

Systems Medicine of Metabolic-Signaling Networks – A New Concept for Breast Cancer Patient Stratification



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Comparison between stage 1 and stage 2 MESI-STRAT proposal

The proposed project has remained the same in stage 2, both in content and in budget. One adaptation was made in that VHIO replaced EPO as a partner as VHIO has outstanding expertise in BC PDX models and as a clinical BC center. Some wording was changed and text sections rearranged to better convey the concept and goals of MESI-STRAT to the reviewers.

Definitions:

MESI models. Combined computational models of metabolic and signaling (MESI) networks that govern drug response in ER+BC.

MESI marker panels. Panels of metabolites and signaling proteins which in conjunction with MESI computational models predict drug response in distinct patient subgroups. Initially, proteins and metabolites will be measured in tumor tissue and body fluids. Ultimately, we aim to develop MESI marker panels measurable solely in body fluids to stratify ER+BC patients to targeted drug therapies, and continuously monitor drug response without the need for tumor tissue.

Abbreviations

BC	breast cancer	L-Kyn	kynurenine
CDK4	cyclin dependent kinase 4	MAPK	mitogen activated protein kinases (MEK-ERK pathway)
DEC	dissemination and exploitation committee	MBC	metastatic breast cancer
DMP	data management plan	MESI	metabolic signaling
EC	European Commission	MP	management plan
ER	estrogen receptor alpha	mTOR	mammalian target of rapamycin (targeted by Everolimus)
ER+	ER-positive	ODE	ordinary differential equations
ET	endocrine therapy	PDX	patient derived xenograft
GC	gender committee	PI3K	PI3 kinase
GSEA	gene set enrichment analysis	RTK	receptor tyrosine kinase
HER2	human EGF receptor 2	SB	strategic board
IAB	international advisory board	SOC	standard of care
IDO1	Indoleamine-2,3-dioxygenase	SOP	standard operating procedure
IIT	investigator initiated trial	Trp	tryptophan
KA	kynurenic acid	TTO	technology transfer office
KID	German Cancer Information Service (CIS) / Krebsinformationsdienst	WOO	window of opportunity

1. Excellence

Summary. Breast cancer (BC) is a complex disease with high prevalence in the EU. 75% of the tumors are estrogen receptor-positive (ER+), and are treated with endocrine therapies (ET). The MESI-STRAT consortium will develop new models for knowledge-based stratification of patients into subgroups with different ET resistance mechanisms. We will establish predictive pipelines for (1) patient stratification prior and during ET; (2) recurrence risk assessment when ending ET; (3) marker panels to guide established targeted therapies for ET-resistant patients; (4) novel ET resistance mechanism-based therapy design.

The unique collection of matched BC tissue, serum, and >10 years follow-up from the patient organization PATH is essential for the longitudinal analysis of ET resistance and relapse. Our team of oncologists, modelers, bioinformaticians and experimentalists will develop new computational models in combination with network analyses and pharmacogenomics, to integrate multi-omics data and explore **metabolic and signaling (MESI)** networks driving ET resistance. Metabolite marker panels measured in biological fluids will enable patient stratification, resistance monitoring and clinical decision-making. This is a new concept as BC metabolism is poorly explored for diagnostics and therapy. Upon successful validation in preclinical models, the predictive marker panels and related treatments will be jointly investigated by our clinical and industrial partners in clinical studies.

1.1 Objectives

Background. BC claims the lives of 92,000 European women per year¹ (33.4/100,000 inhabitants) and accounts for the highest costs of all cancer-related healthcare (€6.73 billion; 13%)². Each year 332,000 new cases are registered (incidence 62.8/100,000)³ with 75–80% of them being ER+. ET, which block ER-related tumor growth show high efficacy⁴. Yet, even though most early-stage ER+ patients receive ET, approximately 30% will eventually relapse with metastatic BC (MBC). 5–10% of patients present with MBC at diagnosis⁵, of which only one-fifth will survive 5 years³. More than 40% of MBC patients do not respond to first-line ET (intrinsic resistance), and a significant percentage experience relapse, despite initial response (acquired resistance)⁴. ER+BC is unique in that recurrence rates remain almost constant for up to 20 years⁶ and this long follow-up is critical to understand relapse. But most cancer registries do not capture relapses and most trials do not follow the patients long enough, leading to a lack of accurate data on ET resistance and recurrence⁵. Mechanisms contributing to ET resistance include loss or mutations of ER, as well as changes in the expression of ER coregulators⁴. As ER is a signaling molecule, research and clinical trials on ET resistance focus on the crosstalk of ER with other oncogenic signaling networks⁴. Among them, the mammalian target of rapamycin (mTOR) and the mitogen-activated protein kinase (MAPK) pathways are major drivers of BC escape from ET⁴. Both pathways control ER phosphorylation and expression, and can endogenously activate the ER (Fig. 1). The mTOR inhibitor Everolimus (Novartis) has been

approved for targeted combinatorial ET with aromatase inhibitors – yet with limited success as the number of non-responders is high⁷. One reason for this limitation may be that Everolimus enhances MAPK activity⁸. The contribution of MAPK to ET resistance is currently not therapeutically exploited although genetic alterations of HER2 (13%) and PIK3CA (36%) are frequent in BC. Both drive activity of PI3 kinase (PI3K) and AKT, activators of mTOR and MAPK, which are currently explored as clinical targets in BC (Roche, Novartis & others). Cyclin dependent kinase 4 (CDK4) has recently emerged as another promising clinical BC target (Novartis, Pfizer & others). Importantly, CDK4 not only drives cell cycle progression and ER signaling but also mTOR activity, by inhibiting the mTOR suppressor tuberous sclerosis complex (TSC1/2)⁹. As PI3K and CDK4 concomitantly activate mTOR via TSC1/2,

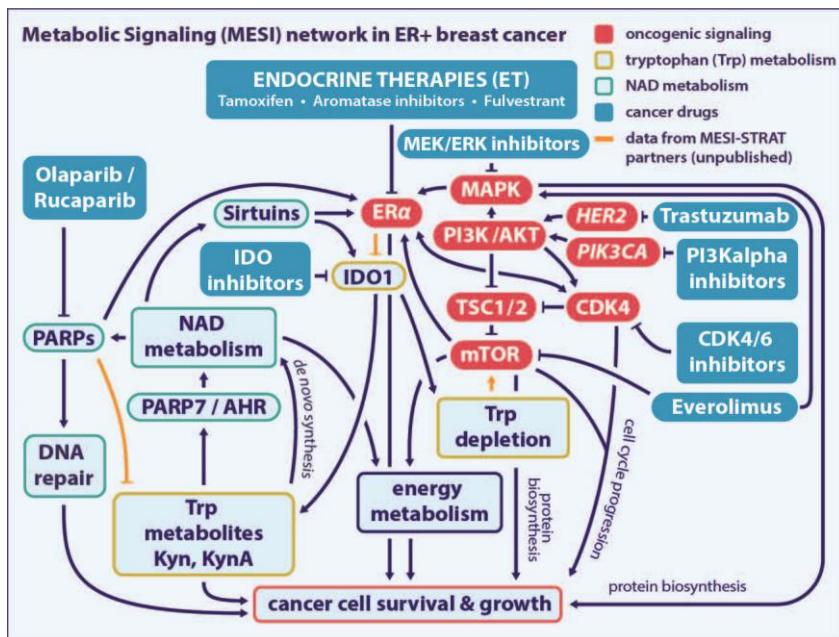


Figure 1. Metabolic Signaling (MESI) network in ER+ breast cancer (BC). The estrogen receptor (ER α) is a central BC driver which is inhibited by endocrine (hormonal) therapies (ET). Central oncogenic signaling (red) and metabolic (light blue) routes are closely intertwined, suggesting that tumor metabolism contributes to endocrine resistance. We propose to exploit this to derive new concepts for patient stratification to endocrine therapies and other targeted cancer drugs (dark blue). See "background" section for details.

CDK4/6 inhibitors sensitize PIK3CA-mutant BC for PI3K inhibitors¹⁰, and raise hope for combinatorial use with ET^{5,11}. Although ER levels and oncogenic mutations are markers in BC therapy, their predictive power regarding targeted therapy response remains limited. In recent years, intense sequencing efforts, such as the METABRIC study¹², provided new insights, but did not improve therapy response.

Therefore, **new concepts for decision-making** are urgently required at several stages during ET.

(i) **At therapy onset:** overtreatment with additional chemotherapy is a concern, as it offers only ca. 4% absolute gain in survival¹³, has short- and long-term side effects and a considerable socioeconomic burden. Multigene prognostic tests¹⁴ can identify good-prognosis patients who might safely omit chemotherapy, but are limited to intrinsic ET resistance and unable to identify alternative targeted therapies. We propose MESI marker panels to stratify patients based on their individual ET-resistance mechanism(s), which would significantly improve this.

(ii) **During endocrine therapy:** it is currently impossible to predict the time-point of acquired resistance and, if it occurs, no markers are available to identify which ET resistance mechanism(s) are active, in order to determine which of the available targeted therapies would be superior to chemotherapy.

(iii) **When ending the therapy:** no method is available to predict which patients will relapse and might profit from prolonged treatment beyond the current standard of 5 years. Tumor material is typically only available at therapy onset and cannot be used to monitor acquired resistance and recurrent disease.

Furthermore, marker panels measured in body fluids would represent primary and metastatic tissues throughout the body. MESI-STRAT will develop such marker panels, measurable in body fluids, to assess the mechanism(s) of ET resistance and guide the stratification of patients with ET resistance for targeted (combinatorial) therapies. The combinatorial inhibition of ER and mTOR and/or MAPK appears promising but the limited success of combinatorial aromatase inhibitor/Everolimus therapies suggests that we are missing important aspects of BC biology. One reason is certainly that mTOR and MAPK are embedded in a highly dynamic kinase signaling network with complex wiring by multiple feedback mechanisms (**Fig. 1**). Moreover, the dynamics of these networks differ in a tumor- and patient-specific manner. Given this enormous complexity, the outcome of drugs targeting the oncogenic signaling network cannot be intuitively predicted. Rather, dynamic modeling techniques are required, to quantitatively capture the context- and patient-specific network tuning and predict therapy outcome. Such predictive models hold great promise for patient stratification at all stages of ET.

Given the tremendous efforts to identify new therapeutic targets and reliable markers for therapy monitoring in BC, it is surprising that tumor metabolism is only rarely considered. PI3K, MAPK and mTOR are all critical regulators of central metabolic pathways. Two key metabolic molecules, tryptophan (Trp) and NAD, have recently emerged as major tumor-promoting players^{15,16}. The enzyme indoleamine-2,3-dioxygenase 1 (IDO1) catalyzes the degradation of Trp to kynureneine (Kyn) (**Fig. 1**), an onco-metabolite which enhances cancer aggressiveness by suppressing anti-tumor immunity and enhancing cancer cell motility¹⁵. The CO laboratory recently showed that the ER status determines IDO1 levels in BC¹⁷, and that ET enhances IDO1 expression in ER+BC cells (unpublished, **Fig. 2 A-C**; similar observations with Fulvestrant, not shown due to space constraints). Hence, IDO1 derepression may contribute to ET resistance. Indeed, we observe elevated Kyn levels in the serum, and increased *IDO1* levels in tumor tissue of ER+BC patients on ET (**Fig. 2D, E, CO**, unpublished), suggesting that Trp metabolites can serve as markers to monitor developing ET resistance. In keeping with this, high *IDO1* levels correlate with worse outcome in ER+BC patients on ET (**Fig. 2F, CO**, unpublished), suggesting that IDO1 contributes to ET resistance. Enhanced IDO1 activity deprives cells of Trp. Trp deprivation in ER+BC cells enhances mTOR activity, as assessed by phosphorylation of the mTOR substrate 4E-BP (**Fig. 2G, KT**, unpublished), and mTOR inhibition increases IDO1 levels (**Fig. 2H, CO** unpublished), suggesting multiple feedback regulations between them. Of note, mTOR is known to activate the ER¹⁸ (**Fig. 1**), indicating that the effects of mTOR inhibitors on IDO1 are mediated in part by the ER. Of note, we also find that ERK kinase is required for IDO1 suppression by the ER agonist estradiol (E2, **Fig. 2I, CO**, unpublished), suggesting that the MAPK pathway mediates ER effects on *IDO1*. For all these reasons, we propose that Trp metabolism is a hitherto neglected mediator of ET resistance in ER+BC. Hence, Trp metabolites could serve as diagnostic markers (explored by the partnering SME NIN), and IDO1 inhibitors are currently being developed and investigated for cancer therapies by the BE laboratory and his pharma partners.

Trp and NAD metabolism are closely linked (**Fig. 1**), as NAD is synthesized *de novo* from Trp, and Trp metabolites activate NAD metabolism via the aryl hydrocarbon receptor (AHR, **Fig. 1**)¹⁵, which is counteracted by poly (ADP ribose) polymerase 7 (PARP7)¹⁹. NAD-dependent enzymes including PARPs and sirtuins (protein deacetylases) affect ER signaling^{20,21} and are promising drug targets for ER+BC therapy. PARP inhibitors, such as Olaparib, are used to induce synthetic lethality in tumors with mutations in the DNA repair protein BRCA1/2 (occurring in 5-10% of all ER+ and ER- BC cases²²). We find that the Trp metabolite kynurenic acid (KA) is reduced in Olaparib-treated BC cells (**Fig. 2J**, unpublished, GW, Harvard), indicating that PARPs influence Trp metabolism, possibly

via the NAD pathway, and that Trp metabolites can serve to monitor therapy response to PARP inhibitors. 36% of BRCA1/2 mutated patients are ER+²³; even though they are rare, studying them will provide general insights into the interplay of NAD and Trp metabolism in ER+BC patients. NAD metabolism is closely linked with glycolysis and mitochondrial activity, which are central to energy metabolism and are closely intertwined with the mTOR pathway²⁴. Indeed, we find alterations in TCA cycle metabolites in serum of Everolimus-treated BC xenograft models (BB and HJ, unpublished), suggesting that energy metabolism is affected as well.

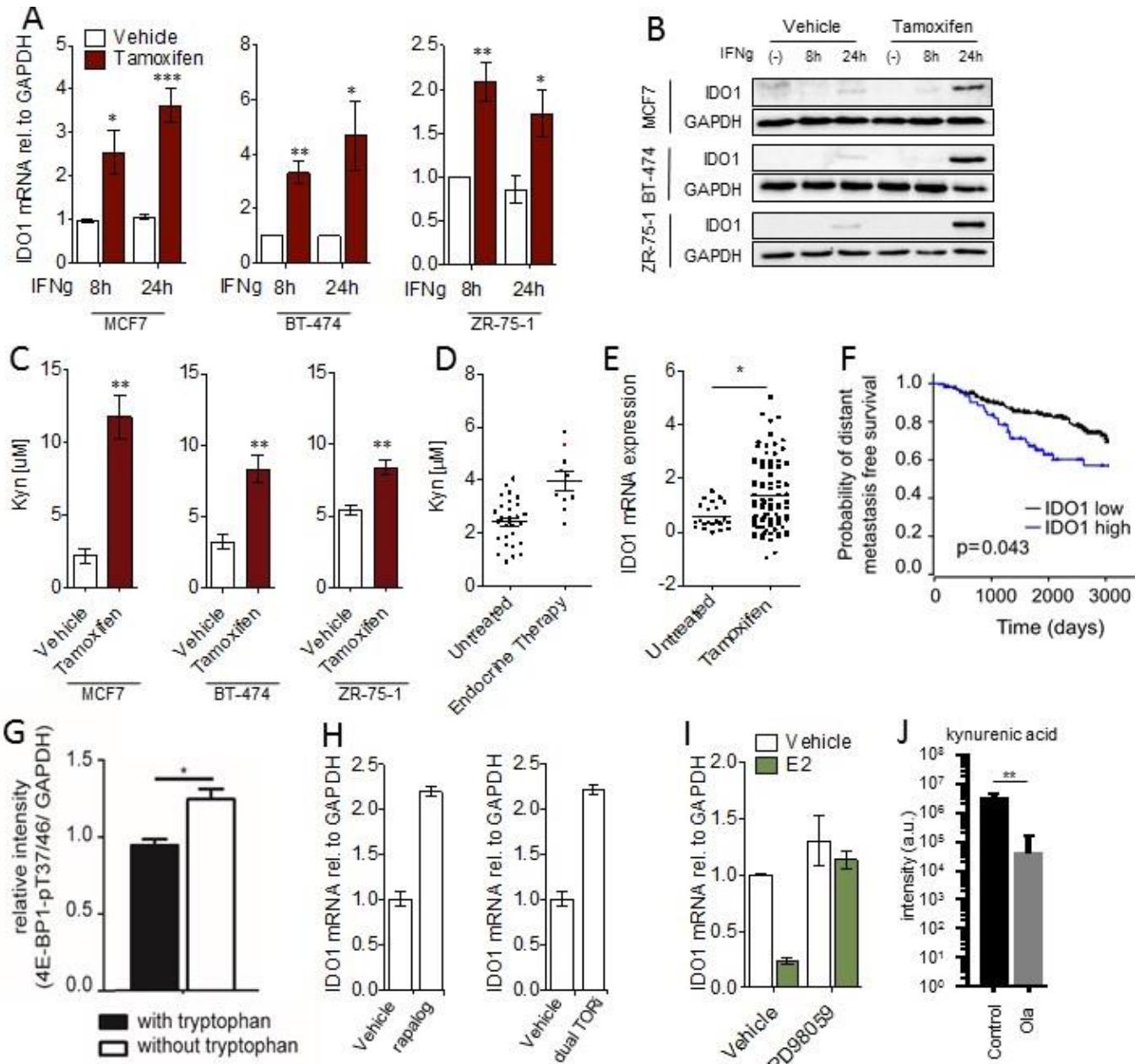


Figure 2. Preliminary data of the MESI-STRAT consortium. (A-F) Endocrine Therapy (ET) enhances Trp degradation by IDO1 in ER+BC cells and patients. (A) 48 h Tamoxifen (100 nM) + IFNγ (1000 U/mL, 8 or 24 h) increases IDO1 mRNA (qRT-PCR, n = 3–4); (B) IDO1 protein (immunoblot, n = 3); (C) and kynurenine (Kyn) release (HPLC, n = 3). Similar results observed with Fulvestrant (not shown). (D) 3 weeks neoadjuvant ET with Tamoxifen (n = 8) or Letrozole (n = 2) increases Kyn (ELISA) in sera of ER+ BC patients (control: 30 untreated ER+ BC patients). (E) IDO1 mRNA (microarray) is increased in Tamoxifen-treated (n = 87) ER+ BC patients with an inflammatory tumor microenvironment (upper quartile of IFNγ expression) (control: untreated ER+BC patients, n = 22, Mann-Whitney U test). (F) High IDO1 mRNA expression (upper quartile of tamoxifen-treated ER+ BC cohort, n = 258) reduces the probability of distant metastasis-free survival (microarray, GEO datasets GSE9195, GSE6532). (G-H) Low Trp enhances mTOR activity, and mTOR inhibitors induce IDO1 in MCF-7 ER+BC cells. (G) 24 h Trp starvation enhances mTOR substrate 4E-BP1-pT37/46 (quantified immunoblot signal, n = 3). (H) 48 h rapamycin (20 nM) or dual mTOR inhibitor Torin1 (250 nM) enhances IDO1 mRNA (qRT-PCR). (I-J) MAPK and PARP inhibitors alter Trp metabolism. (I) 48 h MEK inhibitor PD98059 (20 μM) prevents suppression of IDO1 expression (qRT-PCR) by estradiol (E2, 10 nM). (J) 3 h Olaparib (Ola) reduces kynurenic acid secretion (mass spectrometry analysis). Results are expressed as means, error bars indicate s.e.m. Statistical significance was determined by Student's t-tests; *p < 0.05, **p < 0.01, ***p < 0.001.

Objectives. The main MESI-STRAT aim is to develop new models for the disease-mechanism-informed stratification of ER+BC patients into ET responsive or resistant subgroups, based on their clinical/molecular phenotypes. Using advanced mathematical modeling approaches, we will include not only qualitative but also *quantitative* changes of network parameters to establish predictive pipelines for (i) patient stratification prior to ET; (ii) assessment of resistance development during ET; (iii) recurrence risk assessment when ending ET; and (iv) personalized (combinatorial) therapy approaches for patients with intrinsic or acquired ET resistance.

The clear, realistic and measurable MESI-STRAT objectives are:

- MESI-REPOSITORY - a searchable database of matched fresh frozen tissue, serum, and pseudonymized patient data (treatment and follow-up) of BC patients from clinical cohorts (PATH, UHH, UMCG, VHIO, and collaborators), and patient-derived model systems (matched cultures & patient-derived xenograft (PDX) models, VHIO + EurOPDX). – WP1
- A MESI-SEEK data management platform for integration of experimental, preclinical and clinical data with computational models, network analyses, and pharmacogenomics. – WP2
- Datasets covering ER+ BC signaling and metabolism for model parameterization, including MESI marker panels specific for ER+BC subgroups with different drug response mechanisms. – WP3 and WP4
- Mathematical small-scale dynamic models of integrated metabolic signaling (MESI) networks parameterized for ER+ BC, and modified for individual patients and patient subgroups; network analyses and genome scale models to identify metabolic alterations beyond Trp and NAD in ER+BC subgroups; model-based prediction of targeted (combinatorial) therapies for patient subgroups with different drug response mechanisms that can be stratified by MESI marker panels. – WP5
- Clinical trials without drug treatments to determine MESI marker panels that detect at diagnosis high- versus low-risk patients, during ET stable disease versus relapse, and at diagnosis stable disease versus future relapse; and model predictions on improved targeted drug therapies for all these situations. These trials rely on existing BC patient cohorts at clinical partner centers, and a longitudinal sample collection of blood and urine of patients from the PATH Biobank for up to 10 years after diagnosis. – WP6
- Trials with drug treatments including preclinical trials in subgroup-matched patient-derived cultures, tissues, and PDX models, as well as clinical trials: a Window Of Opportunity (WOO) Trial and an ET Termination Trial in the PATH cohort, to identify and validate MESI marker panels which stratify patients into subgroups with different resistance mechanisms, and predict improved targeted drug therapies. Links with 20 ongoing clinical trials including 3 male cohorts to conduct Intervention Validation Trials to clinically test the predictive value of MESI marker panels for subgroup specific drug responses. Design of investigator initiated (IIT) and umbrella trials to assess individualized (combinatorial) therapies and novel therapeutic targets. – WP7

1.2 Relation to the work programme

Table 1. Relation of MESI-STRAT to the challenge and scope in the SC1-PM-02 work programme.

Note: The relation with the **expected impact** is addressed at the beginning of section 2.1 (Impact).

SC1-PM-02 Challenge & scope	Relation with MESI-STRAT activities and objectives
Patient stratification: more effective therapeutic interventions tailored to (groups of) individuals with common molecular phenotypes	Stratification of ER+ BC patients into subgroups with common, better defined metabolic and signaling (MESI) phenotypes enables selection of ET-resistant patients for targeted therapies, based on their individual ET resistance mechanisms. This will allow the timely identification of ET resistance and stratification of patients to established targeted therapies. MESI-STRAT will also develop novel concepts to target metabolic vulnerabilities in ER+BC.
Novel concepts for disease-mechanism based patient stratification	Metabolic changes are closely linked to known signaling alterations in BC (Fig. 1) but are currently neglected as BC biomarkers. Model-informed approaches for BC patient stratification are not available. MESI-STRAT implements both aspects to address the need for novel concepts for patient stratification to targeted therapeutic interventions in BC.
Integrate multidimensional and longitudinal data	The data integrated by MESI-STRAT spans multiple dimensions including the genome, transcriptome, proteome, metabolome, and clinical cancer phenotypes in model systems and patients. Our longitudinal clinical cohorts allow long term studies that are so crucial to understand ET resistance and relapse in ER+BC. The integration of this multidimensional and longitudinal data is achieved by computational model and network analysis (WP5) and our MESI-SEEK data management platform (WP2, Fig. 4).
Harness the power of omics, including pharmacogenomics, systems biomedicine approaches, network analysis and of computational modelling.	Our comprehensive systems bio-medicine approach entails the following steps: computational dynamic MESI network models will predict MESI marker panels correlating with ET response in distinct ER+BC patient subgroups. For MESI marker panels predictive of ET resistance, targeted therapies (depicted in Fig. 1 & other relevant drugs) will be explored <i>in silico</i> . Network analyses will widen the marker panels, by mapping them onto subgroup-specific genome, transcriptome, and proteome data to identify correlating alterations in ancillary metabolic networks. As a result, we derive comprehensive MESI marker panels (measurable in blood/urine) and matched computational models, which predict ET and targeted drug response in distinct patient subgroups (pharmacogenomics).
Validate in pre-clinical and clinical studies	Predictive MESI marker panels will be measured in samples from the longitudinally collected PATH cohort, and our WOO and ETTermination Trials (WPs 6 + 7) to validate their predictive value for intrinsic and acquired ET resistance, and relapse after ending ET. MESI marker panels and predicted targeted therapies will be preclinically and clinically validated in patient-derived cultures, tissues, and PDX

Table 1 (continued)

	and acquired ET resistance, and relapse after ending ET. MESI marker panels and predicted targeted therapies will be preclinically and clinically validated in patient-derived cultures, tissues, and PDX models, and in Intervention Validation Trials for which MESI-STRAT links with 20 ongoing clinical ER+ BC trials. MESI marker panels validated in these clinical trials and novel therapy concepts successful in preclinical tests will be taken further by initiating IIT and umbrella trials (WP7).
Take into account sex and gender differences	While BC accounts for 25% of all cancers in women, male breast cancer is rare (less than 1% of all BCs). The majority of male BC is ER+ and despite the fact that some differences have been detected between the molecular phenotype of female and male BC, we expect that some of the MESI-STRAT results will also be applicable to male BC. The 20 ER+ BC trials linked with MESI-STRAT include 3 male cohorts which will be a precious resource to test whether the MESI marker panels with strong predictive value in female ER+ BC also apply to male ER+BC.
Actively involve patient associations	The patient organization PATH co-coordinates MESI-STRAT (Leader WPs 1 + 9). PATH harbors a unique BC collection of over 9,200 matched blood/serum, tumor, and adjacent normal tissue samples, and follow-up data. Clinicians and patient experts at PATH support patient recruitment for clinical trials (WP7), communicate the MESI-STRAT mission and results to the BC patients, promote the continuous investigation and implementation of BC patients' interests into the MESI-STRAT strategy and actions, and promote the implementation of successful new stratification concepts (WP9).
Consider regulatory aspects of clinical practice and commercialisation opportunities	Three SMEs will be active at different levels of our value-creation chain (diagnostics, computational models for the pharma sector, data/model management). The partnering clinical centers and PATH have longstanding experience with clinical practice and trials for BC patients. The SMEs and clinical partners will continuously guide the adherence to regulatory aspects for the clinics and commercialization.
Focus on complex diseases having high prevalence and high economic impact	ER+ BC is a highly complex disease consisting of diverse, yet incompletely understood molecular subtypes which define the response to ET. BC has a 5-year prevalence of 1,814,572 in the EU, 75% of which are ER+. BC accounts for the highest healthcare costs (€6.73 billion; 13%) of all cancer-related healthcare in the EU.

1.3 Concept and methodology

(a) Concept

The MESI-STRAT consortium (**Fig. 3**) is organized around the **central idea** that patient-individualized, quantitative model-based simulations of the metabolic and signaling (MESI) networks converging on ER will allow us to predict the individual response to ET and further targeted drugs. This will enable:

- continuous response monitoring by measuring ET resistance-mechanism-specific MESI panels in body fluids;
- stratification for existing therapies, targeting signaling molecules which mediate ET resistance (**Fig. 1**);
- new concepts for combinatorial targeting of metabolic and signaling (MESI) networks.

Our modeling strategy (outlined in **Fig. 4**) is a hypothesis-driven bottom-up approach, which relies on the **basic concept** that differences in the molecular networks, which are directly targeted by cancer drugs, define the drug response. We directly reconstruct the networks that are targeted by ER+BC drugs, including ET, mTOR, CDK4/6, PI3K, and HER2 inhibitors, and include signaling networks often altered in ER+BC such as MAPK. Markers currently considered for drug response prediction are mostly direct components of these signaling networks but they can only be measured in tumor tissue, which is not available for monitoring purposes. Molecules measurable in body fluids, such as metabolites of the Trp and NAD pathways, would be much better suited for patient stratification. They can be expected to represent tumor signaling network tuning because tumor metabolic and signaling networks are multiply intertwined. However, due to the complex cross-regulation within and between them, it is not possible to intuitively deduce signaling network behavior and related drug response from metabolite panels. To deal with this complexity and to account for the highly dynamic and nonlinear response behaviors of signaling and metabolic networks, we will apply dynamic computational modeling in iterative cycles of model calibration and experimental verification. Using ordinary differential equations (ODEs) calibrated with experimental and clinical data, our models will provide a quantitative framework for systematic analysis of network components whose properties (levels, activity) govern MESI marker panels and associated drug responses in different ER+BC subgroups. To complement our hypothesis-driven strategy, we will also take advantage of top-down approaches by applying network analyses of omics wide data and genome-scale modeling to identify and cover additional networks, such as energy metabolism, that potentially harbor additional features that distinguish our MESI subgroups. This combination enables a comprehensive pharmacogenomic approach, which allows us to (*i*) predict drug responses based on expression levels and activities of tumor proteins that form part of the targeted signaling networks, and (*ii*) detect the tuning of the tumor networks and related drug response based on secreted metabolite marker panels measurable in body fluids.

The MESI-STRAT consortium: interdisciplinary and stakeholder focused

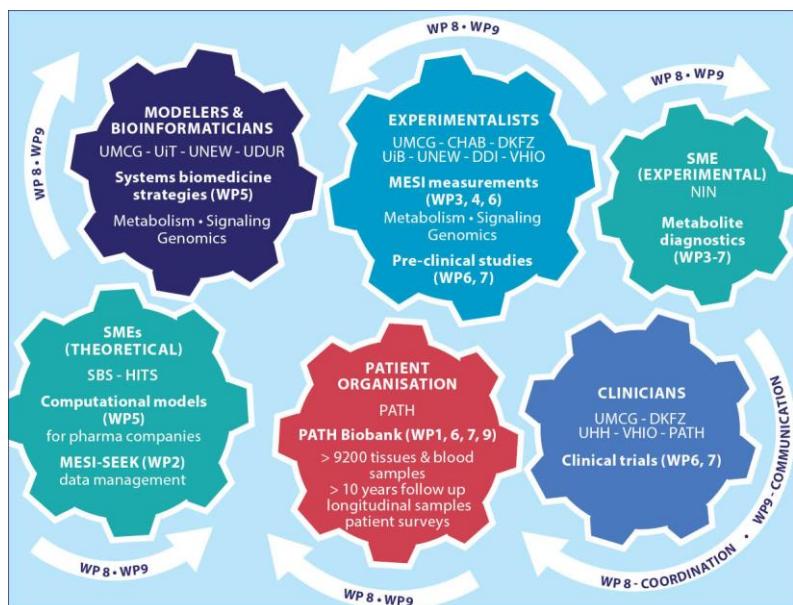


Figure 3: The MESI-STRAT consortium. Roles and expertises of MESI-STRAT partners.

The MESI-STRAT coordination team combines **systems biology (KT)** and **clinical BC expertise (TA)** with the perspective of a **patient organization (PATH)**, which runs the only BC biobank led by an executive board of three BC survivors. Hence, we develop our systems medicine strategy with a special focus on patient and societal involvement. **Clinicians (TA, CSC, HJ, LV, SS, AS, CSA, MP, MO, MB)** from the **PATH Biobank**, **three European clinical BC centers (UHH, UMCG, VHIO)**, and **clinical collaborators (EB, GW)** provide access to patients' data, samples, biobanks, and clinical follow-up; and enable transfer of findings to clinical trials. **Preclinical testing** is done by VHIO (VS) and DDI (BE) in ER+BC PDX, and by DKFZ (CO) and UMCG (SJ) in cultures and tissues. **Expert teams combine modeling and experimental expertise** in signaling (KT, CS, NC, DPS, BA/SBS) and metabolism (CO, MZ, EV, IH, BB). The **MESI-SEEK platform** (WM, HITS) will enable **omics wide data integration** by network analysis (RF, UMCG & SG, UDUR) and small- and genome-scale modeling (DPS, BMB, IH, BA). **Three SMEs** play pivotal roles in MESI-STRAT and ascertain timely implementation of commercialization opportunities: HITS (WM) implements the MESI-SEEK platform; SBS (BA) guides the development of MESI-STRAT models to ensure high commercial value, and commercializes the end product; NIN (KK) develops and commercializes diagnostic assays for metabolite markers; **Novartis** (PI3K, CDK4/6 inhibitors, Everolimus) collaborates and acts on the MESI-STRAT advisory board (see letters); **Pfizer** and **Celgene** are linked with MESI-STRAT through AS (UHH, PRAEGNANT), and **Merrimack Pharmaceuticals** is on our IAB. Partners at DDI (BE) and UNEW (NC) work closely with **pharma partners** Clovis, Astra Zeneca, Pfizer, Abbvie, and Tesaro to explore the therapeutic potential of targeting Trp metabolism and PARPs in translational and preclinical studies and by developing novel compounds. They will mediate collaborations in terms of access to samples and data from trials and for joint trial design.

Positioning of the project (innovation stage)

MESI-STRAT is situated early in the spectrum of “idea to application”. Data, samples, and cell models (WP1) are available to start WPs 1-7. Thus, analysis and construction of computational MESI-STRAT models are already within reach. The output of our objectives would categorize WPs 2-5 within **TRL 2** (basic technology research), and WPs 6-7 within **TRL3-4** (research to prove feasibility). Commercial systems biology and diagnostics services by the partnering SMEs SBS, HITS, and NIN are on the market, which will enable MESI-STRAT to recognize and implement the commercialization potential of newly developed models and metabolite marker panels for patient stratification. Section 2.2.1.2 (commercial exploitation) details the specific measures with all three SME partners that will lead to commercialization of MESI-STRAT results, including a **pipeline for computational MESI model development and customization** for the pharma market.

Our experience with patent protection of relevant computational models, target molecules, and diagnostics provides substantial background IP and will ensure timely patenting of MESI-STRAT results, thus improving the European innovation potential for systems medicine. MESI-STRAT consortium partners have patented (1) dynamic mTOR models and applications thereof for patient stratification in clinical studies and personalized medicine (KT+DPS, WO2012163440); (2) novel clinical targets modulating mTOR in cancer (KT, WO2014108532); (3) diagnostic Kyn assays (SME NIN, EP11797208.3 and 10 2015 104 088.3) – a multiplex method to measure Trp and its metabolites by chemical labeling with isobaric mass tags and LC-MS/MS (CO, PCT/EP2016/076265) – and PARP-based cyto- & histochemical detection kits (MZ, US 20100196909, A1/EP2214020). In addition, our experience in conducting clinical trials, our close ties with pharma companies (see 1.3.a, consortium description), and the support by our local tech transfer offices (TTOs, see letters) will enable rapid translation of our results into clinical practice.

Linked national or international research and innovation activities

Leading European systems medicine centers in Germany (**DKFZ & CHAB**), UK (**CISBAN, UNEW**), and the Netherlands (**SBC-EMA, UMCG**) partner MESI-STRAT. DKFZ and **EMBL-EBI** are members of Infrastructure for Systems Biology Europe (**ISBE**), providing access to collaborations and resources for infrastructure, data management and modeling such as **Elixir Human Data management, Biomodels, ENA, and IntAct**. BC PDX models will be shared via the **EurOPDX** consortium, with SJ as board member and VS as member. Our data will be integrated with existing large-scale omics data, e.g., from **TCGA** invasive BC, **METABRIC**, and **DKTK** (German Cancer Consortium). Coordinators of two German systems medicine consortia (CHAB, MAPTorNET; DKFZ, GlioPATH) and an ERA-NET TRANSCAN-2 consortium (DKFZ; PROMETOV) partner MESI-STRAT. These three projects build resources connecting oncogenic signaling to metabolism in different cancers and will be highly complementary with the MESI-STRAT focus on BC: **PROMETOV** analyses ovarian cancer heterogeneity with a focus on Trp metabolism; **MAPTorNET** connects mTOR and MAPK signaling networks in neuro-endocrine tumors (NET); **GlioPATH** connects NAD, Trp, and mTOR models to derive novel treatment paradigms in glioma. The coordinators of the **EPIPREDICT** consortium are on the IAB and provide links to European systems research into epigenetic changes in ER+BC. Also, the **European Working group of Breast Cancer Research** and the EU-funded **BASIS** project (breast cancer somatic genetic study), that is part of the **ICGC (International Cancer Genome Consortium)** and linked to **METABRIC**, are represented by our IAB (see 3.2.1).

(b) Methodology

The MESI-STRAT patient cohorts, and patient-derived model systems

With PATH as co-coordinator, the MESI-STRAT consortium (**Fig. 3**) has the outstanding opportunity to exploit a unique collection of fresh frozen tumor tissues, matching normal adjacent tissues and sera, as well as longitudinal therapeutic and follow-up datasets from **>9,200 BC patients** (out of which **>7,400 ER+**), processed and stored according to strict SOPs. For metabolite and phosphoprotein measurements, immediate freezing and storage at ultralow temperatures are essential, making this extremely high-quality repository central to our project. The PATH cohort is complemented by the WOO and ET Termination Trials, and linkage of MESI-STRAT with 20 ongoing clinical ER+BC trials by partners and collaborators investigating ET and targeted drugs including PI3K, mTOR, CDK4/6, HER2, and PARP inhibitors. The trials are detailed below (p.12, ff), in WPs 1,6-7, clinical annex.

Patient samples will be matched with a wide range of **experimental BC model systems** (cultures, PDX, tissues). VHIO (VS) has 9 ER+BC PDX models with matched cultures (detailed in WP1) and currently develops further models. The 9 models are available at project onset to immediately start WPs 3-4. 35 additional ER+BC models can be accessed via the EurOPDX consortium (partnered by VHIO and UMCG). If specific subtypes, identified by MESI-STRAT, are not represented by the available PDX models, they can be generated by VS (VHIO) from selected patients. BE (DDI) has developed mice with a humanized immune system into which selected PDX representative of different MESI subtypes will be transferred. This will enable preclinical testing of anti-tumor immune responses. The PDX models will be complemented by tissue bioreactor experiments (DKFZ, CO; UMCG, SJ/KT; and the collaborating SME CELLEC BIOTEK, Basel, CH) in which drug interventions can be tested for up to three weeks in freshly cultured ER+BC patient tissues, enabling analysis of the drug effects not only on the tumor cells but also on tumor-infiltrating immune and stromal cells.

Approach and project phasing

In a stepwise, iterative approach (**Fig. 4**), we will match biochemical, phenotypic, and omics analyses of BC model systems and patient samples with existing large-scale sequencing data (TCGA, METABRIC) to establish a set of predictive MESI markers for disease monitoring and patient stratification for targeted therapies (pharmacogenomics). Our staggered phased plan ensures that the MESI-STRAT goals are reached within 51 months.

Phase I – Data and samples from clinical cohorts, patient-derived cell and PDX models will be compiled and longitudinal samples will be collected (WP1). The WOO and ET Termination Trials (WP7) will be initiated. MESI-SEEK data and model management platform will be set up (WP2). Small-scale metabolic and signal transduction models (WP5) will be integrated, parameterized and validated stepwise, based on experimental time course measurements and cancer-relevant phenotypic data (proliferation, migration, survival) (WPs 3,4) in ER+BC cell lines. **Deliverables:** data, samples, cell/PDX models distributed; MESI-SEEK platform; theoretical models addressing cross talks and parameterized for cell lines.

Phase II – Model setup and validation for patient-derived cultures & supernatants, and PDX models & sera/urine (WPs 3-7). Iterative cycles of experiment-model refinement using metabolic and signaling data. A critical step will be validating that models parameterized on protein and metabolite levels – as measurable in patient samples – accurately simulate individual dynamics of signaling and metabolism in cultures and PDX (WP5, task 5.2). Our

preliminary (KT) and published²⁵ data indicate feasibility. If not, patient-specific models will be parameterized based on dynamic time course data from patient-derived cultures and PDX. To define the minimal datasets necessary for model parameterization, sensitivity analyses in our MESI models will determine critical proteins and metabolites whose changes lead to different drug response and MESI marker panels. These components will be measured from patient tissues & biological fluids, and used for individual model parameterization, subsequent identification of patient subgroups with similar drug response mechanisms, and related marker panels differing between the subgroups. RNA-seq, panel-seq & discovery proteomics (expression) of patient samples representative of MESI subgroups with different ET response will be performed (WP3). **Deliverables: pharmacogenomics I:** identify subgroups with different MESI-signatures (component levels) in patient cohorts & correlate with ET response (ER and oncogene activity, onco-metabolites, clinical outcome); predict intervention strategies involving signaling and metabolism for non-responders.

Phase III – Preclinical testing and validation of marker panels and related therapies in subgroup-matched patient-derived cultures/PDX and samples (WPs 6, 7). PDX models representative for subgroup-specific MESI signatures will be established in immune-competent animals to test anti-tumor immune responses. If necessary, new representative PDX models and matched cell cultures will be obtained from EuroPDX or established from selected patients (WP1). Predictive MESI signatures will be mapped onto RNA-seq and proteome expression and panel mutation data from different MESI subgroups; these analyses will be extended to RNA-seq data in TCGA and from the METABRIC¹² study. Network analyses entailing Gene set enrichment (GSEA) and Bayesian network analyses will overlay mutations, expressed transcript isoform balance and protein sets in the multilevel omics data, and identify major altered metabolic pathways and their network structure. For up to three of them, ET-resistant-subgroup specific genome-scale and/or small-scale dynamic models at isoform resolution will be setup to predict specific metabolic alterations leading to characteristic secreted metabolite signatures, to enrich our subgroup-specific MESI marker panels (WP5). **Deliverables: pharmacogenomics II** – preclinical testing and validation of subgroup-specific drug responses and related secreted metabolite marker panels.

Phase IV – Validation of predictive MESI signatures in patient body fluids and tissues: in the longitudinal PATH cohort (WP6), WOO, ET Termination, and Intervention Validation Trials (WP7). Design and initiation of IIT and umbrella trials (WP7). The clinical trial strategy is detailed in **Table 2**, in WPs 6-7, and the clinical annex. **Deliverables:** validation in clinical trials; patent applications for predictive biomarkers and models.

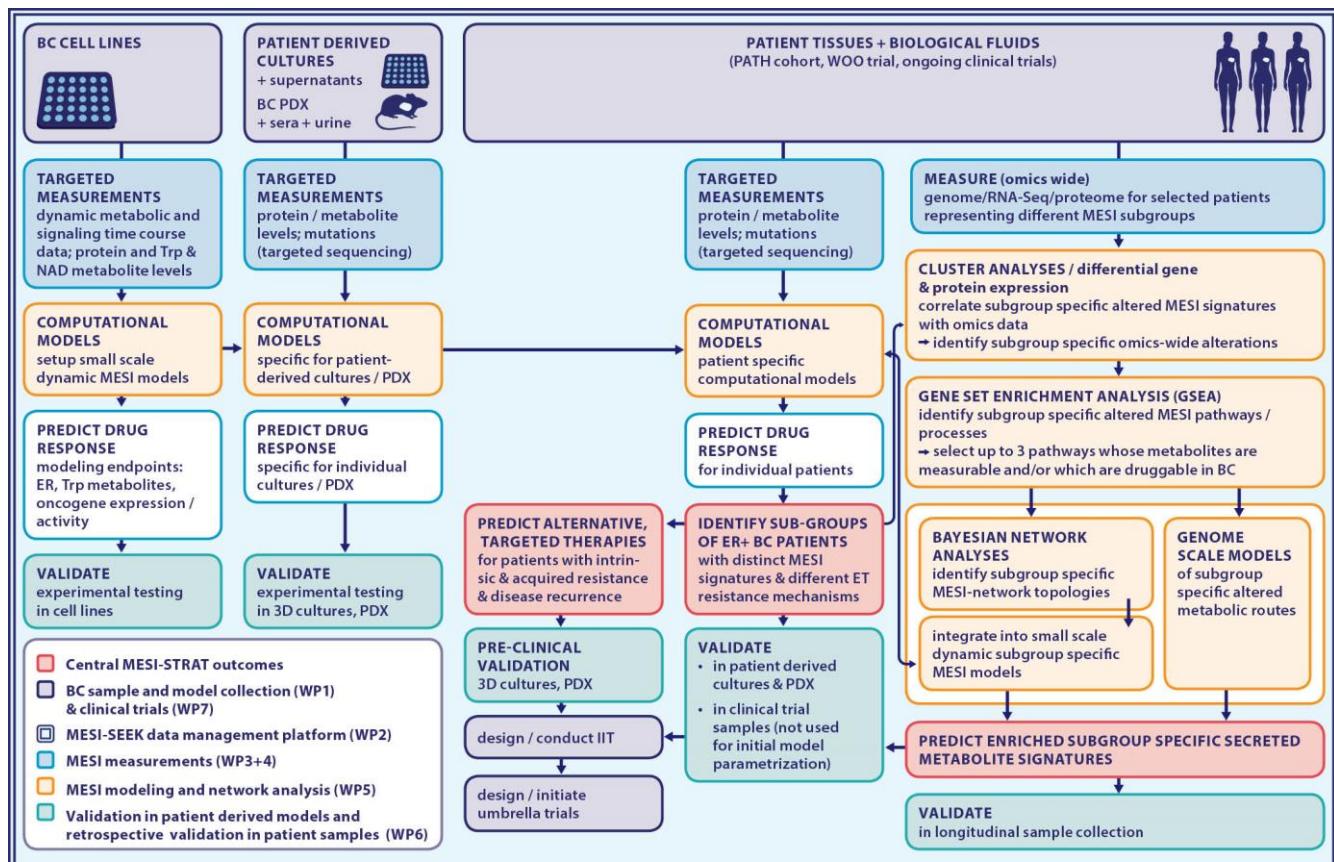


Figure 4 - The MESI-STRAT strategy.

Work package (WP) Overview

The clinical WPs (1, 6, 7) will be led by clinicians; the systems WPs (2-5) will be led by modelers, experimentalists and data management experts; and the coordination and impact WPs (8, 9) are led by the coordinator UMCG, the co-coordinating patient organization PATH, and the SME SBS. A short overview is given here, and the detailed descriptions are provided in section 3 (implementation).

WP1 Data survey of existing patient samples and materials (Leader PATH, TA)

MESI-REGISTRY: pseudonymized clinical and follow-up data, protocols, patient materials (PATH and other sources); patient-derived models; continuous integration of clinical data on newly collected samples (WPs 6, 7).

WP2 Setup and maintenance of MESI-SEEK data and model management platform (Leader HITS, WM)

MESI-SEEK: computational models; signaling, metabolic, omics, and phenotypic data generated by WPs 3&4, data from external sources (TCGA, METABRIC, others), scientifically relevant pseudonymized clinical data.

WP3 Assess SIGNALING NETWORKS for model parameterization & validation (Leader CHAB, CS)

Measurements of ER, mTOR²⁶ and MAPK²⁷ network components in cultures, PDX, and patient samples (tissues, body fluids): Biplex assays, targeted proteomics, reporter assays, and proximity ligation assays. Phenotypic readouts include cell cycle distribution, apoptosis sensitivity, clonogenicity, and proliferation.

- Monitor signaling dynamics and conduct intervention screens by combining metabolite & growth factor stimuli (e.g., Trp, IGF, EGF) with drugs targeting signaling and metabolism (Fig. 1; drug panels and signaling molecules to be measured detailed in WP3).
- Quantitative measurement (targeted proteomics²⁸) of signaling proteins that determine subgroup-specific drug responses in patient samples, predicted by WP5
- Quantitative omics analyses: discovery proteomics (expression), deep RNA-seq + panel-seq to complete existing omics data for selected cell & PDX models and patients, representative of predicted MESI subgroups (WP5).
- Analysis of patient-derived ER+BC models and samples, treated with model-predicted drug combinations.

WP4 Assess METABOLIC NETWORKS for model parameterization and validation (Leader UiB, MZ)

Analyze Trp, NAD, and energy metabolites, pathway component expression and function. Samples and perturbations of metabolic networks as in WP3, to assure identical conditions required for modeling.

- Quantitation of enzyme concentrations & activities by targeted proteomics²⁸ & semi-automated activity assays.
- Quantitative measurements of intracellular and extracellular metabolites (ESI-MS/MS, ELISA).
- Metabolic flux analyses: metabolite consumption, production; stable isotopes incorporation²⁹.

WP5 Integrative MESI network modeling and network analyses (Leader UNEW, DPS)

- Build integrated MESI models by connecting existing and newly developed ODE-based dynamic network models of Trp³⁰ and NAD metabolism with mTOR³¹ and MAPK²⁷ models. Integrate other metabolic pathways (e.g., energy metabolism³²) depending on network analysis (see below).
- Parameterize integrated models using detailed molecular & biochemical time course and cellular & high-throughput data (WPs 3, 4).
- Incorporate biological and clinical data (patient samples, outcome) into computational models using the MESI-SEEK platform (WP2), employing model reduction techniques for sparse data as necessary.
- Global and local sensitivity analyses and systematic combinatorial perturbations with inhibitors to dissect network dynamics, characteristic metabolite profiles, and minimal parameterization data sets (WPs 3,4,6,7) for patient subgroup specific models.
- Guide validation of predictive MESI markers in cells, PDX and patient samples (WPs 6,7) and selection of models and patient samples representative for distinct MESI subgroups for in depth omics analyses (WP3).
- Network analyses to predict and guide validation of extended metabolite panels characteristic of different MESI subgroups measurable in body fluids (WPs 4,6,7).
- Predict targeted (combinatorial) therapies for ET resistant subgroups and guide validation in preclinical and clinical studies, and IIT and umbrella trial design (WP7).

WP6 Preclinical and clinical trials without drug treatments in ER+BC patient-derived models and the longitudinal PATH cohort (Leader DKFZ, CO)

- Longitudinal PATH collection of biological fluids for the MESI-STRAT Risk Detection, Relapse Detection, and Relapse Prediction Trials (detailed in Table 2, WP6, and clinical annex).

- Validation of computationally predicted MESI-signatures (a) in preclinical models (cultures and PDX) with known therapy outcome; (b) in patient samples and correlation with risk and relapse; identify patient subgroups with different ET resistance mechanisms, different drug responses, and distinctive MESI marker panels.

WP7 Preclinical and clinical trials with drug treatments: preclinical drug tests, WOO, ET Termination and Intervention Validation Trials (Leader UHH, SS)

- Preclinical testing of predicted therapies (WP5) in cultures and PDX models; analysis of immune effects of promising therapies in immunocompetent PDX models (established by BE) and tissue bioreactors.
- WOO, ET Termination, and Intervention Validation Trials to identify and validate MESI marker panels predictive of intrinsic ET resistance and response to targeted therapies; details in **Table 2**, WP7, and clinical annex.
- To comprehensively test the predictive power of the identified MESI marker panels for patient stratification for targeted therapies, and for fundamentally new treatment avenues, IIT and umbrella trials will be planned with academic and industrial partners (Novartis and others, see consortium description 1.3.a and letter).

WP8 Project coordination: management and communication (Leader UMCG, KT)

The MESI-STRAT strategic board, supported by the project manager, ensures day-to-day management.

- Coordination of the workflow and exchange of materials within the MESI-STRAT consortium.
- Coordination of knowledge exchange and in depth training among the partners in regular consortium meetings and visits, channeling feedback and ideas.
- Administrative and financial coordination, communication with and reporting to the EC.
- Intellectual property (IP) management plan.
- An international advisory board of patient organizations and external experts (oncology, signaling, metabolism systems medicine, see 3.2.1) will attend the meetings, receive progress reports, and advise MESI-STRAT on the project research, strategic orientations, and project progression.

WP9 Dissemination, exploitation, and communication (Leader PATH, TA; Co-Leader SBS, BA)

- Dissemination & exploitation plan: measures and pipelines to implement MESI-STRAT scientific and clinical results into scientific, clinical, regulatory, and commercial practice and to enhance patient literacy.
- Communication plan: communicates the MESI-STRAT results to and receives input from a broad group of stakeholders including scientists, healthcare professionals & managers and commercial stakeholders plus societal stakeholders such as patients and their families, policy makers, EU citizens.

Sex/gender-related aspects

The CCI of **gender** is consequently implemented at the **consortium level** as 50% of the PIs are female, (p. 1). The annual death rate for BC is 33.4 and 0.5 per 100,000 inhabitants for women and men, respectively. Hence BC in men is rare. ET are used for over 70% of all female BC. Since the majority of male BC is ER+, we expect that some of the MESI-STRAT results will also be applicable to male BC. Thus, developing improved stratification and targeted therapy concepts for ER+BC addresses the largest proportion of BC patients in the EU, and is potentially relevant for both female and male patients.

A **gender committee (GC)** will focus on the consequent consideration and implementation of gender issues at all project levels. This concerns in particular the translation of results from female ER+BC to male ER+ BC. CSC (UMCG) and SS (UHH) both have a strong focus on male BC and access to large male BC sample collections (see clinical annex, Study 7). MESI-STRAT has access to these male BC cohorts, which encompass collections of FFPE tissues, fresh frozen tissues, and body fluids. The male BC sample collections are very precious as male BC is rare. Hence, the GC will evaluate and decide on the validation of strongly predictive MESI marker panels from female ER+BC subgroups in the male cohorts. Furthermore, the GC will constantly evaluate MESI-STRAT actions and results regarding the proper consideration of gender issues.

Clinical trial strategy

Our multilevel clinical trial strategy is designed to account for the biological and clinical traits of ER+BC, which presents several challenges including the long time to relapse, the low percentage of relapsing patients, and the risk for relapse remaining constant over two decades. Consequently, trials with endpoints such as progression-free survival are long-term efforts that cannot be realized within the typical budget and duration of an H2020 project. MESI-STRAT overcomes these limitations by taking advantage of existing cohorts and trials, by conducting own trials where needed, and by collaborating with pharma and academic partners for IIT and umbrella trials, designed based on the MESI-STRAT outcomes. Table 2 provides an overview of all preclinical and clinical MESI-STRAT

studies. Studies No 1–6 are essential, unique and innovative approaches to develop new predictive MESI models and marker panels for stratification of ER+BC into subgroups and to inform clinical decision-making. Study 7 and the prospective design of IIT and umbrella trials (7.5) accelerate the translation of our biomedical and clinical results to medical use by pioneering the application of new MESI models and marker panels for ER+BC therapy monitoring and design.

Table 2. MESI-STRAT clinical and preclinical trials. Trials without any drug treatments are grouped into WP6, and trials with drug treatments into WP7. *The clinical study number corresponds to the numbers in the Clinical Annex, where all study details are provided, incl. a list of all trials in Study No 7. Trial numbers correspond to the task numbers in WPs 6 and 7.

Study category	Study type	Clinical Study No & Trial #*	Short name	Study title	Study design
Trials without drug treatments: WP6	clinical	Study No 1 WP6: Trial 6.1	Risk Detection Trial	Identification and validation of MESI marker panels discriminating high-risk vs. low-risk ER+BC patient subgroups	Samples and clinical data from PATH biobank
		Study No 2 WP6: Trial 6.2	Relapse Detection Trial	Identification and validation of MESI marker panels detecting ER+BC patient subgroups with relapse	Samples and clinical data from PATH biobank and clinical trials associated with MESI-STRAT
		Study No 3 WP6: Trial 6.3	Relapse Prediction Trial	Retrospective identification and validation of MESI marker panels at diagnosis predicting patient subgroups with future relapse or stable disease	Samples and clinical data from PATH biobank
		Study No 4 WP6: Trial 6.4	Tissues for Preclinical Trials	Screening and collection of ER+BC tissues with subgroup-specific MESI marker panels for preclinical models (PDX, bioreactor)	Samples obtained in the frame of ongoing clinical studies at VHIO and UHH
	preclinical	WP6: Task 6.5	Preclinical Model-Subgroup Assignment	Identification of primary cell and PDX models representing novel ER+BC subgroups defined by differential MESI marker expression	Samples and data from preclinical trials at VHIO, UMCG, and DKFZ
		WP7: Task 7.1	Preclinical Intervention Trials	Validation of predictive MESI marker panels in primary cell cultures, cultured primary tissues, and preclinical interventional trials in PDX models	Samples and data from preclinical trials at VHIO, UMCG, and DKFZ
Trials with drug treatments: WP7	clinical	Study No 5 WP7: Trial 7.2	WOO Trial	Prospective window of opportunity trial: two weeks neoadjuvant Anastrozole in postmenopausal women with ER+BC	Interventional clinical trial performed at UHH
		Study No 6 WP7: Trial 7.3	ET Termination Trial	Analysis of longitudinally collected serum and urine from ER+BC patients before and after termination of ET	Prospective clinical trial by PATH, collecting samples/ clinical data from patients on and off ET
		Study No 7 WP7: Trial 7.4	Intervention Validation Trials	Clinically validate predictive MESI models and marker panels for targeted drug interventions	20 clinical trials by partners and collaborators; incl. 3 male cohorts
		WP7: Task 7.5	IIT/Umbrella Trial Design	Design own IIT and umbrella trials, in which ER+BC patients will be stratified to different therapies by MESI-marker panels	For future impact, UHH, UMCG, VHIO will design and initiate IIT & umbrella trials

The longitudinal PATH cohort, initiated and driven by BC patients, is unique because of the timeline (>10 years follow-up) and case numbers (over 9,200 cases), which is not offered by any other existing clinical BC cohort or registry, and which would be impossible to realize within the time and budget of the present H2020 project. Hence, PATH offers us the unprecedented opportunity to design a comprehensive study throughout ET in which we will

- detect at diagnosis: high- versus low-risk patients (**Fig. 5A, Risk Detection Trial**, Study No1);
- detect at relapse: stable disease versus relapse (distant metastasis); this cohort will be enriched with samples from other cohorts, such as the Everolimus/Exemestane (collaborator: EB), IMPACT (UMCG) or the PRAEGNANT cohorts (UHH) (**Fig. 5B, Relapse Detection Trial**, Study No 2);
- predict at diagnosis: patients with future relapse or stable disease under ET (**Fig. 5C, Relapse Prediction Trial**, Study No 3);
- predict at the end of ET: relapse and prevention strategies by targeted drugs (**Fig. 6, ET Termination Trial**, Study No 6).

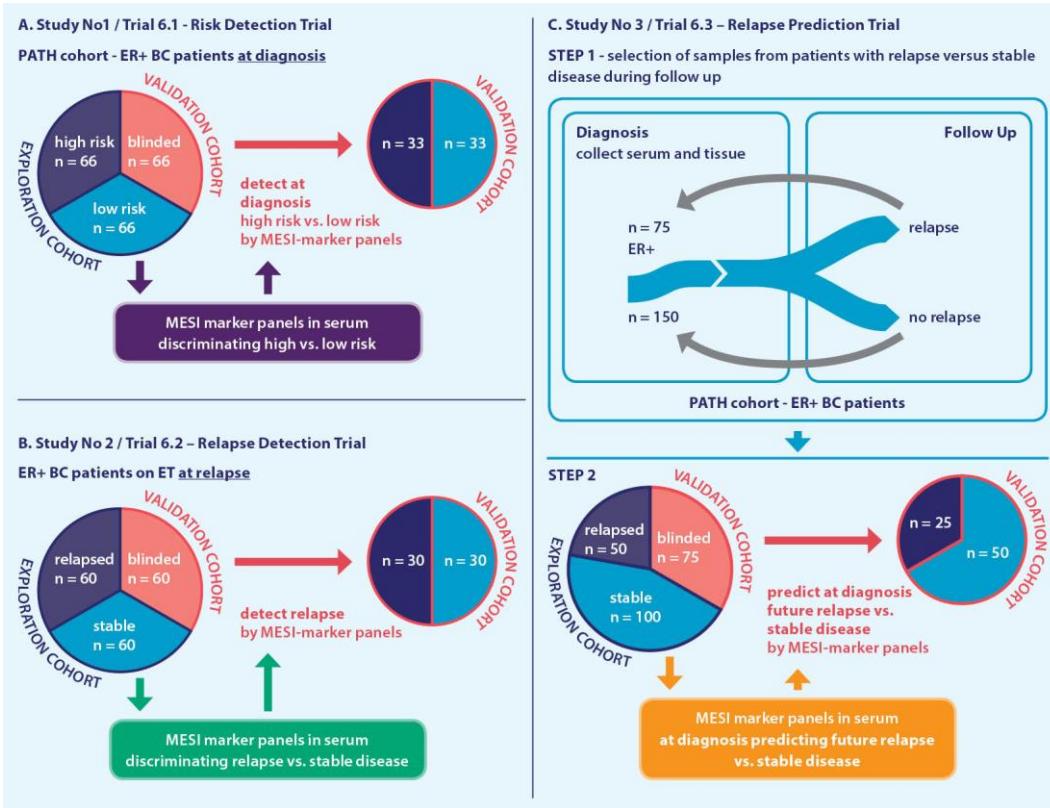


Figure 5. Clinical Studies 1-3 are enabled by the longitudinal PATH cohort.
(A) Risk Detection Trial.
(B) Relapse Detection Trial. **(C)** Relapse Prediction Trial

The WOO Trial (study No 5, Trial 7.2 **Fig. 6**) complements the ET Termination Trial, as we compare longitudinal samples from the same patients at diagnosis before and on ET. This is novel as typically only tissues from patients without ET are used to guide initial therapy decisions, and matched patient samples without and with ET are not available. This is a serious limitation for ER+BC research, as ET itself triggers processes that alter therapy responses. Due to the lack of matched samples without and with ET, ET-inherent effects on MESI networks and the implications for the response to combined targeted therapies are currently neglected. For example, immunomodulatory therapies such as IDO inhibitors are so far not clinically tested in ER+BC as basal immune infiltration is low. However, several studies³³⁻³⁵ and our own data (**Fig. 2**) suggest that ET itself triggers tumor immune infiltration and changes in immunosuppressive Trp metabolites, and that this is associated with ET resistance. Therefore, we need to study the MESI networks and their drug response in preclinical models and in patients under ET. Our WOO trial follows a timely strategy, which will allow us to study ET-induced MESI panels. The combination of our WOO trial with the PATH cohort trials (Studies No 1-3 and 5-6) will be essential to reach our central aim of developing computational models and define MESI marker panels that can stratify ER+BC patients into subgroups with different ET resistance mechanisms, and guide decisions for combinatorial treatment schemes of ET with targeted drugs.

Figure 6. Clinical trials to predict ET response and relapse

(i) **when ending ET** (ET Termination Trial, Study 6) and (ii) **when starting ET** (WOO, windows of opportunity) Trial, Study No 5).

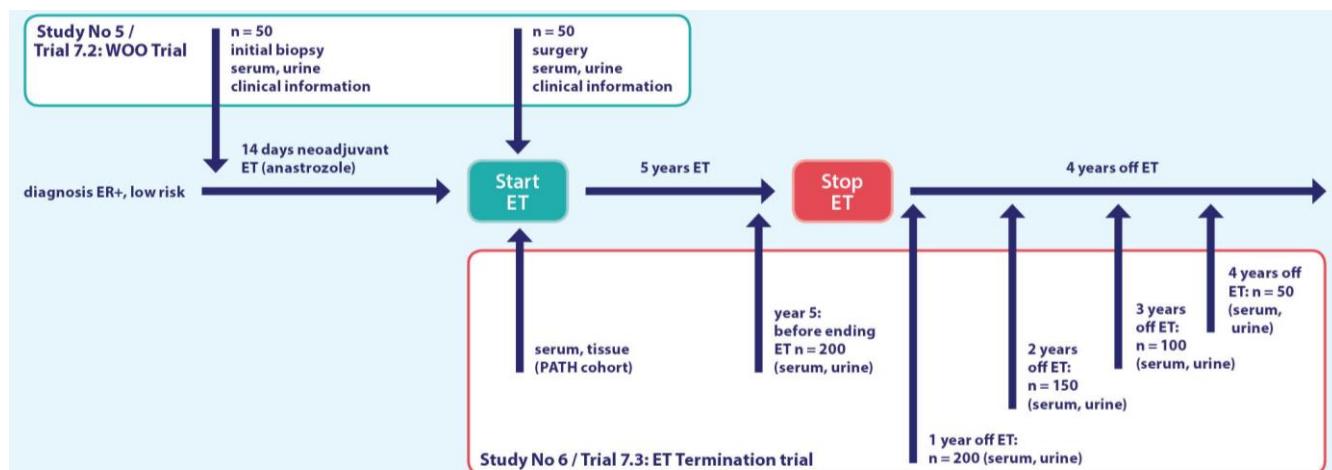
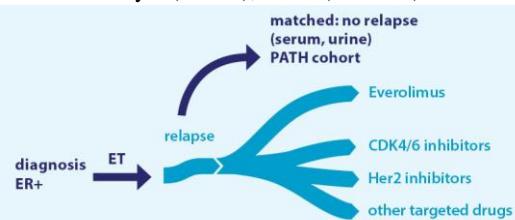


Figure 7. Intervention Validation Trials

Clinical Study 7 (Annex), WP 7 (Trial 7.4).



marker panels also apply to male ER+BC. **This will directly address the criterion of gender-sex analysis.**

To pioneer the application of new MESI marker panels for ER+BC patient stratification, MESI-STRAT will conduct **preclinical tests** in patient-derived cultures, tissues, and PDX models (WP6, 6.4 and 6.5; WP7, 7.1). Furthermore, we will **design own interventional ITT and umbrella trials** (Fig. 8; WP7, 7.5) with academic (UHH, UMCG, VHO and further European centers) and pharma partners (e.g., Novartis, Celgene, Pfizer, and others; see 1.3.a). Due to their long duration exceeding the typical timeline of EU projects, and the necessary funding volume exceeding the MESI-STRAT budget, these trials cannot be conducted as a part of MESI-STRAT, but they will be a direct result from our project. The necessary links with pharma companies who can partner and fund these trials are in place, and initial trial planning has been setup with Novartis (see attached letter) to apply for dry substance and funding following an established pipeline. Furthermore, we will apply for public funding with National and European funding bodies.

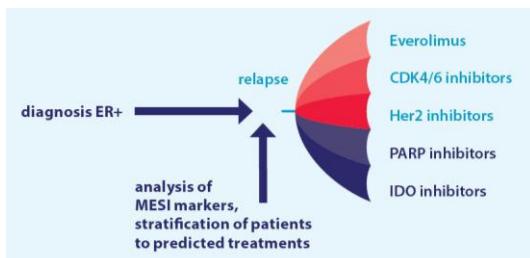


Figure 8. Umbrella trial design. See WP7 for details.

1.4 Ambition

- **Advance beyond the state of the art.** MESI-STRAT is poised for groundbreaking discoveries of subgroup- and patient-specific vulnerabilities. Our findings will identify patients at risk for relapse, and enable evidence-based therapy. Avoiding ineffective treatments by informed ET resistance mechanism-based clinical decisions on targeted therapies will improve overall survival and quality of life of ER+BC patients. Drugs targeting PI3K, mTOR, and MAPK networks are in clinical use for many cancers. And, alterations in MESI networks, including Trp, NAD, and energy metabolism, are major hallmarks in nearly all tumors. Thus, the metabolic and signaling alterations predisposing ER+BC to individual therapy responses may also contribute to specific therapy responses in other cancers, enabling translation of predictive MESI marker panels to other cancer types.

- **Innovation potential.** The PATH cohort is a unique chance to correlate ER+BC therapy responses and relapse in individual patients with input-output maps of detailed dynamic and genome-scale MESI models. This will allow us to identify patient subgroups with distinct MESI marker panels and ET response. MESI-STRAT will develop MESI marker panels measurable in body fluids and matched MESI models that predict the risk of progression, AND stratify patients for targeted therapies.

No prognostic test predicting response to targeted therapies is available for BC. Current multigene prognostic tests (e.g., Mammaprint, OncoDX) predict the risk of ET resistance only at ET onset, as they require tumor tissue. Patients classified by these tests as medium/high risk undergo chemotherapy with considerable costs for therapy and treatment of short- and long-term adverse effects. In contrast, subgroup-specific MESI marker panels do not only predict the risk of ET resistance, but they allow ET resistance mechanism-based stratification of patients at risk for targeted therapies with fewer adverse effects. Furthermore, no tumor tissue is required for MESI marker panels, which are therefore detectable at any stage of the disease and throughout ET.

Current efforts to identify circulating markers focus almost exclusively on circulating tumor DNA (e.g., by the supporting pharma company Novartis) which have been correlated with the risk of disease progression. However, they are not used in conjunction with computational models predictive of disease and resistance mechanisms. Therefore, our MESI marker panels will reach beyond these efforts: in combination with our MESI models, the MESI marker panels will allow to determine individual ET resistance mechanisms, and to simulate and predict responses to multiple alternative drug interventions.

New marker panels and related MESI models will be patented, building the basis of effective development of MESI-STRAT outcomes for the market. At project start patent landscaping and freedom to operate analyses will be provided by the partner TTOs at UMCG and DKFZ. According to our own patent search there are currently

no competing patents in our field (MESI models for patient stratification). Relevant patents for diagnostics, targets and computational models for patient stratification are held, and models (services, SysBioSim) and diagnostics (products, NIN) are marketed by MESI-STRAT partners (see “Positioning of the project”).

2. Impact

2.1 Expected impacts

2.1.1 MESI-STRAT impact

a) Expected impacts set out in the work programme

Table 3. Expected MESI-STRAT impacts in relation to the work programme.

Expected impact	Relation with MESI-STRAT activities and objectives
1. New models for patient stratification to inform clinical decision making	MESI-STRAT will deliver new computational models that enable individualized assessment of body fluids and/or tumor tissue, to stratify patients for ET and/or targeted treatments based on the individual ET resistance mechanism(s). Therapy response throughout ET and the risk of recurrence when ending ET can be assessed. This enables model-based clinical decisions during ET and timely switching to established or new targeted therapies. The concept of considering circulating metabolites for computational-model-informed therapy decisions is innovative, as current BC marker research focuses mainly on tumor and circulating DNA. Hence, MESI-STRAT will reach beyond other efforts in academia (e.g., TCGA, METABRIC) and pharma (e.g., Novartis) and will deliver new concepts and models for patient stratification.
2. Accelerate the translation of biomedical and clinical research results to medical use	Preclinical and clinical trials run by MESI-STRAT partners and collaborators, and IIT and umbrella trials designed by MESI-STRAT will validate (1) MESI marker panels for patient stratification, and (2) individualized, new therapy concepts for ET resistant patients. These trials will place the MESI-STRAT results at the threshold of clinical and commercial implementation by our SME partners and the pharma industry (see 4 below).
3. Increased cost-effectiveness of the novel concepts in comparison to already established practices	MESI model-based stratification concepts will increase cost-effectiveness in three principal ways: (1) The lack of markers for stratification of ER+BC patients to targeted therapies results in high rates of non-responders. Ineffective therapies cause high costs (i) as the medications themselves are expensive, (ii) due to the management of potential adverse events, and (iii) due to increased risk of progression to severe metastatic disease with high health care and socioeconomic costs (e.g., hospitalization, inability to work, etc.). MESI-model-informed stratification to treatments efficacious for specific subgroups, will reduce these costs by avoiding ineffective treatments. (2) The use of metabolite marker panels, measurable in body fluids which are readily accessible without surgical intervention, will significantly reduce diagnostic costs. (3) In conjunction with informative MESI markers, our disease-mechanism-based models will allow response prediction for compounds targeting the MESI networks. Hence, our models can be used for clinical trial design, to stratify cohorts, based on MESI marker panels, into predicted responders and non-responders. Inclusion of only predicted responders into the trials increases the expected response rate. This enhances cost-effectiveness by reducing the numbers of participants and the related costs for the trials.
4. Increased research and innovation opportunities in this innovative industries-driven field, particularly small or medium-sized enterprises (SMEs)	Our 3 SME partners will closely co-develop and conduct the MESI-STRAT research, and directly exploit our results in the sectors of diagnostics (NIN), computational modeling services for efficient and fast drug development catering to the pharma sector (SBS), and systems medicine data and model management (HITS). To maximise innovation opportunities, we implement dedicated pipelines with our partnering SMEs to develop MESI-STRAT results toward industrial application (section 2.2.1.2). Several SMEs and pharma companies collaborate with MESI-STRAT for diagnostics and compound development, services to pharma industry, and clinical trials (see consortium description in 1.3.a, and letters of support). The partnering and collaborating companies are main commercial MESI-STRAT stakeholders and will guide our efforts in annual meetings and workshops toward the development of stratification and therapy concepts with high clinical & commercial potential.

b) Improving innovation capacity

In a pan-European effort, MESI-STRAT will - for the first time - computationally link kinase signaling and Trp/NAD metabolic networks in ER+BC (WPs 3-7). MESI-STRAT will explore the clinical feasibility of systems-driven biomarker and therapy design by validation in preclinical PDX and tissue models, in longitudinal patient cohorts (WP6), in preclinical and clinical interventional trials (WP7), and by designing and initiating IIT and umbrella trials for prospective validation (WP7). Specifically, MESI-STRAT will enhance:

- ***Scientific innovation capacity***

of its academic and clinical partners and of the systems medicine field in general by developing a new bottom-up systems biomedicine concept for marker panel development: new MESI models for ER+BC patient stratification will inform clinical decision making for ET and targeted therapies.

- We will develop and connect dynamic computational models of metabolism and signalling in ER+BC across longitudinal time scales, and use network analyses to integrate multidimensional omics data types (genome, transcriptome, signalling/ proteome, metabolome). This is innovative as signalling and metabolism have hitherto only been analysed separately by systems approaches.
- We will enable stratification of ER+BC patient subgroups based on their metabolic makeup and related marker panels in biological fluids, which is innovative, as so far BC metabolism has been poorly explored and metabolite marker panels have not been considered for BC patient stratification.
- Because our strategy is broadly applicable to any cell type or organism, MESI-STRAT will pioneer a modeling-enhanced integrated understanding of how changes in signalling networks influence metabolic phenotypes, and vice versa.

→ Scientific innovation will be measureable by invited conference presentations, the number of scientific papers, their impact in terms of citations, and the standing of the journals in which they are published.

- ***Clinical innovation capacity***

of our clinical partners and clinical research by accelerating the translation of our biomedical and clinical research results to medical use.

- We will implement clinical trials at all levels; observational and longitudinal cohorts and trials, an interventional WOO trial, participation in numerous trials with targeted drug interventions, and the design of IIT and umbrella trials to prospectively test the predictive power of our MESI marker panels for patient stratification for targeted therapies. MESI-STRAT also has access to male BC cohorts for testing predictive marker panels, addressing the cross-cutting priority of gender. Clinical testing under strict implementation of regulatory aspects (see 2.1.2) ensures the quality and medical usability of the MESI marker panels and aids swift implementation in the clinic.
- We will develop new, modeling-enhanced concepts for clinical trial design and management. MESI models can be used to stratify patients for novel drugs that target the networks covered by the MESI models, in addition to stratification for existing therapies. For instance, IDO inhibitors are currently in clinical trials, and PARP inhibitors may have uses beyond BRCA mutated patients as they affect NAD metabolism, which is closely intertwined with Trp, ER, mTOR and MAPK networks. Our MESI models can be used to individually stratify patients by their MESI markers. This increases the response rate and improves the statistics, thereby decreasing the necessary cohort size for clinical trials, accelerating patient inclusion into clinical trials and decreasing trial costs, as compared to current practise.
- We introduce a novel concept for disease-mechanism-based tumor characterization by MESI model-derived metabolite marker panels in body fluids, which complements classical histology and tumor marker based phenotyping in BC. This will allow for the first time to predict tumor progression, monitor therapy response and relapse, and enable optimal therapy design without the need for tumor tissue; therefore, ER+BC patients can be closely monitored over years in a non-invasive and cost efficient manner, thus improving their outcome by earlier detection of progression to metastatic disease, and marker-guided therapy decisions for targeted drugs and ET prolongation. This is innovative, as currently no markers exist for this purpose in ER+BC.
- Initially developed here for ER+ BC, the MESI-STRAT bottom-up approach is broadly applicable to most cancers and thus has the potential to not only innovate BC therapy but also cancer therapy as such.

→ Clinical innovation will be measureable by the predictive strength of the MESI marker panels, as assessed in our clinical trials, and by the number of trials, which will be designed for prospective marker testing.

- ***Commercial innovation capacity***

Increased research and research and innovation opportunities in the innovative industries-driven field of systems medicine will be created particularly for our partner SMEs by

- implementing an innovation pipeline with SBS (detailed in 2.2.1.2) to educate young scientists for MESI

model development at our academic sites, who will subsequently customize our models at SBS for the pharma industry (e.g., Novartis). The innovation potential is dual: SBS wins highly educated employees with the tailor-made profile fitting SBS' requirements; and there is a clear development strategy for the MESI-STRAT models from earliest setup to customization and use by the pharma industry. All entities involved in this value creation chain are partners or collaborators of MESI-STRAT.

- deriving metabolite marker panels to guide targeted therapies in ER+BC,
 - that can be the basis for the development of new diagnostic devices by the MESI-STRAT partner NIN and collaborator QuantumDX (see letter);
 - that can be marketed together with drugs interfering in signaling networks, developed by our collaborating pharma companies (1.3.a, consortium description)
 - improving and developing services provided by SMEs to systems medicine, creating a business case in particular for the data/model management platforms developed by HITS.
 - deriving a novel bottom-up strategy for mathematical model based analyses for faster and efficient drug development in preclinical research, and for predictive marker-panel development and improved clinical trial and therapy design for the pharma industry
 - as developed and marketed by SBS,
 - and as a basis for own industry engagement (see below, exploitation).
 - quick and cost-efficient monitoring of emerging ET resistance and relapse in patients using MESI marker panels in biological fluids. Currently no marker panels are available for this purpose in ER+ BC.
- Commercial innovation will be measureable by the number of students educated and hired by SBS, patents applied for and granted for MESI models, marker panels and diagnostic tests licensed to partnering or external SMEs and companies, and the outcome of the feasibility studies for a possible own industry engagement.

c) Integration of new knowledge: giving new life to old drugs

Our bottom-up approach can (i) identify predictive MESI network marker panels to select patients at ET onset for additional drugs or combinations thereof, (ii) detect marker panels that correlate with progression under ET, and (iii) predict drug combinations to target the associated ET resistance mechanisms. A broad range of drugs interfere with the mTOR and MAPK signalling and metabolic networks depicted in **Figure 1**. Among these are inhibitors of PI3K, Akt, CDK4/6, MEK-ERK, and mTOR (everolimus and other rapalogs), and metabolic modulators, such as IDO and PARP inhibitors, and metformin, which interfere with Trp degradation, NAD and energy metabolism, respectively. Several of these drugs have been in clinical use, with known safety and toxicological profiles. Thus, the model-enhanced identification of MESI-STRAT marker panels for repurposing these drugs for specific ET-resistant BC patient subgroups and new indications can considerably speed up clinical trials and use in patients. Drug repurposing can also prolong the drug lifecycle for the companies holding the patents.

d) Other environmental and socially important impacts

Improvement of **patient welfare and literacy** is a major goal of MESI-STRAT. The BC patient organisation PATH has directly contributed to our research direction since the earliest project planning and the BC patient expert and communication coach Doris Schmitt and the European BC patient organisation Europa Donna are on the MESI-STRAT advisory board. PATH also collaborates with the MESI-STRAT partner German Cancer Information Service (SW, KID/DKFZ) and its European partners to act as multipliers communicating MESI-STRAT findings to BC patients and their families, and implementing patients' feedback into the MESI-STRAT programme. They will also raise public awareness for the issues and opportunities that systems medicine offers for BC therapy.

2.1.2 Barriers and obstacles

A general obstacle is imposed by variation in the ethical regulations for patient data and sample handling in the countries covered by our European consortium. For example, the required level of anonymization and accessibility of data can differ. Also the lack of clear comparative guidelines (i.e. lack of knowledge about such differences) can slow down patient data and sample collection, and usage by MESI-STRAT. Our consortium will address this challenge by strictly implementing the national regulations that apply in each partner country (see Section B5 and annex). However, we deem it an important EU regulatory task to harmonize ethical and data security regulations to enhance opportunities for pan-European data exchange for systems medicine and clinical study efforts.

Another issue is the accessibility of patient tissues and biological fluids, which is often in the hands of

single pathologists or clinicians. The lack of clear legislation to make such materials accessible to other researchers, and to document sample quality for follow up studies, often limits the possibilities for validation of findings in larger sample panels. MESI-STRAT overcomes this issue by conducting own clinical trials and by associating with trials conducted by the partnering BC biobank PATH, partnering and collaborating clinical scientists and pharma companies. Strict SOPs, as developed by PATH, UHH/NCT (see letter), and UMCG, will be applied for sample collection and biobanking. Again, harmonization of clinical sample access and standards of handling by the EU would greatly enhance the opportunities for pan-European systems medicine. The European Biobanking and BioMolecular resources Research Infrastructure Consortium (BBMRI-ERIC, <http://www.bbmri-eric.eu/>) is a step towards this by providing tools to share aggregate information on European biobanks. PATH, UHH and UMCG all contribute to BBMRI (as detailed in WP1), thus ensuring that the biological samples collected in the frame of MESI-STRAT will remain available to the scientific community.

Pan-European trials are possible but require extensive administrative efforts to account for different regulations in different partner countries. Therefore, MESI-STRAT will be supported by the partnering clinical trials competence center KKS Heidelberg, which is part of UHH. As a small full-service contract research organisation (CRO), KKS provides support for our interventional clinical trials including protocol development, monitoring, data management, and reporting. Thus, KKS will manage the MESI-STRAT WOO trial, advise on IIT and umbrella trial design, and ensure their conduct in accordance with European regulations. KKS will also advise on regulatory issues regarding usability of our longitudinal sample collection (PATH) and other collected samples across the pan-European MESI-STRAT consortium. We will also use the EC initiative on breast cancer (ECIBC) Guidelines Platform, another resource targeted at harmonizing the care for BC patients across Europe. Thus, MESI-STRAT activities and trials will conform with high quality, evidence-based guidelines at the European level.

2.2 Measures to maximise impact

The **MESI-STRAT dissemination and exploitation plan** (2.2.a/2.2.1) maximises impact through dissemination to a broad range of professional users in science and healthcare management (2.2.1.1), and through exploitation in research, healthcare, and industry (2.2.1.2). The **MESI-STRAT communication plan** (2.2.b/2.2.4) complements this by focusing on scientific, medical, and commercial stakeholders as well as societal actors and non-specialist stakeholders. Our **data management plan (DMP)** (2.2.2) and **intellectual property rights (IPR) management plan** (2.3) are key to maximise dissemination while protecting patients' rights, observing regulatory aspects, and preserving exploitation opportunities by timely protection of IPR. Furthermore, **internal communication** is seminal for effective innovation management, and is implemented by the MESI-STRAT management plan (WP8, and Section 3.2).

Table 4. Measures to maximise impact of MESI-STRAT

Measure to maximise impact	Aims	Target audience
1) Dissemination and non-commercial exploitation (2.2.1)	- Maximise use of MESI-STRAT results by scientific and medical stakeholders, and health care management (e.g., in hospitals, insurances)	Scientific experts and professionals from basic and medical sciences and health care management, companies in diagnostics, systems medicine and pharma, patients and families
2) Commercial exploitation (2.2.1)	- Maximise use of MESI-STRAT results by commercial stakeholders - Conduct feasibility studies for own commercial engagements	Companies in the fields of pharma development, diagnostics, and services for systems medicine
3) Management of data and knowledge (2.2.2) & intellectual property (2.2.3)	- Promote effective innovation management - Respond to external or internal opportunities	MESI-STRAT partners and collaborators: - Internal communication - Regulations: grant and consortium agreements
4) Communication (2.2.4)	- Communicate MESI-STRAT results to scientific, medical, and commercial stakeholders, societal actors and non-specialists - Implement the goals named under responsible research and innovation (RRI) and public engagement, incl. the gender dimension in the MESI-STRAT research team, decision making, and R&I content	- All previously mentioned stakeholders - Healthcare providers and healthcare professionals - Patients and families - Policymakers - EU citizens

a) Dissemination and exploitation of results: MESI-STRAT Draft Dissemination and Exploitation Plan

2.2.1 Dissemination and exploitation, and data to monitor impact

Central to the MESI-STRAT dissemination policy and actions is the dissemination and exploitation plan for the MESI-STRAT results outlined below and in WP9. This plan is jointly led by the partnering SME SBS and the patient organization PATH, and regularly reviewed by the strategic board (SB) to reflect results obtained during the project. It contains the following elements: (i) key goals; (ii) target audiences; (ii) mediums and means for dissemination and exploitation; (iv) dissemination frequency/timing; (v) impact monitoring. The **key goal** of MESI-STRAT's dissemination and exploitation activities is to achieve a significant and durable impact on EU citizens' health by the implementation of MESI model based stratification.

2.2.1.1 Plan for dissemination and non-commercial exploitation of MESI-STRAT results

The basis for all dissemination and exploitation of the MESI-STRAT results is the systematic validation of the predictive power of our MESI models and marker panels in clinical studies in ER+BC patients, laid out in section 1.3.b (Methodology / Clinical Trial strategy) and implemented through WPs 6 and 7.

Dissemination of MESI-STRAT results will further

- **basic and medical research**

- MESI model construction as a new bottom-up approach for marker panel development (WP5)
- Novel concepts and methods to connect metabolic and signalling computer network models across different time scales and omics scales (genome, transcriptome, signalling/ proteome, metabolome) (WP5)
- Novel methods to parameterize multiscale MESI network models based on multiscale data (genome, transcriptome, proteome, metabolites) (WPs 3-5)
- MESI marker panels to stratify patient cohorts, and correlate the subgroups with multilevel omics scale data to identify ET-resistance mechanism-based MESI marker panels in biological fluids (WPs5-7) that
 - predict tumour progression
 - enable optimal therapy design
 - allow monitoring of therapy response and relapse.

- **clinical research and cost effectiveness for health care management**

- A novel, pharmacogenomic approach for ET resistance-mechanism-based tumor characterization by MESI model-derived metabolite marker panels, which complements classical histology-based phenotyping
- New, modeling-enhanced concepts for clinical trial design and management
- Modeling-derived and bottom-up approach-driven MESI-STRAT marker panels that can guide therapy design, ET response and relapse monitoring. Measuring the MESI marker panels in biological fluids allows quick and cost-efficient monitoring of arising ET resistance and relapse in patients where no tumor material is available (i.e., at any time following initial surgery). Currently no marker panels are available for this purpose in ER+BC.

The main target groups

of the MESI-STRAT dissemination strategy will be medical and clinical researchers and health professionals including policy makers for health care management, e.g. at hospitals and insurances.

The activities, detailed in Table 5, will generally include the following:

- i. Open Access publishing and Open Research Data Pilot (see 2.2.2 Data management plan and 2.2.3 Management of knowledge and IP, and WP2)
- ii. close interaction with other consortia active in the field of systems medicine of BC and inclusion of consortia representatives in the MESI-STRAT international advisory board (IAB)
- iii. presentations of interim and final results at national and international conferences;
- iv. active involvement of policy makers at insurances and hospitals who decide on health care management to raise awareness of the increased cost effectiveness offered by MESI models (see Table 3).
- v. information about the project and its aims to scientific stakeholders via a continuously updated MESI-STRAT website and press releases
- vi. a concluding symposium at the end of the project, with presentation of the results and hands-on workshops aimed at the scientific community active in systems medicine and BC research.

Major instruments in MESI-STRAT's dissemination policy will be to

- **publish advances beyond the state-of-the-art** in open access peer-reviewed scientific journals, and to share them at high impact scientific meetings. Several MESI-STRAT partners, including AS & SS (UHH), DS (PATH), CSC

(UMCG), NC (UNEW) regularly attend major national and international BC conferences (detailed in table 5), both as invited speakers and attendees. We anticipate that disseminating our research in high-impact papers will seed and strengthen research lines in systems oncology focusing on the interactions between signalling and metabolism to target cancer by individualized therapies. MESI-STRAT strives to implement open access and open data wherever possible. However, we need to consider the boundaries set by the requirement to protect patients' data, and to enable exploitation by allowing IPR protection prior to dissemination of our results. Hence, a trade-off needs to be made between the requirement of open data and the protection of patient privacy and IPR. A dissemination and exploitation committee (DEC, WP9) – led by the SME SBS and the patient organisation and co-coordinator PATH – will oversee this process and will take decisions on timelines of keeping data non-public.

- **closely collaborate with other BC systems consortia** to optimally exploit synergies and enhance the national and European investments in BC systems medicine. Representatives of BC systems medicine consortia are on our IAB to maximise the synergies among our projects: the European Working group of Breast Cancer Research, the EU funded BASIS project (breast cancer somatic genetic study), the ICGC (International Cancer Genome Consortium), the Oslo2 study (> 2500 samples incl. molecular data), METABRIC, and the ITN EpiPredict (epigenetic regulation of ET resistance in breast cancer and systems medicine approaches to predict ET outcome). Based on the knowledge of these other consortia, our IAB will foster synergies with MESI-STRAT by identifying and initiating opportunities for exchange and collaboration.

Table 5. MESI-STRAT draft plan: scientific dissemination and non-commercial exploitation (/a = per year)

Key goals	Target audience	Medium and means *one-way **two-way interaction	Timing of implementation	Impact monitoring = reaction of target audience
• Promote new models for patient stratification to inform clinical decision making. • Set new method standards to connect metabolic and signaling models for systems oncology (methodological approaches, data/modelling standards)	systems biologists & systems medicine scientists	**present new models at systems biology conferences, e.g., ICSB, SBHD, and others	throughout and after project	- conference invitations ($\geq 2/a$) - size of audience (≥ 50)
		* open access publications in high-impact systems biology journals, e.g. Nat. Commun., MSB, Science Signal., PLoS Comput. Biology, EMBO J., etc.	start in year 2, throughout and after project	- number of papers ($\geq 3/a$) - number of citations, impact of citing journals
		**final symposium at the end of MESI-STRAT project including hands-on workshops	end of project	number of attendees (≥ 200)
• Accelerate the translation of MESI-STRAT biomedical and clinical research results to medical use, in particular new MESI marker panels and models for clinical decision making	BC researchers (basic and clinical) BC healthcare professionals BC patients	**present ER+ BC diagnostics/therapy results at cancer conferences: • Annual European Breast Cancer Conference (EBCC) • Annual meeting of the German Society for Senology (DGS). AS, UHH is vice president 2017 (senologiekongress.de). • SABCS, ABC (Advanced Breast Cancer) and annual meetings of AGO (AG Gyn. & Oncology), GBG (German Breast Group), St. Gallen Consensus Conference, etc.	throughout and after project	- conference invitations ($\geq 5/a$) - size of audience (≥ 100)
		* open access publications in high-impact and highly visible medical journals, on clinical studies and new routes to BC therapy, e.g., NEJM, Clin Breast Cancer, Eur J Cancer, etc.	start in year 2, continued after project (partners, collaborators)	- number of papers ($\geq 3/a$) - number of citations, impact of citing journals
		**identify and use synergies with other consortia and initiatives on BC systems medicine, e.g., European Working group of BC Research, BASIS (breast cancer somatic genetic study), the ICGC	since early project preparation (project representatives invited for MESI-STRAT IAB)	- joint publications with other BC related consortia and initiatives (at least 3 during the project)

Table 5 (continued)

		(International Cancer Genome Consortium), the Oslo2 biobank, METABRIC, ITN EpiPredict, etc.	continued throughout and after project	- number of citations, impact of citing journals
• Promote the emerging field of systems oncology	cancer researchers/ oncologists (basic and clinical)	**present new strategies in oncology research at cancer conferences , e.g. ASCO, ESMO-ECCO, AACR & Cell Symposia, DKK (German Cancer Congress) open data access publishing in cancer journals: 1) new strategies in systems oncology, e.g., in Nature, Science, Nat. Comm. 2) new molecular mechanisms in cancer, e.g. in Cell, Cancer Cell, Cancer Discovery	throughout and after project	- conference invitations ($\geq 2/a$) - size of audience (≥ 50)
• Promote the implementation of novel MESI-STRAT concepts (models and marker panels) by the health care sector to increase cost effectiveness in comparison to already established practices	health care managers and policy makers (insurances, hospitals, politicians)	** invite health care managers from the partnering hospitals to the annual MESI-STRAT meetings. Increase network inviting further hospital representatives in subsequent years ** invite health insurance representative and politicians active in health politics to annual meetings and final conferences	start in year 2, continued after project (partners, collaborators) annually, start in year 2 year 4+5	- number of paper ($\geq 3/year$) - number of citations, impact of citing journals - number of participants from health care policy at conferences ($\geq 2/a$; at least 5 at concluding conference)

2.2.1.2 Plan for commercial exploitation: Increased research and innovation opportunities for SMEs

The MESI-STRAT activities for the commercial exploitation plan are detailed in table 6, and ensure effective commercial exploitation of MESI-STRAT results. Having adequate IP protection is imperative both for licensing and internal development and therefore the consortium will seek patent protection of new results and findings (foreground IP). This will be supported by the TTOs within the MESI-STRAT consortium (e.g., UMCG, DKFZ), who have extensive experience in licensing procedures. The IPR management plan is outlined in 2.2.3.

MESI-STRAT results suitable for commercial uptake will be

- new computational models for marker development, clinical trial, and therapy design for the pharma industry, as developed and marketed by SBS, and/or as a basis for a possible own industry engagement.
- New marker panels for targeted therapy of ER+BC, which can be
 - marketed together with drugs interfering in signalling and metabolic networks – as done by pharma companies with whom the MESI-STRAT partners collaborate for clinical BC trials and/or which are on the MESI-STRAT IAB (see 1.3 (a), The MESI-STRAT consortium)
 - the basis for the development of new diagnostic devices as developed by the MESI-STRAT partner NIN and collaborator QuantumDX (see enclosed letters)
- services provided by companies to systems medicine, such as data/model management platforms by HITS.

The main target groups of the MESI-STRAT exploitation strategy are

its partnering SMEs NIN, SBS and HITS, several collaborating SMEs including QuantuMDX and CELLEC BIOTEK, and its contacts with the pharmaceutical industry (see 1.3.a).

Major activities in MESI-STRAT's exploitation policy will be implemented with our partnering SMEs:

(i) Pipeline for customization of computational models for use by pharma industry, and assessment of their marketing potential by SysBioSim B.V. (SBS)

Jointly with the partnering SME SBS, MESI-STRAT will setup a pipeline, which allows us to systematically assess and customize the computational models developed within MESI-STRAT for the pharma industry. This pipeline also entails the transfer of expert knowledge and personnel from the academic partners to the partner SMEs. Currently there is a limited workforce in the field of systems biology/systems pharmacology, which is a serious bottleneck for SBS, and the field. The major commercial interest of the MESI-STRAT academic partners is to license their computational models to the industry. This will be greatly enabled by the experience of SBS in customizing and marketing computational models for customers in the pharma sector. Due to confidentiality agreements, SBS

cannot detail its pharma customers in this proposal, but will be able to act as a mediator linking the MESI-STRAT academic partners with pharma industry.

Our pipeline will consist of the following steps:

1. ***Training of master students in computational biology*** in the frame of model development within MESI-STRAT at the academic partner sites UMCG, CHAB, UNEW, UiT. The training will be led by UMCG, and students will be sent for internships to the partner institutions, which conduct modelling work in the frame of MESI-STRAT (CHAB, UNEW, UiT).
2. ***MESI-STRAT model customization by trained modelers at SBS***. The modelers who earned their master of science in computational biology under the supervision of MESI-STRAT modelling PIs (UMCG, UNEW, CHAB, UiT) and have been trained in the development and use of MESI-STRAT models will join SBS. Under the supervision of a senior modeler at SBS (funded by MESI-STRAT), the newly trained modelers will customize the MESI-STRAT models for use in the pharma industry.
3. ***Trial licensing of MESI-STRAT models to SBS***. While customizing MESI-STRAT models for pharma stakeholders, SBS will constantly assess the marketing potential of these models. SBS will be granted trial licenses at favourable conditions for limited time periods. SBS will initiate contacts with pharma companies to develop and execute projects utilizing the MESI-STRAT models. During this time, SBS will assess the market potential of the MESI-STRAT models based on measures such project prospects and acquisitions, the time and cost for customization, licence fee and the market estimation of project fee. In case the trial period confirms a positive market value and viable commercial opportunities, parties will evaluate the market potential and decide on a licence fee that is beneficial for both parties.
4. ***For models with positive licensing trials***, SBS will obtain long-term royalty bearing licenses from each partner to provide services to the pharma industry for patient stratification for targeted therapies.

(ii) Development and commercialization of diagnostic tests to detect MESI marker panels in patients' body fluids by Neuroimmun GmbH (NIN)

The partnering SME NIN develops ELISA-based diagnostic tests for various disease markers relating to amino acids and biogenic amines, including Trp and its metabolites (for products see www.neuroimmun.com). MESI-STRAT will explore metabolites in biological fluids as markers for ER+BC therapy response, progression, relapse, and patient stratification for targeted therapies. Strong evidence in the MESI-STRAT consortium suggests that Trp metabolites may drive ET resistance and ER+BC relapse. If the predictive power of Trp metabolites is validated, they will be patented by MESI-STRAT partners (see 2.2.3 Management of knowledge and IP). NIN has the diagnostic tests and related patents for the detection of the metabolic marker panels and can directly market them and develop multiplex tests for this application. MESI-STRAT will likely also identify additional metabolites that are predictive of ER+BC therapy response to be evaluated by NIN for commercial assay development. NIN will receive trial licenses for candidate markers and develop diagnostic tests; if successful, long-term licenses will be obtained. Developed ELISAs will be validated according to criteria defined by the GMP guideline ICH Q2.

(iii) Additional developments to openSEEK providing a test case for the long-term business model of Systems Medicine data management activities at HITS gGmbH

The openSEEK software used for MESI-SEEK in the FAIRDOMhub is open source under BSD license. While the software is free to end users, funding is needed for maintenance and extension. HITS expects to build extensions to SEEK that directly benefit the project and the broader systems medicine community, improving FAIRDOM services, as well as SEEK's use in the context of systems medicine projects. The resulting code will be open-source while it is developed. The long-term business model is based on charging users for administration, service, advice, and training. Service includes contract development, and curation, which is crucial for the long-term vision of this investment for the community. Being part of MESI-STRAT is an important step for HITS towards elaboration of such a business model.

(iv) Commercialisation, licensing and marketing by academic partners

In addition to specialist computational models and diagnostic tests for ER+BC, MESI-STRAT will derive

- clinically validated MESI marker panels for ER+BC therapy monitoring and design.
- preclinically validated MESI target molecules for potential new drugs.

Given our novel approach to identify metabolite-based MESI markers and targets for ER+BC, their added value is potentially clinically significant. New MESI marker panels and targets will be evaluated for a direct fit with project partners for advanced development or for sale to third parties. The related IP protection and licensing will be coordinated by the TTOs within the MESI-STRAT consortium.

The main objective of the licensing strategy is to benefit ER+BC patients, and for this purpose two main options will be considered:

- **Licensing to third parties**

Foreground IP on developed MESI-STRAT models, biomarkers, and their associated assays, shall be licensed to the partnering and collaborating systems medicine, diagnostic, and pharma companies, but may also be of interest to companies outside MESI-STRAT. Partnering companies will have the first option as detailed in the consortium agreement. A license to a third party can include a license to multiple results (foreground IP) in order to increase the interest of parties. Potential licensees should have a significant presence in the field of BC (e.g., Novartis, Celgene, Pfizer, Roche, and others, see 1.3.a), services for systems medicine (e.g., SBS), or development of diagnostic tests (e.g., NIN, QuantuMDX). Our licensing vision is that we will license to parties (internal and external to the MESI-STRAT consortium) who are interested in establishing further research collaborations with the MESI-STRAT partners to combine state-of-the-art science with the requirements of the envisaged application and market. Combining the diagnostic opportunities with drug development will also provide licensing opportunities and enable the licensee to monitor and improve the therapeutic effect of their drugs and new lead compounds. As outlined above (in “(ii) Development and commercialisation of diagnostic (...) by NIN”), there is already serious interest for the development of diagnostics by the partnering SME NIN, based on the to-be-identified biomarkers (see partner description NIN). QuantuMDX is also interested in applying the new markers for diagnostic devices, which, for example, would enable BC patients to monitor their therapy responses by themselves.

- **Evaluate the potential of an own industry engagement**

In certain cases, it might be more effective to advance development by creating a spin-off company, ensuring full dedication and offering an opportunity to attract funds and venture capital to support pre-clinical development and the initial clinical trials. Depending on the results of the clinical proof of concept, a larger industrial partner would potentially be involved during the clinical studies to enable late clinical studies and marketing of the drugs or biomarkers. This route has been shown to be successful and is supported by the TTOs of UMCG and DKFZ (see letters). The decision to license foreground IP to third parties or to advance the development through spin-off companies depends on the MESI-STRAT outcome and will be subject to evaluation by the dissemination and exploitation committee (DEC, WP9) and on feasibility studies conducted by the partner TTOs to evaluate the added value of combining the technology and findings of MESI-STRAT with a corporate approach. The study will include the value proposition, both patient and market perspectives, a financial evaluation of investments required and potential revenues, as well as IPR position, business strategy and potential partners.

Table 6. MESI-STRAT draft plan for commercial exploitation (/a = per year)

Key goals	Target audience	Medium and means *one-way **two-way interaction	Timing of implementation	Impact monitoring = reaction of target audience
<ul style="list-style-type: none"> • increase research and innovation opportunities, particularly for SMEs active in diagnostics and pharma companies → gain internal and external partners for out-licensing and commercialization → evaluate own commercial involvement 	companies in pharma sector & diagnostics	<p>** develop diagnostic tests/devices/services with partner NIN and collaborators QuantuMDX and CELLEC BIOTEK</p> <p>** work/collaborate with companies that sell relevant drugs and conduct large clinical trials (e.g., Novartis, see letter), and contacts already established via the clinical trials in which MESI-STRAT participates (see 1.3.a, WP1,6,7, clinical annex)</p> <p>** approach new collaborating companies <ul style="list-style-type: none"> - via existing networks (of TTOs and of partners) - by presenting patents at patent information fairs and conferences - via feasibility study by partner TTOs </p>	from the beginning, throughout and after the project	<p>number of interactions with companies ($\geq 10/a$)</p> <p>- number of patent applications (≥ 4)</p> <p>- number of licensing requests ($\geq 2/a$)</p>

Table 6 (continued)

<ul style="list-style-type: none"> Increase research and innovation opportunities, particularly for SMEs active in systems medicine 	partnering SMEs: service providers for systems medicine	<p>**HITS partners a broad range of systems biomedicine infrastructure projects. Participation in MESI-STRAT gains sustainability experience and promotes use of open source and open data.</p>	since early project planning, throughout and after the project	functionality customization and first-hand advice (≥ 10 interactions/a within MESI-STRAT and with external customers)
		<p>**SBS develops and customizes computational models for the pharma sector. In MESI-STRAT, SBS customizes models for pharma use and hires students educated at academic partners, licenses models, and commercializes them with pharma partners.</p>	since early project planning, throughout and after the project	request for development of customized computational model-based analyses for drug development/ Drug Candidate/Target Assessment Projects (≥ 10 interactions/a within MESI-STRAT and with external customers)

2.2.2 Data management plan (DMP)

Table 7. MESI-STRAT draft data management plan: Data types, standards, sharing/accessibility, curation/preservation

Citations for resources mentioned in the table are as follows: PSI³⁶, MSI³⁷, SBML³⁸

Data types & estimated volumes	Description	Standards	Data sharing & accessibility for verification and re-use	Curation and preservation
Patient data Relational searchable database 10 MB	Pseudonymized, organized as patient history, samples (blood, urine, tissue), associated cell lines and PDX models	We conform to EU directives, NFU (Netherlands Federation of University Hospitals), and law of the protection of personal data (LDLG-BW) directives in Germany, agreed by the ethical councils of all data holders involved. All clinicians will be GCP trained, all department procedures will be defined in SOPs available on a protected website.	Shared with consortium members via MESI-SEEK platform, always following patient and data privacy regulations including the new REGULATION (EU) 2016/679, and other specific limitations based on the informed consent obtained.	Entries will be manually curated by WP1 and entered into the database.
Raw omics data, computational models, and relevant clinical data 10-20 TB	Output of high-throughput experiments: sequencing, proteomics, metabolomics Computational models Scientifically relevant pseudonymized clinical data, depending on the informed consent obtained and data privacy regulations	<ul style="list-style-type: none"> Sequencing data: MINSEQE standard for data clarity and reproducibility. Proteomics data: PSI standards. Metabolomic data: Metabolomics Standards Initiative (MSI), which combines SMRS, ArMet and MIAMET. Computational models: Model formats will adhere to COmputational Modeling in Biology' NEtwork (COMBINE) standards http://co.mbine.org/. SBML is a free and open interchange format which will mainly be used for MESI-STRAT models. SED-ML will be used for storing numeric model experiments in a reproducible manner. 	We expect medium to large amounts of data. To ensure distribution within MESI-STRAT and open data access after publication, EMBL-EBI are our chosen collaborators. Data will be uploaded to their servers as generated, and made publicly available and open access at the time of publication via EMBL-EBI. De-identified clinical and demographic data will form part of these datasets to allow re-use by future systems oncology studies. <u>Controlled access</u> applies to all data types that may be unique to an individual and requires user certification, following established TCGA procedures https://tcga-data.nci.nih.gov/docs/publications/tcga/datatype.html . This applies, for example, to RNAseq data.	Data annotation/ curation will be performed at the point of submission. Data will be securely hosted as follows: <ul style="list-style-type: none"> sequencing data in ENA, RNAseq data in ENA & Expression Atlas, proteomics in PRIDE & IntAct, metabolomics in MetaboLights, published and patent-protected computational models at BioModels. In addition, RNAseq data will be deposited in NCBI/GEO https://www.ncbi.nlm.nih.gov/geo/ . This redundancy further ensures long-term accessibility.

To maximise access to and re-use of MESI-STRAT data, our consortium participates in the *Open Research Data Pilot*. As detailed in section 2.2.3, the decision on mode and timing of open access publishing of documents and data must include protection of patient privacy, and IPR protection of MESI-STRAT findings, in keeping with regulatory aspects for clinical studies and commercialization. The MESI-STRAT data management plan will follow guidelines specified under **FAIR data management** ('F' stands for findability, 'A' for accessibility, 'I' for interoperability, 'R' for reusability of data). Our data management strategy is fully compliant with the H2020 drive towards FAIR data management. It has already been agreed across the consortium, and will be formally delivered as a data management plan in month 6 of MESI-STRAT. Our data management platform, MESI-SEEK will be based on the FAIRDOM (FAIR Data, Operations, Models) project, which provides the openSEEK platform, and the FAIRDOMHub site for data sharing. Our openSEEK platform MESI-SEEK is characterized by a transparent, searchable structure, interlinking processed data to raw data, metadata and computational models according to ISA standards. MESI-SEEK will contain all experimental data including analysed omics data, shared protocols, and mathematical models and relevant pseudonymized clinical information. The ownership of uploaded data and models will remain with the groups that produced them. All the uploaded data will be accessible to all project partners. Standard Operating Procedures (SOPs) for experimental procedures will also be stored and linked to the data.

WP2, dedicated to the implementation of the MESI-STRAT DMP, is led by the SME HITS, one of the core FAIRDOM partners that have contributed to the FAIRDOM guidelines document. We will collaborate closely with the FAIRDOM data management project for issues concerning data and metadata management and storage, data curation and annotation, technical modeling support, and publication of project data. **Preservation of the data** stored in MESI-SEEK will be guaranteed for at least 10 years beyond the end of the project. Raw data will be submitted to **public repositories**, and we will collaborate with EMBL-EBI to guarantee open access upon publication of MESI-STRAT results (see attached letters). EMBL-EBI databases are member projects of ELIXIR, which will guarantee wide reusability of our data. We will also interact with the ELIXIR Human Genomics and Translational Data team to ensure that our methods evolve with current best practice. **Clinical data** will be kept in the MESI-REPOSITORY set up at the central DataCenter of the IT Core Facility of the DKFZ, which complies with all regulations of data safety necessary for storage of personal data and the protection of confidentiality of individual records (for details see 5.1.4 Personal Data). Access to sequencing data that potentially allow identification of individuals will be restricted as detailed in Table 7.

Costs for data curation and preservation

Data and models will be annotated and curated by the MESI-STRAT partners who generate and publish them (WP3-7). This process and adherence to strict SOPs and data standards (Table 7) will be overseen by HITS, the leader of WP2. Setup, maintenance, and extension of the MESI-SEEK data and model management platform is covered by MESI-STRAT budgets during the running time of our project. HITS guarantees long-term maintenance of the data stored centrally. Long-term maintenance of data stored at other partner institutions will be provided by the respective partner centers. The data management plan will detail contingency plans emphasizing the longevity of the data. Deposition of all published data and models in open access databases at EMBL-EBI and NCBI/GEO is covered by public funding and ensures adherence to data standards, long-term accessibility and re-use of MESI-STRAT data by future systems oncology efforts.

2.2.3 Plan for management of knowledge and Intellectual Property (IP) protection

The management of knowledge and intellectual property will be monitored by the Coordinator subject to the decision of the Strategic Board (SB, WP8), the dissemination and exploitation committee (DEC, WP9) led by SBS and PATH, and the Consortium Agreement. The Consortium Agreement, and the Grant Agreement will be based on the DESCA model, which is the most broadly used consortium agreement model for H2020 projects. The model will be adapted to accommodate MESI-STRAT related principles such as data management, the terms for favourable licensing for evaluating the market potential of MESI-STRAT models by SBS and other potential licensees, and the details on collection, storage and use of patient data.

At the critical times of (1) patenting, (2) licensing, or (3) own industrial initiatives, the contributions of all involved consortium members up to this point will be documented and frozen to define what the object of the license will be. The efforts of the leading institutions for patenting, licensing, feasibility analyses for establishing a possible spin-off will be continuously documented, to reward continuing investment. The following aspects will be the basis for detailing the DESCA model to the specific needs of MESI-STRAT:

Ownership of MESI-STRAT Results (i.e., Foreground): All MESI-STRAT participants shall keep laboratory books showing ownership, date of generation and progress in generation of knowledge. These notes will also

reflect contributions to jointly developed foreground (i.e., results). Where no individual ownership can be ascertained, partners will establish their respective shares of ownership based on contribution, for example through effort. The consortium agreement will further detail management issues such as sharing of the costs arising from legal protection procedures and exploitation of the jointly owned foreground.

In case of transfer of ownership, the assignor shall conclude appropriate arrangements to ensure that its contractual obligations with respect to dissemination, use, and the granting of access are passed on to the new owner, as well as by the latter to any subsequent assignee. In certain cases, prior notice shall be given to other participants and to the European Commission.

Protection of MESI-STRAT Results (i.e., Foreground): Foreground with potential for commercial exploitation shall be protected in an adequate and effective manner. MESI-STRAT participants shall aim at making collective decisions concerning the best strategy to protect foreground, which may include a decision not to protect the foreground, for example, due to a lack of commercial opportunity. In all cases and in an appropriate time frame (making sure protection is not harmed), outcomes shall be disseminated via journal publication or other means of putting foreground in the public domain. When necessary, an invention shall be kept confidential with postponed application and dissemination activities.

The consortium agreement will include further details on:

- the number of days (at least 14) prior to the intended publication to inform all partners;
- procedures on the method and content of informing the other participants;
- procedures on the methods and content of written objections by other partners to the intended publication.

Access rights for implementing the project: Access rights to background and foreground needed for implementing the project will be granted on a royalty-free basis.

Access to another participant's results (foreground) or background: The access shall be granted if the requesting participant needs it in order to carry out the project or to exploit one's own foreground. Any additional conditions such as the definition of 'needed', and if necessary, exclusion of specific background from the obligation to grant access, will be stated in the consortium agreement. In case of sublicensing, the terms and conditions shall be agreed in writing.

Measures to provide open access and open data

The decisions on IPR protection in relation to timing of open access publishing will be taken by the SB (WP8), supported by the dissemination and exploitation committee (DEC, WP9), together with the partners involved. In accordance with Horizon 2020 regulations, MESI-STRAT will grant free-of-charge access to peer-reviewed scientific publications and underlying data to the end user. Implementation of open access data sharing will occur via the grant agreement. All data generated, whether primary raw data or analysed secondary data, will be documented in a traceable manner in the MESI-SEEK database (see section 2.2.2 Data management plan and WP2) to aid further meta-analysis and reuse within and outside the consortium.

Data dissemination will occur principally in conjunction with open access scientific publications in national and international specialized publication organs and peer-reviewed journals. Publication of datasets and models in structured, searchable and citable databases at EMBL-EBI or NCBI/GEO will enhance reuse of data and models (see section 2.2.2 Data management plan and WP2). DOI references to data guarantee long-term citability. Open access scientific publications will be achieved by self-archiving in institutional repositories, following a possible embargo period after journal publication of 6 - 24 months (green open access). Several partner institutions and nations have come to agreements with publishers ensuring that costs for gold open access publishing are waived or reduced for publications co-authored by members of these institutions (see e.g., <http://www.rug.nl/library/open-access/oa-finances>). HITS has ring-fenced internal funds for open access. Where possible, costs for gold open access will be applied for at the partner institutions and national funding bodies (e.g. Stimuleringsfonds Open Access, NWO, NL). To further ensure gold open access publishing in our consortium, a dedicated budget for additional costs of open access publishing will be reserved by the coordinator and can be distributed to the partners if they cannot access other funding support.

b) Communication activities

2.2.4 The MESI-STRAT Communication Plan

Our comprehensive communication plan, central to WP9 jointly led by the patient organisation PATH and the SME SBS, complements our dissemination and exploitation strategy by communicating MESI-STRAT results to

and receiving input from a broad group of stakeholders including scientists, healthcare professionals and commercial stakeholders - also addressed by our dissemination and exploitation measures - plus societal stakeholders such as patients and their families, policy makers, and EU citizens. Table 8 shows MESI-STRAT's draft plan for communication, including measures for monitoring the impact of the communication activities.

MESI-STRAT key communication activities

(1) **MESI-STRAT patient days** for patients participating in the clinical studies of MESI-STRAT will be organized by the patient organization PATH and the German Cancer Information Service (KID) at DKFZ. Our partner DS is PATH co-chair and BC patient expert, communication coach, and advisory board member for several clinical trials (see letter and DS's CV in the annex). The impact of the patient days will be proportional to the number of attendees. Therefore, MESI-STRAT representatives (PATH, KID), SB, partnering scientists and clinicians will visit each of the clinical partner centers (Heidelberg, Groningen) and clinical centers collaborating with PATH at least twice during the project for on-site patient days to present and discuss the MESI-STRAT results, to receive input from patients on their urgent needs (e.g. guidance on targeted therapies, treatments of co-morbidities or therapy side effects), to discuss how to implement these needs in the MESI-STRAT studies, and to motivate patients to continue participating in clinical studies. Patient days will provide patients with high-quality reliable information essential to empower patients and their families to deal with their fears and to actively take part in the decision making on their therapies. Addressing fear by informed decision making is also important to strengthen therapy adherence. Patient-centered communication is key to reach this goal. Therefore, SW (DKFZ / KID) and DS (PATH) will advise on frequent questions that are relevant to BC patients and on how to best communicate the results of MESI-STRAT. For example, it is important to clarify to patients the timeline for a MESI-STRAT result to be translated to the clinic, and to choose ways of communication that motivates patients to be involved in decisions regarding their own therapy.

In particular, we aim to

- inform patients about the latest MESI-STRAT results, and receive their input on therapies to take to the next steps in the MESI-STRAT work plan;
- listen to the needs of patients and what they expect from MESI-STRAT;
- translate this information into measures to further specify deliverables in the course of the project.

We will interact and collect feedback from patients in two ways:

(i) *through discussion rounds* of MESI-STRAT partners – including patients, patient experts, communication experts, clinicians and scientists (e.g., SW+CO, DKFZ; DS, PATH; KT+CSC, UMCG; SS+AS, UHH), to be documented and implemented by the Strategic Board (SB) into the scientific objectives, policy, and strategic orientations of MESI-STRAT;

(ii) *through questionnaires* designed with KID/DKFZ and PATH in WP9 and distributed during patient days and as electronic polls via the MESI-STRAT website to collect opinions and feedback in a formalized manner.

In addition to the patients themselves, MESI-STRAT will also actively invite other stakeholders from the public (patient families and partners, other patient organisations for cancer/tumour diseases, public invitation via internet to attract wider audience), the scientific community (professional networks from other medical centers, to be contacted via email), and the political community (e.g. invitation of clinical opinion leaders and representatives from national funding bodies for basic and clinical science) in order to ensure that results of the project are endorsed by multiple stakeholders throughout Europe.

(2) **A European telephone survey on frequent questions by breast cancer patients, relevant to MESI-STRAT.** KID (DKFZ) supports 6,000 breast cancer patients per year by phone counselling, and ER+BC patients are also counselled by KID's European partner organizations in the UK, Norway and The Netherlands who collaborate via the International Cancer Information Service Group (<http://icisg.org/>). KID and its European partners will monitor questions relevant to MESI-STRAT by questionnaires for their co-workers who offer phone counselling for BC patients. SW (KID, DKFZ) will analyse them and report on the results at the annual MESI-STRAT meetings.

The questionnaires will collect information on • type of caller (patient with diagnosed BC, patient under suspicion of BC, relative), • sex of caller and/or patient, • age of caller and/or patient, • origin of caller and/or patient (country), • disease localization (breast), • specification of disease (DCIS, LCIS, breast cancer), • situation of the patient (diagnosis, first treatment block, maintenance therapy, relapse, palliative situation, etc.), • tumor receptor status (ER, PR, Her2, not known), • endocrine therapy, • prognostic markers, • predictive markers

Based on the results of this survey, SW will advise the MESI-STRAT strategic board as well as health care professionals and patient organizations in and associated with MESI-STRAT on how to implement the patients' questions into the project, and how to effectively communicate MESI-STRAT results to patients.

(3) Communication of MESI-STRAT results at patient events and platforms, incl. those for male BC (cross cutting priority gender). MESI-STRAT aims and results will be communicated at BC patient events for female and male BC at partner and external institutions and internet platforms. This includes events such as the NCT patient day (Heidelberg School of Medicine) at UHH and DKFZ with 500-750 patient attendees per year, the yearly Patient Congress BOOG-BVN-Pink Ribbon (NL) with 200 attendees, and the annual symposium of the Dutch Breast Cancer Patient Society (Borstkanker Vereniging Nederland, BVN) with 200 attendees. **CSC (UMCG)** is chair of the Dutch Male Breast Cancer Consortium, steering board member of the EORTC-BIG Male Breast Cancer Consortium, and on the BVN scientific board. She regularly presents at the BVN symposia, patients events, and Public Academy lectures on BC at UMCG. **AS and SS (UHH)** regularly speak at the NCT patient day. SS is part of the board of the German Society of Gynaecology and Obstetrics (DGGG) and has organized the congresses every second year with patients' contributions over the last 6 years. Being the head of the hereditary center at the women's clinic, she has organized meetings with the BRCA e.V network with an upcoming event in September 2017. Patient platforms for communication of MESI-STRAT results include the project "diploma patient" (www.diplompatientin.de, **DS (PATH)** is a member), run under the umbrella of the patient advocacy group mamazone e.V., and the dialogue around breast cancer (www.dialogrunde-brustkrebs.de), a cooperation of Pfizer with MammaMia which focuses on patients with MBC. Importantly, DS is executive board member of the European patient academy EUPATI (<https://www.eupati.eu/>). EUPATI aims to support the integration of patient involvement over the entire process of drug development through a training tool box for patient involvement in setting research priorities.

(4) Website, electronic newsletters, podcasts and YouTube films, and social media. MESI-STRAT will create a public website and electronic newsletters with project information and results. WP9, led by PATH and SBS, will pro-actively work with the other partners to provide content for both. The website will also host the feedback collection from patients (see 1), and aid in newsletter distribution. Key lectures during MESI-STRAT annual meetings, patient days and at external events (see 3, and dissemination) will be available as podcasts and YouTube films and will be promoted via the MESI-STRAT website and social media (Facebook, Twitter). Access and download rates from the website and social media will be monitored to measure the impact.

(5) Professional training and patients' literacy

- **medical professionals and basic scientists.** Open workshops will be held at the annual MESI-STRAT meetings, announced via the MESI-STRAT website, electronic newsletters, social media, and professional and patient networks, where MESI-STRAT partners and collaborators will share their expertise and knowledge on recent developments in MESI-STRAT and systems oncology.
- **students studying to become medical or basic scientists.** All MESI-STRAT partners are actively involved in academic teaching at universities, medical centers, and international events such as workshops and summer schools (e.g. organised by SB@NL, or the DKT <https://dktk.dkfz.de/en/training/dktk-summer-school/introduction>).
- **patients, their families and relatives, and EU citizens (i.e. 'the public')** who are interested in learning about the perspectives of systems oncology. The partners will disseminate developments in MESI-STRAT and systems oncology in the context of public lectures and discussion rounds, such as the *studium generale* at Heidelberg University (DKFZ, UHH), and the Medical Public Academy (Medische Publieks-academie) at UMCG (www.umcg.nl/NL/UMCG/medische_publieksacademie), the project diploma patient and the European patient academy EUPATI (see 3).

(6) General measures to raise public awareness for systems oncology in Europe will be achieved by joint press releases of partner institutions for annual meetings, patient days, and scientific publications. In addition to scientific publications for an expert audience, we will also write reviews and give interviews in lay language to be published in national and regional newspapers as we have done in the past; such as *Telegraaf* (CSC, 02/2015, 'Oh, I am sorry, you are a man!' on male breast cancer), *Dagblad van het Noorden* (CSC: 02/2016, 'Breast cancer without a lump'; 11/2015 'No breast cancer is the same'), *NRC* (CSC, 10/2016 'Madam, you do/don't need chemotherapy'), *Deutsche Ärztezeitung* (AS, 10/2011, 'Tailored therapies for breast cancer'), *Facebook* (CO, 2011, Lindau Nobel laureate meeting, 'A Tough Balance: Cancer Research and Motherhood'), national internet platforms such as *bio-pro.de* (KT, 05/2015, The art of deciphering signalling), and broadcasted on TV, e.g. *ZDF* (AS, 02/2016, 'Volle Kanne, Focus topic Cancer'), or *YouTube* (AS, 03/2013, 'Gene signatures in clinical practice', interview with patient organization Mamazone e.V.).

Table 8. MESI-STRAT draft plan for communication. (/a = per year)

Key goals	Target audience	Medium and means *one-way **two-way interaction	Timing of implementation	Impact monitoring = reaction of target audience
<ul style="list-style-type: none"> • Accelerate the translation of MESI-STRAT biomedical and clinical research results to medical use, guided by patients' needs. • Promote rapid and better use of systems medicine research results • Support mutual learning, science literacy • Target MESI-STRAT to patients' needs • Perform participatory research 	cancer patients specifically ER+ BC patients	<p>**MESI-STRAT patient days implemented by BC patient organisation PATH and the Cancer Information Service KID (DKFZ)</p> <p>**Presence at patient days and patient-internet platforms to present project results and new directions in BC therapy, e.g. at partner BC centers, Breast Health Day (Europa Donna, on our IAB) http://www.breasthealthday.org/, 'diploma patient' congress focused on breast cancer patients. http://www.diplompatientin.de/index.html</p> <p>**European telephone survey of BC patient needs related to MESI-STRAT, implemented by European Cancer Information Services (led by KID, DKFZ)</p> <p>**questionnaires on needs of patients related to MESI-STRAT, circulated at patient days and on internet by PATH and KID</p> <p>**publications in journals and on websites read by BC patients, e.g., EUPATI European Patient Academy www.eupati.eu, MammaMia (ca. 20,000 copies, https://mammamia-online.de), mamazone MAG, Alliance again BC</p>	from project start on, throughout/ after project, at least twice at each partnering and associated BC center	≥200 patient attendees and partners/a
			throughout and after project at least 3 times/a	≥300 patient attendees and partners/a (200 at UMCG, 500 at UHH/ DKFZ)
			from project start on, throughout project	≥6,000 patient contacts/a
			start in year 2, throughout and after project	≥100 responses of patients and partners/a from 2nd year on
			from beginning throughout the project	at least 2/a
<ul style="list-style-type: none"> • Align MESI-STRAT with needs & expectations of society in accordance with responsible research and innovation (RRI), with particular emphasis on gender issues • Foster socially relevant research and innovation outcomes of MESI-STRAT 	European citizens (including all other stakeholders, see section 2.2.1)	<p>* MESI-STRAT website</p> <p>* Newsletters electronic MESI-STRAT newsletters PATH print newsletter</p> <p>**polls/surveys on interest in systems oncology, understanding of principles, expectations regarding research goals and ethical aspects via the MESISTRAT website</p> <p>**MESI-STRAT gender committee (GC) will discuss results relevant to male BC at meetings incl. those of the EORTC-BIG Male Breast Cancer Consortium</p>	<p>throughout the project</p> <p>during project, 2 newsletters/a</p> <p>before, during and after project, 1 newsletter/a</p> <p>from beginning throughout the project</p> <p>start in year 2, throughout the project</p>	<ul style="list-style-type: none"> - monitor traffic on website (count clicks, downloads, divided by country) <p>monitor download rates by country</p> <p>7,500 copies</p> <p>response rate per country ≥200/ a</p> <p>→ present/ implement results at annual meetings</p> <p>≥2 presentations for male BC / a</p>

Table 8 (continued)

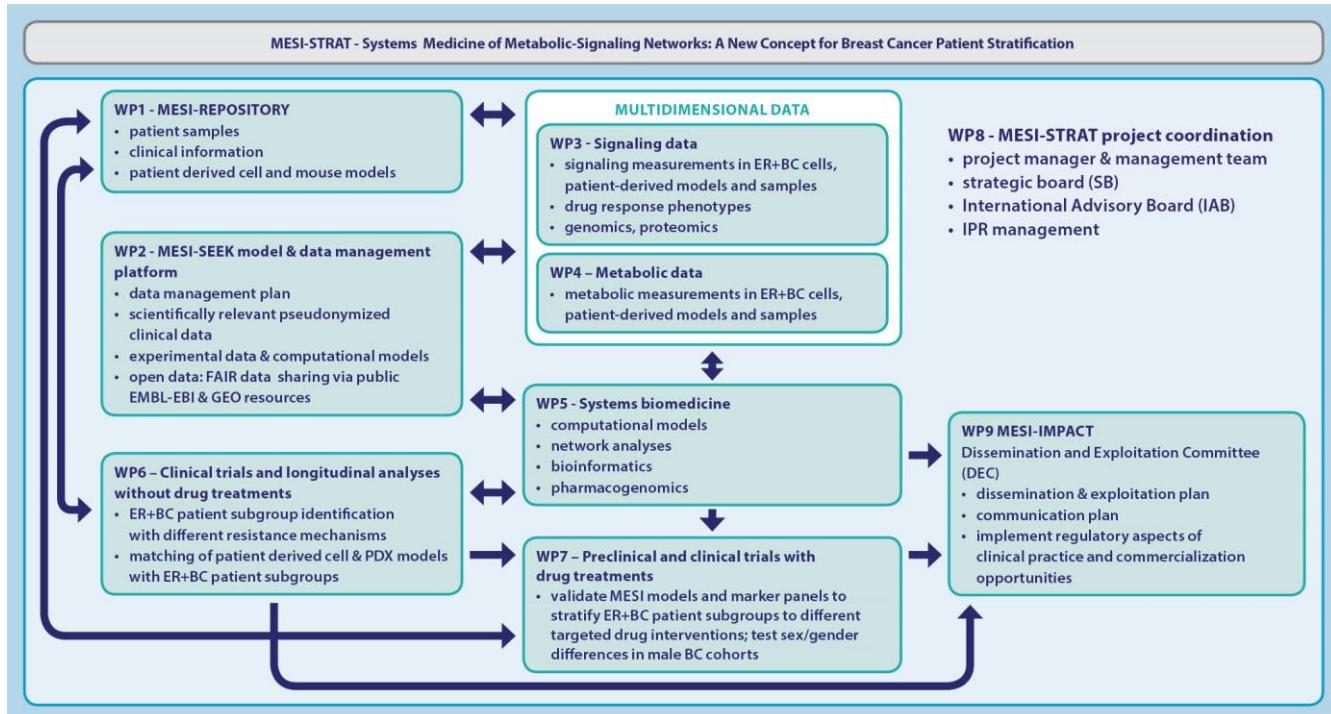
Key goals	Target audience	Medium and means *one-way **two-way interaction	Timing of implementation	Impact monitoring = reaction of target audience
Present project results with focus on chances, risks, and implementation of systems oncology to foster <ul style="list-style-type: none"> • awareness • mutual learning • science literacy • easier access to scientific results 	healthcare providers, health professionals	skills & training: **open workshops as part of annual MESI-STRAT meetings	each year throughout the project	≥15 attendees/a
	medical and basic science students at partner institutions and beyond	skills & training: **implement strategies and research of MESI-STRAT in academic teaching (courses and internships in partner's laboratories)	throughout and after the project	≥50 attendees/a
	skills & educational training for <ul style="list-style-type: none"> - patients and their families - EU citizens 	**invited lectures of MESI-STRAT partners in the context of public lectures at partner universities, medical centers and patient initiatives such as: diplompatientin.de	from beginning throughout the project	≥2 public lectures/a
Convey added value of transnational cooperation to lever systems oncology for patient stratification to inform clinical decision making	EU citizens	joint press releases of partner institutions for annual meetings, patient days, scientific publications from the consortium	from beginning throughout and after the project	≥3 joint press releases/a
		EU and MESI-STRAT logo and/or project number on all dissemination/exploitation/communication actions		≥20 releases/a that carry the MESI-STRAT/EU signature
Consideration of regulatory aspects of clinical practice: <ul style="list-style-type: none"> • Promote systems oncology in clinical guidelines • Enable implementation of systems oncology in disease management • Consider MESI-STRAT results for future grant programmes and industrial engagement 	policymakers: clinical opinion leaders	invitation of clinical opinion leaders <ul style="list-style-type: none"> - to annual MESI-STRAT meetings - to patient days 	from beginning throughout and after the project	≥3 opinion leaders participate in meetings and patient days

3. Implementation

3.1 Work plan — Work packages and deliverables (incl. Gantt and Pert charts)

3.1.1 Brief presentation of the overall structure and work plan

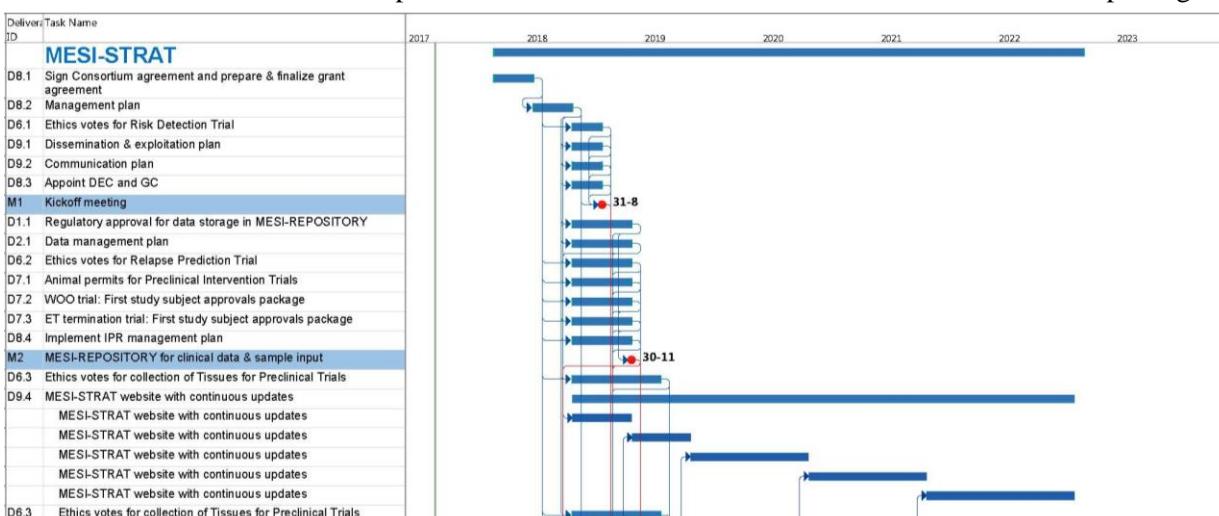
An overview of the MESI-STRAT strategy and summary of the WPs are given in section 1.3 (b). The strategy is shown in **Fig. 4**. WP8 will coordinate the work. The clinical WPs 1, 6, 7 will be led by clinicians, the theoretical WPs 2 and 5 are led by modelers, bioinformaticians and data management experts, and the experimental WPs 3 and 4 are led by experimentalists. WP9 focuses on maximizing the impact and is led by the partnering patient organization PATH and the SME SBS. The WPs provide technology platforms on which we run our work plan, following an iterative straightforward systems biomedicine concept.



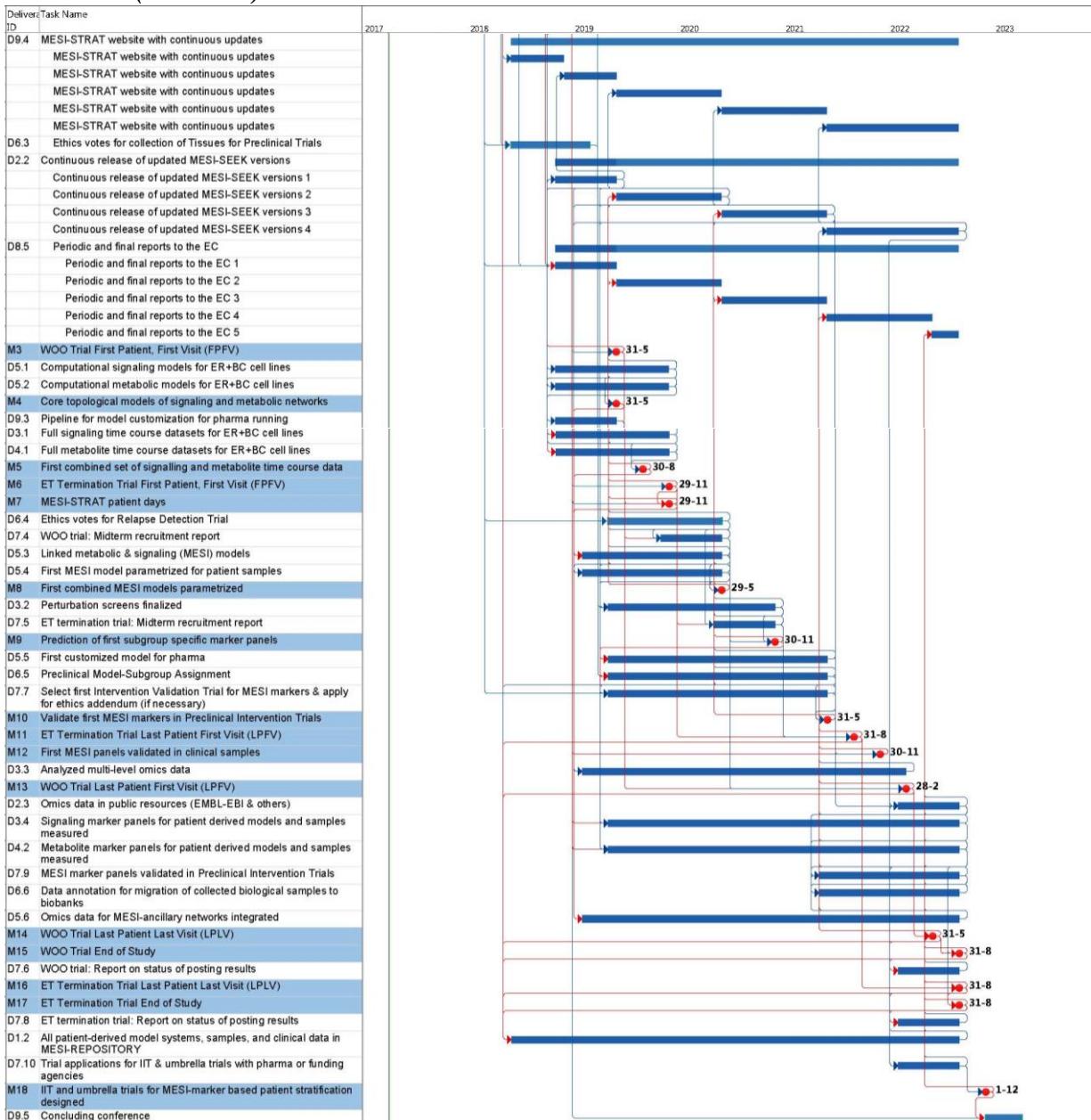
3.1.2 Timing of the different work packages and their components (Gantt chart)

The project will operate on the core basis of 51 months active scientific and clinical work. To ensure a fast start, there will be a preceding period of 8 months for preparation and signature of the grant agreement, timely application for ethics votes, staff recruitment, working and business rearrangements, planning meetings. At the end there will be further three months for writing the final report and for a public conference.

Timeline: Full project duration: 51 months: months -8 to -1: preparatory work; months 1 to 51: active clinical and scientific work; months +1 to +3: public dissemination/communication conference and final reporting.



Gantt chart (continued)



3.1.3 Detailed work description

Work package number	1	Lead beneficiary					PATH
Work package title	Data survey of existing patient samples and materials						
Participant number	1	2	3	4	5	6	15
Short name of participant	UMCG	PATH	UHH	DKFZ	VHIO	DDI	HITS
Person months per participant:	1	9	10	1	3	1	2
Start month	1					End month	51

Objectives. The overall aim of WP1 is to provide the biological samples, pre-clinical models, clinical samples (for details see clinical annex), and data to derive model-based MESI marker panels allowing the prediction and monitoring of ET responses, and the validation of established and novel (combinatorial) therapies for newly identified ER+BC subgroups.

The specific objectives of WP1 are to provide

1. a comprehensive overview of pseudonymized clinical data, patient materials and patient-derived cell and PDX models in the MESI-REPOSITORY

2. ER+BC cell lines, PDX, matched cultures and patient material for preclinical trials
3. matched fresh frozen ER+BC tissue, serum, and pseudonymized patient data from the PATH collection.
4. longitudinally collected serum and urine from ER+BC patients at relapse and under intensified treatments (PATH and clinical studies performed by partners and collaborators)
5. biobanking of samples and pseudonymized clinical data from our WOO and ET Termination trials

Description of work. Lead partner: PATH. **Further partners:** UHH, UMCG, VHIO, DKFZ, DDI, HITS

WP1 will provide a searchable database (MESI-REPOSITORY) of fresh frozen and FFPE tissue, serum/plasma, urine, and pseudonymized patient data (treatment and follow-up) from BC patients from existing clinical cohorts (PATH, observational studies UHH, UMCG, VHIO), blood, and urine from longitudinal collection in the framework of MESI-STRAT and from relevant BC clinical trials. In addition, this repository will incorporate patient-derived model systems (matched cultures of primary human BC cells and PDX models) (VHIO & DDI). WP1 thus provides the basis for the analyses performed in WPs 2–7 of MESI-STRAT and will also incorporate pseudonymized clinical data and biological samples of WP7 into the MESI-REPOSITORY.

As the MESI-STRAT marker panels to be derived are hitherto unknown, our sample collection will need to allow a variety of measurements. Therefore, patient cohorts will be built up in a comprehensive way using the biobanks (fresh frozen and FFPE tissue, blood, serum, urine) and sample collections from studies for later validation of all potential markers. Clinical data to be collected will/can include: (i) clinical-pathological characteristics of the patients (age, tumor type, grading, staging, imaging, TNM status, Ki67 status); (ii) therapy and response (follow-up); (iii) amount and type of material present at either the clinics or biobanks; (iv) sufficient material to determine molecular features (e.g., mutations status by panel sequencing).

MESI-STRAT partners and collaborators will provide patient samples for MESI-STRAT

(see **clinical annex** for details on the clinical trials). Their existing sample collections include:

- **PATH BC biobank:** 4022 matching fresh frozen ER+BC tissues, adjacent normal tissues and sera from BC patients with full documentation; >80 postmenopausal and >50 premenopausal with high-risk BC, (as defined below, task 1.1) respectively; 106 samples of patients who developed distant metastasis during follow-up; 1847 patients stable >5 years; 451 patients having received 5 years of ET in the MESI-STRAT study period.
- **UHH:** >300 longitudinal serum/plasma samples from the PRAEGNANT study (patients with advanced, incurable, metastatic breast cancer under intensified treatments, sampled at each progression); samples from 5 interventional clinical trials with ET, CDK4/6 inhibitors or HER2 inhibitors, >70 serum, plasma, and urine samples as well as tissues of patients from the GEKKO study (at diagnosis); **Male cohorts:** approx. 100 FFPE samples from male BRCA mutant BC patients.
- **UMCG:** 200 samples from the IMPACT Breast trial (non-rapidly progressive MBC patients eligible for first-line systemic therapy undergoing molecular imaging); 20 samples from FDHT PET AND BICALUTAMIDE IN MBC trial; **Male cohorts:** 200 serum/plasma samples & fresh frozen BC tissue prospectively collected from males with BC; 800 FFPE samples retrospectively collected from males with BC.
- **VHIO:** samples from 9 interventional clinical trials with CDK4/6 inhibitors, PI3K inhibitors, Akt inhibitors, tyrosine kinase inhibitors, or mTOR inhibitors (Everolimus, dual inhibitors)
- **Prof. E. Boven (VUMC, Amsterdam, NL):** 175 serum/plasma samples + clinical follow-up of patients before Everolimus treatment combined with Exemestane (NCT02109913, Everolimus/Exemestane study) (see letter).
- **Prof. G. Wulf (Beth Israel Deaconess Medical Center, Harvard, Boston, USA):** longitudinal plasma samples (before, on and off treatment) of patients treated with PI3K-inhibitors and/or Olaparib (n = 50) (see letter).

Feasibility of sample provision and biobanking

PATH Biobank, UHH/NCT, and UMCG have longstanding expertise in biobanking and contribute to the European Biobanking and Biomolecular Resource Research Infrastructure (BBMRI-ERIC) initiative. Thus, our partnering biobanks will immediately contribute to our project by providing retrospective samples and actively banking the prospective samples collected in the frame of MESI-STRAT (see letter from the UHH/NCT biobank). This will ensure that the biological samples collected in the frame of MESI-STRAT will be available for the scientific community.

Task 1.1 Provision of sera and selected BC tissues at diagnosis from high-risk and low-risk ER+ BC patients
Partners: PATH

High-risk and low-risk ER+BC patients will be selected according to the criteria below. 50 sera of each pre- and postmenopausal high- and low-risk ER+BC patients will be provided (200 sera in total) and corresponding BC tissues of approx. 5 patients per group (20 BC tissues in total).

Selection of ER+BC patients

Low risk	High risk
Tumor size less than 2 cm in diameter (pT1)	Premenopausal: tumor size more than 2 cm in diameter (\geq pT2-3) Postmenopausal: tumor size more than 5 cm in diameter (\geq pT3)
No cancer cells in any nearby lymph nodes (pN0)	Cancer cells have spread to 4 to 9 axillary lymph nodes, or have enlarged the internal mammary lymph nodes (\geq pN2)
Well-differentiated, slower growing cells (G1)	Less differentiated, faster growing cells (G 2-3)
No metastasis (M0)	No metastasis (M0)

>80 postmenopausal and >50 premenopausal patients that fulfil these criteria are available per group in the PATH cohort. To harmonize the collections, we will include the following most common histopathological tumor types: invasive carcinoma of no special type (NST, also known as invasive ductal carcinoma) and invasive lobular carcinoma.

Task 1.2 Provision of sera and selected ER+BC tissues at diagnosis of patients who remained stable for >5 years and of patients who developed relapse/distant metastasis Partners: PATH

75 sera of ER+ BC patients who developed distant metastasis during follow-up and 150 sera of patients who remained stable for >5 years will be provided. The tumors at diagnosis will be matched for tumor size, lymph node affection, and grade. Representative BC tissues, approx.10 for the ones with distant metastasis during follow-up and 20 for the controls, will be provided for the analysis of the mechanisms underlying the MESI marker panel differences in BC tissue.

Task 1.3 Biobanking and provision of sera and urine from ER+BC patients before and after termination of ET and from ER+BC patients at relapse and under intensified treatment Partners: PATH, UHH, UMCG, VHIO, E. Boven, G. Wulf

Provision of serum and urine and pseudonymized clinical data of patients at relapse and under intensified treatments in the frame of clinical studies performed by partners and collaborators of MESI-STRAT (UHH, UMCG, VHIO, E. Boven, G. Wulf). from above

Task 1.4 Survey of ER+BC cell lines and of matched patient-derived cell and PDX models and corresponding clinical samples Partners: VHIO, DDI, UMCG

ER+ BC cell lines: Suitable ER+BC cell lines are widely used in cancer research and will be used by MESI-STRAT for the basic parameterization of dynamic computational models specific for ER+ BC (WPs 3-5). They will be obtained from ATCC and distributed across the MESI-STRAT consortium and will be regularly genotyped to ensure that all partners work with the same material. We will work primarily with MCF-7, ZR-75-1, and BT474 cells. Further ER+BC cell lines such as T47D and others will be considered as needed.

ER+BC PDX models, matched cultures, and patient samples: Nine ER+BC PDX models from VHIO (2 from primary, 5 from metastatic and 2 from both primary and metastatic tumors) (VS), 35 PDX models from the EuroPDX consortium (SJ, VS), matching 3D cell cultures for 9 ER+BC PDX models (VS), immunocompetent ER+BC PDX models (DDI, BE). Further ER+BC models can be established by VS if needed.

Task 1.5 Setup of the MESI-REPOSITORY encompassing all patient materials, pseudonymized clinical data, and models Partners: DKFZ, PATH, UHH, UMCG, VHIO, HITS

The MESI-REPOSITORY will enable all consortium members to gain insight into the available patient-derived cell and PDX models, pseudonymized patient samples and clinical data. WP1 will document pseudonymized patient data including age, BC type, grading, staging, receptor expression, clinical parameters and partner site from existing collections as well as from patients that will be prospectively recruited (WP7). The available data will be used by other WPs for selection of tissue/serum/urine samples for signaling, omics (WP3) and metabolic analyses (WP4), and of tumor samples/PDX for model verification and clinical validation analyses (WPs 3, 4, 7). The MESI-REPOSITORY will be set up at the central DataCenter of the IT Core Facility of the DKFZ, which complies with all regulations of data safety necessary for storage of personal data and protection of confidentiality of individual records (for details, see section 5.1.4 Personal Data).

Deliverables

D1.1 Regulatory approval for data storage in MESI-REPOSITORY (month 6)

D1.2 All patient-derived model systems, samples, and clinical data in MESI-REPOSITORY (month 51)

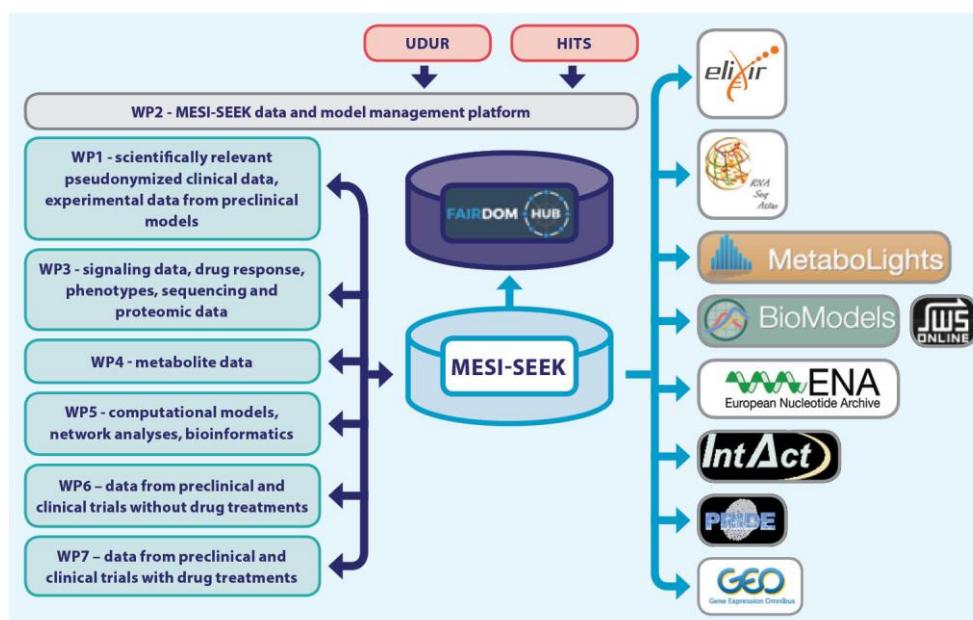
Work package number	2		Lead beneficiary					HITS	
Work package title	Setup and maintenance of MESI-SEEK data and model management platform								
Participant number	1	2	3	4	7	9	10	11	14
Short name of participant	UMCG	PATH	UHH	DKFZ	UiB	UNEW	CHAB	UDUR	HITS
Person-months per participant:	1	1	1	1	1	1	1	6	18
Start month	1			End month		51			

Objectives. Key property of MESI-STRAT data management is the adherence to the H2020 Programme Guidelines on FAIR Data Management, and participation in the open data pilot. The **overall aim** of WP2 is to curate and preserve the generated data and make it available to all MESI-STRAT partners and external users in a user-friendly and securely accessible format.

The **specific objectives** of WP2 are the

1. creation of the MESI-STRAT **data management plan (DMP)**, outlined in section 2.2.2), according to the consortium agreement
2. setup and maintenance of the **MESI-SEEK data management platform** which implements the DMP and allows compliant and simple management of scientifically relevant pseudonymized patient data; of raw data from sequencing, proteomics, and metabolomics (WP3 and 4) including their deposition into publicly hosted databases; and analyzed data from sequencing, proteomics, metabolomics, molecular kinetic measurements and computational models, using MIBBI-compliant templates

Description of work. **Lead Partners:** HITS, co-leader UDUR. **Support from:** DKFZ, CHAB, UMCG, and all WP leaders, and in collaboration with EMBL-EBI and FAIRDOM, as well as ELIXIR where appropriate.



WP2 interacts with all other WPs and coordinates the management of research data arising from MESI-STRAT. All WPs have the primary responsibility to manually upload their data and models according to agreed standards as soon as they are available. This will be supported by the FAIRDOM team, and the scientists appointed at HITS gGmbH and UDUR. They will represent MESI-STRAT in the FAIRDOM PALS team and attend the PAL focus group meetings that FAIRDOM holds twice a year. Furthermore, they will have visitor status at EMBL-EBI (see letters of support), and will interact closely with the IntAct and ATLAS teams at EBI.

Task 2.1 Setting up the data management plan (DMP)

The agreement with all WP leaders on data management, detailed in section 2.2.2 and formalized in the consortium agreement, is already in place and will be implemented upon project start. Within the first six months a detailed **DMP** will be setup together with the other WP leaders, to establish a data management framework tailored to the needs of each WP. The DMP will (i) ensure efficient and accurate standardized data exchange within MESI-STRAT and facilitates data release and sharing with the scientific community in line with patient privacy regulations and H2020 guidelines on open access to research data; and (ii) coordinate the primary analysis of all omics datasets and their conversion to formats easily accessible to modelers and bioinformaticians.

The **MESI-STRAT DMP** is based on the outcomes of the FAIRDOM project (FAIR Data Operations Models, <http://www.fair-dom.org>) and will be run by HITS, one of the core FAIRDOM partners. FAIRDOM provides the FAIRDOMHub site for data sharing and the openSEEK platform – a user-friendly, public hosted service which allows sharing of data and computational models. The **FAIR (Findability, Accessibility, Interoperability, Reusability) data management guidelines** will be the basis for our DMP and require definition of *(i) the data standards applied* (see 2.2.2, Table 7); *(ii) data exploitation and sharing/ accessibility for verification and reuse*. As detailed in 2.2.2 MESI-STRAT will provide open access whenever possible legally, ethically, and without compromising IPR and patient privacy regulations. The data will be citable using DOIs, Research Objects and Combine Packages can easily be generated and exported into OpenAIRE Zenodo website. *(iii) data curation and preservation*. Curation services is a HITS core activity, and data will be preserved for at least 10 years beyond the end of the project.

Task 2.2 Setting up the openSEEK database MESI-SEEK

The DMP will be implemented into the openSEEK instance for the MESI-STRAT project: MESI-SEEK. HITS will set up MESI-SEEK as well as a project area inside the FAIRDOM Hub. MESI-SEEK is characterized by a transparent, searchable structure, interlinking processed data to raw data, metadata and computational models according to the ISA standard (i.e., structuring as Investigations, Studies, and Assays). SOPs will be stored and linked to the data. In particular, SOPs that will be used by multiple partners, like protocols for cell cultivation and analytical methods (metabolomics, transcriptomics, proteomics) for harmonized signaling (WP3) and metabolic (WP4) data acquisition for model parameterization, will be standardized and shared via FAIRDOMHub.

- **Long-term data preservation:** FAIRDOMHub offers management facilities, secure and long-term storage of up to 1.5 TB, the ability to link data with external resources and publications, and data and model curation support that are funded within this project. The data availability will be guaranteed 10 years beyond the end of the project. This is a prerequisite for making the data citable. The ownership of uploaded data and models remains with the groups that produced them and is clearly visible within openSEEK. By agreement of the MESI-STRAT partners, the uploaded data will be accessible to all partners. The PIs take the role of “gate-keepers” who decide if and when data sets are opened to the outside world, typically in conjunction with a scientific publication. DOI references to data guarantee long-term citability. Publication of data sets and models via a structured, searchable, and citable OpenSEEK database will enhance reuse of data and models.
- **Linkage with public data:** Next to MESI-SEEK, we will also refer to available public data and link them with data generated by MESI-STRAT to enable and facilitate their integrated processing and analysis. The following data sets are currently publicly available and will be integrated with MESI-SEEK upon project start:

1) TCGA and METABRIC data sets for untreated tissues of ER+ BC patients

(*Provisional data; ** Sequencing, CNA, RNAseq-V2, microarray, miRNA, Methylation & RPPA)

Study	Samples	ER+ BC	Data Type	Patient Status	PMIDs
METABRIC	2509	1498	Sequencing	Patients were either naive to treatment, or treated in accordance to their corresponding clinical group.	22522925; 27161491
TCGA-BRCA*	1105	813	Multiple platforms**	Naive to treatment	23000897; 26451490

2) GEO data sets with data from patient's BC tissue after receiving targeted treatments (*Anastrazole, Letrozole, Exemestane). The Tamoxifen data sets are selected examples of studies comprising > 100 samples.

Targeted treatment	Studies	Samples	Data Type	Accessions	PMIDs
Tamoxifen	9	2105	RNA arrays	GSE6532; GSE17705; GSE22219; GSE22216; GSE9893; GSE37405; GSE12093; GSE12665; GSE6577	17401012; 18498629; 20479250; 20697068; 21737487; 21737487; 18347175; 22623953; 18821012; 21947828; 17404078
Fulvestrant	4	253	RNA arrays	GSE76040; GSE71791; GSE48905; GSE33658	27185372; 24916694; 24505287; 21792626
Trastuzumab	8	861	RNA arrays; SNP genotyping	GSE22358; GSE66399; GSE50948; GSE76360; GSE58984; GSE42822; GSE55348; GSE44272	21373875; 26245675; 27484801; 24443618; 26842237; 23158478; 25164009; 26334217; 25330188
Aromatase inhibitors*	12	995	RNA arrays	GSE20181; GSE5462; GSE59515; GSE16391; GSE10281; GSE55374; GSE35186; GSE48906; GSE71791; GSE18378; GSE26544; GSE41994	20697427; 20646288; 17885619; 20646288; 26033813; 19573224; 19666588; 25100562; 26033813; 22722193; 24505287; 24916694; 20428938; 21777924; 24242068

3) Data sets from GEO reporting data sets generated using male BC tumor tissue:

Accession	Samples	Data Type	PMIDs
GSE57087	75	Genome variation profiling by genome tiling array	26355282
GSE31259	74	Expression profiling by array	22333393
GSE50512	56	Genome variation profiling by genome tiling array	-
GSE17155	38	Non-coding RNA profiling by array	19664288
GSE23891	25	Genome variation profiling by array	21045282; 21547577

Task 2.3. Build adaptation tools to simplify DMP adherence

Tools for DMP adherence will be based on available tools including RightField for creation of ontology-rich data templates; iPython for web-based interactive software; OpenRefine interactive tool for spreadsheet transformation. The priorities will be based on the data flow in the project and the needs of the partners.

Task 2.4 Integration of pseudonymized patient data

In collaboration with WP1 and in line with the DMP and informed consent for the respective patients and all applicable regulatory constraints concerning data security and privacy (see DMP, section 2.2.2), MESI-SEEK will use the specimen/sample support of SEEK to set up a data collection containing pseudonymized patient information relevant for the scientific analyses within MESI-STRAT in a user-friendly and extendable format.

Task 2.5 Management of unprocessed and analyzed omics data

WP2 will coordinate collection of sequencing, proteomics, and metabolomics data, molecular kinetic measurements and biomodels (WP3-5), using MIBBI compliant tables. Analyzed data will be catalogued and deposited in MESI-SEEK to make it a one-stop shop for project members that visualizes the available data and databases managing them, and enables integration of the multidimensional data types in WP5.

Raw data that presents potential issues regarding data security and privacy will be securely stored, and sharing will be restricted, according to the informed consent, the applicable legal regulations, and best practice as established by TCGA (see section 2.2.2, Table 7). Raw data that does not fall under regulatory constraints regarding patient data safety and privacy will be catalogued in MESI-SEEK. The raw data will remain stored at the host institutions generating them, and will be regularly registered with MESI-SEEK following a standardized protocol. This is particularly important upon publication following open access standards, in line with the DMP, to provide a transparent workflow and ensure long-term mining. To ensure accessibility and long term preservation our raw data will also be submitted to repositories linked to EMBL-EBI (letters included from IntAct, PRIDE and ATLAS, ENA). All these databases are member projects of ELIXIR, which guarantees wide reusability of our data. We will interact with the ELIXIR Human Genomics and Translational Data team to ensure that our methods evolve with current best practice.

Task 2.6 Provide the required input to WP5 (computational modeling and network analyses).

Model hosting. OpenSEEK includes the JWS Online tool (<http://www.jji.bio.vu.nl/>) which hosts computational models in an interactive way allowing partners to run the models directly from OpenSEEK without needing specialized programming expertise. This improves interaction between experimentalists and modelers. JWS is SBML compliant, and the FAIRDOM team provides technical curation of the models (e.g., for standard identifiers). We will deposit ODE models and genome-scale metabolic models together with selected simulations. The modelers will document which data have been used for designing them. Model annotation will follow MIRIAM standards, and model versions will be distinguished by different names and precise annotation of updates. The project will contribute to JWS Online cost on a per-model basis.

Integrated omics platform. Once the bulk of the omics data sets are available, we will develop an integrative platform which handles transcriptomics, proteomics, and metabolomics data under a unified schema. It will then be visualized in Expression Atlas, the EMBL-EBI value-added resource for functional omics data, alongside other comparable studies.

Deliverables

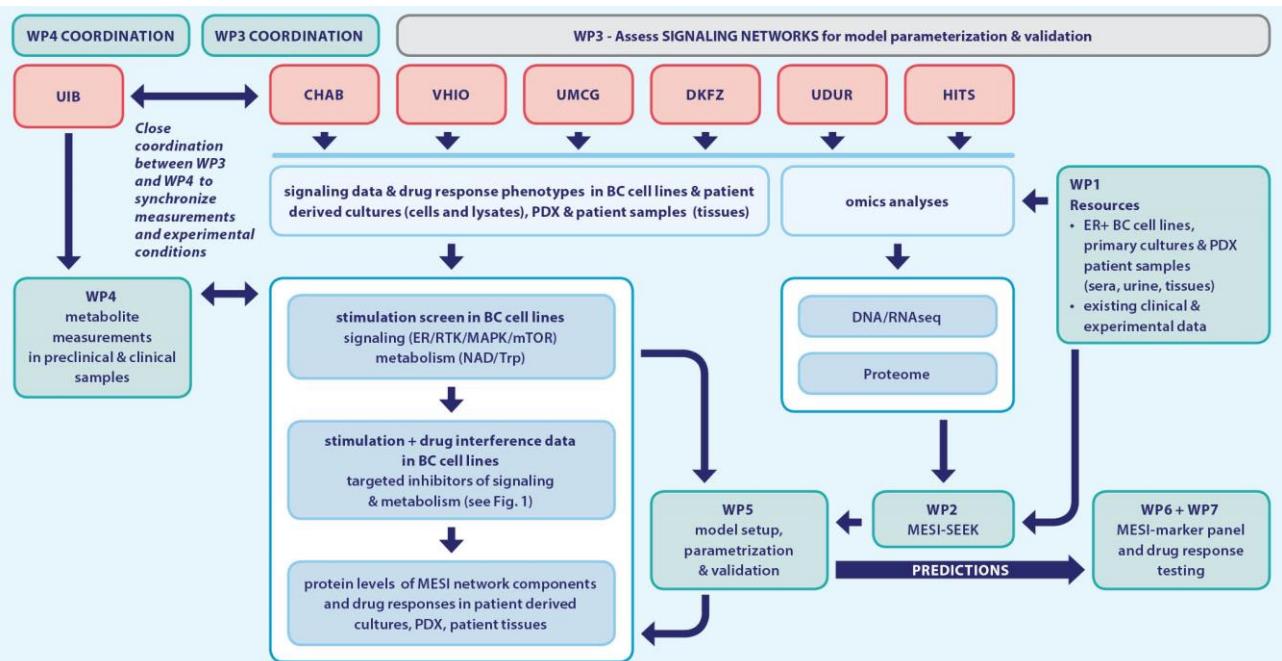
- D2.1 Data management plan (month 6)
- D2.2 Continuous release of updated MESI-SEEK versions (months 12, 24, 36, 51)
- D2.3 Omics data in public resources (EMBL-EBI & others) (month 51)

Work package number	3	Lead beneficiary			CHAB
Work package title	Assessment of SIGNALING NETWORKS for model parameterization and validation				
Participant number	1	4	5	10	11
Short name of participant	UMCG	DKFZ	VHIO	CHAB	UDUR
Person-months per participant:	69	4	3	35	8
Start month	7	End month			51

Objectives. The overall aim of WP3 is to generate quantitative and time-resolved data for network reconstruction (WP5), focusing on the MAPK/mTOR/ER signaling network and its connections to metabolic networks (Trp, NAD, and energy metabolism, WP4). The specific objectives of WP3 are

1. time-resolved multiplex measurements of signaling components (level, activation state) in ER+BC cell lines. Protocols for signaling analyses will be harmonized with those for metabolic analyses (WP4), to provide matched signaling and metabolic data for model parameterization (WP5).
 2. perturbation screens to gain information on metabolic and signaling network connectivities as a basis to establish MESI network topology for models in WP5.
 2. analyses of protein levels and phosphorylation for individualized network adaptation and verification of model predictions in cell cultures, patient-derived cultures, PDX, and patient samples.
 3. sequencing and proteomic analyses of selected samples representative of ER+ BC patient subgroups (cell lines, PDX + matched cultures, patient samples), if data is not available from public sources.

Description of work. Lead partner: CHAB. Further partners: UMCG; DKFZ; VHH; UDUR, HITS



Task 3.1 Signaling data for dynamic model parameterization. Partners: CHAB (CS) with UMCG (KT).

To derive parameters required for model parameterization, perturbation studies with stimulators and inhibitors of the MAPK, mTOR, and ER networks will be performed, as described by CS^{27,39} and KT/DPS^{31,40,41}.

Task 3.1.1. Time course data. ER+BC cell lines (defined in WP1, task 1.4) will be analyzed regarding receptor expression and activation (e.g., ER, HER2, IGF-R). Afterwards, stimulators for signaling networks in ER+BC cells will be identified by testing several **activators/inhibitors of MESI networks**, such as

- (a) growth factors: estradiol (E2), insulin/IGF, EGF family members;
 - (b) metabolites: Trp deprivation/supplementation, nicotinamide deprivation, re-addition of Trp metabolites (including L-Kyn & KA).

We will measure over time the response dynamics of the targeted **signaling network components**: RTK; adapters, such as Shc and IRS; mTOR and MAPK upstream regulators, incl. PTEN, Akt, and TSC1/2; mTORC1/2 itself; mTOR modulators, incl. PRAS40 or Deptor; mTOR substrates, incl. p70S6K, 4E-BP, and ULK1; MAPK pathway components, incl. CRAF, BRAF, HRAS, KRAS, NRAS, MEK, ERK, p90RSK p38 and p42/44 MAPK, JNK, MKKs; common MAPK and mTOR targets incl. MNK and eIF4E; and others).

This analysis will be performed on a short time scale (between 2 and 90 min) to determine (*i*) the activation kinetics of downstream messengers and (*ii*) a window of stable activation for later perturbation screens with the drugs listed below (step 2). Measurements of pathway activation will be performed via Bioplex assays, immunoblotting, and ELISAs for semi-quantitative analysis of kinase phosphorylation and protein levels, and (where necessary) by established MAPK and mTOR reporter assays, and proximity ligation assays^{26,42} to quantify kinase substrate interactions *in situ*. Absolute quantification of critical network components that define drug response will be established by targeted proteomics²⁸, which is a standard technology at the UMCG MS core facility. Targeted proteomics measurements will also be adapted and used for the analysis of patient samples. Outcomes of this first step will be discussed and adapted in close collaboration with WP5 to determine the most informative investigations for modeling, and with WP4 to derive protocols that allow observation of signaling and metabolism kinetics on similar time scales, and/or network connections via input/output modules (see WP5). In addition to short term signaling measurements we will also record cancer-relevant phenotypic responses such as cell proliferation, survival and migration, to be integrated as endpoints into the computational models. For this purpose, we will use Incucyte technology for highly parallelized live cell imaging, established in the CS and KT labs.

Task 3.1.2. Perturbation screens will be performed with the ER+BC cell lines using combinations of pathway activators (defined in 3.1.1) and inhibitors. The latter are predicted based on simulations in WP5, and include **clinically relevant compounds** (singly and in combination) for

- (i) ET: Tamoxifen, Fulvestrant
- (ii) Signaling network interference: RTK inhibitors (Sunitinib, Lapatinib), mTOR inhibitors (Everolimus, Temsirolimus; dual inhibitors such as AZD8055), PI3K inhibitors (Taselisib/GDC-0032, Alpelisib/BYL719, Buparlisib/BKM120), AKT inhibitors (Afuresertib/ASB183), CDK4/6 inhibitors (Ribociclib, Palbociclib), HER2 therapies (Trastuzumab, Pertuzumab), MEK inhibitors (Trametinib, Cobimetinib), ERK inhibitors (SCH772984), RAF inhibitors (PLX7904)
- (iii) Metabolic network interference: IDO inhibitors (Epacadostat), PARP inhibitors (Olaparib, Rucaparib), Metformin (targets energy metabolism).
- (iv) In the absence of specific inhibitors, RNA interference, CRISPR/Cas9, and fragment libraries, in place at UMCG and CHAB, will also be used to suppress network components and to have the possibility to explore networks beyond the before mentioned signaling and metabolism pathways.

Stimulators and inhibitors will be applied in a defined order, and network response will be extracted by multiplexed measurements of protein levels and phosphorylation states. Phenotypic features (proliferation, migration, survival) will be measured by automated, parallelized Incucyte live-cell-imaging screens. The observed dynamics across the network will be compared to computational simulations (WP5) and appropriate adjustments in model topology and/or generation of further data will be addressed. Altogether, this approach will provide data to derive ER+BC-specific network model topologies and dynamics in WP5.

Task 3.2 Linking signaling pathways and metabolic networks. Partners: UMGC (KT), CHAB (CS).

In parallel to the analyses outlined above, we will work closely with WP4 to determine links between signaling and metabolic networks. Optimally, we aim to apply the same experimental conditions and timescales for signaling (WP3) and metabolic analyses in WP4 (MZ, BMB, CO). To this end, an experimental routine will be set up together by WP3 and WP4 to allow parallel and SOP-directed sample processing for signaling measurement (BioPlex, Western Blot, proteomics) and metabolic measurements (see WP4), and phenotypic analyses (proliferation, survival, migration etc., see task 3.1.1). To determine links between signaling and metabolism from both directions, we will exchange and jointly adapt protocols for perturbation experiments with signaling activators (growth factors, amino acids) and subsequent measurement of metabolic pathways, and *vice versa* (e.g., perturbation with metabolic intermediates and measurement of signaling responses). If it turns out that we have to work at different timescales for certain inputs and/or metabolites, we will specifically detect molecular entities that allow connecting the metabolic and signaling models via input-output modules (e.g., expression changes of enzymes in MESI networks).

Task 3.3 Data to parameterize computational models for patient-derived cultures/PDX, & individual patients. Partners: UMCG (KT, BMB), DKFZ (CO), CHAB

To enable parameterization of our computational models for patient-derived cultures, PDX, and individual patient samples (Task 5.2), we will measure the protein levels of critical MESI-network components which control signal propagation and fluxes through the MESI-networks. To allow accurate, absolute quantification of proteins within the MESI-networks from minimal amounts of sample such as from primary 3D cultures,

PDX, patient tissues, and biopsies, we will apply targeted proteomics using peptide standards and single/multiple reaction monitoring²⁸ as routinely used by the UMCG partners (KT, BMB). If not yet available, mutation analyses of critical network components will be analyzed by BC panel sequencing at CHAB and DKFZ (<https://dktk.dkfz.de/en/research/core-facilities/genomics>).

Task 3.4 Omics-scale characterization of selected subgroup-specific ER+ BC cell lines, patient-derived primary cultures, PDX, and patient samples. Partners: DKFZ (CO), UMCG (KT), UDUR and HITS, CHAB

For selected cell lines, patient-derived cultures, PDX and patient samples, representative of ER+BC subgroups with distinct MESI marker panels and different drug responsiveness, we will perform quantitative omics-wide RNA and protein expression analyses. We will run these analyses for up to 3 subgroups with up to 20 patients, 4 PDX models, and 4 matched cultures per subgroup.

For RNAseq, 100 ng to 1 µg (depending on sample type) of total RNA will be used to prepare strand-specific libraries and carry out library QC according to established protocols at the DKFZ genomics facility to generate a minimum of 200 million reads of 2x 100 bases of paired end reads per sample. Basic data analysis will be carried out at DKFZ following existing and established pipelines.

Quantitative shotgun proteomics will be conducted label free or by chemical (TMT) labeling following established procedures at the UMCG interfaculty MS Center and in KT's laboratory^{31,43}.

Analyzed data will be stored and curated within MESI-SEEK (see WP2) to develop an integrative platform which handles transcriptomics, proteomics, and metabolomics data under a unified scheme. It will then be visualized in Expression Atlas, the EMBL-EBI value-added resource for functional 'omics data, alongside other comparable studies, and provided to WP5 for network analyses.

Deliverables

- D3.1 Full signaling time course datasets for ER+BC cell lines (month 18)
- D3.2 Perturbation screens finalized (month 30)
- D3.3 Analyzed multi-level omics data (month 45)
- D3.4 Signaling marker panels for patient derived models and samples measured (month 51)

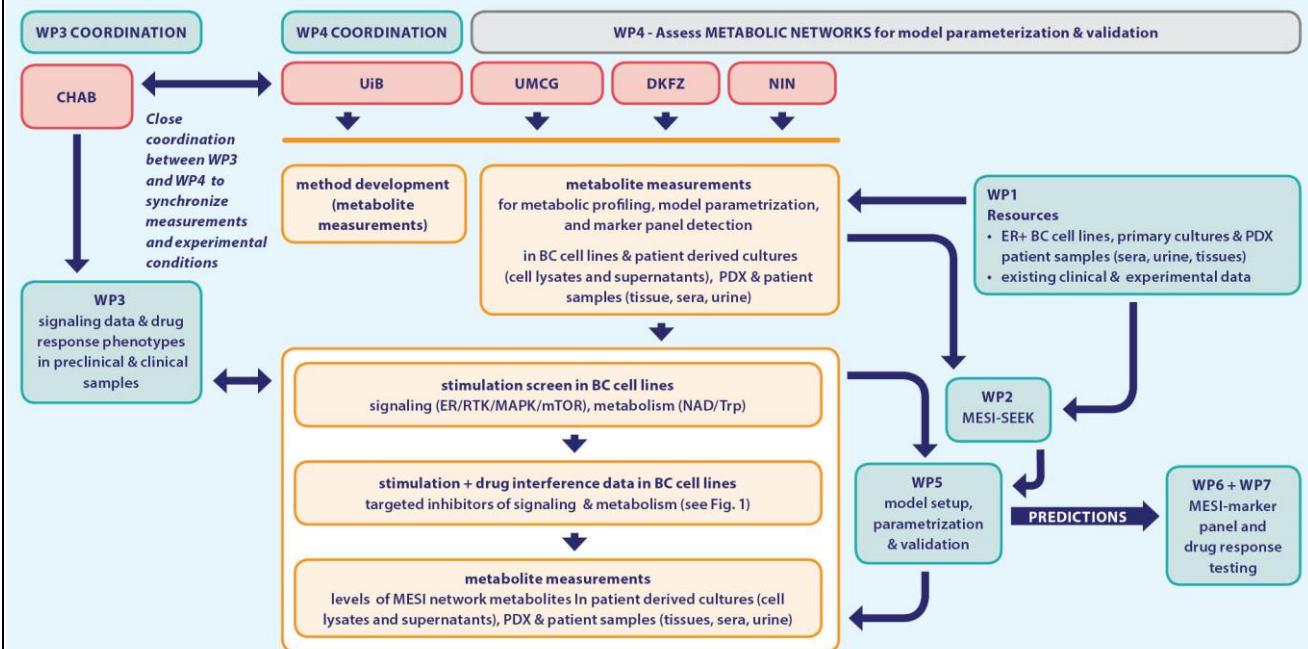
Work package number	4	Lead beneficiary	UiB		
Work package title	WP4 - Assess METABOLIC NETWORKS for model parameterization and validation				
Participant number	1	4	7	10	12
Short name of participant	UMCG	DKFZ	UiB	CHAB	NIN
Person-months per participant:	12	51	33	1	15
Start month	7		End month		51

Objectives. The overall aim of WP4 is to identify distinct metabolic alterations in experimental models (ER+BC cell lines, patient-derived cultures, PDX) and patients' body fluids and tumor tissues. This includes characterization of various model systems regarding quantitative measurements of metabolites and metabolic fluxes, and iterative parameterization and validation for the mathematical models generated in WP5.

The specific objectives of WP4 are to:

1. Optimize and standardize reproducible procedures for metabolite extractions and analyses;
2. Establish protocols and provide quantitative metabolic data for parameterization of computational models (WP5), in close conjunction with WP3, through; comprehensive, dynamic metabolic profiling of ER+BC cell lines; identification of metabolic shifts in a signaling and metabolic intervention screens; detection of metabolic fluxes; metabolic characterization of patient-derived matched cultures and PDX models and patient samples
3. Validate models (WP5) by targeted analyses of critical pathways/metabolites
4. Based on model predictions (WP5), establish metabolic profiles (MESI marker panels) in body fluids from patient-derived models and patients, representative of ER+BC subgroups with different drug responsiveness.

Description of work. Lead partner: UiB (NAD and general bioenergetics). **Further partners:** DKFZ (CO, Trp, and NAD metabolites), NIN (Trp metabolite ELISAs), UMCG (BMB, energy metabolism)



WP4 will perform metabolomics analyses of the Trp and NAD networks, and of other key metabolites identified by network analyses in WP5 as being relevant for ER+BC patient stratification. All necessary protocols are in place in the partner laboratories. In collaboration with WP3, protocols and culture conditions will be optimized to measure signaling and metabolite dynamics under the same stimulation conditions with growth factors and/or metabolic intermediates, and inhibitors of signaling and metabolism (detailed in WP3). Comprehensive profiling of intra- and extracellular Trp and NAD metabolites and abundance of key metabolic enzymes by targeted proteomics (WP3) will be performed to provide initial metabolite parameters to build the mathematical models in WP5. In addition, metabolic fluxes for the Trp and the NAD networks will be measured by stable isotope labeling (DKFZ, UiB). Depending on the outcome of network analyses (WP5) and feasibility, other relevant metabolic pathways will be analyzed, such as energy metabolism (UMCG).

Model development (WP5) for individual patients and identification of ER+BC subgroups with similar MESI marker panels and drug responsiveness will be supported by metabolic profiling of PDX and patient samples. The results of these analyses will be critical to select (WP5) MESI marker panels for validation in patient samples from observational and interventional clinical studies (WPs 6 and 7).

Task 4.1 Development and validation of standardized procedures to extract a comprehensive set of metabolites from biological samples

This task will establish validated standard protocols for the reproducible, quantitative extraction of the **metabolites to be analyzed**. Initially, we will consider the following metabolites:

- Trp metabolism:** Trp, Kyn, kynurenic acid, anthranilic acid, hydroxykynurenone, formylkynurenone, hydroxyformylkynurenone, hydroxytryptophan, serotonin, anthranilic acid, hydroxyanthranilic acid, quinolinic acid, xanthurenic acid, cinnabarinic acid, tryptamine, indole-3-pyruvate, indole-aldehyde, indole lactate, indole acetic acid, 5-hydroxyindole acetic acid;
- NAD metabolism and adenine nucleotides:** nicotinamide, N1-methylnicotinamide, nicotinic acid, NAD, NADH, NADP, NADPH, nicotinamide riboside, nicotinamide mononucleotide, nicotinic acid mononucleotide, ATP, ADP, AMP.

A novel multiplex method to measure Trp and its metabolites by chemical labeling with isobaric mass tags and LC-MS/MS has been developed and patented by CO, DKFZ (filed October 31 2016, Application n° PCT/EP2016/076265). This setup will be employed for the analysis of the Trp metabolome, and allows high precision and time efficiency by comparison of compounds in up to ten different samples in the same run (e.g., of time course analyses, biological replicates or multiple sample comparisons *in vitro* and *in vivo*). UiB has also recently developed measurements of NAD metabolites and the related metabolic fluxes based on isotope labeled metabolites. We will also measure other relevant metabolites identified by network analyses in WP5 to be significantly altered in different ER+BC subgroups. For example, we are prepared to measure additional metabolites

from energy metabolism: acetyl-CoA, succinyl-CoA, and glycolytic (phosphorylated sugars, pyruvate, lactate), TCA cycle (citrate, alpha-ketoglutarate, succinate, fumarate, malate), and pentose phosphate pathway intermediates; likely some of them will emerge as critical from the analyses in WP5.

Currently, different extraction procedures are used in the different partner laboratories for the before mentioned metabolites, due to different chemical instabilities. These procedures will have to be optimized and harmonized, because (i) for some of the samples (in particular patient samples) multiple extractions may not be feasible, (ii) it will be necessary to combine data sets from independent cultures or even from different laboratories.

Task 4.2 Medium-throughput metabolic profiling of ER+ BC cell lines for model parameterization

Dynamic, quantitative analyses of metabolites (detailed above) will be conducted by CO (Trp) and MZ (NAD) using established quantitative UPLC-ESI-MS/MS technology.

Further analyses are designed to quantify metabolites, enzyme concentrations and activities in a physiological range. These include

- intracellular Trp measurements using a genetically encoded Trp sensor based on the Trp-activated repressor protein, TrpR (CO)⁴⁴;
- semi-automated activity assays for selected enzymes in NAD (MZ) and Trp (CO) metabolism;
- measurements of enzyme concentrations in NAD, Trp and other metabolic pathways by quantitative, targeted proteomics with labeled standards, as detailed in WP3;
- flux measurements using stable isotopes (MZ, CO, BMB): determine metabolite consumption and production by analyzing time-dependent conversions of isotope-labeled metabolites in the various pathways;
- ELISA-based measurements of Trp metabolites (KK, NIN), incl. Trp, Kyn, QA, KA, allowing analysis of a broader set of samples and translation of MESI panels to clinical applications.

The data from these measurements will be provided to WP5 for model parameterization.

Task 4.3 Linking signaling pathways and metabolic networks

To connect signaling and metabolic responses, it is critical that treatments are conducted under the exact same conditions in WP3 and WP4 (WP3 and above). In addition, the timescale of the experiments will be optimized together and a stimulation and perturbation screen (described in WP3) will be performed to reveal not only connections *within* signaling and metabolic pathways, but also *between* signaling and metabolism. If different timescales turn out to be required for some metabolic measurements, molecular connection points that can serve as hubs for input–output model connections between metabolic and signaling models will be identified (WP5) and included in our measurements.

Task 4.4 Data for model refinement and validation of model-derived subgroup-specific MESI marker panels and drug responses

The computational models (WP5) describing the MESI-networks in ER+BC cell lines will be refined by re-assessment or extension of the set of metabolites, as needed. Analyses for refinement will include, e.g., kinetic analyses of key enzymes and/or measurement of additional enzyme activities important in the respective networks. Modification of flux measurements might also be needed (e.g., use of different/additional time points). Model-predicted ER+BC subgroup-specific MESI panels (WP5) will be validated by targeted metabolite measurements in cell lines, cultures, PDX and patient samples (WPs 1, 6, 7) with or without relapse, and treated with pharmacological modulators of signaling and metabolism (outlined in WP3).

Deliverables

D4.1 Full metabolite time course datasets for ER+BC cell lines (month 18)

D4.2 Metabolite marker panels for patient derived models and samples measured (month 51)

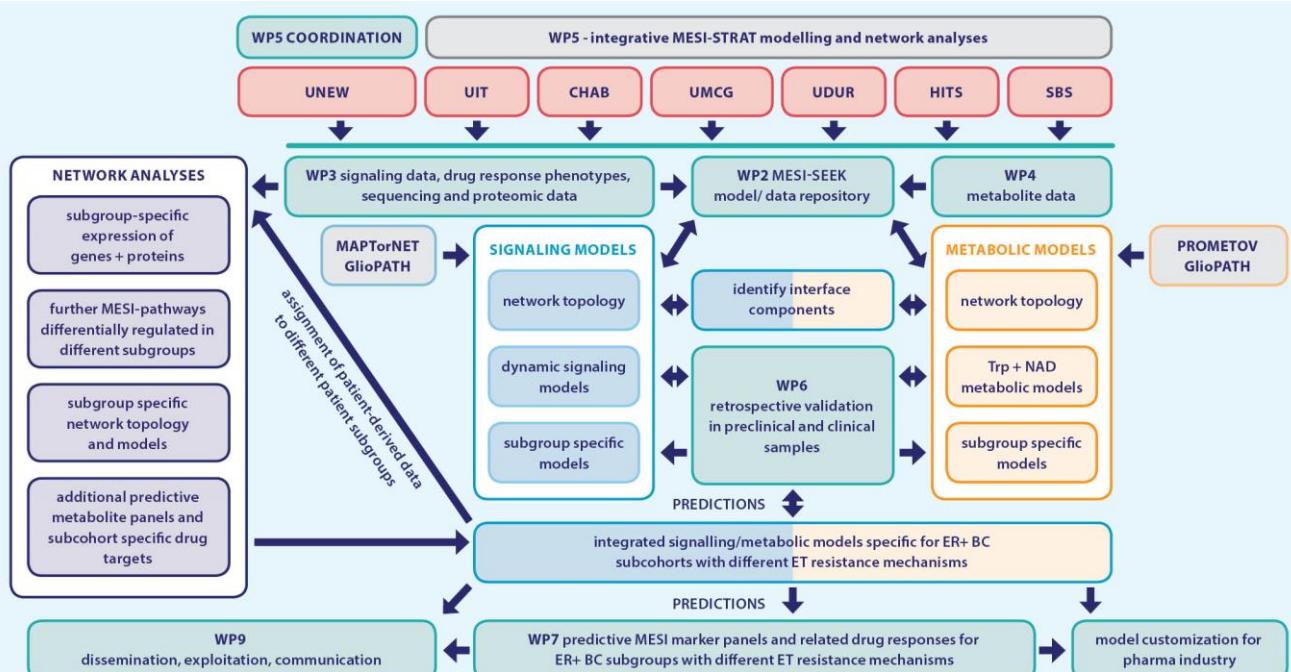
Work package number	5	Lead beneficiary	UNEW				
Work package title	WP5 - Integrative MESI network modeling and network analyses						
Participant number	1	8	9	10	11	13	14
Short name of participant	UMCG	UiT	UNEW	CHAB	UDUR	SBS	HITS
Person-months per participant:	56	35	42	6	20	12	1
Start month	7	End month			51		

Objectives. The overall aim of WP5 is to use mathematical modeling and network analysis to (i) stratify ER+BC patients into subgroups with different aberrations of metabolism and signaling, (ii) predict related differences in MESI-marker panels, (iii) predict and understand the subgroup-specific mechanisms mediating response or resistance to ET and targeted drugs, and (iv) identify alternative combinatorial treatment options.

The specific objectives are to:

1. adapt existing dynamic models of kinase signaling and metabolism for ER+BC cell lines.
2. outline the connection topology and scale the component levels for MESI networks in ER+BC cells.
3. adapt computational models to patient-specific data from PDX, primary cultures, and clinical samples.
4. predict MESI marker panels to stratify ER+BC patients into sub-groups with different therapy response and drug resistance, and monitor their drug response and arising resistance; alternative established treatments for ER+BC patients with drug resistance; and new treatments and targets for improved (combinatorial) drug targeting of MESI networks.

Description of work. Lead partner: UNEW. Further partners: UiT, CHAB, UMCG, UDUR, HITS, SBS



We will build predictive dynamic mathematical models of integrated MESI networks, which will be initially parameterized using ER+BC cell line derived semi-quantitative time course data (WP3, 4), and will be adapted step-wise based on quantitative measurements of protein and metabolite levels to patient derived cultures, PDX, and patient samples (WP1). Network analyses will serve to identify potential additional subgroup-specific metabolites to enrich our predictive MESI marker panels. The predictive power of our models regarding MESI-marker panels and related drug response or resistance will be validated and refined by preclinical and clinical trials (WP6, 7). Validated models will be customized for pharma partners. All data will be received from and models will be hosted by the MESI-SEEK data and model management platform (WP2).

Task 5.1 Describe the topologies and build and connect dynamic models of ER-mTOR-MAPK signaling and Trp-NAD metabolism. Partners: UNEW, UiT, CHAB, UMCG

First, we will build separate core dynamic models of MAPK and mTOR signaling. At the same time, we will extend and refine existing computational models of Trp and NAD metabolism. A combination of COPASI⁴⁵ and Data2Dynamics⁴⁶/MATLAB software, designed by our IAB member A. Raue, will be used for these tasks. Using available information and expertise of MESI-STRAT partners and related consortia (GlioPATH, MAPTOrNET,

PROMETOV) on the network structures and dynamic properties of metabolic and signaling (MESI) models, we will work out the topologies and create dynamic models of ER-MAPK-mTOR signaling (UNEW, CHAB, UMCG) and Trp-NAD metabolism (UiT). Based on quantitative time course signaling and metabolite data from ER+BC cell lines (WP3, 4) we will parameterize and validate the MESI models. For model fitting to data, parameter identifiability will be assessed using methods such as profile likelihood⁴⁷ and estimation will use both global and local optimisation algorithms. Perturbation screens in which signaling or metabolic network components are inhibited and the effects on all parts of the MESI networks are monitored will reveal ER+BC specific network connections within and between the signaling and metabolic networks, and will serve to connect the separate network models either by merging them into one model, or by input-output schemes.

Task 5.1.1 Dynamic ER-MAPK-mTOR network models for ER+BC cells. Partners: UNEW, UMCG, CHAB
 We will develop dynamic ODE-based models of signaling pathways in ER+BC cell lines (detailed in WP1), as described earlier by the partners for computational mTOR^{31,40,41} and MAPK^{27,39} models. We will incorporate information on mutational profiles if their effects on the activity of network components are known (e.g., PIK3CA or PTEN mutations activate the PI3K-Akt pathway). Experimental data for steady-state and dynamic, time-course responses of RTKs, adaptors and downstream signaling proteins (WP3), to growth factors and metabolites (WP3, 4), and inhibitor perturbation data (WP3) will be used to calibrate the models in a modular approach, as data from WP3 becomes available. Powerful model analysis techniques (e.g. global and local sensitivity analyses, systematic combinatorial perturbations simulating effects of RNA interference, inhibitors and drugs) will enable us to determine critical nodes and dissect the network response dynamics. Robust global estimation methods such as adaptive simulated annealing will return large ensembles of permissible fitting parameters, which will allow characterizing the uncertainty of the model predictions. The predictive value of the dynamic models will be validated by comparing model simulations to drug perturbation data (detailed in WP3) that has not been used for model parametrization.

Task 5.1.2 Computational models of Trp and NAD metabolism for ER+BC cells. Partners: UMCG, UiT
 We will refine and extend existing models of Trp- and NAD-metabolism, using available information on the network topology and interface components with signaling models^{17,30,48}. Experimental data obtained for ER+BC cell lines under steady state and dynamic conditions in WP4 will be used to calibrate the models based on measured expression levels of components (expression data, protein concentrations, V_{max}) and fitting the models to metabolic fluxes and metabolite time courses. Where possible primary biochemical data, such as affinity constants for substrates, products, inhibitors and activators, will be used. Initial validation of the predictive value of the models will essentially be conducted as in 5.1.1, e.g., by predicting the effects of metabolic interventions (e.g., IDO inhibitors, Olaparib/Rucaparib), on the concentration of Trp or NAD metabolites (detailed in WP4).

Task 5.1.3 Link signaling and metabolic network models. Partners: UNEW, CHAB, UMCG, UiT
 Once separate models for signaling and metabolism have been built, parameterized, and validated, they will be connected. To facilitate the integration of our metabolic and signaling models, the signaling data (WP3) and the metabolic data (WP4) will be measured under identical conditions. Initial information on integration of mTOR-MAPK signalling with Trp-NAD metabolism networks will come from literature and collaborating consortia (GlioPATH, MAPTor-NET, PROMETOV), and be communicated to WP3 and 4 to be included in experimental analyses. Perturbation screens with drugs targeting signaling or metabolism (detailed in WP3) and RNAi or Crispr-Cas9 libraries, and measurement of signaling components (detailed in WP3) and metabolites (detailed in WP4) will deliver information on ER+BC specific connectivities within and between ER-mTOR-MAPK signaling and Trp-NAD metabolic networks. Iterative rounds of modeling, experimental testing and model refinement will be needed to identify potential missing connections and mutual influences of the pathways. For fully integrated MESI models, model complexity might have to be reduced to ensure identifiability of the model parts that are critical for predictions of drug response and associated MESI marker panels. Time-scale separation between signal transduction and metabolic adaptation may be an issue impeding the setup of fully integrated dynamic MESI models. Therefore, an alternative approach will be to use an input-output coupling to combine metabolic and signaling models. For this, the metabolic models will be tuned for different metabolic conditions (such as high and low Trp due to different IDO levels), and the related metabolite levels will be predicted. These metabolite levels will be used as inputs to the signaling models to define the activity of the metabolite-sensitive signaling network components, determined by the perturbation screens (WP3, 4). Likewise, the metabolic models can be tuned to different signaling conditions (e.g., high versus low ER, PI3K or mTOR activity) and the effects on MESI marker panels and their response to drug interventions can be predicted. Once the models are linked we will also include quantitative phenotypic data from WP3 (cell proliferation, survival, migration etc.)

to integrate cancer-relevant phenotypic modeling endpoints. To achieve this, we will consider the phenotypic processes as additional nodes in the networks and infer the specific connections and their relative importance by applying model selection strategies as we have carried out with molecular component^{31,41}. Confidence in putative connections will be enhanced with gene ontology analysis of omic data from Task 5.5.

An initial quality check will be performed by analysing model predictions for our observations (see section 1, preliminary data, **Fig. 2**) that ET induces IDO and reduces Trp levels, and that an mTOR inhibitor phenocopies this effect. To pass the quality check, the models have to correctly predict these observations. For further validation, data from experiments (WP3, 4) in which metabolism will be monitored upon interventions in signaling and *vice versa* (see WP3 for detailed lists of metabolic and signaling inputs and perturbations) will be compared to model simulations. Based on the resulting data from WP3 and 4, and their fit with model simulations, we will suggest model improvements and initiate a next round of experimental testing.

Next, we will perform sensitivity analyses of our MESI models to identify components whose levels/activity alter responses to ER+BC drugs (listed in WP3) for **modeling endpoints** including oncogene activity (e.g., Akt, PI3K, mTOR, MEK/ERK and phenotypic features (proliferation, survival, migration). The resulting list of critical network components (proteins/metabolites) will be provided to WPs 3 and 4 for measurement in PDX and patient samples to enable individualized model parametrization in Task 5.2.

Task 5.2 Adapt dynamic models to patient derived cultures and PDX. **Partners:** UMCG, UiT, UNEW, CHAB A critical step will be the adaptation of our models from ER+BC cell lines to patient samples from which dynamic time course data can usually not be obtained. Our approach is based on the idea that enzyme activities *per se* are the same in all systems (cell lines, PDX, patient tumor tissues), and are modulated by differences in enzyme abundance and mutations in individual systems or patients. This means that dynamic models, parametrized based on time course data from cell lines, can be parametrized for other samples in which time course data are not easily measureable, by altering protein levels and accounting for mutations in central network components. This concept is frequently used in metabolic studies and recently shown to be applicable²⁵ to signaling networks. Thus we will adapt the models based on ER+BC cell lines (task 5.1) to patient derived cell cultures and PDX based on differences in protein levels, measured by targeted proteomics (WP3), and mutations with known effects on signaling and metabolic networks analysed here. We will validate that the dynamic simulations properly reflect signaling and metabolic dynamics and perturbation responses in patient-derived systems. For this we will measure basal levels of signaling proteins and metabolites in four PDX and matched 3D cultures, of which always two show similar drug responses as determined by the VS lab (VHIO). For model validation, the MESI network response dynamics to perturbations with growth factors, metabolites, and drugs (detailed in WPs 3+4) will be simulated and compared to measured time course data (cultures) and drug responses (PDX).

Task 5.3 Pharmacogenomics I: derive patient-specific models, and predict ER+BC subgroups with distinct drug response mechanisms and different MESI marker panels. **Partners:** UNEW, UMCG, UiT, CHAB Next we will parametrize the models based on protein (WP3) and metabolite (WP4) levels and mutation analyses for individual patient samples. To do this, we will analyse our models parametrized for ER+BC cell lines, patient cultures and PDX and predict signaling proteins and metabolites that critically alter metabolite marker panels or drug responses. Model parametrization based on these protein & metabolite panels will be validated and refined by measurements in up to three matched PDX and 3D cultures. The same refined panels will then be measured in patient tissues and body fluids for subsequent model parametrization. Based on the dynamic simulations of growth factor/metabolite stimulation and drug response dynamics for individual patients, we will group the patients into subgroups with common drug response mechanisms and MESI panels.

Based on this we will predict:

- patient subgroups with common therapy response/resistance mechanisms to ET and targeted drugs (listed in WP3), and MESI marker panels to distinguish between these subgroups
- optimized treatments for combinatorial targeting of signaling and metabolism with established drugs
- new targets for improved (combinatorial) drug targeting

For example, for our Relapse Prediction Trial (WP6, trial 6.3) metabolite panels or signaling network components (detailed in WPs 3+4, and narrowed down as described above) will be measured in the exploratory cohort. Based on model parametrization with the measured protein levels, we will predict metabolite panels that distinguish stable disease from future relapse, and we will validate these predicted markers by comparison to our metabolite measurements in the exploration and validation cohorts. Next we will computationally analyse the mechanistic differences in signaling networks that potentially lead to ET resistance. By simulating drug perturbations, we can propose combinatorial targeted drug interventions which sensitize patients with different ET resistance mechanisms for ET.

Similar schemes will be run for model-based analyses of the Risk and Relapse Detection (trials 6.1 and 6.2) as well as the WOO and ET Termination Trials (trials 7.2 and 7.3) to predict and validate MESI-marker panels to detect progression risk at the start of ET (6.1), ET response at the start of ET (7.2, WOO), and relapse during ET (6.2) and after ET (7.3); and to predict suitable alternative targeted therapies for high risk patients and ET resistance at all these stages.

Task 5.4 Pharmacogenomics 2: guide experiments to validate ER+BC subgroups with distinct drug response mechanisms and different MESI marker panels in preclinical trials and clinical cohorts

Partners: UMCG, UiT, UNEW, CHAB. We will validate MESI marker panels predicted to correlate with different ET resistance mechanisms and related different responses to targeted therapies in our Intervention Validation Trial (WP7, trial 7.4). For example, if our models, based on data from the the Relapse Prediction Trial (6.3), suggest that a given MESI marker panel predicts at ET start a later relapse (i.e., ET resistance) we will measure this panel in samples from the PRAEGNANT study and analyse correlation with time of relapse. If the MESI model, parametrized based on the MESI panel, predicts sensitization of the tumor to ET by a given targeted treatment, e.g., CDK4/6 inhibitors or mTOR inhibitors, the panel will be tested in the respective cohorts (mTOR: Exemestane/Everolimus study; CDK4/6: Parsipal, Ribecca). Based on the validated MESI marker signatures, we will assign our ER+BC PDX models to the different patient subgroups (Preclinical Model - Subgroup Assignment, WP6, trial 6.5). In the subgroup-matched PDX models we can run prospective preclinical trials to test if the drug responsiveness of the PDX models corresponds to the responsiveness predicted by the computational models (WP7, trial 7.1, Preclinical Intervention Trials). Ultimately, prospective clinical IIT will need to be designed and initiated, and the panels and related therapies may be also included in umbrella trials to be designed and applied for in WP7 (task 7.5).

Task 5.5 Network analyses to identify MESI-ancillary networks and broaden MESI marker catchment for ER+BC patient subgroups. **Partners:** UMCG, UiT, UNEW, CHAB, UDUR, HITS

It could be that not only the signaling (ER-mTOR-MAPK) and metabolic (Trp-NAD) networks tested here, but also other networks differ between the patient subgroups. To identify such differences and refine our MESI-marker panels and computational models predictive of the drug responses, network analyses of omics wide expression data at RNA and protein level for cell cultures, PDX models and patient samples representative of the previously identified ER+BC subgroups will be conducted.

We will examine public resources of ER+BC expression data, e.g., from TCGA and METABRIC, but based on earlier reports⁴⁹, we expect that although useful to identify tumour-specific pathways, the datasets are too complex to identify patient subgroups without further information. We will tackle this issue by searching our ER+BC subgroup-specific patterns of differently expressed MESI network components in RNA expression data, and use this information to stratify the public datasets into our subgroups to identify additional candidates.

Of note, proteomic data is very scarce in public resources. Therefore, our own analyses will mainly focus on protein expression (WP3) which we will analyse in conjunction with RNAseq (expression) and panel seq (mutation) data to identify expression differences at protein level between the subgroups and correlate proteomic information (KT⁴³) with RNA expression, splicing (SG^{50,51}) and mutation information (RF⁵²) (proteogenomics). RNAseq data will be mapped to the GrCh38 release and tools such as TopHat2⁵³ and cufflinks⁵⁴ will be used on our high depth sequencing data to identify potential differentially regulated isoforms that could be matched to our proteomics data. Further normalization and gene differential expression will be carried out using different tools limma (Voom)^{55,56}, DESeq2⁵⁷ and edger⁵⁸, applying univariate and multivariate analyses. We will also collaborate with EMBL-EBI (see letters) in using integrative network analysis of RNAseq and proteomics data to identify genes interacting with the MESI network (via IntAct), as well as whose expression profiles are kinetically correlated. This will involve integration of the data sets at multiple levels for systematic identification of cross-talk among key MESI pathways by using random walk on protein interaction networks to identify ancillary MESI network regulator genes bridging these cross-talking pathways.

Additional lists of candidates with subgroup-specific expression patterns will be followed using a step-wise bioinformatic network analysis pipeline entailing supervised and unsupervised data driven methods. To group candidates into pathways, we will use a combination of both GSEA and GSA on the MSigDB⁵⁹ gene sets, especially C2 (KEGG & REACTOME), C5 (GO) and C7 (Immune signatures). Additional unsupervised consensus clustering will be applied to refine unique features of our previously defined subgroups. To overlay different dataset types (mainly RNA expression and mutation profiles), eQTL analyses will be performed. Methods such as Bayesian network analysis and tools such as Cytoscape⁶⁰ and Ingenuity Pathway Analysis and MetaCore will be used for robust network mapping and reconstruction with the multi-omics datasets overlay. Subgroup-specific additional, ancillary MESI networks can be analysed by genome scale modelling where, in the absence

of detailed kinetic data, methods such as constraint-based modelling and flux balance analysis can be used to predict related differences in the abundance of secreted metabolites between the subgroups, as described by BMB⁶¹. This can be validated experimentally (WP4) by measuring the newly predicted metabolites in cultures, PDX models, and patient samples representative of the different subgroups. Alternatively, if the size of the ancillary networks allows it (max. 20 components) we will directly integrate them into our dynamic ODE-based subgroup specific MESI-models. For some pathways frequently altered in cancer, such as energy metabolism, we already have detailed dynamic models for glycolysis³² and fatty acid oxidation⁶² (BMB) that can be integrated into our dynamic MESI models. For others we may need to develop new models. As this is a considerable effort, we will limit the number to maximum of three newly identified networks which encompass subgroup-specific drug targets and MESI markers. For these, dynamic data from cell lines and protein and metabolite levels in PDX models and patient samples will be acquired to integrate these networks into the sub-group specific models predictive of drug response and specific MESI marker panels. The widened MESI models and marker panels will have the advantage that more MESI metabolites can be measured in patient samples, enhancing the level of confidence for the predictive value of the marker panels.

Task 5.6 Model Customization for pharma clients. Partners: SBS, UMCG, UNEW, UiT

SBS will contact potential clients to introduce the MESI-STRAT models and their potential use, discuss clients' strategies for the development of new BC therapies and identify parallels between the capabilities of the models and client's needs. Frequent interaction will be made, e.g., with Novartis and Merrimack (both on our IAB) and with the pharma companies involved in our linked trials (see 1.3.a) for feedback on model development and prioritization of therapeutics to be considered for model development. Together with the pharma stakeholders, SBS will identify the interactions between the drug candidate of the client (pharma) company and pathway elements such as elements of mTOR pathway in MESI-STRAT models to analyse the potential effect, efficacy and mode of action of the drug candidate. The customization is done in a step-wise approach where SBS performs data mining on the drug candidate and its potential interaction within the cell, literature search, data integration, model writing, model integration and simulation runs. In case a cross talk with other relevant pathways is observed during the data mining and literature search steps, SBS will integrate the models of the additional pathways to MESI-STRAT models within its capabilities and capacity. SBS adopts hybrid modelling strategy utilizing logical and kinetic modeling of signaling and metabolic pathways at intracellular level (including flux balance analysis, rule-constraint based approaches and alike). Parametrization of the models will be done based on the data from WP1, publicly available resources, as well as from clients' proprietary data if available. Based on the developed models and simulations, SBS analysis produces outputs such as predictions about the dosage, drug efficacy, drug efficiency, mode of action and advice towards downstream research such as animal and clinical studies, which can be done in a more cost and time efficient based on results from simulations.

Deliverables

- D5.1 Computational signaling models for ER+BC cell lines (month 18)
- D5.2 Computational metabolic models for ER+BC cell lines (month 18)
- D5.3 Linked metabolic & signaling (MESI) models (month 24)
- D5.4 First MESI model parametrized for patient samples (month 27)
- D5.5 First customized model for pharma (month 36)
- D5.6 Omics data for MESI-ancillary networks integrated (month 51)

Work package number	6	Lead beneficiary	DKFZ			
Work package title	Preclinical and clinical trials without drug treatments in ER+BC patient-derived models and the longitudinal PATH cohort					
Participant number	1	2	3	4	5	6
Short name of participant	UMCG	PATH	UHH	DKFZ	VHIO	DDI
Person-months per participant:	2	8	8	35	3.5	2
Start month	1	End month			51	

Objectives. The main aim of WP6 is to derive MESI marker panels that identify clinically relevant ER+BC patient subgroups and to find preclinical models (matched primary cells and PDX models) representative for the distinct ER+BC subgroups. Our specific objectives are

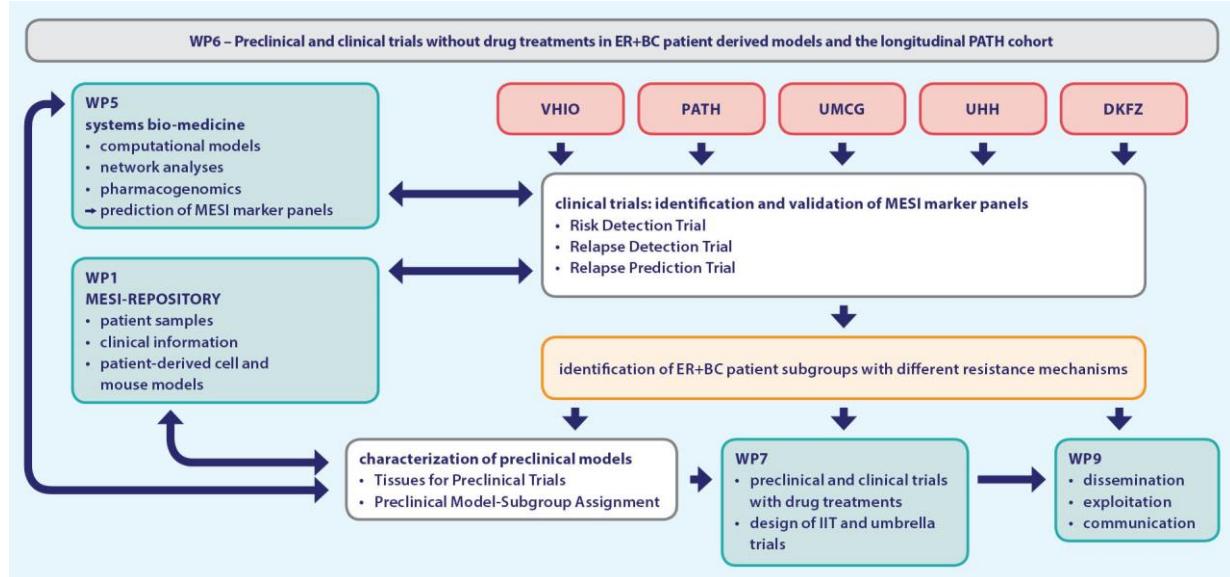
1. Identification and validation of MESI marker panels to discriminate high risk versus low risk ER+ BC

patient subgroups; detect ER+BC patient subgroups with relapse; and predict at diagnosis patient subgroups with future relapse

=> Identification of ER+BC patient subgroups with different resistance mechanisms

2. Identification of preclinical models representative for different ER+BC patient subgroups

Description of work: Lead partner: DKFZ, further partners, PATH, UHH, UMCG, VHO



Task/Trial 6.1 Risk Detection Trial: Identification and validation of MESI marker panels discriminating high risk versus low risk ER+ BC patient subgroups. Partner: PATH

Analysis of 66 sera collected at diagnosis from women with high and low risk ER+ BC, respectively, (for high/low risk criteria see WP1) for signalling pathways (WP3) and metabolites (WP4) will allow identification of MESI marker panels (WP5) detecting high/low risk in ER+BC patients. To validate if the identified markers indeed discriminate between high and low risk patients, 66 sera of women with high and low risk ER+BC, respectively, will be analysed in a blinded fashion and assigned to high and low risk groups according to their MESI marker panels. Comparison with the known clinical characteristics will allow validation of the discriminatory power of the identified MESI markers (see Figure 5A). In addition, selected tumor tissues representative for specific MESI markers signatures will be analysed in detail regarding their signaling and metabolic networks to gain a better understanding of the molecular mechanisms underlying the MESI marker panels discriminating high and low risk patients.

Task/Trial 6.2 Relapse Detection Trial: Identification and validation of MESI marker panels detecting ER+BC patient subgroups with relapse. Partners: PATH, UHH, UMCG, VHIO, collaborator: E. Boven

60 prospectively collected sera of stable patients from the PATH cohort matched to 60 sera collected from ER+BC patients at relapse/distant metastasis in the frame of studies (appropriate baseline samples from the PRAEGNANT, IMPACT or Everolimus/Exemestane studies) will be employed. Analysis of signalling pathways (WP3) and metabolites (WP4) in these sera will allow identification of MESI marker panels (WP5) detecting relapse in ER+BC patients. To validate if the identified markers indeed detect relapse, 60 sera of matched stable or relapsed women, respectively, will be analysed in a blinded fashion (WP3&4) and assigned (WP5) to relapsed vs. stable groups according to their MESI marker panels. Comparison with the known clinical characteristics of the patients will allow validation of the discriminatory power of the identified MESI marker panels to distinguish between stable and relapsed patients (see Figure 5B).

Task/Trial 6.3 Relapse Prediction Trial: Retrospective identification and validation of MESI marker panels at diagnosis predicting patient subgroups with future relapse or stable disease. Partner: PATH

Analysis of 50 sera obtained at diagnosis from patients that developed relapse/distant metastasis during follow-up compared to 100 sera from patients that remained stable for > 5 years for signalling pathways (WP3) and metabolites (WP4) may allow identification of MESI marker panels (WP5) predictive for future relapse/distant metastasis. To validate if the identified markers indeed are able to predict future relapse/distant metastasis, 75 sera will be analysed in a blinded fashion (WPs 3-5) and assigned to deriving either from stable or relapsed

patients according to their MESI marker panels. Comparison with the known clinical characteristics of the patients will allow validation of the predictive power of the identified MESI marker panels for future relapse. Comparison with the markers identified in Task 6.1 and 6.2 will determine whether the markers for high risk ER+BC at diagnosis and acute relapse overlap with those for developing future relapse (see Figure 5C). In addition, selected tumor tissues representative for MESI markers panels of patients that remained stable or relapsed during follow-up will be analysed in detail regarding their signaling and metabolic networks (WPs 3&4) to investigate the molecular mechanisms underlying the MESI markers predictive for future relapse.

Task/Trial 6.4 Tissues for Preclinical Trials: Screening and collection of ER+BC tissues with subgroup-specific MESI marker panels for preclinical models (PDX, bioreactor). Partners: VHIO, UHH

Fresh human ER+BC tissue for cultivation in perfusion-based bioreactors or the generation of PDX-models (in case a preclinical model for a relevant, newly defined ER+ BC subgroup is missing) will be collected in ongoing clinical studies at VHIO and UHH.

Task 6.5 Preclinical Model - Subgroup Assignment: Identification of primary cell and PDX models representing ER+BC subtypes defined by differential MESI marker expression. Partners: VHIO, DKFZ, DDI

Comparison of the MESI networks and the expression of MESI marker panels (WPs 3-5) in our 9 matched primary cell and PDX models (including at least one immunocompetent model) with the MESI-marker defined ER+BC subgroups to identify which models best represent specific subgroups of patients for preclinical analyses. If a preclinical model for a relevant, newly defined ER+ BC subgroup is missing, available models from EurOPDX will be analysed or up to two novel PDX models will be established at VHIO.

Deliverables *see comment next to Table 3.1c (Deliverables) on mandatory deliverables for clinical trials.

- D6.1 Ethics votes for Risk Detection Trial (month 3)
- D6.2 Ethics votes for Relapse Prediction Trial (month 6)
- D6.3 Ethics votes for collection of Tissues for Preclinical Trials (month 9)
- D6.4 Ethics votes for Relapse Detection Trial (month 24)
- D6.5 Preclinical Model-Subgroup Assignment (month 36)
- D6.6 Data annotation for migration of collected biological samples to biobanks (month 51)

Work package number	7	Lead beneficiary	UHH			
Work package title	Preclinical and clinical trials with drug treatments: preclinical drug tests, WOO, ET Termination and Intervention Validation Trials					
Participant number	1	2	3	4	5	6
Short name of participant	UMCG	PATH	UHH	DKFZ	VHIO	DDI
Person-months per participant:	21	3	25	23	12	20
Start month	1	End month		51		

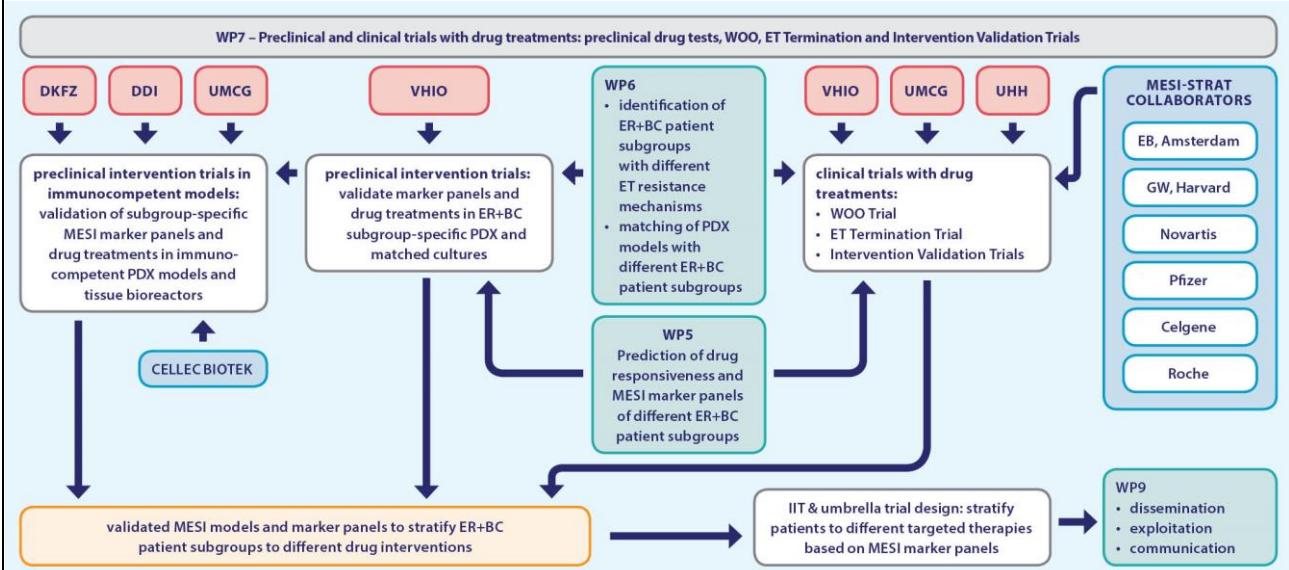
Objectives. The overall aim of WP7 is to validate the predictive power of the MESI models and marker panels derived from WPs 5+6 for stratification of ER+BC patient subgroups for drug interventions.

Clinical studies in ER+BC present several challenges including the long time to relapse, the low percentage of relapsing patients, and the risk for relapse remaining constant over two decades. Hence, trials with endpoints such as progression free survival are long-term efforts that cannot be carried out within the typical budget and duration of a H2020 project. MESI-STRAT overcomes this limitation by taking advantage of existing cohorts and trials, by conducting own trials where needed, and by collaborating with pharma companies for IIT and umbrella trials to be designed based on the MESI-STRAT outcomes.

The specific objectives of WP7 are to

- 1) Validate predictive MESI marker panels in primary cell cultures, cultured primary tissues and preclinical interventional trials in PDX models. (Task 7.1)
- 2) Identify the alterations in MESI marker panels through ET and their association with therapy response to enable stratification for ET. (Tasks 7.2 & 7.3)
- 3) Validate MESI-models and marker panels in clinical trials performed by partners and collaborators and test if the identified MESI marker panels also apply to male ER+BC (Task 7.4)
- 4) Design own IIT and umbrella trials, in which ER+BC patients will be stratified to different therapies by MESI marker panels. (Task 7.5)

Description of work. Lead partner: UHH; Supporters: UMCG, DKFZ, DDI, VHO



Task 7.1 Preclinical Intervention Trials: Validation of predictive MESI marker panels in primary cultures, cultured primary tissues and preclinical interventional trials in PDX models.

Partners: VHO, DDI, DKFZ, UMCG. The therapy schemes predicted by our computational MESI-STRAT models will initially be validated *in vitro* using primary cell cultures and primary human ER+BC tissues cultured in perfusion-based bioreactors. As the primary cell cultures are matched to our PDX models, results obtained in cell culture can swiftly be translated to the PDX models.

However, using primary cell cultures the effects of the tumor microenvironment on therapy responses cannot be assessed. In addition, some ER+BC subgroups may not grow as primary cell cultures and have very poor take rates in mice. Therefore, freshly excised ER+BC tissue will be cultured in perfusion-based bioreactors developed for tissue engineering purposes, which successfully maintain viable tumor, immune and stromal cells in ER+BC tissues for up to 21 days. This platform will be used in addition to primary BC cells to test the targeted therapies predicted (WP5) for specific ER+BC subgroups (WP6).

We will investigate at least 8 interventions relevant for the MESI network (tamoxifen, fulvestrant, trastuzumab, mTOR inhibitors, PI3K inhibitors, CDK4/6 inhibitors, MEK inhibitors, PARP inhibitors and IDO1 inhibitors) and combinations thereof in appropriate primary cultures or cultured tissues. The most promising therapies for specific subgroups identified by this approach (up to 4 per PDX) will be validated in up to 6 PDX models representative for different subgroups. In the case of validation of therapies requiring the immune system, e.g., IDO1 inhibitors, up to 4 immunocompetent PDX models will be established. If no PDX model exists for a subgroup of interest, we will try to establish it in the course of the project, by screening patients for the respective MESI marker panels before surgery and trying to derive primary cultures, cultured tissues and PDX from the fresh tissue. If the tumor cells do not grow as primary cultures and PDX we at least will be able to study their drug response in primary tissue cultured in perfusion-based bioreactors.

Treatment schemes for PDX mice, representing clinical cancer therapy schemes, are regularly conducted at VHO. Samples for several interventions (P13K, CDK4/6 inhibitors and combination thereof) are already available for analysis; other relevant regimens, based on MESI model predictions will be easily implemented.

Samples for several interventions are already available for analysis (see table below); other relevant regimens, based on MESI model predictions will be easily implemented. While some tumors will recapitulate the model-predicted therapy responses, others are expected to escape immediately, or therapy resistance will arise with time in the initial responders. We will take advantage of this phenomenon to elucidate the differences between the responding tumors versus immediate and emerging resistant tumors. PDX tissues will be taken to molecular characterization of the response differences by panel sequencing and RNASeq (WP3) as well as metabolic measurements (WP4) of therapy-resistant versus responsive PDX. We will establish kinetic MESI networks and their response to drug perturbation in different ER+BC PDX-derived genetic backgrounds as a novel means of ER+BC phenotyping. We expect that MESI-models will display a higher predictive power regarding individual ER+BC therapy response, as compared to established histology or expression-based tumor-phenotyping. The knowledge gained by this approach will again feed into analyses of patient material to enable an iterative optimization of MESI marker panels for prediction of therapy response.

Task/Trial 7.2 WOO Trial: Prospective Window Of Opportunity Trial: 2 weeks neoadjuvant Anastrozole in Postmenopausal Women with ER+BC. Partner: UHH

The WOO Trial will allow us to study the patients' response to ET, by comparing BC tissue, serum and urine from patients at diagnosis without and with ET (for details see Study No.5 in the Clinical Annex)

The following primary endpoint will be assessed:

- association of MESI networks & MESI marker panels with biological/pathological responses.

The following secondary endpoints will be assessed:

- percent change in Ki67 expression from baseline to the core biopsy 2 weeks after the start of treatment;
- pCR defined as absence of invasive cancer in the breast and sampled regional lymph nodes;
- clinical response of the breast tumor to therapy as assessed by histopathology;
- radiologic response of the breast tumor as assessed by radiologic or ultrasound assessment.

Study design: We will perform an investigator-initiated monocenter, open label, prospective non-randomized neoadjuvant trial investigating the biological effects of 2 weeks of Anastrozole monotherapy in tumor tissue, serum, plasma and urine in 70 postmenopausal women with ER+ breast cancer. After routine biopsy postmenopausal patients predicted to receive an Aromatase inhibitor after surgery will receive Anastrozole therapy for 2 weeks prior to surgery. The primary objective of the trial is to investigate the biological effects of Anastrozole monotherapy in serum, urine and tumor tissue of ER+ BC patients by assessing the percentage of change from the baseline value in Ki67 expression after 2 weeks of therapy and analyzing gene expression, protein expression and metabolites in the tumor tissue (before and after Anastrozole treatment) as well as analyzing MESI marker panels in the sera and urine of the patients. The clinical research organization (CRO) KKS will support SS and AS in conducting this clinical trial (see budget included in UHH and letter of support).

Diagnosis and Main Criteria for Inclusion and Exclusion: The study will enroll 70 postmenopausal women with early-stage ER+ breast cancer. Study participants will be required to have breast cancers with a tumor size >1 cm. Patients enrolled shall not have received prior therapy for their BC and will not have inflammatory cancers.

Test Product, Dosage, and Mode of Administration: The trial medication Anastrozole will be supplied as tablets administered orally, 1 mg daily (for details on neoadjuvant Anastrozole see Study No.5 in the Clinical Annex).

Criteria for Evaluation: Efficacy: • percent change in Ki67 expression from baseline to the core biopsy 2 weeks after the start of treatment • pCR defined as absence of invasive cancer in the breast and sampled regional lymph nodes • clinical response of the breast tumor to therapy as assessed by histopathology • radiologic response of the breast tumor as assessed by radiologic or ultrasound assessment. Safety: • adverse events Health Outcomes:

• EORTC QLQ-C30. Biomarkers: • Plasma, serum, urine and fresh frozen tissue samples will be tested for MESI markers

Task/Trial 7.3 ET Termination Trial: Analysis of longitudinally collected serum and urine from ER+ BC patients before and after termination of ET. Partner: PATH

PATH will coordinate the collection of longitudinal sera and urine from PATH cohort patients (for details see Study No. 6 in the Clinical Annex). ER+ PATH patients in the last year of endocrine treatment as well as in the following years without endocrine therapy will be invited to donate serum and urine every year. This will allow us to obtain serum and urine in the presence and absence of ET. Analysis of serum/urine from patients before and after termination of ET and comparison to clinical follow-up may allow identification of MESI marker alterations predictive of recurrence after termination of ET. Patients with these markers might benefit from prolonged treatment beyond the current standard of 5 years.

Task/Trial 7.4 Intervention Validation Trial: Clinically validate predictive MESI-models and marker panels for targeted drug interventions. Partners: UHH, PATH, UMCG, VHI

Due to the long time to relapse, the low percentage of relapsing patients, and the risk for relapse remaining constant over two decades, trials with endpoints such as progression free survival are long-term efforts that cannot be carried out within the typical budget and duration of a H2020 project. MESI-STRAT overcomes this limitation by taking advantage of existing trials to analyze MESI-models and marker panels in patients receiving targeted drug interventions. MESI-STRAT will investigate the effects of targeted drug interventions on MESI marker panels and their association with drug response in human ER+ BC patients in appropriate clinical studies performed by partners and collaborators (for details see Study No. 7 in the Clinical Annex). If enough patients of a MESI-marker defined ER+BC subgroup predicted to respond to the investigated drug intervention are available in a clinical trial, the predictive power of the MESI marker panel to stratify patients for the targeted drug

intervention will be validated by analyzing if the patients of this subgroup respond better to this drug than the rest of the study population. Furthermore, by analyzing three male BC cohorts it will be investigated whether the MESI markers identified for female ER+BC also apply to male ER+BC.

Task 7.5 IIT/Umbrella Trial Design: Design own IIT and umbrella trials, in which ER+BC patients will be stratified to different therapies by MESI marker panels. Partners: UHH, UMCG, VHIO

Our clinical partners (UHH, UMCG, VHIO) will design IIT and umbrella trials to prospectively validate the identified MESI marker panels, predicting response or resistance to (combinatorial) targeted therapies. We will seek support from public funding agencies and our collaborating pharmaceutical companies (see 1.3.a, consortium description). Links with pharma partners are in place to apply for dry substance and funding following established pipelines which involve step-wise review of trial design, required budget, and the study protocol (see, e.g., letter of support by Novartis); public funding will be applied for with National and European funding bodies, e.g., the DFG, NWO, KWF, and EC.

Deliverables *see comment next to Table 3.1c (Deliverables) on mandatory deliverables for clinical trials.

- D7.1 Animal permits for Preclinical Intervention Trials (month 6)
- D7.2 WOO trial: First study subject approvals package (month 6)
- D7.3 ET termination trial: First study subject approvals package (month 6)
- D7.4 WOO trial: Midterm recruitment report (month 24)
- D7.5 ET termination trial: Midterm recruitment report (month 30)
- D7.6 WOO Trial: Report on status of posting results (month 51)
- D7.7 Select first Intervention Validation Trial for MESI markers & apply for ethics addendum (month 36)
- D7.8 ET termination trial: Report on status of posting results (month 51)
- D7.9 MESI marker panels validated in Preclinical Intervention Trials (month 51)
- D7.10 Trial applications for IIT & umbrella trials with pharma or funding agencies (month 51)

Work package number	8		Lead beneficiary							UMCG	
Work package title	Project coordination: management and communication										
Participant number	1	2	3	4	7	9	10	11	13	14	
Short name of participant	UMCG	PATH	UHH	DKFZ	UiB	UNEW	CHAB	UDUR	SBS	HITS	
Person-months per participant:	29	1	1	1	1	1	1	1	1	1	
Start month	-8				End month			51+3			

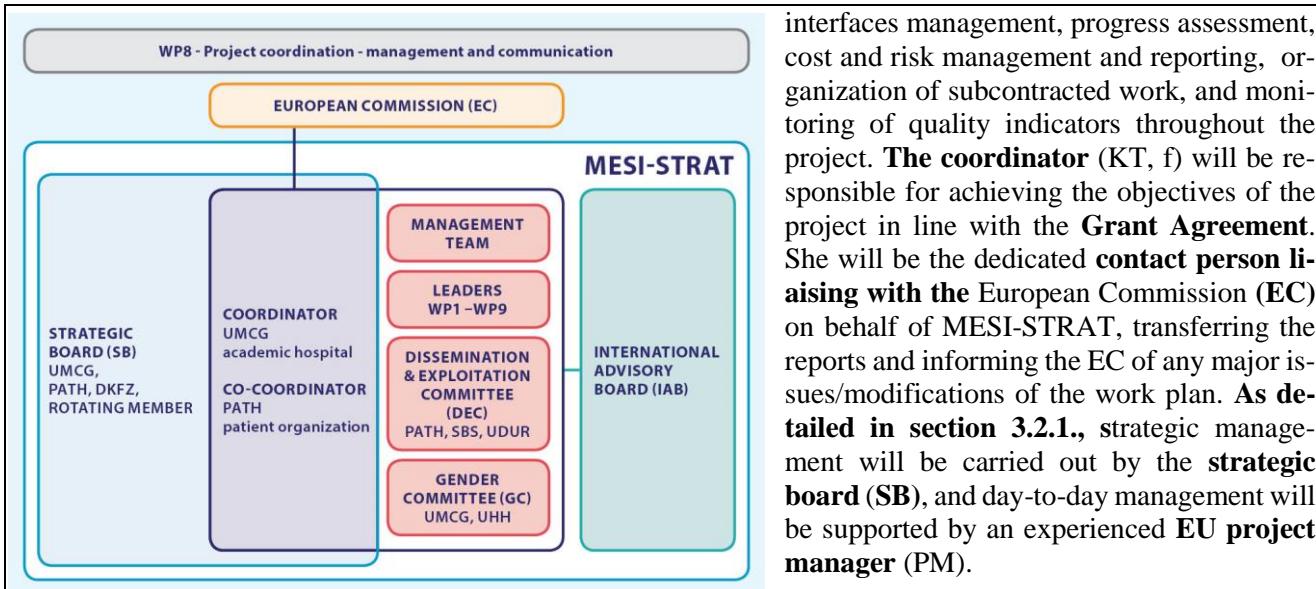
Objectives. The overall aim of WP8 is the implementation of the **management plan** to coordinate all MESI-STRAT actions. Iterative procedures are inherent to systems bio-medicine. The MESI-STRAT management will adhere to this iterative principle throughout to achieve continuous improvement of its processes.

The **specific objectives** for WP8 are the

1. administrative and financial coordination, and communication with and reporting to the EC
2. coordination of the MESI-STRAT workflow, risk and gender issues.
3. coordination of knowledge exchange between the MESI-STRAT partners.
4. **IPR management plan:** Innovation and intellectual property (IP) management and protection..

Description of work. Lead partner: UMCG (KT); **co-leader** PATH (TA). **Partners:** all WP leaders WP8 is dedicated to MESI-STRAT management and organization, and will be led by the project coordinator (KT, UMCG) and the co-coordinator (TA, PATH), supported by a project manager and by the facilities of the coordinating host institution (UMCG). The tasks are outlined here, details can be found in **section 3.2**.

Task 8.1 Management plan, administrative/financial coordination, and communication / reporting to the EC
Based on the **management plan** (drafted in 3.2), a **project management manual** will be put in place and continuously adapted where necessary, to guide all further implementation, incl. the decision-making processes,



interfaces management, progress assessment, cost and risk management and reporting, organization of subcontracted work, and monitoring of quality indicators throughout the project. **The coordinator** (KT, f) will be responsible for achieving the objectives of the project in line with the **Grant Agreement**. She will be the dedicated **contact person liaising with the European Commission (EC)** on behalf of MESI-STRAT, transferring the reports and informing the EC of any major issues/modifications of the work plan. As detailed in section 3.2.1., strategic management will be carried out by the **strategic board (SB)**, and day-to-day management will be supported by an experienced **EU project manager (PM)**.

Task 8.2 Coordination of the workflow

As **coordinator**, KT will oversee the interfaces between the various WPs as well as the preparation and dissemination of the project reports using information provided by partners. As the chairperson of the SB, KT will ensure optimal coherence between decision-making bodies to minimize constraints. Any risk issues will be addressed as detailed below in section 3.2.3.

The **SB** will set the annual scientific objectives, policy and strategic orientations of the project in accordance with the project program, our Consortium Agreement and the rules of the Grant Agreement. It will coordinate the workflow and exchange of materials. A prime task of the SB is to ensure, jointly with the WP9 leaders PATH and SBS, the implementation of the plans for dissemination and exploitation, and for communication (Section 2.2 and WP9). In these tasks the SB will be supported by the **international advisory board (IAB)**, the **dissemination and exploitation committee (DEC)**, and the **Gender Committee (GC)** (detailed in section 3.2.1, management).

The **PM** will collect information at least every 6 months to monitor the progress of the project towards its objectives, deliverables and milestones. The PM and KT will ensure compliance of the project with the technical annex and, if necessary, propose modifications to the SB. Accordingly, KT and the PM will prepare the agendas of SB meetings, propose decisions, and will be responsible for the proper execution and implementation of the decisions of the SB.

Task 8.3 Coordination of knowledge exchange between the partners, and meeting schedule.

Information that can influence the project implementation will be communicated and implemented by a communication and meeting structure that relies on iterative management strategies that have proven successful in previous and ongoing collaborations. Specifically, the partners work in interdisciplinary **MESI-STRAT expert teams** of modelers/informaticians and experimentalists/clinicians, who specialize in different aspects of cancer signaling, metabolism, computational modeling/network analysis and data handling (see Section 3.3). The expert teams will be the smallest internal communication units who develop their joint research foci and report to their mutual WP groups. MESI-STRAT expert teams will come together in bi-weekly individual online and on-site meetings. A dedicated meeting schedule, detailed in 3.2.1, will ensure smooth information flow at all levels of our consortium and the speedy identification and resolution of any risks arising.

Task 8.4 Innovation and intellectual property (IP) management and protection.

This **MESI-STRAT innovation management** (detailed in Section 3.2.2) follows well-established iterative practice in systems approaches and implements the guiding principle of **continuous improvement through iterative development** within all WPs and their interactions. This gives MESI-NET the possibility for self-correction, enabling it to respond to an external or internal opportunity. The **understanding of other market and technical problems** is ensured by the open communication between the SMEs, and scientific and clinical partners in our consortium.

The management of knowledge and IP will be monitored by the Coordinator subject to the decision of the SB, advice by the DEC, and the Consortium Agreement. Based on the ownership of MESI-STRAT Results (i.e., Foreground), and evaluation by the DEC and the coordinator, foreground with potential for commercial

exploitation shall be protected in an adequate and effective manner as laid out in section 2.2.3.

The decision on whether to publish open access documents must come after the more general decision on whether to go for a publication directly or to first seek protection using IPR, as it is crucial to maintain the option to exploit research results commercially, e.g., through patenting. Following our consortium agreement, MESI-STRAT will implement the **IPR management plan** (outlined in section 2.2.3) through the grant agreement. The academic partner institutions have dedicated TTOs that ensure proper handling and protection of IP. The TTO of the coordinating institution (UMCG) has been actively involved in the MESI-STRAT proposal preparation to shape the measures of the IPR management plan.

WP8 will work in synergy with each individual WP and partner to facilitate each WP's own dissemination of specific technical activities, innovations, and findings, with due recognition of the IP management and ownership. At the critical times of (1) patenting, (2) licensing, or (3) own industrial initiatives, the contributions of all involved consortium members up to this point will be documented and frozen to define what the object of the patent/license will be. The efforts of the leading institutions for patenting, licensing, feasibility analyses for establishing a possible a spin-off will be continuously documented, to reward continuing investment.

Deliverables

- D8.1 Sign Consortium agreement and prepare & finalize grant agreement (month -4)
- D8.2 Management plan (month 1)
- D8.3 Appoint DEC and GC (month 3)
- D8.4 Implement IPR management plan (month 6)
- D8.5 Periodic and final reports to the EC (months 12, 24, 36, 48, 52)

Work package number	9		Lead beneficiary		SBS (BA); Co-Lead PATH (DS)									
Work package title	MESI-IMPACT: Dissemination, exploitation & communication													
Participant number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Short name of participant	UMCG	PATH	UHH	DKFZ	VHIO	DDI	UiB	UiT	UNEW	CHAB	UDUR	NIN	SBS	HITS
Person-months per participant	12	2	1	1	1	1	1	1	1	1	1	1	5	1.5
Start month	1							End month		51				

Objectives. The overall aims of WP9 are to (i) achieve a significant and durable impact on EU citizens' health, in particular BC patients and their families, by promoting the use MESI-STRAT's new models for ER+BC patient stratification to inform clinical decision making, (ii) accelerate the translation of the biomedical and clinical research results of MESI-STRAT to medical use, (iii) promote the use of MESI-STRAT's models and marker panels by health care professionals and the industry on the basis of increased cost-effectiveness in comparison to current practice, (iv) increase research and innovation opportunities, particularly for SMEs active in system medicine. **Specific objectives:** WP9 coordinates measures to maximize impact, by implementing the (1) Dissemination and exploitation plan, (2) Plan for open access and open data, and (3) Communication plan.

Description of work. Lead partner: SBS (BA); co-leader PATH (TA)

WP9 is dedicated to the dissemination, exploitation and communication of the MESI-STRAT results. The SME SBS (BA) and PATH (DS, patient expert) will jointly lead WP9 to ensure optimal scientific and commercial dissemination, recognition and implementation of opportunities for exploitation, and communication of the MESI-STRAT programme and its results to all stakeholders. SBS and PATH will be supported by the MESI-STRAT management team (UMCG), the Cancer Information Service KID & its European partners, and the IAB.

Task 9.1 Dissemination and exploitation plan, and implementation

As detailed in Section 2.2.1, this plan ensures the promotion of MESI-STRAT's scientific and clinical results as well as commercial outcomes and opportunities to all stakeholders. WP9 will work closely with WP8 (coordination) in liaison with all WP leaders to ensure that all key interim and final results are disseminated appropriately – with a balance between dissemination of results by WP leaders and expert teams, and IP protection as outlined in WP8 and section 2.2.3. Scientific dissemination will be through conference presentations, workshops,

peer-reviewed journal publications, and own workshops and symposia. Effective commercial exploitation of new MESI models and MESI marker panels will be achieved with (i) MESI-STRAT's SMEs providing systems medicine services (SBS, HITS) or developing diagnostic tools (NIN, QuantuMDX, CELLEC BIOTEK); (ii) collaborating pharmaceutical industry (see 1.3.a); and (iii) TTOs of the academic partners to evaluate the potential for an own industrial engagement.

Task 9.2 Open access and open data

MESI-STRAT will adhere to the goals set by the EC for **open science** feeding into the policy for RRI, by actively pursuing **open access publishing** and participating in the **Open Research Data Pilot**, in line with restrictions imposed by patient confidentiality and IPR protection. As detailed in section 2.2.3, **measures to provide open access and open data** ensure accessibility of MESI-STRAT results by the entire scientific community, commercial entities and the public. Exploiting the opportunities of upcoming EU legislation for publicly funded research, MESI-STRAT papers will be made accessible from 2018 on (when the according legislation becomes effective) 6 months after their publication in scientific journals via open repositories of the partner institutions (**green open access**), and linked to the MESI-STRAT website. Publication costs for **gold open access** will be either covered by institutional or university funds, or will be applied for at National funding agencies. For cases where none of these options exist, there is a budget reserved to be allocated to MESI-STRAT partners in need. For participation in the **Open Research Data Pilot**, MESI-STRAT will deposit research data in repositories to enable third parties to access, mine, exploit, reproduce and disseminate these data (2.2.2). EMBL-EBI will support deposition and accessibility of MESI-STRAT raw and curated data in EMBL-EBI-hosted repositories.

Task 9.3 Communication plan and implementation

MESI-STRAT seeks to promote its results to a broader group of stakeholders in order to have a significant impact on future health-related societal challenges. **The comprehensive MESI-STRAT communication plan**, detailed in section 2.2.4, is core to WP9 and complements our dissemination and exploitation strategy. Next to scientific, commercial, and societal stakeholders including patients and their families, our communication plan also addresses clinical opinion leaders involved in deciding future health policies, and the public.

As detailed in section 2.2.4, MESI-STRAT will promote its results and receive feedback at own patient days, by a European telephone survey, and by participating in local and national patient days, and presentations at events and initiatives aimed at patients' literacy, integration, and involvement in the process of medicines research and setting research priorities. Materials on the website will be important, as will be electronic newsletters, podcasts, YouTube films and social media, and publications in lay journals.

WP9 will work by initially commissioning the design and delivery of a common web portal, then it will link with other WP leaders and other project partners to set the pattern of the initial formative communication programme. Feedback and coordination by the partnering patient organization PATH during internal meetings, and feedback systematically collected by questionnaires and electronic polls, will help to direct the MESI-STRAT strategy based on the medical needs of patients to improve quality of life as well as survival. Awareness about new standards in the implementation of systems medicine approaches will be raised by educational training for medical professionals and basic scientists and students, offered by the partnering experts, and for the public (e.g., Medical Public Academy, Studium generale at partnering institutions and the European patient academy EU-PATI). Joint press releases, reviews, and interviews in lay language, and a lay accessible public version of MESI-STRAT's final report, distributed via the project portal and linkage to other key websites, will complete our communication plan, which will be topped off by a large audience conference at the conclusion of the project to promote MESI-STRAT's findings on systems medicine of metabolic signaling networks to treat ER+BC, and derive future implications thereof for other cancer diseases.

Deliverables

- D9.1 Dissemination & exploitation plan (month 3)
- D9.2 Communication plan (month 3)
- D9.3 Pipeline for model customization for pharma running (month 12)
- D9.4 MESI-STRAT website with continuous updates (months 6, 12, 24, 36, 51)
- D9.5 Concluding conference (month 52)

Table 3.1b: List of work packages

WP No	Work Package Title	Lead Participant No	Lead Participant Short Name	Person-Months	Start Month	End Month
1	Data survey of existing patient samples and materials	2	PATH	27	1	51
2	Setup and maintenance of MESI-SEEK data and model management platform	14	HITS	31	1	51
3	Assessment of SIGNALING NETWORKS for model parameterization and validation	10	CHAB	121	7	51
4	Assess METABOLIC NETWORKS for model parametrization and validation	7	UiB	112	7	51
5	Integrative MESI-STRAT modeling and network analyses	9	UNEW	172	7	51
6	Preclinical and clinical trials without drug treatment in ER+ BC patient- derived models and the longitudinal PATH cohort	4	DKFZ	58,5	1	51
7	Preclinical and clinical trials with drug treatments: preclinical drug tests, WOO, ET Termination and Intervention Validation Trials	3	UHH	104	1	51
8	Project coordination: management & communication	1	UMCG	38	-8	51+3
9	MESI-IMPACT: Dissemination, exploitation & communication	13/2	SBS/PATH	30,5	1	51
Total person- months				694		

3.1.4 Graphical presentation showing how the components interrelate (Pert chart) and list of deliverables.

Figure 9. PERT diagram: relationship between WPs and tasks. Shown are the most important connections between MESI-STRAT WPs and their deliverables and milestones.

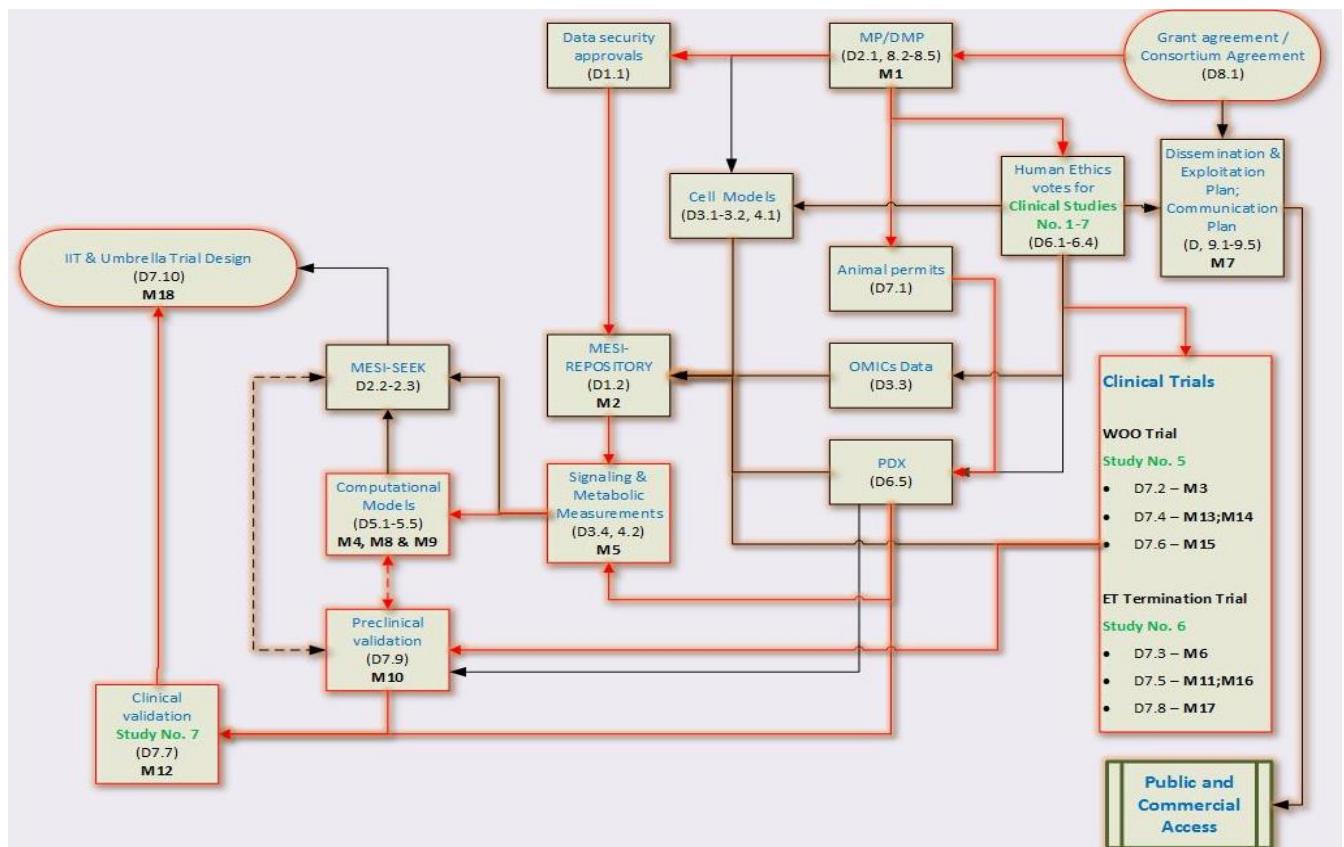


Table 3.1c: List of Deliverables.

(PU=public; CO = confidential, *CO/PU: these deliverables are initially confidential but will be made public upon publication, subject to regulations and considerations regarding patient data privacy and IPR protection. **The mandatory deliverables for clinical trials** incl. “First study subject approvals package”, “Midterm recruitment report” and “Report on status of posting results” are provided for clinical trials, in which MESI-STRAT recruits patients (WOO trial, ET Termination trial, see table 2 in section 1). All other trials rely on existing cohorts or trials sponsored by MESI-STRAT partners or collaborators. Therefore, no patients will actively be recruited by MESI-STRAT and the mandatory deliverables are not applicable. However, in some cases ethics votes or addenda to ethics votes may be required and are included as deliverables for each of the associated trials. The **mandatory deliverable “data management plan”** is in month 6.

<i>Deliverable #</i>	<i>Deliverable name</i>	<i>WP</i>	<i>Lead Partner</i>	<i>Type</i>	<i>Dissemination level</i>	<i>Delivery months</i>
D8.1	Sign Consortium agreement and prepare & finalize grant agreement	8	UMCG	R	CO	-4
D8.2	Management plan	8	UMCG	R	PU	1
D6.1	Ethics votes for Risk Detection Trial	6	PATH	R	CO /PU*	3
D8.3	Appoint DEC and GC	8	UMCG	R	PU	3
D9.1	Dissemination & exploitation plan	9	SBS	R	PU	3
D9.2	Communication plan	9	PATH DKFZ	R	PU	3
D1.1	Regulatory approval for data storage in MESI-REPOSITORY	1	DKFZ	R	CO	6
D2.1	Data management plan	2	HITS	R	PU	6
D6.2	Ethics votes for Relapse Prediction Trial	6	PATH	R	CO /PU*	6
D7.1	Animal permits for Preclinical Intervention Trials	7	VHIO + DDI	R	CO /PU*	6
D7.2	WOO trial: First study subject approvals package	7	UHH	R	CO	6
D7.3	ET termination trial: First study subject approvals package	7	PATH	R	CO	6
D8.4	Implement IPR management plan	8	UMCG	R	PU	6
D9.4	MESI-STRAT website with continuous updates	9	UMCG	DEC	PU	6, 12, 24, 36, 51
D6.3	Ethics votes for collection of Tissues for Preclinical Trials	6	VHIO UHH	R	CO /PU*	9
D2.2	Continuous release of updated MESI-SEEK versions	2	HITS	OTHER	CO /PU*	12, 24, 36, 51
D8.5	Periodic and final reports to the EC	8	UMCG + all	R	CO	12, 24, 36, 48, 52
D9.3	Pipeline for model customization for pharma running	9	SBS	DEM	CO /PU*	12
D3.1	Full signaling time course datasets for ER+BC cell lines	3	CHAB	R	CO /PU*	18
D4.1	Full metabolite time course datasets for ER+BC cell lines	4	UiB	R	CO /PU*	18
D5.1	Computational signaling models for ER+BC cell lines	5	UNEW	OTHER	CO /PU*	18
D5.2	Computational metabolic models for ER+BC cell lines	5	UiT	OTHER	CO /PU*	18
D5.3	Linked metabolic & signaling (MESI) models	5	UNEW, UiT	OTHER	CO /PU*	24
D6.4	Ethics votes for Relapse Detection Trial	6	PATH DKFZ	R	CO /PU*	24
D7.4	WOO trial: Midterm recruitment report	7	UHH	R	CO	24
D5.4	First MESI model parametrized for patient samples	5	UNEW, UiT	OTHER	CO /PU*	27
D3.2	Perturbation screens finalized	3, 4	CHAB	R	CO /PU*	30
D7.5	ET termination trial: Midterm recruitment report	7	PATH	R	CO	30
D5.5	First customized model for pharma	5	SBS	OTHER	CO /PU*	36
D6.5	Preclinical Model-Subgroup Assignment	6	DKFZ	R	CO /PU*	36
D7.7	Select first Intervention Validation Trial for MESI markers & apply for ethics addendum (if necessary)	7	UHH UMCG DKFZ VHIO	R	CO /PU*	36
D3.3	Analyzed multi-level omics data	3	CHAB	R	CO /PU*	45
D1.2	All patient-derived model systems, samples, and clinical data in MESI-REPOSITORY	1	DKFZ	OTHER	CO	51
D2.3	Omics data in public resources (EMBL-EBI & others)	2	HITS	OTHER	CO /PU*	51
D3.4	Signaling marker panels for patient derived models and samples measured	3	CHAB	R	CO /PU*	51

D4.2	Metabolite marker panels for patient derived models and samples measured	4	UiB	R	CO /PU*	51
D5.6	Omics data for MESI-ancillary networks integrated	5	UDUR HITS UMCG	R	CO /PU*	51
D6.6	Data annotation for migration of collected biological samples to biobanks	6	DKFZ	R	PU	51
D7.6	WOO trial: Report on status of posting results	7	UHH	R	PU	51
D7.8	ET termination trial: Report on status of posting results	7	PATH	R	PU	51
D7.9	MESI marker panels validated in Preclinical Intervention Trials	7	UHH, DKFZ	R	CO /PU*	51
D7.10	Trial applications for IIT & umbrella trials with pharma or funding agencies	7	UHH UMCG DKFZ VHO	R	CO /PU*	51
D9.5	Concluding conference	9	PATH DKFZ	DEC	PU	52

3.2 Management structure, milestones and procedures

3.2.1 Organisational structure and the decision-making in relation to the complexity and scale of the project

The MESI-STRAT project brings together 14 partners. **The Project Coordinator** Prof. Dr. Kathrin Thedieck (f) was unanimously appointed by all MESI-STRAT partners. She is a young high profile and highly active female scientist in systems medicine, and has the full support from WP leaders in the MESI-STRAT consortium, representing an experienced panel of high profile systems biologists and medical scientists.

WP8 is dedicated to the **management and organization of the overall project**, led by the Project Coordinator, supported by a project manager (PM) and the host institution (UMCG). Project management covers a range of activities including project planning, and implementing deviations from planned actions where necessary; gathering intelligence to promote the generated results; and preparing and undertaking the exploitation.

Our project has a strong **team commitment** to the tasks of MESI-STRAT. The partners are closely linked by previous and ongoing collaborations in systems biology and medicine, evidenced by multiple joint publications and funding (Section 3.3, Consortium as a whole). **Each WP** has been designed by its own team, facilitated by meetings and close interactions across the consortium throughout the proposal preparation. The support for the successful stage 1 submission, and the composite set of plans for all WPs continues to be endorsed by all involved. Thus, MESI-STRAT starts with the advantage of shared vision and commitment, and mutual support for the leadership of KT and for the partner work streams.

The MESI-STRAT consortium structure and procedures as laid out below is further detailed in the Consortium Agreement which will be the basis for the Grant Agreement to be signed between the coordinator and the EC. The Grant Agreement will be based on the DESCA model and includes provisions, procedures for convening ad hoc meetings and dealing with extraordinary events such as conflicts, partners leaving or entering the project.

Project management will be implemented at a **strategic level** and an **operational (day-to-day management)**. The **management structure is graphically shown in WP8**.

- **Strategic management**

In accordance with the consortium size and the project structure, the strategic management includes one governance body: the **MESI-STRAT Strategic Board (SB)**, consisting of the coordinator (KT, systems biologist, UMCG), the co-coordinator (TA, clinician & patient organization PATH), a clinician and cancer metabolism expert (CO, DKFZ), and a fourth rotating member. The balanced representation ensures the efficient implementation of our systems bio-medicine project and strong involvement of patients as a main group of stakeholders. The rotating member will be appointed according to the major tasks or emerging risks at the different project phases. For example, in the beginning, the setup of the MESI-SEEK platform will be of major importance both to MESI-STRAT and to join the EC Pilot on Open Research Data by implementing a data management plan. Therefore, the leader of WP2 (data management) HITS will join the SB in this initial project phase. Decision-making will be supported by regular online meetings with all WP leaders and co-leaders, and by polls if decisions by vote are required. The SB sets annual scientific objectives, policy and strategic orientations of the project in accordance with the rules of the Consortium and Grant Agreements and the project programme; ensures proper administrative, legal and financial operations; monitors the progress of the project towards its objectives, deliverables and milestones, and prepares the reports to the EC; decides on the distribution of the EC pre-financing/interim payment; funding for open access publishing (open data); and modifications to the consortium such as inclusion/exclusion of beneficiaries, funding redistribution, corrective measures, and modification to the research programme.

The SB will be assisted by the **International Advisory Board (IAB)** providing strategic input and supervision of the activities of the MESI-STRAT program. The IAB is composed of global experts recognized in the fields of basic and clinical BC research, signaling & metabolism in BC, systems medicine, companies active in the pharma sector and systems medicine, and patient representatives. Together with the partnering and collaborating SMEs, the IAB will ensure scientific excellence as well as compliance with the needs of industry and society. It will promote dissemination, exploitation, and communication and provide visions for the structuring and operation of activities of **MESI-STRAT**.

Expertise	Name and Affiliation	m/f
Integrated Genomics of BC	Prof. emerita A.-L. Borresen-Dale, Dept. of Cancer Genetics, Inst. for Cancer Research, The Norwegian Radium Hospital , Oslo, NO	f
BC liquid biomarker discovery and trials	Prof. E. Boven, MD, PhD, Medical Oncologist, VU University Medical Center , Amsterdam, NL	f
BC metabolism	G. Wulf, MD, PhD, Assoc. Prof., Dana-Farber Harvard Cancer Center, Boston, US	f
BC patient expert	Doris Schmitt, PATH co-chair , executive board member of the European patient academy EUPATI , and patient-doctor communication coach , Konstanz, DE	f
European BC patient organization	Susan Knox, CEO, EUROPA DONNA – The European Breast Cancer Coalition , Milan, IT	f
BC drug development pharma	Dr. Eric Hoedemaker, Medical Director Oncology, Novartis , NL	m
Systems modeling for pharma drug discovery	Dr. Andreas Raue, Lead early stage immune-oncology development and biomarker discovery, Merrimack Pharmaceuticals , Cambridge, MA, US	m
Metabolic modeling and systems biology of epigenetics in BC	ITN EpiPredict Coordinator: Dr P.J. Verschure, University of Amsterdam, NL Prof. H. V. Westerhoff, Director Manchester Centre for Integrative Systems Biology (MCISB), University of Manchester, UK	f m

A **committee for exploitation and communication of results (DEC)** will be built by the SME SBS, the patient organization PATH, the clinical partner UHH and the academic partner UDUR to represent all interest groups and expertise within the MESI-STRAT consortium. In regular meetings the DEC will evaluate the potential of MESI-STRAT results for scientific and commercial exploitation and advise on the next steps. The DEC will take care that access rights to background and foreground IP needed for implementing the project will be granted, and will monitor MESI-STRAT actions and results (i.e., foreground) to identify IP that requires protection prior to publication, always observing patients' interests and privacy regulations.

A **gender committee (GC)**, headed by our clinical experts CSC (UMCG) and SS (UHH), both specializing on male BC, will focus on the consequent consideration and implementation of gender issues at all project levels. This concerns in particular the translation of results for female ER+BC to male ER+BC. CSC (UMCG) and SS (UHH) both have a strong focus on male BC and access to large sample male BC collections (see WP1, 7). These are very precious as male BC is rare. Hence, the GC will evaluate and decide on the validation of strongly predictive MESI marker panels from female ER+BC subgroups in the male cohorts. Furthermore, the GC will constantly evaluate MESI-STRAT actions and results regarding the proper consideration of gender issues and consideration and validation for male BC wherever possible.

- **Day-to-day management**

The coordinator (KT)

- is the dedicated contact person liaising with the EC on behalf of the MESI-STRAT consortium, transferring the reports and informing the EC of any major issues/modifications of the work plan,
- is responsible for the achievement of the project in line with the Grant Agreement,
- is the chairperson of the SB, ensuring coherence between decision-making bodies and to minimize the risk of bottleneck
- acts as the primary conduit between the SB and the IAB,
- mediates and settles disputes within the consortium.

The PM (project manager, to be appointed), is responsible for implementing the decisions of the SB and the day-to-day management of the project. The PM will provide an administrative link with the Commission Desk Officer, and partner institutions' administrations. The PM will oversee administrative, legal, and financial operations and reporting, and effective and efficient implementation of the project.

The Management Team (KT together with the PM)

- prepares the meetings, proposes decisions and prepares the agenda of the SB,

- is responsible for the proper execution and implementation of the decisions of the SB,
- monitors the effective and efficient implementation of the project;
- collects information at least every 6 months on project progress, examines that information to assess the compliance of the project with the technical annex and, if necessary, proposes modifications to the SB.

Meeting schedule

- monthly video conference of the SB + MT, including identification and discussion of any problems arising on progress, resources or other matters.
- monthly work and progress returns to the project office.
- bi-monthly video conference between all WP Leaders, to discuss progress, identify and resolve arising issues, and an alternating presentation on scientific progress in the partner labs.
- six-monthly face-to-face meeting of WP Leaders and co-leaders.
- annual meeting with the whole consortium.
- ad hoc meetings for addressing unexpected events.
- all WPs will hold monthly meetings, chaired by the WP leader or co-leader, to ensure progress and achievement of their milestones and deliverables and for timely identification and resolution of emerging risks.

The PM will prepare agendas for the listed consortium meetings, and promptly produce memoranda on agreements and actions. The **annual meeting** with the whole consortium will be held as a one-day event involving all MESI-STRAT partners. For efficiency, but also to enable good inter-personal linkage, the meeting will be preceded by half-day meetings of the WP Leaders and of the IAB; each WP will then have the opportunity to hold its own working meeting, and inter-WP discussions will be led by the tandems. Most members will thus attend for two days. To tighten the links and ensure commitment between the partners, the annual meeting will be organized and hosted by a different partner each year.

Table 3.2a: List of milestones

To monitor the progress of the WPs and to ensure compliance with the deliverables (Table 3.1c) the following milestones have been identified. The mandatory milestones for clinical trials incl. “First Patient, First Visit (FPFV)”, “Last Patient, First Visit (LPFV)” and “Last Patient, Last Visit (LPLV)” are provided for clinical trials, in which MESI-STRAT recruits patients (WOO trial, ET Termination trial, see table 2 in section 1). All other trials rely on existing cohorts or trials sponsored by MESI-STRAT partners or collaborators. Therefore, no patients will be actively recruited by MESI-STRAT and the mandatory milestones are not applicable.

Mile-stone #	Mile-stone name	Related WPs	Due date	Means of verification
M1	Kickoff meeting	8 & 1-9	3	Meeting held
M2	MESI-REPOSITORY for clinical data & sample input	1	6	MESI-REPOSITORY up and running
M3	WOO Trial First Patient, First Visit (FPFV)	7	12	Informed consent signed
M4	Core topological models of signaling and metabolic networks	5 & 3,4	12	Model structure approved by WP leaders and uploaded in MESI-SEEK
M5	First combined set of signalling and metabolite time course data	3,4	15	Data set uploaded in MESI-SEEK
M6	ET Termination Trial First Patient, First Visit (FPFV)	7	18	Informed consent signed
M7	MESI-STRAT patient days	9	18	First patient day held
M8	First combined MESI models parametrized	5 & 2-4	24	Parametrized models uploaded in MESI-SEEK
M9	Prediction of first subgroup specific marker panels	5 & 2-7	30	Model predictions in MESI-SEEK
M10	Validate first MESI markers in Preclinical Intervention Trials	6 & 2-5	36	Data sets uploaded to MESI-SEEK
M11	ET Termination Trial Last Patient First Visit (LPFV)	7	39	Informed consent signed
M12	First MESI panels validated in clinical samples	6 & 2-5	42	Data sets uploaded to MESI-SEEK
M13	WOO Trial Last Patient First Visit (LPFV)	7	45	Informed consent signed
M14	WOO Trial Last Patient Last Visit (LPLV)	7	48	Samples in NCT tissue bank (UHH)
M15	WOO Trial End of Study	7	51	Data analysis complete
M16	ET Termination Trial Last Patient Last Visit (LPLV)	7	51	Samples in PATH biobank
M17	ET Termination Trial End of Study	7	51	Data analysis complete
M18	IIT and umbrella trials for MESI-marker based patient stratification designed	7	51	Trial protocols in place

3.2.2 Effective innovation management in the management structure and work plan

The MESI-STRAT WPs are technology, expertise, and delivery centred. The technical expertise resident in the WPs is a constant that we draw upon to implement our **highly iterative systems medicine strategy**. In this approach, existing knowledge is formalized into mathematical models, and simulations are turned into predictions, which are then tested against experimental data. At each step, the test results will feed back into the theoretical models, where any discrepancies may reveal a missing knowledge and/or need to change our hypotheses. Resolution of such discrepancies typically requires multiple cycles of open-minded systematic analysis of existing data and brainstorming sessions with experimental, theoretical, and clinical partners. Followed by evaluation and prioritization of new ideas, implementation into models and further testing against experimental data.

The **MESI-STRAT innovation process**, implemented in WPs 8 and 9 and detailed in sections 2.2.1 (scientific dissemination, commercial exploitation), 2.2.2 (data management plan), 2.2.3 (IPR management & open data), and 2.2.4 (communication) follows this well-established iterative practice in systems approaches.

Collaborations, open internal communication, and mutual trust and respect are key to innovation management. These key aspects will be ensured by

- frequent virtual and on-site meetings (see previous section)
- a consortium whose partners have communicated closely and collaborated productively for many years, familiar with projects that involve cross-disciplinary communication, requiring the willingness to listen to partners from other disciplines and to try out their ideas.
- the **understanding of both market and technical problems, including commercial and medical regulatory aspects** due to the open communication between the SMEs, and scientific and clinical partners and patient experts in our consortium.

As a result, the guiding principle of all MESI-STRAT efforts will be the **continuous improvement through iterative development** within all WPs and their interactions. This gives MESI-STRAT the possibility for self-correction and the ability to respond to an external or internal opportunity.

3.2.3 Critical risks, relating to project implementation, and risk mitigation measures

Risk management will be given a high priority. (i) Risk identification: foreseeable risks identified by the consortium during the elaboration of the project are stated below; (ii) Risk treatment: identified risks (see below) and/or alternative plans to limit their impact will be drawn up, and followed up by the SB. Milestones will serve as an assessment tool, enabling risks to be monitored and corrective measures to be implemented. The WP leader(s) will monitor their milestones and report to the Coordinator and the SB for recommendations on continuation/implementation of contingency plans. The management team will ensure that relevant information and knowledge circulates efficiently between partners (see WP8).

Conflict resolution will be approached by consensus at the point of origin; if consensus cannot be reached, the SB and ultimately the coordinator will make the final decision: for any problems arising with individual partners we will first try to find a solution within the institution; secondly within the WP to try to cross-cover any work problems; and thirdly at project level to find a solution within the WP Leaders' meeting. As the last resort the Project Coordinator will have final decision. No partner will be allowed to exceed their budget without appropriate approval and resource transfer. If any partner seeks to (or does) undertake work not in the project plan, or not in accord with agreed procedures (especially with regard to ethics) that work will not be funded.

Decision-making process concerning the evolution of the consortium (entry of a new party, withdrawal of a party, change of coordinator) or suspension of the project will be made by unanimous vote. All other decisions within the consortium will always be made by a qualified majority of two-thirds (2/3) of the partners'.

Table 3.2b: Critical risks for implementation

Management risks

Description of risk. "Low/Medium/High" risk without mitigation.	WP(s) involved	Proposed risk-mitigation measures
Delayed start up due to resource or communication problems (medium)	WP8 + all	The mitigation measures will render the likelihood of all risks <u>low</u> .
Shortage of resources and financial changes (medium)	WP8 + all	Partner will have to continue the work with the available resources, and seek to supplement with internal resources. If necessary tasks will be adjusted.
Lack of communication between the consortium partners (medium)	WP8 + all	Virtual meetings and on-site meetings at different partner sites will regularly take place, to maintain contact and enhance collaboration.
Delays on reporting/deliverables (high)	WP8 + all	The Project Coordinator is responsible for solving any procedural problems that will appear. Conflict resolution mechanisms in place.
Illness of WP leader (high)	WP8 + all	A system of co-leaders and deputies is already in place.

Scientific and Impact Risks

Description of risk.	WP(s) involved	Proposed risk-mitigation measures.
“Low/Medium/High” risk without mitigation.		The mitigation measures will render the likelihood of all risks <u>low</u> .
Data sharing restricted due to legal restrictions arising during the project (medium)	WP2 + WP1, 6, 7	Wide range of algorithmic possibilities, tools & techniques for creating aggregated, sharable, pseudonymized data
Too rigid database design might limit data exploitation as research questions evolve (low)	WP2	Choice of database strategy supporting expansion, and regular consultation with coordinators to anticipate required additions.
Insufficient adoption of MESI-SEEK platform (medium)	WP2 + all	Teaching and training of partners by HITS and UDUR high quality easy-to-use software
Insufficient data quality (medium)	WP2 + all	Curation support, training, & software that helps creating high quality data; Strong support by coordinator for such measures.
Issues with time scale differences in metabolic and signaling responses to perturbations (medium)	WP3, 4, 5	Adaptation of time-series measurements; change of time points; adaptation of measured biological level (e.g., RNA in addition to protein); measurements taken under steady-state conditions
Insufficient tumor material in samples for molecular characterization (medium)	WP3	Collect additional samples; repeat the analysis and optimize them for lower amounts of proteins or metabolites
Unexpected network interactions discovered by network interference studies (WP3, 4) not covered by the models (WP5) (high)	WP3-5	Meet the network specialists (WPs3, 4, 5) and discuss potential adaptation of the model
Design of experimental approaches (WP3, 4) not optimal for perturbation studies (WP5), as initially selected time points may be unsuitable or inhibitors too unspecific (high)	WP3, 4 & WP5	Run small experimental tests to quickly re-design the experimental approaches, to adapt time points, extraction protocols or perturbation regimens
Setup of standardized metabolite extraction protocols delayed, as some metabolites might be unstable or LC-MS may not be accurate enough for their quantification (low/medium)	WP4	Use metabolite-specific extraction protocols or biochemical kits (rather than, for example, LC-MS-based methods) for measurements of key metabolites
Calibration and validation issues with computational models of MESI networks (medium).	WP5	Structural and practical identifiability analyses will ensure that model parameters can be estimated from data. Arising issues will be addressed by additional data or by model reduction. Problems of model validation (i.e., differences in model predictions and experimental testing) will be addressed with modifications in topology, i.e., model reduction, or model enrichment and additional data.
The initial models covering ER-mTOR-MAPK and Trp-NAD networks may not cover sufficient components to distinguish between different patient subgroups (low).	WP5 + WP3 & 4	Omics and network analyses will be expanded to identify RNAs/ proteins whose differential expression correlates with MESI networks to strengthen their predictive power.
The available ER+BC PDX models of VS are not representative of critical subgroups identified by MESI-STRAT (medium)	WP6	Agreements via EurOPDX are in place to share available ER+BC PDX models among consortium partners. VS can generate additional models for critical subgroups for which no PDX is available.
Poor engraftment rate of ER+BC PDX in immunocompetent models (medium)	WP6+7	Collection of additional patient material; adaptation of xeno-transplantation protocols
Poor recruitment or high dropout for WOO Trial (Study 5) (low)	WP7 + WP1	Our estimates on recruitment rates are low and already entail 20 possible dropouts. We will provide high quality training and information about the MESI-STRAT studies to support the recruiting physicians.
Poor recruitment or high dropout for ET Termination Trials (Study 6) (low)	WP7 + WP1	Enhance measures to promote awareness and motivation by information, e.g., at patient days, via letters, networking with patient organization and patient information platforms etc. (see impact, 2.2.4).
Insufficient stakeholder engagement (low)	WP9 + all	Improve workflow with other WPs and the efficiency of the process.
Lack of stakeholder availability at annual meetings and workshops (medium).	WP9 + all	Early planning, early availability assessment, and early notification of potential workshop dates.

3.3 Consortium as a whole

3.3.1 Match of the consortium with MESI-STRAT objectives, expertise and complementarity of the members, and value chain

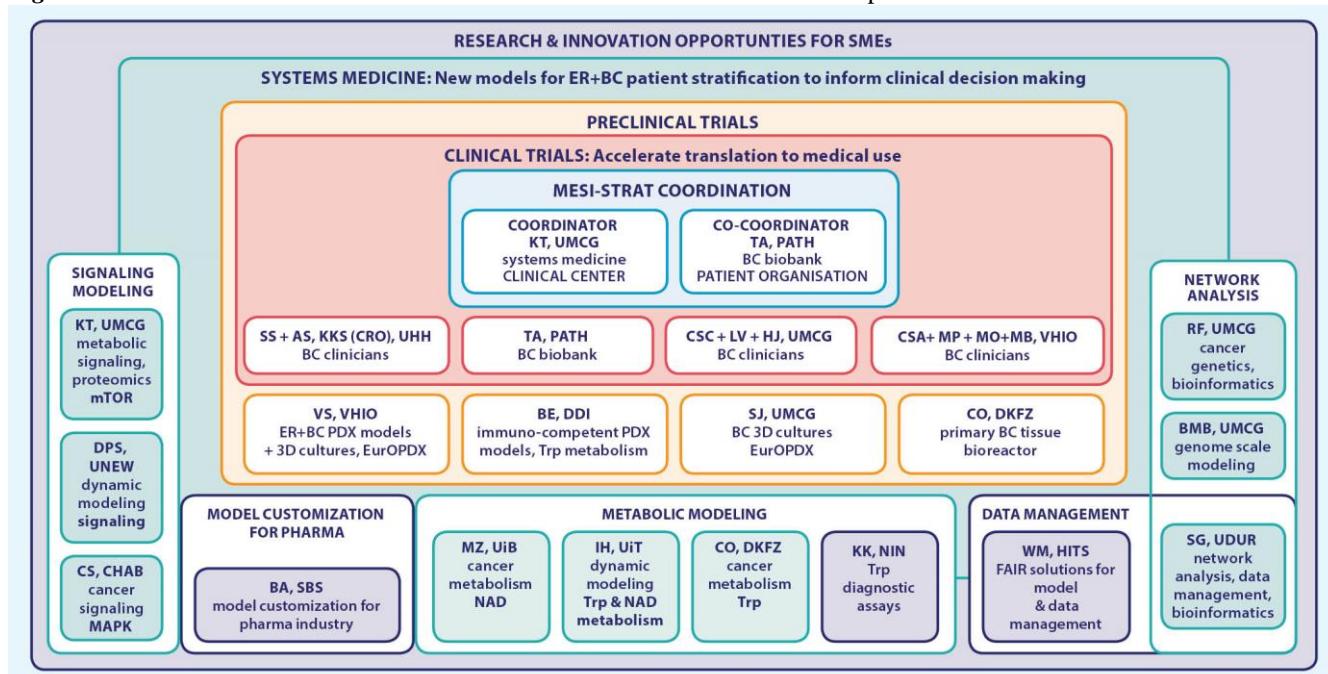
MESI-STRAT assembles the scientific excellence and essential multidisciplinary expertise (biology, medicine, mathematical modeling, bioinformatics, biobanking, and patients' expertise) to implement MESI-STRATs systems medicine approach to deliver and to preclinically and clinically validate novel concepts for ER+BC patient stratification (see also Section 1.3.a for short description). Key complementary expertise is assembled into **multidisciplinary expert teams** that link the work across the work packages and pan-European institutions (detailed in 3.3.2). The interconnection of academic and clinical partners with the partnering SMEs SBS, NIN, and HITS and collaborating SMEs and pharma companies ensures the implementation of the multi-level **MESI-STRAT value chain**, further detailed in 3.3.3.

As evidenced by joint papers and grants, the **MESI-STRAT consortium has close internal links**. Several partners cooperate within the GlioPATH (CO, IH, KT), MAPTorNET (CS, DPS, KT), PROMETOV (CO, KT) and EuroPDX (VS, SJ) consortia, which will deliver data and models to MESI-STRAT. Many partners are linked by a strong joint publication history (e.g., DPS+KT; IH+MZ; KT+SG; CO+KK+SS; VS+CS; VS+SJ; TA+AS, etc., see partner descriptions in section 4 for details). MESI-STRAT will link our separate initiatives into an integrated, synergistic effort that enables European systems biology (CISBAN, SBC-EMA, DKFZ) and BC (VHIO, UHH, UMCG, DKFZ) centres to develop new models for disease-mechanism-based stratification of ER+BC patients for targeted therapies.

3.3.2 Roles and resources of the project partners

The **coordination tandem** encompasses systems medicine (KT, UMCG) and clinical BC and biobanking expertise (TA, PATH). The role of the patient organization PATH as a co-coordinator not only ensures the timely, longitudinal collection of ER+BC patient tissues to reach our scientific and clinical aims; but the PATH patient expert DS together with the cancer information service (SW, DKFZ) also ascertain that the patients' perspective is embedded at all project levels, from the earliest planning throughout implementation and actions for dissemination, exploitation and communication.

Figure 10. The MESI-STRAT consortium as a whole. Roles of the consortium partners.



The **interdisciplinary MESI-STRAT expert teams** consist of modelers and experimentalists/clinicians who specialize in different aspects of cancer signalling and metabolism, and preclinical and clinical BC research. The expert teams will focus on (*i*) **signaling** (KT, DPS, CS), (*ii*) **metabolism** (IH, CO, MZ, KK, BB), and (*iii*) **network analysis** (RF, BMB, SG, WM), (*iv*) **preclinical trials** (VS, BE, SJ, CO), (*v*) **clinical trials** (SS+AS, TA, CSC+LV+HJ, MP+MO+MB), and (*vi*) **model customization for pharma industry** (BA + MESI modeling expert teams) (Figure 10). Relying on their joint biological/clinical expertise and their synergistic methodological expertise, the expert teams will bridge the method-focused WPs to run and develop the

MESI-STRAT systems medicine project plan. Effective cooperation is ensured by the MESI-STRAT management plan (WP8) and continuous information management (described above). The bioinformatics **MESI-SEEK platform** (WM, SG) will collaborate closely with EMBL-EBI (see enclosed letters) and the **dissemination and exploitation committee** (DEC, headed by TA and BA). This will enable the integration of biological and medical research data with computational modeling and to make MESI-STRAT data openly accessible (open data) while ensuring commercial exploitation opportunities and patients' interests and privacy. The **gender committee** (GC, headed by CSC, UMCG + SS, UHH) will ensure the implementation of gender aspects throughout, particularly focusing on the validation of MESI panels, identified in female ER+BC, in male BC cohorts.

As detailed in 3.4 all partners have **significant resources** that are tailored according to their respective tasks. This is particularly important for the clinical partners, as the mandatory clinical trials are costly and their implementation is key to the success of MESI-STRAT; and for the patient organization PATH and the partnering SMEs who all have key roles in our consortium, which is also highlighted by their leadership or co-leadership in WP1 (PATH), WP2 (HITS), WP8 (PATH), and WP9 (SBS, PATH).

3.3.3 Industrial/commercial involvement in MESI-STRAT: structure of the consortium to support exploitation and to build the MESI-STRAT value chain (see also Section 2.2).

The combined clinical and scientific expertise provide a unique opportunity for synergistic efforts to develop and implement a pipeline that covers the earliest steps of model development for ER+BC patient stratification up to validation in preclinical and clinical trials, and customization of our MESI-STRAT models for the pharma industry. Our pipeline entails (i) the education of students for model development at the academic partner centers; (ii) their subsequent employment at **the SME SBS** where they further develop and customize the MESI-STRAT models for pharma clients, under the supervision of experienced computational SBS experts; and (iii) trial licensing of MESI-STRAT models by SBS to explore their potential for expanding the SBS services to the pharma industry. All **stakeholders** of this **value chain** including the SME SBS, pharma clients (Novartis, Merrimack, and others), clinicians and patients are integral parts and/or close collaborators of MESI-STRAT. Moreover, the MESI-STRAT IAB covers the full stakeholder chain (clinicians, patients, pharma, and systems oncology companies) and can hence effectively assist in implementing our value chain. Our new models for clinical decision making will increase cost-effectiveness for clinical trial design (due to smaller cohorts necessary to observe statistically significant effects) and ER+BC therapies (due to avoidance of ineffective treatments and side effects by stratifying patients for targeted therapies). Hence, the commercial value of well-characterized predictive computational models for ER+BC patient stratification is potentially high – resulting in a market advantage for SBS. Initially tailored for ER+BC our models can be adapted to all cancers that affect the molecular networks addressed by MESI-STRAT. As most cancers show dysregulated mTOR/MAPK signaling and/or energy and Trp metabolism the MESI-STRAT models will be broadly applicable and hence have a market potential far beyond ER+BC. In addition, the implementation of our value chain will help SBS gain new well-educated computational personnel – which is currently a serious bottleneck for all companies active in the computational sector.

The SME HITS gGmbH has partnered with MESI-STRAT to support data management and to further develop its own platforms and customer services. Participating in MESI-NET constitutes a reference collaboration for HITS gGmbH in systems medicine for the openSEEK platform and the FAIRDOMHub, and enables the enhancement of systems biology infrastructure business models.

The SME NIN develops diagnostic assays for amino acid metabolites. Thus NIN can directly exploit the MESI-STRAT results to design new diagnostic assays for MESI marker panels with known and new prognostic metabolites. Like SBS, also NIN will profit from favourable conditions for trial licensing to explore the potential of MESI-STRAT results for commercial exploitation, and, if successful, will strive to acquire long-term licenses.

Wherever possible the MESI-NET consortium will involve additional industrial partners and/or advisors to ensure that the developments stay in line with market demand and requirements. The level of involvement will depend strongly on the stage of development and the time required to reach the market. The **SMEs QuantuMDX** and **CELLEC BIOTEK** are contributing to the programme as associate partners (see enclosed letters). Their expertise in diagnostics and tailored solutions for preclinical drug testing will be valuable in evaluating the MESI-STRAT results and in obtaining the proper focus to match our results with the market demand. For therapeutic developments, MESI-STRAT will work closely with pharma companies already involved in clinical cooperation with UHH, UMCG, VHIQ, DKFZ, UNEW and DDI (see section 1.3.a) to maximize the window of opportunity for commercial exploitation (Section 2.2).

3.3.4 Other countries and international organizations

All MESI-STRAT partners are based in countries eligible for EU funding.

3.4 Resources to be committed

All partners involved in the MESI-STRAT project are committed to achieve a significant breakthrough in the field of systems medicine by developing new concepts and models for BC patient stratification. The resources and facilities that each organization dedicates to the project (see B4 partner descriptions) further demonstrate their determination to successfully implement the tasks ascribed to this project. The following figures provide an overview of the resources to be committed within the project.

Figure 11: Total effort (PM) distributed per Work Package

The total project effort is of 694 person months (PM), out of which 90,13% are fully dedicated to RTD (Research and Technological Development, WP1-7) activities. All together the 7 WPs dedicated to RTD represent a significant effort of 625,5 PM. 68,5 PM (9,87% of the total) are devoted to management activities (including IP management, WP8) and to the MESI-STRAT impact (dissemination, exploitation, and communication, incl. regulatory issues for commercialization, WP9). WP1 and WP2 (9% of total PM) are dedicated to management of samples and clinical data (MESI-REPOSITORY) and scientific data (MESI-SEEK), and WP6 and WP7 (23% of PM) are dedicated to clinical trials, incl. ethics and medical regulatory issues.

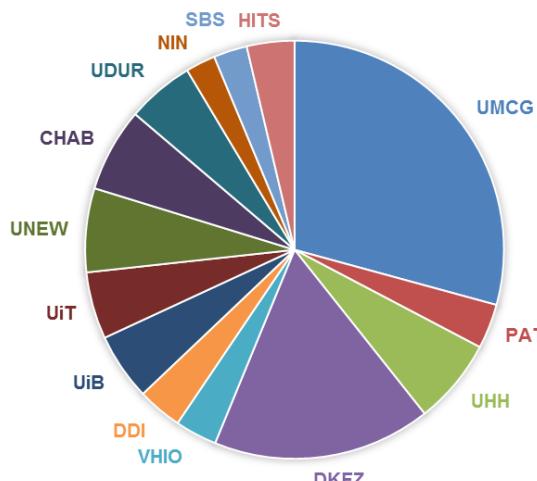
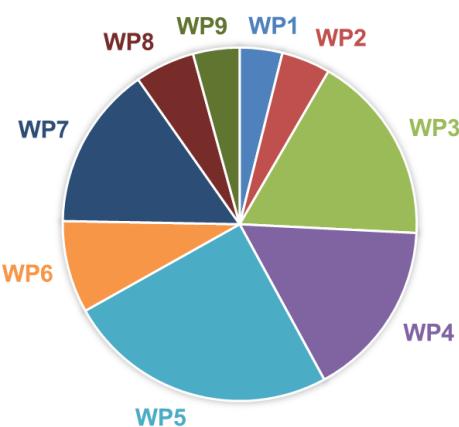


Figure 13 Budget distribution per partner (in EUR)

The total budget of MESI-STRAT distributed among 14 partners amounts to 5,949,964 EUR. The partners UMCG (NL) and DKFZ & UHH (DE) receive 51% of the total requested EU budget for the same reason as indicated in figure 12.

Figure 12: Total effort (PM) distributed per partner

The total staff effort to the MESI-STRAT project is divided among 14 partners. Partners UMCG (NL) and DKFZ & UHH (DE) are allocated 52% of the total staff effort. This is due to the fact that these partners host the clinical studies and multiple measurements and modeling efforts, which require different expertise from experimentalists, clinicians, and computational scientists. Furthermore, UMCG hosts the project coordination (WP8).

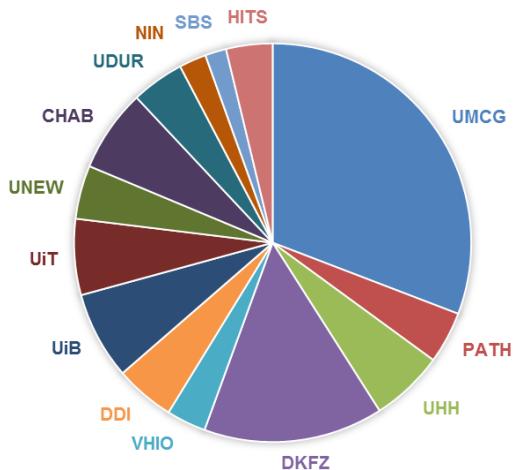


Table 3.4a: Summary of staff effort

WP leaders are identified by showing the relevant PM figure in bold.

	WP1	WP2	WP3	WP4	WP5	WP6	WP7	WP8	WP9	Total Person/Months per Participant
1. UMCG	1	1	69	12	56	2	21	29	12	203
2. PATH	9	1				8	3	1	2	24
3. UHH	10	1				8	25	1	1	46
4. DKFZ	1	1	4	51		35	23	1	1	117
5. VHIO	3		3			3,5	12		1	22,5
6. DDI	1					2	20		1	24
7. UiB		1		33				1	1	36
8. UiT					35				1	36
9. UNEW		1			42			1	1	45
10. CHAB		1	35	1	6			1	1	45
11. UDUR		6	8		20			1	1	36
12. NIN				15					1	16
13. SBS					12			1	5	18
14. HITs	2	18	2		1			1	1,5	25,5
Total PM	27	31	121	112	172	58,5	104	38	30,5	694

Table 3.4b: ‘Other direct cost’ items

This table justifies the costs for ‘travel’, ‘equipment’, and ‘goods and services’ where the total sum exceeds 15% of the personnel costs for a given participant (see budget table in form A).

1. UMCG	Cost (€)	Justification
Travel	20,000	Travel budget for coordinator, physicians, and research postdocs to consortium + WP leader meetings, patient days, and European systems medicine conferences.
Equipment	19,900	Specialised computer for modeling (5,000 EUR), bioreactor for cancer tissue culture (14,900 EUR).
Other goods and services	288,250	Standard consumables. Extra budgets for proteomics and metabolic measurements (WP3, 4: 80,000 EUR); MESI-STRAT consortium management (WP8, 87,500 EUR) incl. organisation of consortia and WP leader meetings, IAB travel costs; measures to raise impact (WP9 24,750 EUR) incl. open access publishing, daily rates patient organisation Europa Donna, feasibility studies, website
Total	328,150	
2. PATH	Cost (€)	Justification
Travel	8.000	Travel budget for PI and medical scientist to join consortium meetings.
Other goods and services	25,000	Extra budget for the organization of 3 European patient days in WP9 (Dissemination).
Total	33,000	
3. UHH	Cost (€)	Justification
Travel	8,080	Travel budget WOO trial manager, and PI and medical scientists to consortia and WP leader meetings
Other goods and services	56,625	Standard consumables. Extra budgets for clinical trials incl. sample and data processing for 70 patients (36,261 EUR), competent and local authoritie(s) fees (4,425 EUR), trial insurances (4,200 EUR), ethics committee and national institution fees (6,300 EUR), trial audit costs (2,000 EUR) for WOO Trial.
Total	64.705	
4. DKFZ	Cost (€)	Justification
Travel	12,000	Travel cost for PI and 2 scientists to join consortia and WP leader meetings.
Equipment	29,800	2 Bioreactors for primary culture of cancer tissue.
Other goods and services	170, 080	Standard consumables. Extra budgets for primary cell culture media and reagents (32,000 Euros); metabolomics columns /consumables; panel seq & RNaseq (25,480 Euros), NCT tissue bank (2,000), bioreactor consumables (21,600 Euros), and audit costs (5,000 EUR).
Total	211,880	
5. VHIO	Cost (€)	Justification
Travel	4,000	Travel cost for PI to join consortia meetings
Other goods and services	85, 000	Standard consumables. Extra budgets for the collection of tumor tissue and blood; animal costs (60,000 EUR) incl. chemosensitivity tests, and establishing two new ER+BC PDX models (20,000).
Total	89,000	
6. DDI	Cost (€)	Justification
Travel	8,000	Travel costs for 2 persons to all consortia meetings.
Other goods and services	60,000	WP7 requires budget for establishing up to four immunocompetent PDX BC mouse models, and running up to two preclinical therapy trials in these models (animal housing for long term in vivo studies, and anticancer therapeutics, incl. IDO 1 inhibitor (Epacadostat).
Total	68,000	
10. CHAB	Cost (€)	Justification
Travel	8,000	Travel budget for PI and postdoc to join consortium meetings.
Other goods and services	42,000	The amount of 40,000 Euros for consumables exactly equals 15% of the personnel costs. This is needed to carry out signaling studies in WP3 (cell culture reagents incl. media, fetal calf serum, additives, antibiotics); compounds and reagents (RNAi, CrisprCas9) for perturbation screens and analyses; antibodies, BioPLEX analysis (multiplex kits), Incucyte consumables for growth/survival/migration analysis; general lab consumables (plastic, chemicals etc.). Furthermore 2,000 EUR audit costs apply.
Total	50,000	
12. NIN	Cost (€)	Justification
Travel	3,900	Travel budget for researcher to join consortium meetings.
Other goods and services	29,500	Standard consumables. Additional budget for materials for L-Kyn, Trp, Serotonin, KA, QA metabolite diagnostic assays (15,000), IDO1 protein assays (2,500), and multiplex assay development (12,000).
Total	33.400	

- **Please note:** No costs of large research infrastructure are requested

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