DISCOVERIE

Project No. 848228

DISCOvERIE

Development, dIagnostic and prevention of gender-related Somatic and mental COmorbitiEs in iRritable bowel syndrome In Europe

Workpackage 7
Deliverable D7.3

Report on effect of approved drugs on candidate mechanisms

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Consortium

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17	Cliclab Technologies Inc	ES	Cliclab
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1. Executive Summary

Background

Focus of analysis is the pathophysiology of Irritable Bowel Syndrome (IBS) and comorbidity conditions including Depressive Disorder, Major (DDM), Anxiety Disorders (ANX), Fibromyalgia (FM) and Fatigue Syndrome, Chronic (FSC). Work package 7 (systems biology and integromics) aims at integrating molecular and clinical data for contributing to the understanding of pathophysiology of comorbidity onset and development, and to propose biomarker candidates for testing molecular mechanistic hypotheses, in translation adding to tools for improving comorbidity diagnosis and prognosis.

The given patient population is frequently under drug treatment. In consequence, drug Mechanism of Action - exhibiting effect on pathophysiology and linked biomarker expression levels - needs to be taken into consideration.

Specific research goals of this deliverable

Research contributing to this deliverable focused on evaluating drug Mechanism of Action on top of comorbidity pathologies and embedded biomarker candidates. Goals of analysis were **i)** identify drugs effectively prescribed in the patient population potentially exhibiting effect on IBS and one (or more) of the comorbidities in focus, and **ii)** identify other drug candidates potentially exhibiting an effect on IBS and one (or more) of the comorbidities. While the first aspect analyses effect of drugs prescribed in the patient population, the second aspect shall propose alternative drug candidates for adding to understanding pathophysiologies (drug effect for probing underlying molecular mechanisms), and if applicable promising beneficial effect. Analyses are executed in-silico, hence all results are to be considered research hypotheses, to be further evaluated in experimental settings.

Research procedures

In-silico analyses grounded in molecular process models (interactomes) for IBS and comorbidity pathologies, together with models of drug Mechanism of Action also represented on the level of interactomes. This setting allowed integrated analysis of pathology details in relation to a drug's effect on molecular processes. Research procedures integrated a variety of data sources for concluding on respective interactome models together with project foreground data on drug prescription.

Results

According to the project workplan, two aspects were covered on the results level: **i)** a shortlist of prescription drugs identified via molecular process analysis and being identified in clinical medication data, and **ii)** a shortlist of prescription drugs identified via molecular process analysis potentially exhibiting effect in IBS comorbidity conditions. Further, biomarker candidates were assigned to both drug lists.

Utilization

All results data are organized in spreadsheets, linking pathologies, biomarker and drug effect, and were provided to project partners for clinical and pathophysiological interpretation in the realm of project foreground data, complemented by biomarker assay development plans.

The latter aspect is a central mean for hypothesis testing and fostering translation toward clinical use.

2. Introduction

2.1 Activity background

According to the project workplan, research executed in WP7 aims at contributing to three goals: First goal is identification and annotation of candidate molecular processes and mechanisms present in - or triggering development of – comorbidities on top of IBS. Second goal is identification of candidate molecular biomarkers embedded in identified molecular mechanisms. With such embedding, causal biomarkers shall be identified supporting diagnostic and prognostic assays for informing on onset and development of comorbidity conditions.

The given patient population sees drugs prescribed for either IBS or any of the comorbidity conditions, and drug Mechanism of Action needs to be taken into consideration for both, pathophysiology status (of IBS alone as well as of comorbidities) and embedded biomarker expression. In consequence, the third goal is evaluation of drug Mechanism of Action on top of comorbidity pathologies, specifically of drugs prescribed in this patient population.

Research contributing to this deliverable 7.3 focused on drug effect evaluation. Specific activities span elements as defined in the project task T7.6 (Identify drug Mechanism of Action (MoA) interference with mechanistic pathology links) of the workplan. The task foresees **i**) analysis of approved drugs used in comorbidity cohorts, and **ii**) identification of approved drugs at present not prescribed in comorbidity conditions but potentially exhibiting beneficial effect. Further, for supporting experimental evaluation of findings, candidate biomarkers and respective molecular mechanisms are to be linked with proposed drug effect.

Management of activities was implemented by WP7 lead (Partner 15, EMTEC), including project foreground data generated in WP2 (Case-control recruitment and follow-up), specifically of task T2.2 (Prospective recruitment of IBS patients, disease controls, and healthy controls) driven by the project partners UGOT, KUL, VHIR, UNIBO, UM, UMF, SKU, GUF and SU. Respective data transfer agreements are in place, all data were retrieved and integrated in a fully anonymous form solely using comorbidity assignment, drug names and prescription frequencies.

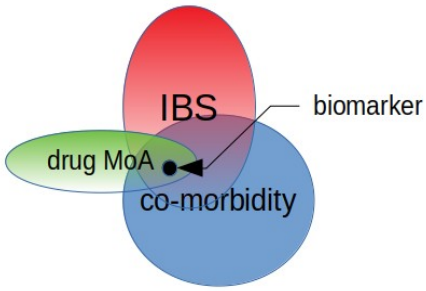
2.2 Research proposition

Research proposition for analyzing drug effect *in-silico* grounds in a model for comorbidity pathophysiology, given as composite molecular processes. Recalling this model assumption according to D7.1, a disease initiates as combination of genetic predisposition and environmental factors (including life style), subsequently displaying a personalized trajectory of disease evolution, manifesting on the level of clinical presentation. Such trajectory is considered as state-transition system, where individual molecular processes of IBS and comorbidities may be shared, or linked (e.g. via mediators), or may resemble a composite (a pathology aspect of IBS is linked with a molecular process also linking with an aspect relevant for a comorbidity or vice versa).

Such molecular model representation served as basis for selecting molecular characteristics for testing toward improved diagnosis, potentially adding to prognosis, and finally adding information on optimal therapeutic means. As a consequence, biomarkers are vital for testing

pathophysiology states along disease evolution, and shall also respect the effect of prescribed drugs.

Integrated analysis of IBS, comorbidities, drug Mechanism of Action and embedded biomarkers is schematically given in Figure 1:



Irritable Bowel Syndrome (IBS)
comorbidity:

- mental:
  - anxiety (ANX)
  - depressive disorder, major (DDM)
- somatic:
  - fibromyalgia (FM)
  - fatigue syndrome, chronic (FSC)

drug Mechanism of Action (MoA)

Figure 1: Integrated analysis of IBS, comorbidities, drug effect and embedded biomarker candidates. Each entity is characterized as interactome models specific for morbidities and drug Mechanism of Action.

Central to computational analysis are molecular process models of IBS and the individual comorbidities. Recalling the deliverable D7.1 (covering identification of candidate molecular processes and mechanisms) these models are given as human protein coding gene interaction networks (including SNPs, miRNA and metabolites being linked to respective protein coding genes as applicable), with nodes coding for molecular features and edges coding for interactions:

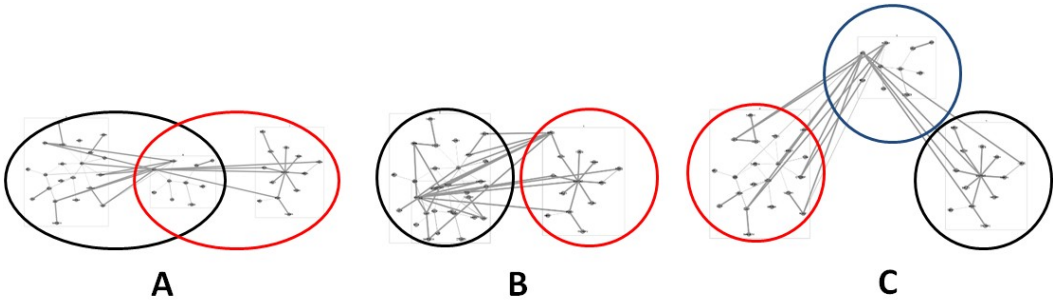


Figure 2: (**A**) Shared disease pathology molecular context; Pathology I (black, e.g. of IBS) and pathology II (red, of a comorbidity) hold common aspects given by the intersection. (**B**) Mediated comorbidity; Pathology I and II integrate in an embedded pathology, indicated by the arrows linking pathology I and II context. (**C**) Composite comorbidity; an additional molecular context (blue) links pathology I and II.

Views on comorbidity pathologies according to Figure 2 served molecular process/mechanism analysis, as well as biomarker candidate selection. With activities toward

D7.3 another perspective came in: Also drug Mechanism of Action (MoA) can be represented as molecular process networks (molecular features being affected by a drug, together with respective interactions), bringing pathologies and drug MoA to a common technical level of description.

This setting allows molecular process interference analysis in analogy to Figure 2A, but now evaluating overlap of pathology (black context) and MoA of a selected drug (red context). With the use of an internal library of drug MoA molecular process models a systematic analysis was executed for individual as well as composite pathology models of IBS and comorbidities.

The result of in-silico screening provided a ranked list of drug interference for IBS and each comorbidities, and finally ordered lists of drugs displaying interference with comorbidity situations. As resolution of interference is given on the level of individual molecular features, also biomarker candidates shortlisted in D7.1 can be analyzed if being part of interference. Accordingly, drug effect-specific biomarker candidates are provided.

2.3 Implementation

Implementing the research proposition outlined in the above section demanded preparation of a series of prerequisite data including background and project foreground.

Prerequisite: Molecular pathology models

Molecular pathology models (protein coding gene interactomes) were established for IBS as well as for comorbidities (of type “mental”: anxiety (ANX), depressive disorder major (DDM); of type “somatic”: fibromyalgia (FM), fatigue syndrome chronic (FSC); technical details were reported in deliverable D7.1). Further, comorbidity models from molecular process composition resulting in an interactome model embedding aspects of IBS and FM (a composite of type “somatic”), and in another interactome model embedding aspects of IBS and DDM/ANX (a composite of type “mental”) were derived. Each model was analyzed for enrichment of molecular processes and mechanisms of potential relevance in each disorder/comorbidity situation.

Prerequisite: Embedded biomarker candidates

Each molecular pathology model was analyzed for embedded biomarker candidates for serving diagnosis/prognosis of comorbidity conditions. I.e., a biomarker candidate was deemed to be part of a joint, mediated or compositionally linked molecular process for IBS and at least one of the comorbidities in focus. The biomarker identification procedure was reported in D7.1, including a consortium-wide consultation.

Prerequisite: in-silico drug mechanism of action (MoA) interference

Applying a method workflow discussed in Curr Pharm Des 23, 29-54 (2017), a proprietary in-silico library of drug/compound Mechanism of Action molecular models (MoA, also presented in the form of protein coding gene interactomes), was used. Each morbidity model was algorithmically overlaid with each entry of the drug/compound library. Relevant overlay (i.e., molecular features together with their interactions identified in a pathology model and a drug

MoA model) was defined by the number of matching protein coding genes, backed by statistical evaluation of enrichment. Result of this procedure was a ranked list of drug/compound interference for each pathology model, as covered in the 2nd periodic project report.

Prerequisite: medication data from clinical practice

From project foreground (WP2; CASTOR study) information on medication for 871 subjects became available. Each subject is either diagnosed with IBS only, or in one of the comorbidity conditions, or serves as reference (healthy controls and individual morbidities). The total set of medication entries is comprised of 2569 (in part redundant) entries.

Workup of this medication item list included a series of *manual* curation steps.

a) assignment to categories

Each unique medication entry was assigned to the categories “drug”, “endogenous compound”, “supplement”, “other”, “ndef”.

A summary on category assignment is given in Table 1:

Total = 2569 (non-unique entries)	**drugs**	**endogenous compounds**	**supplement**	**other**	**ndef**
count	1,840	386	250	46	47

Table 1: CASTOR medication assignment (all counts refer to non-unique entries) to categories.

A drug was defined as specific chemical substance (and respective Mechanism of Action); an endogenous compound was defined as single chemical entity being part of human (patho)physiology (e.g. calcium, amino acids); a supplement was defined as a mix of compounds (e.g. certain nutritional supplements); “other” included e.g. non-systemic medication (e.g. local/topical administration); “ndef” covered compound/drug classes not allowing assignment to a defined chemical entity/category.

b) assignment to generic drug/compound names

CASTOR entries provided a mix of (also country-specific) brand names as well as generic names. Using NCBI MeSH and drugbank as reference (go.drugbank.com) each entry was assigned a standardized reference name. This procedure was possible for all drug entries according to Table 1.

c) matching with in-silico drug MoA library names

The internal drug MoA library uses name spaces from NCBI MeSH and a free version of drugbank. The drugbank name space was matched with the internal drug MoA library name space.

d) drug analog assignment

A certain number of drugs provided in CASTOR did not match with entries of the internal drug MoA library. For these items closely matching items form the same drug class were identified as applicable.

For a small number of drug entries listed in CASTOR no match with the internal drug library was possible: The total set of 1,840 drug entries is comprised of 256 unique entries. Of these, 233 entries were covered in the in-silico drug MoA library and were amenable for analysis; 23 drugs needed to be excluded.

Prerequisite: subject morbidity assignment

From project foreground (WP2; CASTOR study) information on morbidity assignment for 871 subjects became available. For medication analysis particular interest was in subjects with IBS and one (or more) additional comorbidities including ANX, DDM, FM, CFS. Number of subjects are given in Table 2:

total = 524	**IBS only**	**+ ANX**	**+ DDM**	**+ FM**	**+ CFS**
count	226	190	190	105	121

Table 2: CASTOR subjects assigned to morbidities. Subjects assigned to control groups not included.

The total set of patients with IBS and at least one of the comorbidities is 298. The remaining subjects (226) are assigned to IBS only. Subsequent analysis specifically focused on drug medication data listed for the comorbidity situations.

3. Research procedure

3.1 Analysis scheme

With prerequisites in hand the analysis scheme according to the workplan was executed.

Research questions

- Which drugs with identified molecular model interference for IBS and at least one of the comorbidities are also identified in CASTOR as prescribed drugs for IBS or/and a comorbidity condition?
- From the overall screen of drugs given in the in-silico reference library: Which drugs do exhibit statistically relevant interference with IBS and one (or more) of the comorbidities, but not being listed in CASTOR medication data?
- For all identified drugs of interest, which biomarker candidates (from the given list of candidates, further linking to molecular mechanisms) may allow to identify possible drug effect on molecular mechanisms relevant in comorbidities?

While pathology model analysis aimed at identifying joint/mediated/composite molecular mechanisms for comorbidity development, drug Mechanism of Action analysis aimed at identifying the impact of drug effect on comorbidities. Involvement of biomarker candidates

links drug effect and mechanisms, and shall provide experimental means for subsequent testing of drug effect hypotheses.

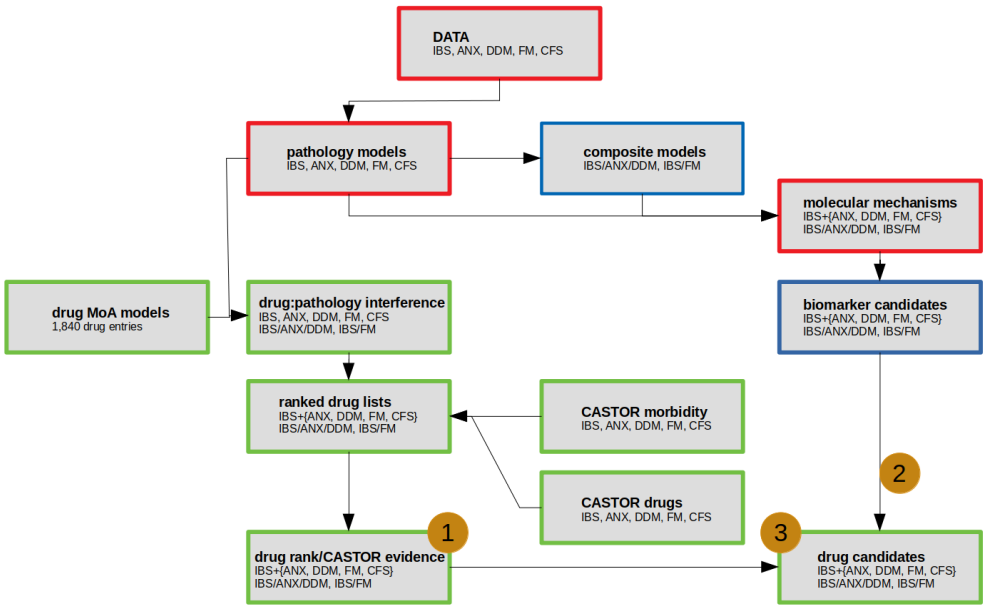


Figure 3: Data flow across involved WP7 activities (red: task T7.3; blue: task T7.4; green: task T7.6), and specific integration of WP2 data (CASTOR study). According to research questions, (**1**) aligns in-silico results and medical prescription data, (**2**) evaluates if selected biomarker candidates allow testing of drug effect, and (**3**) evaluates drug candidates with comorbidity interference but not prescribed in the patient cohort.

3.2 Results

Step 1: ordered drug lists

For IBS, ANX, DDM, FM and FSC, in-silico screening provided pathology-specific, ranked lists of drugs displaying interference according to the interactome models. From these ranked lists pair-wise *ordered* sets were derived covering the morbidity pairs IBS-ANX, IBS-DDM, IBS-FM and IBS-FSC. The order of a drug for an IBS-comorbidity combination is given by the sum of ranks identified for IBS and the respective comorbidity. For composite models inherently covering morbidity combinations (IBS/ANX/DDM; IBS/FM), order of drugs was kept according to interference rank. The result of this step offers ordered drug lists, with the top candidate drug listed first:

	IBS				DDM				
drug name	feature #	p-value	enrichment	*rank*	feature #	p-value	enrichment	*rank*	**order**
									1
									2
									…

Figure 4A: Drug effect annotation scheme, exemplarily given for IBS and DDM. Given is interference (overlap) of a drug MoA model and a morbidity (feature #), statistical evaluation of interference (p-value, enrichment), and rank. The “order” of a drug name is the combined rank.

This list allows to **i**) evaluate if drugs identified via in-silico screening also show up in the medication list of the patient population according to CASTOR study data, and **ii**) may hold drug candidates with potential effect in comorbidity conditions, at present not prescribed in a condition in focus according to CASTOR data.

Step 2: drug effect evidence screening

For adding evidence to identified drug interference, counts of scientific references were included for every drug : morbidity situation. For this, NCBI PubMed was queried via utilizing MeSH annotation regarding reporting of a drug in the context of IBS or any of the comorbidities. Identified references were further classified into translational evidence (coming from in vivo studies or clinical trials) and research evidence (coming from explorative analysis or in vitro studies).

	IBS			DDM			
drug name	translation #	research #	*rank*	translation #	research #	*rank*	**order**
							1
							2
							…

Figure 4B: Drug effect scientific reference annotation scheme, exemplarily given for IBS and DDM. For a drug name, counts on scientific references in the categories “translation” and “research” are provided together with a rank, combined into an “order” for an IBS-comorbidity pair.

Identification of candidates together with reference annotation provided grounds for adding further evidence from medication data seen in clinical practice.

Step 3: adding CASTOR drug frequencies

For each of the IBS-comorbidity conditions and involved drugs identified via in-silico screening, drug prescription frequencies identified in CASTOR were added. The following Table 3 displays the number of drugs being proposed by in-silico analysis and being identified in CASTOR medication (irrespective of prescription frequency) for the different comorbidity conditions.

total	**IBS-DDM**	**IBS-ANX**	**IBS-FM**	**IBS-FSC**
61	53/11	52/11	49/9	46/8

Table 3: Number of drugs identified via in-silico analysis and provided in the CASTOR medication set, and number of drugs in the top 25% of drugs screened according to order (combined rank for IBS and a comorbidity according to interference analysis).

In total 256 unique drugs were identified in the medication set (each with varying frequency), of which 233 were amenable for in-silico analysis. From this number of 233 unique drugs, 61 drugs display some level of molecular model overlap with IBS-comorbidity pathology models,

fairly evenly given for the individual comorbidity pairs. A drug included in this shortlist displayed overlap with IBS and in the majority of cases with all comorbidities to varying extent.

The 61 drugs are only a subset of about 25% of drugs analyzed; 75% of drugs prescribed do not show interference with IBS and at least one of the comorbidity models. In one interpretation according to the underlying model of pathology and drug molecular model interference, the majority of drugs is prescribed due to other morbidities.

However, also the remaining 61 drugs display weak *order* for comorbidity situations (see Figure 4A), even though each drug may come with a high *rank* for either IBS or one of the comorbidities. From the total set of 233 drugs being forwarded to in-silico screening, the mean order for comorbidity situations is about 160 (with a value of 1 being highest order, and 233 lowest order). This finding also holds for composite models of IBS/FM and IBS/DDM/ANX.

One interpretation is that the subset of 61 drugs are biased toward prescription for either IBS or one of the comorbidities, and such drugs come with weak interference with the respective other morbidity. This interpretation is also supported by scientific reference annotation counts, being mostly either with IBS or with a comorbidity.

As overall interpretation (and respecting all limitations of model assumptions), prescription drugs in IBS-comorbidity situations do not come with apparent cross-morbidity effect. Still, according to Table 3, a certain number of drugs is in the top 25% of the drug set according to order. This set of drugs warrants further analysis with respect to potential effect in IBS and a comorbidity.

Step 4: Other drugs with significant interference

The set of drugs discussed in Step 3 is defined by being prescribed according to CASTOR data and displaying overlap on a molecular mechanism level. Next analysis inspected drugs showing overlap with IBS and a comorbidity according to underlying mechanism models, but not being identified in the CASTOR medication set. Table 4 summarizes drug counts according to given criteria.

total	**IBS-DDM**	**IBS-ANX**	**IBS-FM**	**IBS-FSC**
23	21/13	17/13	7/7	10/10

Table 4: Number of candidate drugs showing significant interference as identified via in-silico analysis, and number of drugs in the top 25% of drugs screened according to order (combined rank for IBS and a comorbidity according to interference analysis).

From the total set of ordered drugs not showing up in CASTOR data, a shortlist was generated for each comorbidity situation. Criterion for shortlisting included significant annotation counts in either IBS or a specific comorbidity, but coming with significant overlap of drug MoA model with IBS and a specific comorbidity. As listing in CASTOR is not included in such shortlisting, drugs with high order remain, mostly being within the top 25% of drugs screened.

While such cases are well defined from a prescription perspective (according to annotation counts), the drug effect may also play a role in a comorbidity situation.

Out of the 23 drugs shortlisted, 10 drugs are specifically identified for both, IBS-DDM and IBS-ANX (potentially relevant for comorbidities “mental”), 5 are identified for both, IBS-FM and IBS-FSC (“somatic”). Further analysis of scientific references together with interpretation of molecular interference context may offer hypotheses on effect (beneficial/detrimental).

A second tool for evaluating effect is offered by assignment of embedded biomarker.

Step 5: adding biomarker information

Table 3 and 4 hold drugs with indications of relevance for comorbidity situations (from in-silico analysis and/or effective prescription in the patient cohort). Recalling the level of description of pathologies and drug MoA (see Figure 2), a resolution on the level of individual protein coding genes is provided. Hence, the biomarker candidate list (according to deliverable D7.1) can be matched with pathology:drug interference.

For the biomarker candidates proposed in D7.1, 76 candidates can be mapped to human protein coding genes, being amenable for analysis in pathology and drug Mechanism of Action models. A results data set was generated assigning the set of biomarker candidates to the total set of drugs according to Table 3 and 4.

For supporting biomarker evaluation further annotation data were retrieved, including an update on function (NCBI), class and subcellular location (ProteinAtlas), and scientific reference counts (PubMed MeSH).

3.3 Results transfer

In-silico screening, analysis and interpretation according to tasks T7.3, T7.4 and T7.6 of WP7 resulted in structured data organized in spreadsheets, resembling confidential project foreground for further utilization in project research.

Annex I: drug effect screen summary

- ordered list of 61 drugs (see Table 3) with interference details according to in-silico screening and prescription in CASTOR, including reference annotation
- ordered list of 23 drugs (see Table 4) with interference details according to in-silico screening and no prescription in CASTOR, including reference annotation
- total set of drugs forwarded to in-silico screening

Annex II: biomarker:drug assignment

- extended candidate biomarker annotation
- biomarker:drug assignment, including all drugs according to Table 3 and 4

Integration of results with other WPs is given as follows:

WP7 result	**Project use**	**WP**
Comorbidity mechanism listing	Molecular mechanism analyses; extension/interpretation using project foreground data	WP3-WP6

Candidate biomarker listing (including drug:biomarker assignment)	Assay development	WP8
Prescription drug listing, comorbidity interference	Medical plausibility analysis and interpretation	WP2

Conclusion and outlook

With this result status the analysis concept according to Figure 1 is realized. Certainly, in-silico analyses offer only hypotheses (even though all procedures as executed rest on experimental findings), demanding further verification in the experimental context of IBS and comorbidities.

For this, ongoing tasks in WP7 are in place for **i)** receiving feedback on the given results status from WP2-WP6, and **ii)** receiving further project foreground data to be integrated and analyzed in the realm of the present results status. For this, data specifications have been shared with other WPs, meeting ongoing efforts as defined in project tasks T7.3 and T7.6.

With completion of these activities, WP7 project goals are reached, covering **i)** adding to the molecular mechanistic understanding of IBS and comorbidity development, **ii)** assigning biomarker candidates enabling experimental testing and translational activities toward improving diagnosis/prognosis, complemented by **iii)** deriving hypotheses on prescription drug effect.