeting 2014 (Mainz, Germany) and at the DNA Vaccine Conference 2014 (San Diego, USA) during the session "Introduction of Collaborative Projects and EU Research Programme Horizon 2020". Moreover, the MERIT consortium was presented at the Young Academics Conference of the DKTK School of Oncology in Heidelberg, Germany. In addition, MERIT was presented at the annual meeting of the American Association for Cancer Research in 2015 (Philadelphia, USA) and 2016 (New Orleans, USA). It was further presented at the third international mRNA Health Conference in 2015 (Berlin, Germany). The MERIT consortium was introduced to experts in the field of immunotherapy at the CIMT meetings in 2015 and 2016 (Mainz, Germany). In 2017, the MERIT consortium was presented at the Third CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference (Mainz, Germany) and at the ESMO Immuno-Oncology Congress (Geneva, Switzerland). The publication of MERIT project results in high impact peer review journals was postponed to the end of the trial.

List of consortium participants

No	Name	Short name	Country
1	BioNTech AG (Coordinator)	BIO	Germany
2	Institute Gustave Roussy	IGR	France
3	Vrije Universiteit Brussel	VUB	Belgium
4	University Hospital Zurich	UZH	Switzerland
5	Uppsala University	UU	Sweden

1.5 References

1. Metzger-Filho, O. Dissecting the heterogeneity of triple-negative breast cancer. Journal of Clinical Oncology 30, 1879-1887 (2012).
2. Van der Jeught,K. Intratumoral administration of mRNA encoding a fusokine consisting of IFN-β and the ectodomain of the TGF-β receptor II potentiates antitumor immunity. Oncotarget.(20): 10100–10113 (2014).
3. Tusup, M. Generation of immunostimulating 130 nm protamine-RNA nanoparticles. Methods in Molecular Biology 1499, 155-163 (2017).
4. Schork, N. J. Personalized medicine: Time for one-person trials. Nature 520, 609–611 (2015).
5. Bates, S. Progress towards personalized medicine. Drug discovery today 15, 115–120 (2010).
6. Kreiter, S. et al. Erratum: Mutant MHC class II epitopes drive therapeutic immune responses to cancer. Nature 523, 370 (2015).
7. Katsnelson, A. Mutations as munitions: Neoantigen vaccines get a closer look as cancer treatment. Nature medicine 22, 122–124 (2016).
8. Sahin, U. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature 547, 222-226 (2017).