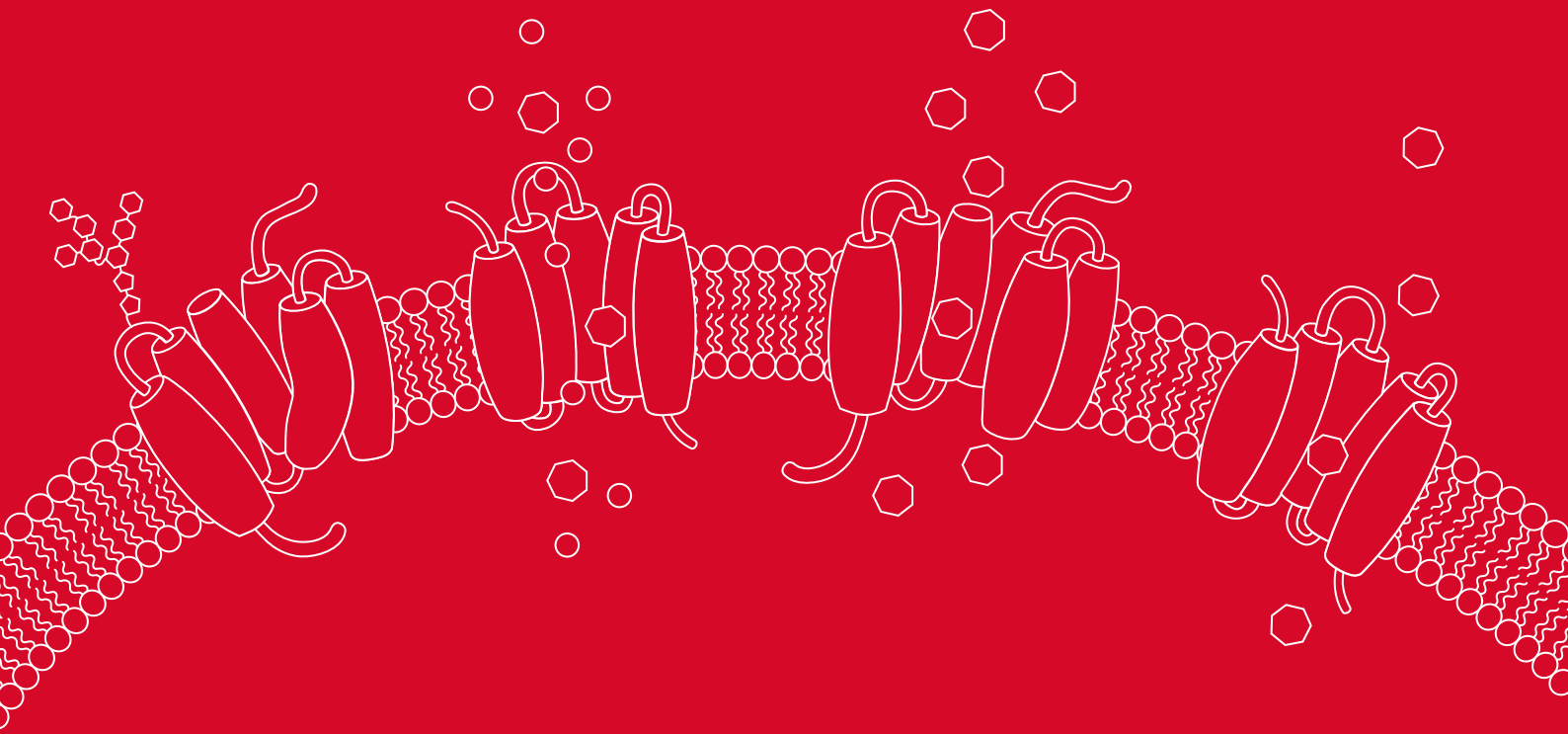

RESOLUTE

Research Empowerment on Solute Carriers

booklet 2020 - public version

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RESOLUTE

Research empowerment on solute carriers

List of participants

Participant No.	Participant organization name	Country
1	CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences (CeMM)	Austria
2	University of Oxford (UOX)	UK
3	University of Liverpool (ULIV)	UK
4	Axxam SpA (AXXAM)	Italy
5	Universiteit Leiden (ULEI)	Netherlands
6	Max-Planck Institut für Medizinische Forschung (MPIMR)	Germany
7	Universität Wien (UNIVIE)	Austria
8	Pfizer Ltd. (Pfizer)	UK
9	Novartis Pharma AG (Novartis)	Switzerland
10	Boehringer Ingelheim Pharma GmbH (Boehringer)	Germany
11	Vifor (International) AG (Vifor)	Switzerland
12	Sanofi Recherche et Développement (Sanofi)	France
13	Bayer AG (Bayer)	Germany

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1. EXCELLENCE

1.1 Objectives

RESOLUTE is resolved to create a decisive advancement in the overall tractability of the Solute Carrier class of protein transporters (SLCs) by providing practical and conceptual advances, and making its research output available openly and pre-competitively to the scientific community. We argue that the ~400 members of this class represent a largely untapped source of new potential drug targets and thus merit the efficiency of scale that can be achieved only through systematic and coordinated efforts [1]. Indeed, because SLC family members operate in an integrated manner over a common metabolic space, we argue that this superfamily can best be approached by a holistic game plan that takes functional networks into consideration. The spectrum of similarities among SLCs that will be identified in the deorphanisation process will offer unparalleled opportunities to infer kinship in mechanism or ligand specificity.

RESOLUTE has one over-arching vision: To **trigger an escalation in the appreciation and intensity of SLC research worldwide and establish SLCs as a tractable target class** (Fig. 1).

RESOLUTE has two non-renounceable high-level objectives:

1. To **create a comprehensive set of long-lasting, intensely characterized tools enabling SLC research and packages of reliable knowledge for the majority of SLCs**.
2. To **develop robust functional assays for at least 50 of the 72 prioritized SLCs**.

RESOLUTE has 16 specific objectives:

1. To create vectors for eukaryotic expression of all human SLC genes.
2. To engineer loss-of-function SLC cell lines as physiologically relevant as possible.
3. To create tagged over-expressed versions of all human SLC cDNAs.
4. To generate and validate renewable and recombinant antibodies and high affinity engineered binding scaffolds against prioritized and selected SLCs.
5. To supply an alliance of structural biology laboratories with cDNA expression systems and protocols able to generate high quality purified SLC proteins.
6. To deorphanise as many SLCs as possible, including intracellularly located SLCs, using an integrated methodology that charts their effects on the accumulation of metabolites and drugs.
7. To define the cellular environment of many SLCs, by determining their subcellular locations and protein interaction partners.
8. To determine the functional dependencies and relationships among SLCs by systematically mapping their genetic and metabolic interactions.
9. To formulate functional assays best suited for any individual SLC, most by direct demonstration and the rest by inference to closely related members likely to operate via similar mechanisms.
10. To test and explore emerging and innovative methodologies to probe for SLC action.
11. To integrate RESOLUTE and publicly available multi-dimensional data into a database, in order to obtain regulatory models highlighting testable hypotheses on the chemical specificity and disease-modifying potential of SLCs.
12. To generate robust assays for most of the prioritized SLCs.
13. To derive chemical insight (natural ligands, chemical probes, initial *in silico* models) for the prioritized SLCs and as many other SLCs as possible.
14. To engage the network of laboratories and community stakeholders in periodic workshops and jamborees to validate data quality and stage ordered and regular public releases.
15. To create open access conditions for RESOLUTE reagents, protocols and databases.

16. To train scientists that will be involved in driving SLC-based R&D in the decades to come in pharma, academia and newly founded companies.

To realize all these objectives, **RESOLUTE is relying on a lean core consortium to operate the central discovery engine with maximum efficiency gains and synergies**. When needed, the consortium will overcome the demanding technical and scientific challenges associated with a SLC-wide 'unlocking' campaign by engaging a formidable network of RESOLUTE Academic Expert Laboratories (in kind against payment) and industrial partners (as subcontractors), each of whom covers unique areas of expertise and in-depth knowledge on specific SLCs and tools. To further extend the impact of the RESOLUTE campaign, we have partnered with a newly formed Structural Biology Alliance, who will (free of charge) exploit the RESOLUTE protocols, vectors and when available, high-affinity binders to elucidate the three-dimensional architecture and molecular mechanisms of this protein group, empowering future drug discovery initiatives (see Fig. 14 for all network partners).

1.2 Relation to the call topic text

RESOLUTE addresses Topic 6 of the IMI2 JU: *Unlocking the solute carrier gene-family for effective new therapies.* The project is streamlined to optimize the likelihood to succeed in **creating the strongest possible unlocking effect** on this large, complex, heterogeneous and functionally interdependent class of potential drug targets. RESOLUTE follows two important principles that have been effective in research undertakings of comparable, seemingly hyperbolic, scope: 1) concentrating activities within a manageable number of operation sites to better gain synergy and efficiency; 2) adopting a largely parallel and biological network-based logic (modules, hit and target-jumping, structural and biological kinship, QSAR clouds) rather than just scaling-up the 'one at a time' paradigm that traditional transporter researchers have followed so far. This philosophy is reflected in the RESOLUTE consortium, that includes the promoters of the Commentary *'Making the case for a more systematic study of SLCs'*, and that is uniquely specialized in large scale biomedical research campaigns that combine pharmaceutical company-grade standards with systems analysis-derived recognition of general principles [1]. RESOLUTE is ideally suited to profit from the synergy that develops with public-private partnerships, as SLCs have fundamental roles in cellular and organismal functions while displaying highly attractive drug target properties. The academic world is interested in identifying the biochemical / biological function and ligand specificity of each family member, while the pharmaceutical industry would integrate this information with available disease association data to cherry-pick those SLCs that would merit a full drug discovery campaign. The synergy also comes into play as the pharmaceutical companies command a set of assays and technologies that are useful for the academic quest to assign function to each SLC transporter. In this partnership all available data on expression, disease association, localisation, transport and protein interactions combine to fill a large, complex but single puzzle.

RESOLUTE adheres to the scope to *'unlock' as much of the SLC family as possible to enable drug discovery efforts to be conducted 'at will' across the whole family of ~400 proteins* by following the proposed **two-level approach:** a broad one applicable to as many SLCs as possible; and a more in-depth one focused on a prioritized sub-list. **These will not be pursued in a temporally distinct fashion.** To save time and increase the chances of delivering, technically related work packages will be operationally integrated, and selected SLCs, either chosen early as part of the priority list (**accelerated targets**), or chosen as representative prototypes of specific families (´**path-finding SLCs**´) will be used as to test assays and pioneer the more innovative approaches. Table 1.1 compares the expected deliverables to the RESOLUTE plan.

Table 1.1 Comparison of the expected deliverables to the RESOLUTE plan.

Expected key deliverables (call topic)	Corresponding RESOLUTE deliverables
Generation of cell systems which express, in functionally competent form, a large majority (>80%) of the ~400 SLCs	CRISPR/Cas9-based inactivation of each SLC gene in a human cell line that naturally expresses it as well as cell lines that overexpress the SLCs (D1.1-1.5)
Generation of a methodology to deorphanise the large majority (>80%) of the ~400 SLCs, and application to rigorously assign endogenous substrates for the vast majority of SLCs	Metabolomic profile of genomically engineered cells, use of chemical probes, chemoinformatics analysis and data integration (D2.1-2.8)
Development of novel, broadly applicable screening methodologies for SLCs	Several methodologies based on impedance, thermal stability, new fluorescence approaches, genetic interactions (D3.1-3.6)
Generation of purified SLC protein and/or cell-free systems containing e.g. proteoliposomes systems, that will facilitate the detailed study of SLCs	Mammalian and insect expression system and troubleshooting workflow to generate milligram quantities of SLC proteins in membranes (D1.6, D5.1-5.3)
Generation of high-quality biochemical reagents and techniques for studying the focused set of 72 family members: 1) highly selective SLC antibodies; 2) techniques to define the interactome of SLCs	RESOLUTE will attempt to obtain high-affinity reagents for the majority of SLCs on the priority targets. Dynamic stimulus and ligand-dependent interactome for at least 50 of the 72 priority targets (D2.6, D5.4-5.5, D7.2)
Generation of high-throughput screening assays for studying a focused set of SLCs	The entire WP6 is dedicated to adapt the assays identified as more suitable to industry-standard robustness and throughput
Directly or via ULTRA-DD: 1) leverage protein reagents to invest in X-ray crystallography of SLC proteins; 2) leverage the SLC assays developed in the project to generate chemical tools that inhibit or modulate the function of SLCs	Partners of the Structural Biology Alliance will take on protocols, plasmids and high affinity binders generated by RESOLUTE and independently mount a significant and concerted effort to elucidate as many structures as possible. RESOLUTE concentrates on creating the conditions for chemical screens, rather than performing them, for prioritization of resources on the core goals and to avoid complexities associated with potential new IP related to chemical structures that could hamper the 'open-access ethos' of the project.

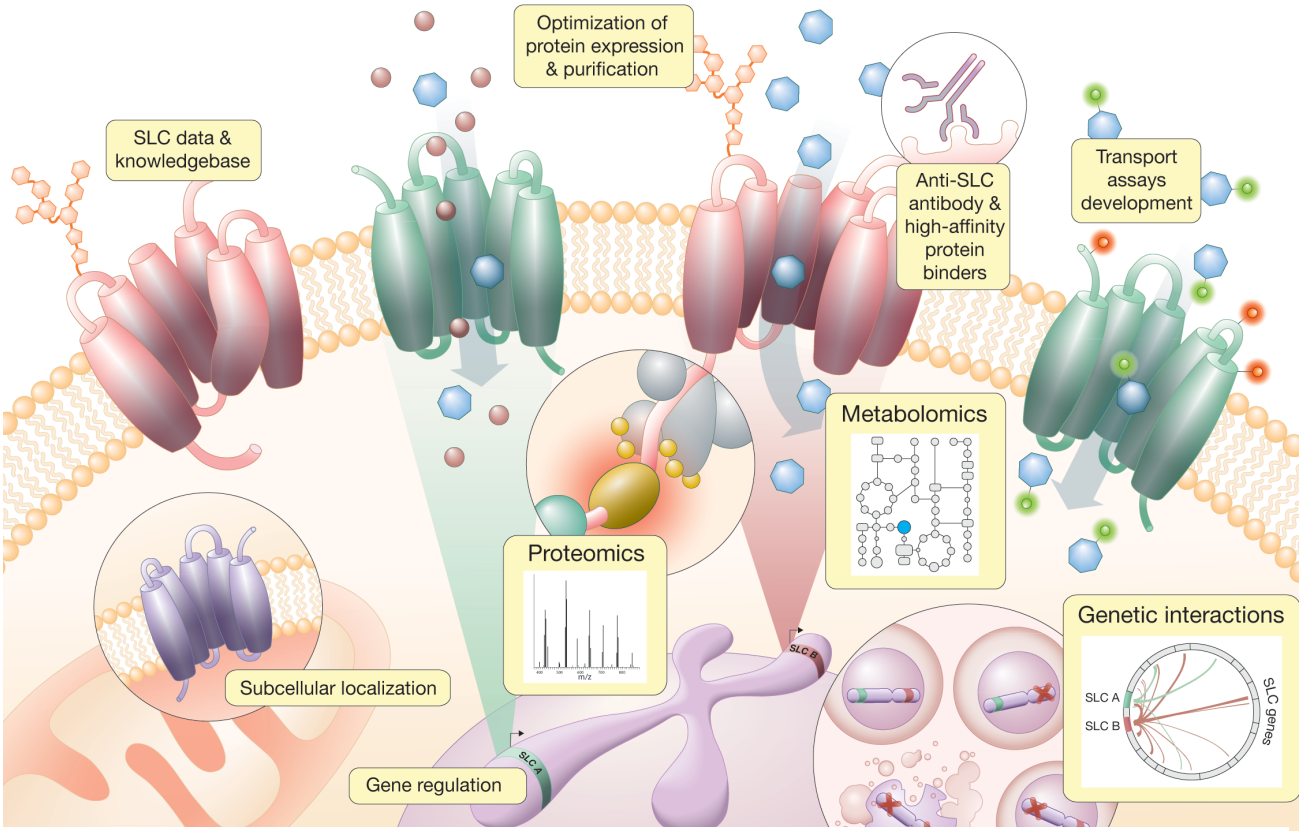

Figure 1: Synoptic representation of the RESOLUTE vision. *The combination of metabolomics and genome engineering forms the core of the deorphanising engine. Genetic and proteomic interactions represent the framework that provides biological context. Transport assays monitoring impedance, fluorescence, mass/charge properties, protein thermal stabilization and changes of voltage all contribute to assess ligand action/engagement. Protein production is used for assays and to generate antibody/ high-affinity ligands. Data is embedded in an integrated knowledgebase that features, among others, genetic variants and disease associations.*

1.3 Concept and approach

The relevance of SLCs for drug discovery is increasingly being recognized and acknowledged [1, 2] and encompasses the entire value chain, from disease-relevant target identification (disease association through >100 mendelian conditions), to drug discovery (all SLCs evolved to engage chemical matter and should therefore be inherently druggable), drug development (flexible and multiple binding modes offer space for ample medicinal chemistry explorations as exemplified by glifozins and SSRIs), toxicology (drugs ending in the wrong organ through SLC-mediated routes, drug-drug and drug-food interactions), and drug disposition (uptake, distribution, extrusion involve SLCs). SLCs may easily represent the class of proteins that covers the most diverse areas of interest of the pharmaceutical industry, and is ideally suited to be studied in partnership in the pre-competitive space.

To date, the biology and pharmacology of SLCs have mainly been addressed on a case-by-case mode. The major motivation behind this call is not only to boost knowledge on SLCs by a big push, but to see whether enough evidence can be provided for the generation of novel concepts that link the target class in unexpected ways and through unexpected principles. We are convinced that such an approach should provide efficiency gains in the quality of the SLC knowledge and justify the establishment of SLC target platforms not unlike GPCRs, kinases and proteases, but perhaps in an even more interesting way. Why potentially more interesting? 1) **Multiplicity of action mode:** SLC knowledge not only provides insight into the proteins as targets, but also reveals important aspects of drug uptake and drug availability, creating information synergies likely to be advantageous (e.g. simultaneous optimization of drug disposition and potency); 2) **Functional interdependence:** the activity of some SLCs may affect the activity of others (e.g. ion exchange, glycosylation and subcellular localization) making an understanding of such dependencies a critical parameter, and offering opportunities to modulate processes in layered fashions; 3) **Physiological**

integration: by transporting metabolites, nutrients, xenobiotics etc., SLC action is linked to metabolism, which is not cell autonomous. SLCs therefore could be considered nodes of physiological integration.

The main challenge of RESOLUTE is to shed systematic light on SLCs in a reliable and efficient way, *while* exploring, discovering and exploiting ways to map the knowledge faster, using multidimensional data (physical and functional interactions, ligand chemical structure, co-expression patterns, 3-D structural features) and while employing a logic that allows for extrapolation and inference.

RESOLUTE merges systematic and focused approaches. It is based on **four pillars** that differ in their risk and innovation profiles.

1) The generation of reliable and de-risked 'hardware' that will be the fundament of everything else, such as cell lines, vectors, proteins, antibodies/high affinity binders, and scalable omics data, both descriptive and functional. This will be done by a few focused laboratories, and will be *guaranteed* to stimulate SLC research worldwide.

2) A central deorphanisation process that combines the power of genomic engineering and metabolomics, and is integrated by orthogonal approaches such as genetic and protein interactions. This should be a potent and close-to-physiology deorphanisation procedure but it is not yet de-risked in terms of SLCome-wide applicability.

3) A parsimonious (non-redundant) process by which the feasibility of an SLC for a particular assay format is tested systematically following a workflow logic that takes phylogenetic and other relationships into account. Such an 'assay-finding engine' is conceived to efficiently advance as many SLCs as possible through a pipeline of different experimental set-ups. As this is probably the most challenging part, it is complemented by a parallel approach for a selected number of SLCs ('path-finding' prototypes selected either to represent families, to represent different subcellular locations, or comprising early elected 'accelerated targets' that are early bets of the priority list). While it is utopian to expect existing or even new 'masterkeys' to unlock all SLC doors, RESOLUTE has assembled a RESOLUTE partner network (see Fig. 14). This is a key strategic choice: we have opted to reserve 5% of the total budget to access RESOLUTE Academic Expert Laboratories (in kind against payment) and engage Subcontractors (selection on best value for money). Given limited resources, we judged it impossible to enrol all potentially relevant technologies and areas of SLC expertise as full consortium partners from the onset. In the overall pipeline, the 'basic engine' will comprise the different laboratories of the core partners, while the 'turbo-boost' will come by engaging additional collaborators in a modular way, if/when required. For example: i) access to the expertise of the RESOLUTE Academic Expert Laboratory of Hartmut Michel (MPI Biophysics Frankfurt) may become decisive to develop an assay for the SLC26 family [3]; ii) the RESOLUTE Academic Expert Laboratory of Eric Geertsma (Goethe University Frakfurt) may be instrumental to develop synthetic binders against specific SLCs in the priority list [4].

4) A collection of linked networks, including i) a network of data linked to other data, ii) a network that represents the relationships among the SLC regulatory circuits, and iii) a network of people.

These four pillars form the structure that combines the solidity of reliable and efficient data generation. The CeMM budget includes subcontracting and in kind contribution against payment. Free of charge services by the Structural Biology Alliance and active participation are drawn in by the excitement of a well-working and good data-delivering network following openness and inclusivity. The long-term success of RESOLUTE will last because of its ability to establish principles, procedures and culture beyond the funding period.

Moreover, there is the **innovation potential** of approaches and assays that are at the embryonic stage and that may or may not represent breakthroughs individually, but collectively will produce new possibilities of assessing SLC function. Several approaches of RESOLUTE are cutting-edge and thus have not yet been validated in the literature. Some are harnessed from other 'target fields' or have recently been developed in the laboratories of the applicants. These include: **1) the use of label-free, impedance-based technology to monitor SLC function; 2) efficient mapping of functional genetic interactions among SLCs; 3) use of mass coded chemical entities and spectrometry to profile SLCs; 4) use of thermal shift to study SLC engagement by a chemical; 5) use of fluorescence interference (FRET, BRET) via fluorescently-tagged compounds.**

Overall, RESOLUTE is designed to form a platform of competent academic and industry partners that will be instrumental for the training of female and male scientists from different career stages in a highly innovative

field that promises to guarantee interesting professional opportunities for the future. We predict that several of the academic, biotech and pharmaceutical leaders of research endeavours on this increasingly popular target class in the future will be traceable to the enlarged RESOLUTE community for which this grant represents a seed. In this highly international consortium, PhD students and postdocs will be exposed to the challenges of project management and learn about efficient reporting, communication, presentation, visualization and data integration while navigating the genomic, biochemical, assay development and chemical world. At the end, RESOLUTE scientists will have enjoyed a privileged access to both high-quality academic as well as industry environments and be comfortable in both professional settings and fluent in a common language. RESOLUTE will have accomplished its ultimate goal if many community members will be able to multiply its initiatives in their future undertakings, creating a snowball effect.

1.4 Ambition

As conveyors of chemical matter in and out of biological systems, transporters have been at the center of attention of pharmacologists and drug discoverers for decades. However, although some members of the group have indeed been exploited successfully, the majority of the SLCs have been difficult to access until now, due to: 1) lack of appropriate research tools; 2) lack of generic assays or assays that probe SLC function in a close-to-physiologically-relevant setting; and 3) lack of appreciation of their redundant and integrated roles. Moreover, the absence of repositories for meta-level annotation and reagents (e.g. linking expression profiles, genetic variants and association to disease, chemoinformatics, structural models) as well as persistence of multiple nomenclatures have added to the problem. **RESOLUTE's ambition is to address these main limitations head-on and transform the risk-reward spectrum across the entire SLC board.** The strategy is to go beyond the state-of-the-art and create a systematic, opportunistic and holistic program that complements the 'one-at-a-time' biochemical approaches that are ongoing in the community and have been very successful, albeit only for a few SLCs. As exemplified by SLC38A9 [5], some SLCs may include signalling properties that may be overlooked if not studied in a natural context. Part of the cutting-edge character of the approach is precisely to consider SLCs as an integrated and interdependent system. How to achieve this while keeping a focus on the SLCs that are a priority for the EFPIA partners? On one hand there is the hope that some SLCs will be prioritized precisely because of an integrated understanding of their physiological connections. On the other hand, priority SLCs will be 'anchored' to the functional network through efforts focused on their interactions at the genetic-functional, proteomic and metabolomics level. Yet, we would consider it a lost opportunity if we would limit our ambitions to the prioritized targets. In fact, the ambition of RESOLUTE is reflected both in quantitative and qualitative aspirations and plans to go beyond the number (72) of high priority SLCs with efforts that are scalable and for which the consortium has considerable experience.

The overall scope of the call is very ambitious; the achievement of even some of the goals would represent a **multiplication of the existing knowledge**. To maximize chances of creating the intensity and focus necessary to create a strong impact, the partners are assembled as a lean consortium of seasoned 'large scale' entities with significant experience in industry standards and collaboration discipline. A meaningful proportion of the budget is allocated to a single entity (the academic coordinator). This is meant to maximize efficiency and provide a sense of purpose, focus, and accountability, while providing leadership and clout to the RESOLUTE consortium. Importantly, this centralized allocation sets aside a significant proportion of the budget to address effectively technological bottlenecks that will be certainly encountered along the way and are not yet foreseeable.

While the full impact of RESOLUTE may take years to affect patients, its innovation potential is very large. We have been arguing in the past that SLC research is likely to represent a considerable gain in knowledge. There are also several shorter-term advantages that will result from the deorphanisation of many SLC transporters: **1) assigning biological function to many SLCs associated with rare disorders and conditions, occasionally suggesting treatment options; 2) providing mechanistically understood biomarkers for patient and disease stratification; and 3) rationalizing desired and undesired side effects of drugs targeting SLCs or being transported by SLCs.** Obviously, the identification of new potential drug targets and drug disposition strategies through the effort spearheaded by RESOLUTE, multiplied by the scientific community, will be the real long-lasting legacy that patients will benefit of on the long run. Once better understood, the SLC-mediated interactions between food uptake, nutrient traffic, environmental toxins, metabolism, disease-

predisposing/causing genetic variations and drug action will offer entire new perspectives in preventive and precision medicine.

RESOLUTE intends to operate an 'open-access ethos' where as many of the results, techniques and reagents developed in the project as possible are made freely available outside the consortium to allow the broad community of SLC research to benefit.

As mentioned above, RESOLUTE will also be a dynamic and diverse environment fostering the growth of young fellows that will become the future ambassadors of RESOLUTE's principles and outcomes, ensuring that the emphasis on SLCs is to stay. To this end, **education and training workshops** will be organized in conjunction with the yearly consortium meetings, initially *for* the fellows, and gradually *by* the fellows.

2. IMPACT

2.1 Expected impacts

Impact on unlocking the SLC target class

RESOLUTE's impact will transcend the funded consortium and funding period by **providing the evidence for the feasibility of turning the SLC group of proteins into attractive drug targets**. It will do so by empowering the community with reagents, research tools, protocols, and databases as well as the necessary 'social and cultural' instruments and exchange platforms (workshops, jamborees, training of scientists fluent in the academic and industry language). Thus, RESOLUTE will become an example of how a relatively understudied and biochemically demanding group of proteins can be 'unlocked' for research and development in public-private partnership. Through the coupling of an inclusive, 'open-access ethos' to the results, techniques and reagents with the highest-possible quality of research output, RESOLUTE expects to accelerate the pace of research in the field of SLCs to the global benefit of basic academic research through to applied research in SMEs and pharmaceutical companies. Specifically, RESOLUTE will deliver step-change advances in the following areas:

- techniques for the production, isolation and characterization of integral membrane proteins,
- techniques for re-incorporating purified proteins into membranes in cell-free vesicle systems,
- metabolomic techniques that may be applied for the characterization of endogenous SLC substrates,
- mass spectrometry-based techniques that allow for the parallel evaluation of mass-coded chemical pools,
- methods to assess SLC engagement by chemical agents, including biochemical and biophysical methods,
- methods for studying SLC interactomes, from tagging strategies to analytical algorithms,
- methods for the detection of membrane transport that involve fluorescence, impedance and sensors,
- methods that may facilitate reliable and cost-effective high-throughput techniques of screening against SLCs,
- gene-editing approaches that may accelerate SLC research through the generation of knock-in and knock-out cell lines for the whole SLC protein family,
- genetic screening approaches that chart functional dependencies,
- genome engineering approaches that exploit the conditionally essential role of individual SLCs for the creation of cell lines and assays suitable for compound screening,
- publications, scientific fora, web portals that link-in the community broadly and in a participatory way,
- and platforms to evaluate the potential impact of frequent and ultra-rare genetic variants in the SLC genome on pathophysiology.

The 'open-access ethos' is a stated intent by all consortium scientists to make freely available, to the broader research community, as many of the results, techniques and reagents as is possible, during and after the completion of the project at no or minimal cost.

Improving European citizens' health and wellbeing and contributing to the IMI2 objectives

RESOLUTE will deliver against many of the key goals outlined in the *Strategic Research Agenda for Innovative Medicines Initiative 2* by providing the scientific basis for the informed choice of SLCs as new drug targets, as well as a rationale for the ADME and pharmacogenomics parameters that, coupled to the molecular profile of patients, could enable precision medicine approaches and help decrease attrition of investigational drugs. Moreover, the tools and approaches we develop to understand the 'SLC transportome' will provide a rationale for the overall traffic disposition of drug and metabolites, and allow us to better *evaluate the holistic impact of new medicines*, which is one of the IMI2 aims. This in turn will help evaluate integrated treatment programs. RESOLUTE will contribute significantly to the *identification of new therapeutic concepts and the tools required to support their systematic validation driving new and innovative approaches to diseases with high unmet need*, part of the R&D expected impact of Axis 1-4 of the program.

Given that many SLCs are potential drug targets, it is evident that RESOLUTE activities will have a large impact on drug discovery and development. Since SLCs are expressed in practically all cell types across all tissues, their therapeutic potential involves many disease areas, including cancer, immune-mediated diseases, neurodegenerative diseases, metabolic diseases and cardiovascular diseases, all of them being part of the European health priorities to be addressed by IMI2. In this regard, it can be anticipated that **RESOLUTE will benefit patients suffering from a range of pathologies as well as society through the accelerated discovery of new chemical entities targeting SLCs**, which will provide the starting point towards effective therapies for many diseases.

Achieving a greater impact with respect to research and innovation by combining Horizon 2020 and private sector funds in a public-private partnership

The benefits of a consortium approach involving publicly-funded and private partner organizations will be substantial in allowing synergistic innovation in the science of SLCs. The scale of this project in both expertise and resources goes well-beyond what any individual research institution could manage. The combination of drug development expertise from EFPIA partners, together with the insights of academic researchers focusing on molecular networks, the capability of AXXAM as an SME, will ensure that the outlined results will be delivered much more rapidly than if single companies or institutions acted alone. In addition, the consortium may be able to further leverage the value of public-private partnerships through collaboration or application of learning of other IMI projects/ consortia, such as: K4DD (via ULEI, UNIVIE); ULTRA-DD (via UOX/SGC); eTOX (via UNIVIE); European Lead Factory (ELF; via UOX/SGC) and Open PHACTS (via UNIVIE).

The project will allow the SME partners (AXXAM and others) to consolidate their core business and to exploit and expand their expertise into new markets. Thus, **SMEs will gain a competitive advantage in an attractive sector for technological and pharmaceutical development** acquiring more strength in the penetration of the international market. The positive impact on the SMEs' revenue that will derive will enable them to provide new jobs and to reinvest in research and innovation. RESOLUTE provides unique know-how to develop novel functional assay systems adaptable to HTS industrial platforms. The project will set the basis for accessing SLC drug discovery through screening of chemical compounds libraries. Novel methodologies developed here to assess SLC activity and new *in vitro* systems will constitute valuable functional assays, otherwise unavailable to the research and industrial community, to discover new modulators of SLC. Through the collaboration between AXXAM and the academic consortium partners, these systems will be manipulated to express suitable functional readout systems and will be transferred to technological platforms useful for high-throughput screening. Moreover, the participating SMEs will benefit by participating in a consortium with world scientific experts in the field of SLC and strengthening their collaboration with the industrial partners.

The EFPIA companies contributing the project will benefit in several ways:

- Unlocking the SLC protein family will increase the number of protein targets considered to be 'readily druggable'. Coupling of the results, techniques and reagents developed in this project with other areas of expertise in pharmaceutical companies (e.g. disease biology, drug design, drug development) is expected to lead to many new opportunities for therapies against most human diseases.
- Working closely with world-leading academic laboratories in the field of SLC research would strengthen the scientific knowledge, experience and capabilities within this important research field inside EFPIA companies.
- Working in close partnership with the SMEs and other pharmaceutical companies in a pre-competitive manner will allow a cross fertilization of knowledge, experience and best-practices in the science of SLCs which will also strengthen scientific capabilities inside EFPIA companies.

Since SLCs provide opportunities for new drug targets and also play a vital role in drug disposition, it is anticipated that advances in science driven by this project will ultimately impact positively the goal of personalized medicine. Overall, RESOLUTE aims to unlock the therapeutic potential within the SLC gene family by generating results, techniques and research tools and making them readily accessible for scientific and industry stakeholders, thus having a share in transforming the IMI2 vision into reality: *delivering the right treatment to the right patient at the right time for priority diseases.*

The scientific advances expected from the project will impact both basic research (increased capabilities to delineate the fundamental roles that SLCs play in health and disease) and drug discovery (accelerated prosecution of drug discovery programs targeting SLCs) alike. Further to the 'open-access ethos' of the results, techniques and reagents developed within this consortium, it is anticipated that these are all used beyond the project in pharmaceutical companies and SMEs in the creation of drug discovery projects or drug discovery services, that ultimately lead to new medicines for the benefit of patients. A roadmap for the dissemination of results is outlined hereafter ensuring the translation of findings into economic gain and, at the same time, fostering the generation of open-access knowledge.

2.2 Measures to maximise impact

a) Dissemination and exploitation of results

RESOLUTE will generate a multitude of new and valuable knowledge such as new high-quality reagents, research tools, techniques and protocols. The overall approach of the consortium is to make its quality-controlled research output as broadly available as possible to the scientific community, inside and outside the consortium, through the application of an 'open-access ethos'. Thus, RESOLUTE will be able to boost the intensity of SLC research worldwide.

The plan for the dissemination and exploitation (DEP) of the RESOLUTE results outlined here (see details hereafter) will be refined and developed further during the overall project duration to ensure that project results will be optimally communicated among different stakeholders and to establish synergies with relevant initiatives and multipliers outside of the consortium. The DEP will undergo a first revision (to be adopted by the Executive Board) in month 6 (carried out within WP9 'Project Management and Dissemination of Results') covering all aspects of a successful communication strategy:

- Definition of the communication objectives,
- Identification of areas and stakeholders that could make use of the project results (key audiences),
- Definition of data and knowledge management,
- Description of concrete and well-timed measures for dissemination of all key results throughout project lifetime and after project end,
- Definition and selection of effective tools, channels and platforms for all groups of potential users and multipliers (stakeholder networks and platforms, domain specific platforms, policy makers platforms, project website, etc.) with long-term perspective, and
- Promoting of each of the impacts aimed at in the work plan.

The dissemination strategy will be adapted in an ongoing process throughout the project duration. The DEP will ensure that promotional materials, scientific papers, poster presentations, conferences, etc. will be disseminated as widely as possible.

Dissemination objectives
The dissemination of project results aims to:

- enhance the visibility of the RESOLUTE project and SLC research as a whole at a local, national and international level,
- attract the interest of potential future partners, neighbouring networks and initiatives,
- encourage talented students and young scientists to engage in the science of SLCs,
- draw the attention of national governments, regional authorities and other public and private funding sources to the need for and ultimate benefits of the RESOLUTE research activities, and
- pave the way for future market demand for the products or services to be developed in the long-term based on the project results.

Key audiences
Key audiences and potential (future) stakeholders include academic and research organizations, EFPIA members and other pharmaceutical companies, SMEs and other companies involved in small-molecule drug discovery and technology development, professional organizations, drug policy makers (e.g. EMA and FDA),

and the general public interested in science and new approaches in drug development. Major multipliers for dissemination of project results are the multitude of scientific (inter)national networks the RESOLUTE partners participate in or have close links to (e.g. European Molecular Biology Organization, Federation of European Biochemical Societies, the International Transmembrane Transporter Society and the Gordon Research Conference in membrane transport proteins community, among others). These links are documented in '3.3 Consortium as a whole' and will be activated and engaged through regular information and collaboration activities.

Data Management

A Data Management Plan (DMP) describing the life cycle for research data to be generated and/or processed throughout the project will be developed in WP8. An initial version will be available in month 6 (D8.1). The DMP is intended to be a living document and will be updated throughout the project whenever significant changes occur. To streamline communication, we identified one individual per partner as interface to data management.

The RESOLUTE data- and knowledgebase plays an essential role in this project by providing a central data repository located at CeMM, collecting and integrating research data generated across the individual work packages and partners. Amongst others, the following types of data will be generated and/or collected during the project duration:

- high-content screening data,
- next-generation sequencing data,
- targeted and untargeted metabolomics data,
- interaction proteomics data,
- small molecule binding, functional bioactivity data,
- scripts, workflows, algorithms and software tools for data analysis and visualization,
- protein homology models and QSAR equations,
- reports on assay development, and
- progress reports on each work package.

The RESOLUTE data- and knowledgebase will be implemented as data warehouse and schema-free database, which proved itself more suitable than classical databases for this kind of multi-discipline research data. A state-of-the-art authentication system on top of the data warehouse and implementation of transport layer security for data transfers will ensure data security. To rule out data manipulation, loss or corruption we will employ checksums, backups and data duplication to public repositories.

A RESOLUTE web portal will provide access to an internal as well as a public section of the RESOLUTE data- and knowledgebase, with the internal section containing data that is not yet ready for publication. In regular workshops, hold in the course of the GAs, data will be curated, annotations will be refined, data quality will be assessed, and data releases to the open access section of the RESOLUTE web portal will be agreed on. Ultimately, all data will be available open access at the end of the project. For scientific publications, we will set up a publication review committee with a 30-day review time, meaning that the data is free to publish if no partner raises objections within a month. All papers published will follow the 'gold' open access policy by submitting the manuscripts to open access journals or by paying for this option in the subscription-only journals.

By ensuring that all generated research data will be findable, accessible, interoperable and reusable (FAIR), the RESOLUTE data- and knowledgebase will efficiently lead to discovery, integration and innovation:

Data will be findable by assigning unique Digital Object Identifiers (DOIs; via DataCite.org) to each dataset and Open Researcher and Contributor Identifiers (ORCIDs) to individuals. We will develop naming conventions, implement a versioning system and choose a suitable keyword and metadata ontology to improve findability.

Data will be accessible at the URL http://re-solute.eu, where the RESOLUTE web portal will provide open access to publications, reports and research data from the RESOLUTE data- and knowledgebase. Scientific,

peer-reviewed publications produced during the project will be available in a machine-readable electronic format and linked to underlying processed data as well as raw data. The web portal will also provide mining tools for optimal data exploitation, e.g. an advanced query system featuring interactive reports and KNIME nodes for database access and data mining. In addition, for increased accessibility, data will be submitted to domain-specific, certified repositories wherever possible (e.g. EBI's Sequence Read Archive for next-generation sequencing data, ProteomeXchange for interaction proteomics data, or ChEMBL for compound/transporter interaction data).

Data will be interoperable by ensuring the use of standard data formats, aiming at maximum compliance with open source software applications. Links to other resources, where possible, and extensive metadata annotation based on domain-specific, established ontologies (e.g. the Minimum Information about a Proteomics Experiment - MIAPE - guideline for interaction proteomics data) will allow inter-disciplinary interoperability.

Data will be re-usable for third parties by choosing a proper open access license model, which will be defined in the initial DMP (D8.1). Data re-usability will also be supported by implementation of automatic, domain-specific data quality control metrics, as well as manual data quality assessments during the data annotation and release workshops. We also plan open access to any algorithms or analysis software tools developed in the course of the project that might be needed to re-use data and re-produce results.

To resource our FAIR data strategy, 2.24% of the budget are assigned to data- and knowledge management. We expect an estimated data production rate of max. 5 TB/month, resulting in a total of up to 300 TB of data produced throughout the project. This implies storage and backup costs of about 200,000€ at a current pricing of about 20€ per TB per month for cloud storage compliant with the EU's General Data Protection Regulation.

The RESOLUTE data- and knowledgebase will be kept online by CeMM for at least 5 more years after the end of the project. For long-term preservation, the diligent use of domain specific standards will ensure that all data will be transferable to corresponding certified public data repositories. A detailed data preservation plan will be part of the initial DMP at month 6 (D8.1).

Knowledge Management and protection

Knowledge Management and protection of results will be defined in the Consortium Agreement and will be monitored from the beginning of the project (specifically supported by WP8 and WP9). The Consortium Agreement will manage amongst other things the ownership and access to key knowledge.

As example, we list hereafter a few points expecting that the Consortium Agreement will certainly consider **knowledge management and protection** in a more detailed manner.

- **Agreement on confidentiality among participants and towards external parties:** The consortium will ensure that dissemination of results will be in line with possibly existing confidentiality obligations. Shared data will be labelled as 'confidential' or 'non-confidential'. Additional appropriate legal agreements will be put in place as, e.g. allowing bilateral access to confidential data, for a specified purpose and subject to special conditions. However, as a general principle, most information and data will be made freely available to the consortium and, when appropriate and quality-controlled, made available to the public.
- **Agreement on dissemination of results:** Each beneficiary shall disseminate its results as soon as possible, unless such dissemination goes against its legitimate interests (e.g. because the results have not yet been protected, etc.) based on a defined review and approval process within the consortium.
- **Agreement on background:** During the project, the beneficiaries enjoy, unless prevented or restricted from doing so by obligations to others, access rights to the background of the other beneficiaries to the extent necessary for undertaking and completing the project as well as required for the purpose of the research use of results under fair and reasonable conditions.
- **Agreement on ownership of and access to project results:** During and after the completion of the project, beneficiaries and their affiliated entities enjoy access rights to the project results for research use under royalty-free conditions; after completion of the project, third parties have the right to request access rights to the results for research use under fair and reasonable conditions.

- **Handling of licence related issues:** The consortium expects that each participant will be holding licences / legal agreements that ensure freedom to operate for technologies that will be used during RESOLUTE. We will solve licencing issues on a case-by-case basis by negotiating directly with vendors, and the consortium will undertake an assessment on whether such issues are affecting the project processes.

b) Communication activities

Based on the outlined plan for the exploitation and dissemination of results, communication activities will focus on scientific interactions that will include:

Publication of scientific papers
Preference will be given to the generation of joint publications of the project results. These will be mainly submitted for publication in high-impact international scientific journals. A 'gold' open access policy will be supported by submitting the manuscripts to open access journals or by paying for this option in the subscription-only journals.

Presentations of scientific results
Project results will be presented at leading conferences in the field such as Gordon Research Conferences, Keystone Symposia and The Bioparadigms Biomedical Transporters meeting series. Further, RESOLUTE intends to establish its own conference series starting in year three (identifying a suitable venue and sponsorship, well in advance). At these events, scientists from the RESOLUTE community at all levels of seniority will be invited to present.

Hosting of scientific and/or training events
Own events (workshops, summer schools, jamborees, training of scientists fluent in the academic and industry language, etc.) will be organised by the RESOLUTE consortium addressing key audiences of project results.

Collaboration with other projects and initiatives relevant to the RESOLUTE results
Where possible and appropriate, specific activities will be carried out to develop and enlarge relationships with other related initiatives (such as K4DD, ULTRA-DD, eTRANSAFE, European Lead Factory and the Open PHACTS Foundation) in order to foster synergies of efforts and mutual leverage of results.

Communication tools to be used will be:

- A **'corporate project design'** (logo, fonts, colours, etc.) as basic framework for the appearance of the project in the stakeholder community.
- **Promotion material** such as leaflets, brochures, project posters, slideshows etc. to facilitate presentations at events and to support meetings of project scientists, etc.
- A **project website** (http://re-solute.eu) which will be the main online access point for the different target groups and which will support and reinforce the rest of the dissemination activities. In an information capacity, the website will highlight project objectives, activities, outcomes and relevant updates. As repository of information, the website will store and make available project resources (including scientific data) and publications to the project participants as well as to the scientific community and the specialized public.
- Use of **social media networks** will provide areas for exchange on various topics related to SLC research and applications and for raising awareness of the research and its results.
- Project results and activities will also be disseminated on a series of **external databases and websites** (see also page 12) for awareness purposes and to foster exchanges on project specific scientific aspects.
- **Press releases** will be addressed to the different stakeholders.

It will be crucial to have the contacts and resources to react in a timely and efficient way whenever something happens that could be relevant to the project. Furthermore, special attention will be given to internal communication (who needs to be informed, of what, how regularly and in which format). Tools for internal communication and collaboration will include among others, a library for document management and sharing, forums for discussion, project calendars, newsletters, WebEx, Skype, etc.

All promotion material generated within the RESOLUTE project, including scientific publications and presentations, the project website, etc. will point out that the project that has elaborated the disseminated results has received funding from the Innovative Medicines Initiative 2 Joint Undertaking.

3. IMPLEMENTATION

3.1 Project plan — Work packages, deliverables and milestones

The proposed architecture of RESOLUTE is presented in Figure 11. The SLC-wide and priority-focused approaches will be carried on in parallel, with a subset of path-finding SLCs used to test and develop technologies that will later be applied to the whole family. WP1, WP2 and WP3 form the core deorphanisation 'engine', which comprises both tool-generating and deorphanisation tasks. WPs 4 to 7 build and extend on the tools and data generated in the deorphanisation phase and apply them to the SLC priority target list. WP8 and WP9 enable data and project management together with outreach activities to support and empower the other WPs. Each of the work packages is broken down into a series of tasks to allow proper structure and follow-up of the work to be completed. The Gantt chart (Fig. 2) shows the estimated timelines.

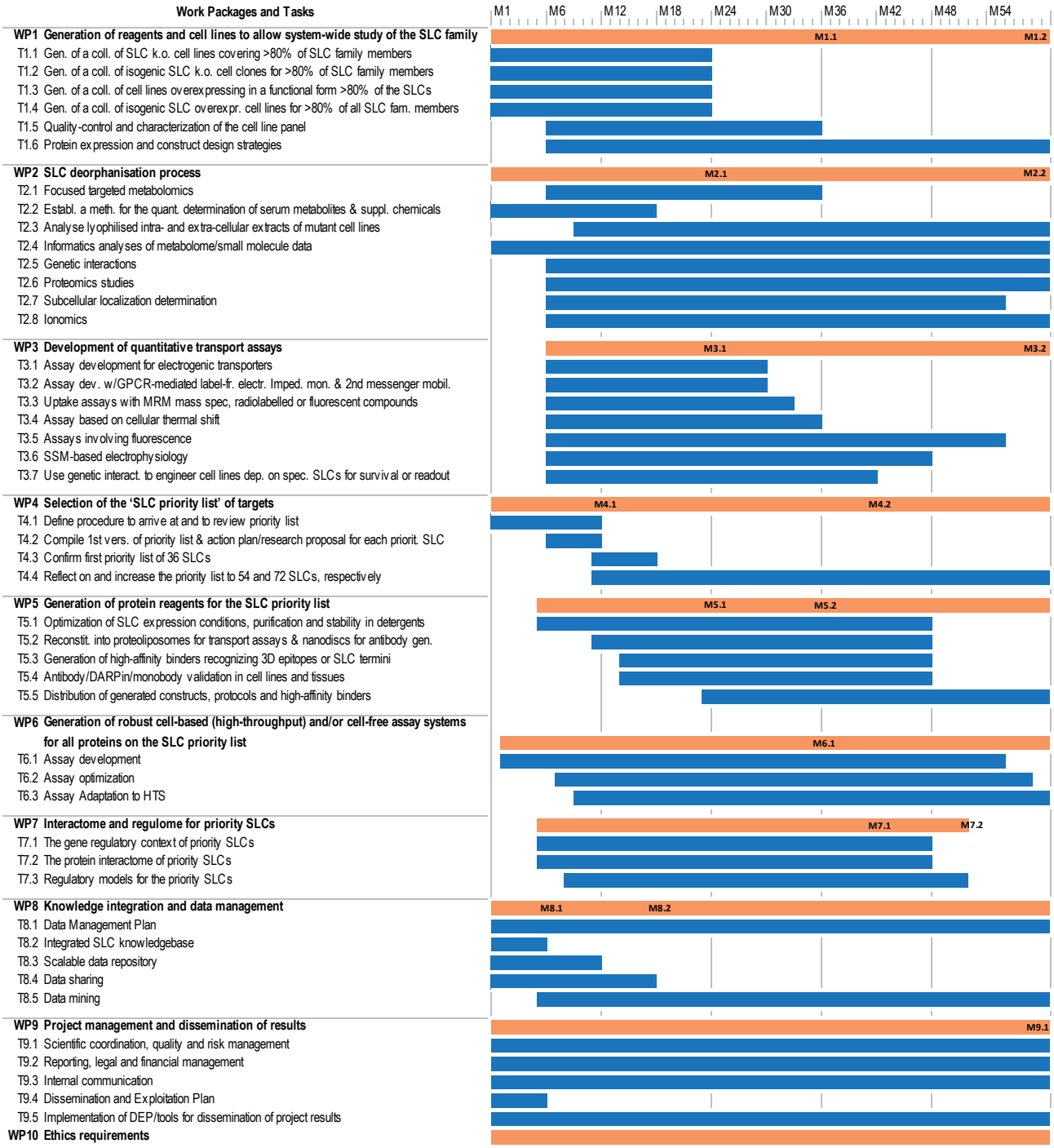

Figure 2: Gantt chart. Timeline for work packages (WP) and corresponding Tasks (T).

Work package number 1	Start Date or Starting Event M1
Work package title	**Generation of reagents and cell lines to allow system-wide study of the SLC family** **Lead: CeMM, Co-lead: Novartis**

Summary

WP1 aims to provide reagents for the deorphanisation and characterization goals set by the call. These include the generation of expression vectors and the creation of a set of cell lines lacking or overexpressing SLC family members in a variety of parental cell lines (Fig. 3). In addition, a first-pass protein expression screen on the entire family will be performed. Together, this large collection of reagents will represent a critical resource for the project itself (i.e. WP2, WP3, WP5, WP6, WP7), the enlarged RESOLUTE network of laboratories, and for the scientific community at large.

Target selection: In order to coordinate the work with the other WPs, a selection process identifying which genes to target first will be implemented within the consortium. We envision that this initial gene set will contain both 'accelerated targets', coming from the priority list, and 'path-finding' SLCs, selected based on their functional and structural diversity, their association with diseases, their amenability to the assay setups available within the consortium, as well as available knowledge on substrates and ligands (as determined by the bioinformatics resources generated in WP8). This initial set will therefore comprise both characterized SLCs for which no assay is available as well as relatively uncharacterized SLCs (e.g. those assigned to a particular class without an experimentally identified substrate) to prime and benchmark the WP2 and WP3 pipelines and, in parallel, the WP5-7 workflows.

Description of work and role of partners

Task 1.1 Generation of a collection of SLC k.o. cell lines covering >80% of SLC family members (M1-M24). Partners involved: CeMM. The membrane transporters that have been ingeniously operationally grouped as secondary transporters and given the rational SLC nomenclature [7] include some ancillary proteins, some channels and many pseudogenes. Taking a pragmatic approach, RESOLUTE will discard pseudogenes, but include a set of additional proteins (some considered 'atypical' but proposed to be structurally related to SLCs, as well as novel members of the SLC family experimentally identified as transporters), for a total of 446 genes [6, 7].

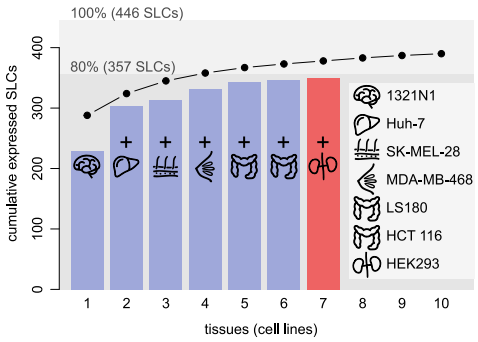

Figure 3: Selection of RESOLUTE cell lines. *As no human cell expresses all SLCs, a limited number of cell lines covering as many SLCs as possible are needed. This saturation plot shows the maximum number of SLCs expressed by increasing numbers of cell lines from different tissues (black line; expression data from Klijn et al. [26]). Cell lines from five tissues are sufficient to express almost 80% of all SLCs. We selected six cell lines (blue bars) to have almost all of the SLC families represented (69/70) with a cumulative expression of 347 SLCs (78%)*

We will generate a panel of cell lines, each carrying a frameshift mutation on a single SLC for >80% of the SLCs by employing the CRISPR/Cas9 technology. We will not use the haploid cell line HAP1 employed in the past, which expresses only some 42% of all SLC genes, but carefully choose cell lines that naturally express the SLC gene to be inactivated. A set of 6 reference cell lines derived from different tissues (central nervous system: 1321N1, breast: MDA-MB-468, liver: HuH-7, gut: HCT116 and LS180, skin: SK-MEL-28) and expressing a total of 347 SLCs representing 69/70 families (65 classical ones plus 5 suggested novel ones [6]) was identified by bioinformatics analysis (see Fig. 3). The cell lines were selected primarily based on their SLC expression profiles, and secondarily on their ease of handling, assay suitability (therefore excluding nonadherent cells) and the extent of usage in the scientific community. For each SLC, k.o. cell clones (two per gene) will be generated by transduction of the cell line expressing high levels of the SLC (to preserve as much as possible

the metabolic environment, interaction partners and post-translational modifications) with lentiviral particles carrying the Cas9 gene and a gene-specific single-guide RNA (sgRNA). A subset of 2-3 parental cell lines will be sufficient to cover the majority of the SLCs targeted. Clones with frameshift mutations introducing early stop codons will be confirmed by sequencing of the targeted gene, their stability monitored over time and their phenotype characterized with a comprehensive multi-parameter analysis (Task 1.5).

Task 1.2 Generation of a collection of isogenic SLC k.o. cell clones for >80% of SLC family members (M1-M24). Partners involved: CeMM, Novartis. We will generate a collection of isogenic SLC k.o. HEK293 Jump-In cells, irrespective of whether HEK293 cells express the targeted SLC. Though HEK293 are aneuploid, we have managed to genome-edit SLC genes efficiently (unpublished data). This panel of isogenic cell lines (1 clone-derived line per gene) will serve as a reference set particularly suited for assay development. Generation of the cell lines will combine the work of CeMM (transduction/transfection and generation of pool of k.o. cells) and Novartis (isolation and characterization of single cell clones).

Task 1.3 Generation of a collection of cell lines overexpressing in a functional form >80% of the SLCs (M1-M24). Partners involved: CeMM. RESOLUTE will order cDNAs for the 446 SLCs targeted in Task 1.1 (codon-optimized and carrying silent mutations to make them gRNA resistant) for expression in human cells under the control of an inducible promoter, i.e. Tet-On system [8]. Lentiviral vectors carrying both untagged and tagged versions (with a HA-TwinStrep tag for proteomics, localization studies and assay development, modifying the tags in case of manifest dysfunction) will be prepared and used to infect the corresponding k.o. cell lines previously generated. A total of two clones (one generated with a tagged construct and one with an untagged construct) will be isolated and characterized.

Task 1.4 Generation of a collection of isogenic SLC overexpressing cell lines for >80% of all SLC family members (M1-M24). Partners involved: CeMM, Novartis. We will generate a collection of isogenic SLC overexpressing HEK293 cells Jump-In cells (1 clone/gene). Transduction will be carried out at CeMM while clone isolation and characterization will be carried out by EFPIA members (Novartis).

Task 1.5 Quality-control and characterization of the cell line panel (M6-M36). Partners involved: Novartis, Boehringer, Sanofi. In collaboration with the EFPIA consortium members, a thorough characterization of the WT and k.o. cell lines will be performed. This will involve a multi-parameter characterization of WT and clonal cell lines, including full-genome sequencing of the WT cells, targeted RNA sequencing for the whole SLC gene family, sequencing of the integration site of the lentiviral construct in the k.o. and overexpressing cell lines as well as a high-content imaging- based phenotypization monitoring parameters such as cell size, organelle number and morphology and intracellular pH [9]. This will represent an in-depth quality control step and initial phenotypization before any of these reagents is used by other WPs or distributed outside the consortium.

Task 1.6 Protein expression and construct design strategies (M6-M60). Partners involved: UOX. Many of the RESOLUTE outputs, including the production of conformation-specific antibodies, transport assays, and some deorphanisation approaches will require purified protein. As part of the SLC characterization effort, a major step is to determine the quantities of purified protein that can be produced from recombinant expression systems, such as insect and mammalian cells. To that end, UOX will employ its high-throughput platforms, which allow to clone, express, purify and analyse a large numbers of construct designs in parallel to perform a first pass expression test, without any further optimization. A key component of these platforms is the use of Baculo- and BacMam viruses, which are highly effective in delivering expression constructs into insect and mammalian cell lines [10], thereby maximizing the expression levels. Over the last 4 years, these platforms have been used to purify milligram quantities of >50 different human membrane proteins, including 14 SLCs ([11-13] and unpublished data). For each member of the SLC superfamily we will screen constructs with GFP inserted between the cleavage site and the affinity tag. GFP-fusion has shown to boost the expression levels of a number of membrane protein targets, presumably by increasing the solubility and/or by facilitating folding (unpublished observations). The GFP-tag will also be used to monitor the subcellular localization in intact cells, and to rapidly screen a number of detergent/lipid conditions using fluorescence-detection size-exclusion chromatography (FSEC), either by analysing whole-cell lysates or using small-scale affinity pull-downs from either insect or mammalian cells. In addition, we have surveyed colleagues and literature with the hope to identify new approaches in the robust production of SLC proteins in a cell-free system, such as cell factory lysates or phage-display. The complexity of the SLC topology and the

role of the posttranslational modification make it particularly difficult and thus not yet applicable as a class-wide strategy. However, once expressed in human or insect cells, we will use a variety of solubilisation strategies to enlarge the spectrum of experimental possibilities. One of these approaches will be the extraction and purification in mild detergents, e.g. digitonin, neopentyl glycol class of detergents [3] or calixarene-based detergents [4]. This procedure permits to purify large amounts of full-length SLCs in detergent micelles, which will be used in thermal shift assays to assess overall stability and the binding of ligands or compounds, as well as other biophysical assays (e.g. Surface Plasmon Resonance, SPR). Another alternative approach will be the detergent-free extraction directly from native membranes with poly-styrene-co-maleic acid (SMA), yielding SMA lipid particles (termed 'SMALPs') which maintain a belt of native lipids around the solubilised membrane proteins. This strategy has been successfully used to extract ion channels, GPCRs and solute carriers, including SLC29A1 [7]. SMALPs could subsequently also be used for the generation of antibodies (WP5) and cell-free assays (WP6).

Participation per partner

Partner number and short name	WP1 effort (%)
1 - CEMM	54.25
2 - UOX	15.34
9 - Novartis	15.27
10 - Boehringer	9.51
12 - Sanofi	5.61
Total	100

List of deliverables

Deliverable Number	Deliverable Title	Lead beneficiary	Type	Dissemination level	Due Date (in months)
D1.1	Vector generation	1 - CEMM	Report	Public	12
D1.2	SLC k.o. cell lines	1 - CEMM	Report	Public	36
D1.3	SLC k.o. in HEK293	9 - Novartis	Report	Public	36
D1.4	SLC overexpressing cell lines	1 - CEMM	Report	Public	36
D1.5	SLC overexpression in HEK293	9 - Novartis	Report	Public	36
D1.6	Protein expression screen	2 - UOX	Report	Public	60

Description of deliverables

D1.1 Vector generation [12]. Collection of lentiviral and Jump-In compatible vectors carrying CRISPR-based Cas9 and sgRNAs targeting all SLCs, together with vectors carrying tagged and untagged cDNAs for >80% of the SLC family

D1.2 SLC k.o. cell lines [36]. Collection of >600 cell lines each carrying frameshift mutations in one SLC gene, quality-controlled and characterized with a multi-parameter approach

D1.3 SLC k.o. in HEK293 [36]. Collection of >300 HEK293 isogenic cell lines, each carrying frameshift mutations in a SLC gene, quality-controlled and characterized with a multi-parameter approach.

D1.4 SLC overexpressing cell lines [36]. Collection of >600 cell lines each overexpressing a specific SLC in untagged and tagged versions, quality-controlled and characterized with a multi-parameter approach.

D1.5 SLC overexpression in HEK293 [36]. Collection of >300 HEK293 isogenic cell lines, each overexpressing a SLC gene, quality-controlled and characterized with a multi-parameter approach

D1.6 Protein expression screen [60]. A family-wide assessment of protein expression yields in recombinant expression systems.

Schedule of relevant milestones

Milestone number	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS1	Collections of SLC k.o. and SLC overexpressing cell lines covering >80% of the family members	1 - CEMM	36	Availability of quality-controlled cell lines to members of consortium; report to GA
MS2	Assessment of SLC protein expression levels	1 - CEMM	60	Family-wide assessment of SLC protein expression levels; Report to GA

Work package number	2	Start Date or Starting Event	M1
Work package title	SLC deorphanisation process Lead: Pfizer, Co-lead: ULIV		

Summary

The aim of WP2 is to develop and implement a generally applicable deorphanisation approach for SLCs. The RESOLUTE engine combines different technologies and employs the 'guilty-by-association' principle. All available data will be used to establish connections: 1) metabolomics, 2) ionomics, 3) genetic interactions, 4) proteomics and 5) subcellular localization (Fig. 1). Results will be integrated with publicly available data as well as with any proprietary dataset made available by any of the partners. The core of the deorphanisation strategy involves genomically engineered cells and metabolomics, while the genetic, proteomic and localization approaches provide for integration within a biological context. Together, the approaches should allow for a synergistic and efficient functional annotation process. The key outputs are an understanding of the substrate (and, as appropriate inhibitor) specificities of each of the chosen SLCs, as well as the genetic interactions between SLCs (see also Fig. 4 and Fig. 5). We have also included a full section (Task 2.8) on ionomics. Furthermore, we do also include fluorimetric (e.g. FLIPR) and fluorescent flow cytometric analyses where either the substrate is fluorescent or it can be made to compete for uptake through an SLC with a fluorescent molecule, or e.g. to vary the fluorescence of a membrane potential dye (see also WP3). Together, data from WP2 will allow the assessment of the likely activity as a substrate of any candidate drug or small molecule.

Description of work and role of partners

Task 2.1 Focused targeted metabolomics (M6-M36). Partners involved: CeMM. To start, we will categorize SLCs for their contribution to steady-state cellular metabolism by systematically comparing the metabolic profiles of cell lysates and culture supernatants from KO and overexpressing cell lines generated in WP1. Initially, we will screen a panel of 196 endogenous metabolites from 11 different compound classes compared to purified standards (amino acids, biogenic amines, acylcarnitines, nucleotides, nucleosides/nucleobases, amino acid derivatives, sugars/sugar derivatives, carboxyl acids, phenyl acids, cofactors, vitamins) using a dMRM based MS/MS detection method with an Agilent Triple Quadrupole Mass Spectrometer. This is a simple, quantitative and highly robust assay that we have established and learned to rely on. Just looking at lipids, the approach has allowed us to propose novel roles for several lipids in cellular processes [15]. The quantitative baseline metabolic footprint resulting from this task, together with the other omics data will be used to cluster SLCs by profile similarity, contributing to a comparative foundation for biological functional assignments to each SLC.

Task 2.2 Establish a method for the quantitative determination of serum metabolites and supplemented chemicals (drugs, diet-derived compounds) (M1-M18). Partners involved: ULIV, Pfizer, CeMM. Our next objective is to screen SLCs for their specific ability to transport drugs and dietary compounds. We must first create standard drug profiles and for this we will use the LC-MS methods we developed for the human serum metabolome [16]. Cocktails of known drugs, metabolites, and diet-derived compounds will be added to the cells either in PBS or pooled plasma. After lyophilization and solvent extraction, we will use high (mass) resolution UPLC-ESMS assays in both ES+ and ES- modes to capture all detectable molecules. Identification will be based on authentic standards with known retention time and MSn data. In parallel, Pfizer will contribute their own integrated metabolomics platforms based on Combi-T analysis [17]. Unbiased global metabolic profiles will be measured from wild type and SLC knockout frozen cell pellets using HRMS coupled with ion-pairing LC. Data deconvolution will be performed using the Combi-T algorithm with databases such as KEGG as the framework for metabolism. The output of the deconvoluted data will be an enriched network map of significant connected metabolites. For the SLC knockouts whose metabolic profiles produced the most interesting observations, RNA-Seq data from duplicate samples will be integrated into the enriched network map. Pfizer will deliver the data to the consortium.

Task 2.3 Analyse lyophilised intra- and extracellular extracts of mutant cell lines (M9-M60). Partners involved: ULIV, Pfizer, CeMM. Cognate pairs of loss- and gain-of-function SLC cells will be incubated in medium augmented with defined amounts of known human metabolites (Recon2: [18], Fig. 4), marketed drugs, and/or selected dietary components. After transport assays are complete, compounds will be extracted from the supernatants, and in some instances from cell extracts, and profiled by MS. Transported compounds will be identified by difference. This 'differencing' procedure was used successfully several years ago [19] to deorphanise SLC22A4, and we now expect it to be even more successful with advances in our hardware and software. The result will be sets of candidate substrates that are at least known to be transported by a particular SLC.

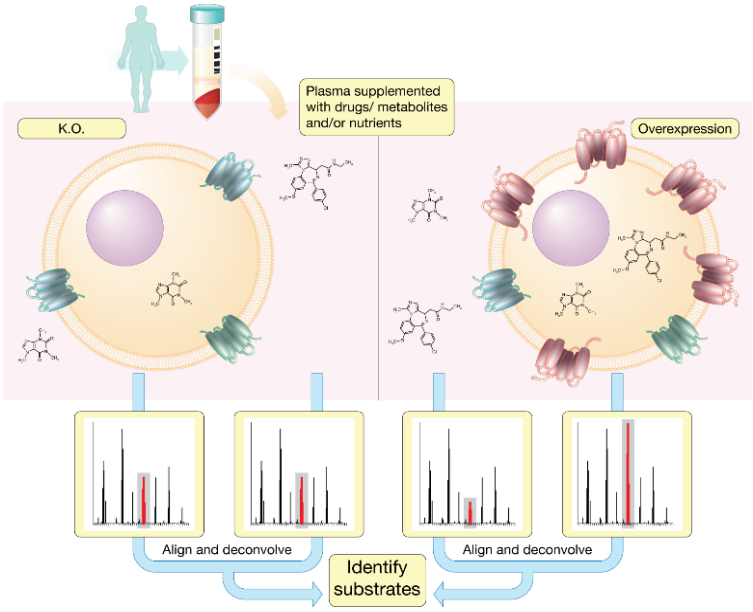

Figure 4: Schematic view of the metabolomics-based deorphanisation approach described in Task 2.3.

Task 2.4 Informatics analyses of metabolome/small molecule data. (M1-M60). Partners involved: ULIV, UNIVIE, Pfizer. Critical to any metabolomics analysis is the informatics processing of the data. A combination of the Progenesis Qi software for small molecule quantification, and KNIME workflows (as in [20]) will be used. QSAR models will be produced for each SLC for which adequate data describing the variance in uptake as a function of a substrate's (or inhibitor's) chemical structure are available. We start with a substrate with any measurable activity, then use cheminformatics methods to search for molecules with a relatively similar chemical structure, and try these iteratively, thereby building up a picture of the basic 'pharmacophore' or kinds of molecule demonstrating activity. Structures (in SMILES or InChI) will be the inputs to KNIME workflows that then encode the structures as binary strings ('2D molecular fingerprints') or vectors of chemical descriptors. Our preferred methods for forming QSARs in KNIME are run in parallel and include multivariate statistics (PLS), random forests, and genetic programming. In very difficult cases, and when the amount of data available allows, we may use Deep Learning algorithms. As KNIME is freely available for desktop use, we will make our KNIME workflows freely available. Where possible (only some algorithms admit this), models will also be converted to the model-independent Predictive Model Markup Language (PMML) and it is these that will be provided as specific models for the QSAR for each SLC. They can be plugged into generic KNIME workflows as specific nodes. In addition, raw data will be provided in the form of conventional Km and kcat values for each substrate (inhibitor), along with the known form of the relevant enzyme kinetics.

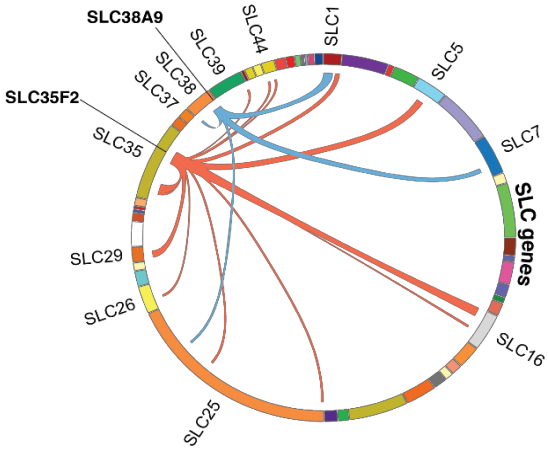

Figure 5: Synthetic lethality interactions map of the SLC38A9 and SLC35F2 genes obtained in human haploid HAP1 cells *(Girardi, Superti-Furga et al., unpublished). SLC38A9 shows synthetic lethal interaction with individual SLC genes known to transport amino acids (of the SLC1, 7 and 38 families). If we knew nothing about the SLC gene function, it would be fair to deduce that it may have something to do with amino acids.*

Task 2.5 Genetic interactions (M6-M60). Partners involved: CeMM. RESOLUTE will exploit the power of somatic cell genetics to assess SLC function. The partners will adopt a strategy that has been successfully employed to determine functional redundancy among SLC genes using human haploid cells, but that also works perfectly in non- haploid cells. Human cell lines express

160 to 240 individual SLC genes (data computed from [21]). Less than 10% of these expressed SLCs are essential for cell growth under normal cell culture condition ([22-24] and unpublished data). The majority of the other SLC genes are likely to be conditionally essential, that is, essential for survival under certain circumstances. One such circumstance is when other SLC genes with redundant function, in a given condition, are eliminated. In a screening process, we will start with one SLC gene knock- out and screen for other SLC genes that confer inability to grow when knocked out additionally. This process is called synthetic lethality and results in a genetic interaction map. Fig. 5 shows such an analysis for the SLC38A9 and the SLC35F2 genes. Although RESOLUTE might embark on a genome-wide synthetic lethal screen, the functional similarity among SLCs supports the idea of focusing first within the gene family, and this will be enabled by the SLC k.o. collection generated in the 6+1 cell lines of WP1. RESOLUTE will systematically determine synthetic lethal genetic interactions between all viable SLC genes. Our established SLC-focused library (~3,000 sgRNAs, roughly 60 times smaller than the commercially available genome-wide CRISPR/Cas9 libraries) is what makes the scale of the screen feasible and cost- efficient. Over time, the project will further extend the set of genetic interactions mapped by using higher complexity libraries targeting two SLCs per construct (currently being developed), and ultimately, we may perform a genome-wide screen if resources allow.

Task 2.6 Proteomics studies (M6-M60). Partners involved: CeMM. We previously employed affinity purification coupled to mass spectrometry (AP-MS) to characterise protein complexes formed by all yeast genes [25, 26], entire human or viral pathways [27, 28] and some SLCs. For example, we found that the previously uncharacterized SLC38A9 protein is a stoichiometric component of the LAMTOR complex regulating mTor [5], and have determined the interactome of several SLC38 family members (unpublished data). RESOLUTE will apply this kind of analysis to all members of the SLC superfamily, using the cell lines that are naturally expressing the gene. Interaction partners of all human SLCs will be identified, using both tagged full-length proteins or tagged domains, as well as any high-affinity antibody available or generated in the project, at least in the validation phase.

Task 2.7 Subcellular localization determination (M6-M56). Partners involved: CeMM. The panel of cell lines expressing tagged constructs will be used to suggest the subcellular localization of the target SLC by confocal microscopy, which will be confirmed by staining the endogenous protein with our validated SLC-specific antibodies. Well-characterized markers (identified from literature and through the input of the Human Protein Atlas project) will be used to identify the most common subcellular organelles, including mitochondria, nucleus, endoplasmic reticulum, Golgi, lysosomes, peroxysomes, endosomes and lipid droplets on a confocal microscope (Leica TCS SP5 or equivalent).

Task 2.8 Ionomics (M6-M60). Partners involved: Vifor. To identify inorganic ions as potential SLC (co-) substrates (possibly as many as one third of the total), supernatants or cell extracts from loss- and gain-of-function SLC cells obtained in WP1 will be profiled by inductively-coupled plasma-optical emission spectroscopy (ICP-OES) or -mass spectrometry (ICP-MS). An ICP-MS combines a high-temperature ICP (Inductively Coupled Plasma) source with a mass spectrometer, which is replaced by an optical emission spectrometer in the case of ICP-OES. The ICP source converts the atoms of the elements in the sample to excited atoms and ions. While both of them may be detected by ICP-OES due to the specificity of the emitted light, only ions are separated and detected by the mass spectrometer. Supernatants or cell extracts from cognate pairs of cells over-expressing or deficient in a particular SLCs will be obtained following transporter assay with or without addition of a mixture of inorganic ions. Detection of e.g. Na^{+}, K^{+}, Mg^{++}, Ca^{++}, Cu^{++}, Fe^{n+} and Zn^{++}, plus a variety of inorganic anions such as sulphate and phosphate, in solutions is either done by ICP-OES or ICP-MS depending on the concentration range to be covered. ICP-MS is usually used for samples with concentrations in the sub-p.p.b. range [29], whereas ICP-OES is applied for concentrations in the sub-p.p.m. range [30]. ICP-OES and ICP-MS are well established technics applied for the analysis of elemental impurities in the analytical laboratories of Vifor (International) Ltd. Both techniques allow multi-element analysis within the same sample, large dynamic linear range and excellent reproducibility. Samples need to be acidified (e.g. for supernatant) and additionally digested (e.g. for cell pellets) at elevated temperatures to ensure any particulate matter is brought into solution. The intensity of the emission or m/z-ratio of the specific element is measured and quantified by comparison against standards with known concentrations of elements. Quantitative differences in the inorganic ion profiles of cognate pairs of cells over-expressing or deficient in a particular SLC transporter will help to identify ions as SLC (co-substrates) and move forward the

deorphanisation process. Overall, we anticipate 800 cell lines in biological triplicates and 2 technical replicates each (~5,000 measurements).

Participation per partner

Partner number and short name	WP2 effort (%)
1 - CEMM	56.23
3 - ULIV	13.04
7 - UNIVIE	0.87
8 - Pfizer	12.67
9 - Novartis	0.57
11 - Vifor	16.59
Total	100

List of deliverables

Deliverable Number	Deliverable Title	Lead beneficiary	Type	Dissemination level	Due Date (in months)
D2.1	Signature metabolic profiles using targeted metabolomics	1 - CEMM	Report	Public	36
D2.2	Methodology for general detection of plasma metabolites and drug/ dietary components	3 - ULIV	Report	Public	18
D2.3	Profile of plasma metabolite changes for each SLC cell line	3 - ULIV	Report	Public	60
D2.4	Informatic analysis of metabolomic data	8 - Pfizer	Report	Public	60
D2.5	SLC genetic interaction map	1 - CEMM	Report	Public	60
D2.6	SLC interactome	1 - CEMM	Report	Public	60
D2.7	Subcellular localization of target SLCs	1 - CEMM	Report	Public	56
D2.8	Determination of SLCs using ions as (co-)substrates	11 - Vifor	Report	Public	60

Description of deliverables

D2.1 Signature metabolic profiles using targeted metabolomics [36]. Robust identification and quantification using LC/GC-MS for some 180 central metabolites

D2.2 Methodology for general detection of plasma metabolites and drug/dietary components [18]. LC/GC-MS coupled to novel deconvolution routines for determining the approximately 3,000 molecules typically observable in serum

D2.3 Profile of plasma metabolite changes for each SLC cell line [60]. Use of methods of Task 2.2 to assess the largest changes between cells lines upregulated/knocked out for particular SLCs

D2.4 Informatic analysis of metabolomic data [60]. Development and deployment of workflows for QSAR analysis of the ability of particular overexpressed SLCs to take up different substrates, and judicious variation of those substrates.

D2.5 SLC genetic interaction map [60]. Production of 'synthetic phenotypes', based on the principle that knocking out both variants of a transporter with redundant function (e.g. loss of ability to transport X) thereby causes a phenotype from which one can infer function.

D2.6 SLC interactome [60]. Physical measurement (by proteomics) of co-expression/co-purification of SLCs with partners.

D2.7 Subcellular localization of target SLCs [56]. Based on optical imaging experiments with tagged SLCs.

D2.8 Determination of SLCs using ions as (co-)substrates [60]. The recognition that many substrates are symporters or antiporters means that one can detect this by assessing the movement of co-ions when transport substrates are added to suitable cell lines.

Schedule of relevant milestones

Milestone number	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS3	Deorphanisation strategy	8 - Pfizer	24	Development and validation of an integrated multi- approach deorphanisation strategy broadly applicable to the SLC family; Report to GA
MS4	Identification of potential substrates for orphan SLCs	8 - Pfizer	60	Identification of potential substrates for orphan SLCs; Report to GA; addition to database; publication in scientific journals

Work package number	3	Start Date or Starting Event	M6
Work package title	Development of quantitative transport assays Lead: Sanofi, Co-lead: MPIMR		

Summary

The aim of WP3 is to develop quantitative transport assays for >50% of the SLC family. This will be done by deploying a consortium-wide effort combining the functional and deorphanisation data/hypotheses generated in WP1 and WP2 with know-how and technologies available to the partners and the network of RESOLUTE Academic Expert Laboratories. We will use established approaches, such as the ones based on voltage-sensing dyes, to tackle electrogenic transporters, followed by a set of 'generic' and innovative approaches including impedance monitoring, MRM (multiple reaction monitoring) mass spectrometry, fluorescence energy transfer, subcellular small molecule sensors or approaches based on genetic interaction information obtained within the project. For the most resilient/idiosyncratic SLCs or SLC family members, RESOLUTE will make use of the specific know-how that is available within the recruited network of RESOLUTE Academic Expert Laboratories (see Fig. 14). WP3 and WP6 overlap and will be started in parallel. We propose a practical workflow, which involves an 'engine' that takes care of an empirical and parsimonious serial funnelling of all SLCs for which we obtain high quality reagents (>300), and a path-finding/accelerated target route (to address the different families and include early priority list members, respectively), which will also be used to reality-check the efficacy of the various assay modules, and optimize the associated technologies (Fig. 6). We expect to identify testable ligands for each SLC through the following possibilities: 1) the ligands are present in the cellular growth medium; 2) ligand candidates have successfully been identified in WP2; 3)

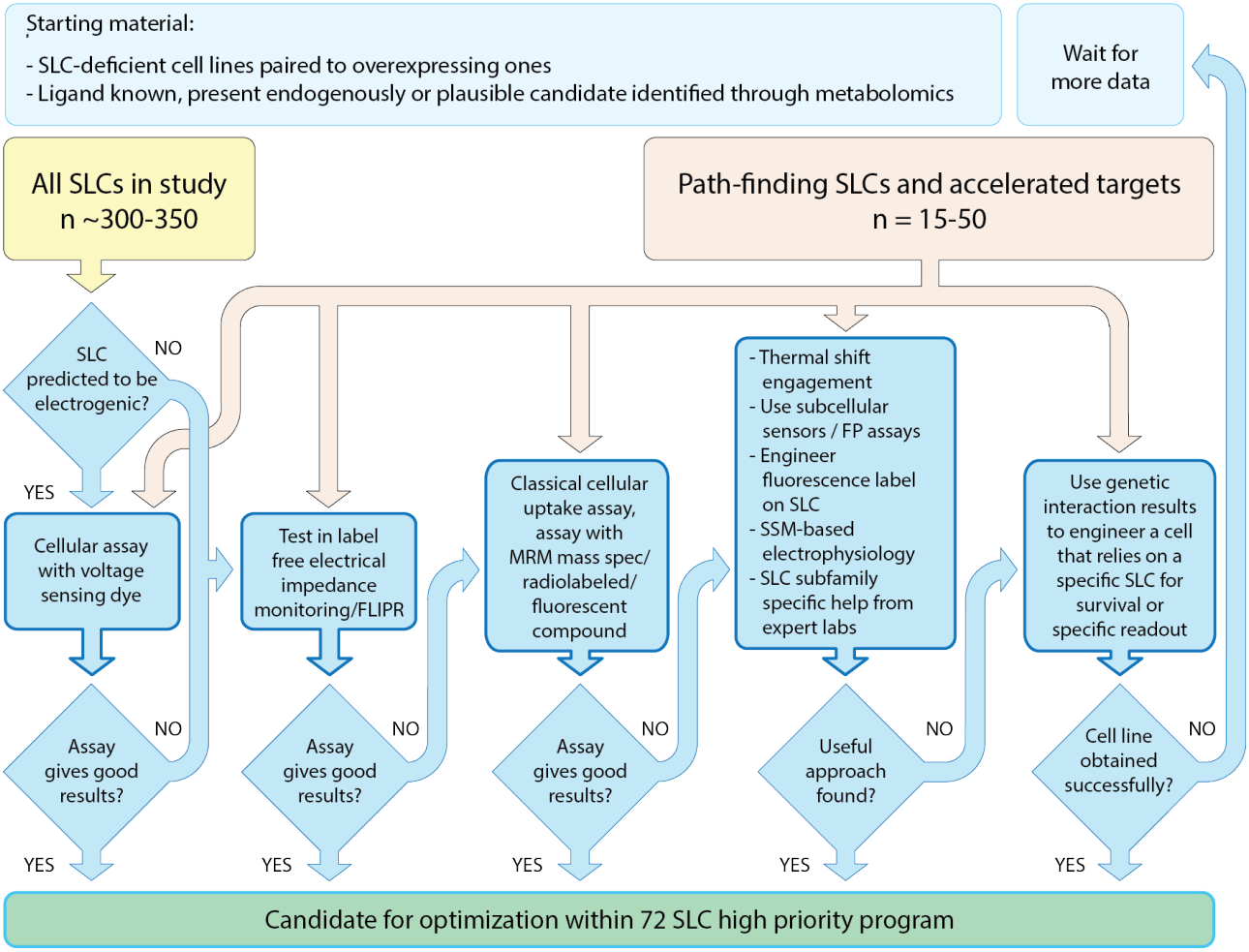

Figure 6: Workflow for the identification of suitable assays for SLCs. *As doing all assays with all SLC mutant cells is neither sensible nor feasible, we opted for a logical workflow taking 'bioinformatic' properties into account.*

ligands are known, or can be inferred from closely related family members. Unless indicated, this ligand 'logic' will be applied to the assay pipeline. All in all, we expect the 'engine' to provide significant clues for assay development for the majority of SLCs interrogated.

As a first level, assays for transporters predicted or known to be electrogenic will be based on setups employing voltage-sensing dyes. Extrapolating from the percentage of known electrogenic transporters in the genome, we expect up to 65% of SLCs to have such properties (Fig. 7), with approximately two thirds of them being at the cell surface.

We are aware that adding large chemical moieties to ligands could indeed affect their binding to the SLCs. While we think that this is still a potentially rewarding effort, we plan to complement this approach with the fluorescent labelling of SLCs and the use of fluorescent sensor proteins to directly measure changes in key metabolite concentrations upon SLC engagement.

The assay pipeline is described in Fig. 6 and, as these are generally broadly used techniques, we limited ourselves to a general description of their implementation within the RESOLUTE workflow.

Description of work and role of partners

Task 3.1 Assay development for electrogenic transporters (M6-M30). Partners involved: AXXAM, Novartis. As a first level, assays for transporters predicted or known to be electrogenic will be based on setups employing voltage-sensing dyes, as done routinely by AXXAM. Extrapolating from the percentage of known electrogenic transporters in the genome, we expect up to 65% of SLCs to have such properties (Fig. 7), with approximately two thirds of them being at the cell surface. The latter should therefore be amenable to assays that measure membrane potential changes, including calcium-dyes, sodium-dyes and membrane-potential dyes. Pairs of cell lines for each gene (i.e. k.o. vs WT or overexpressing lines) will be tested for differential membrane potential response to challenges coming from a small set (1-5) of ligands identified from the output of WP1 and WP2 or from previously available knowledge. We expect that this assay will allow us to establish screens for more than 100 SLCs over the course of the project.

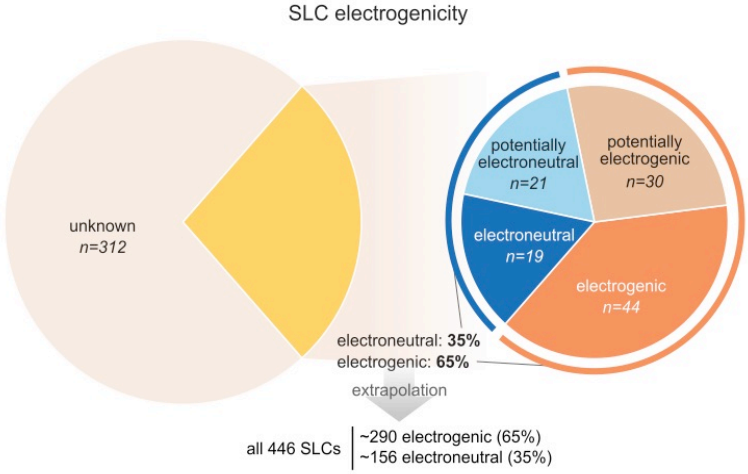

Figure 7: Pie charts showing the proportions of known and potential electrogenic transporters *on an initial set of 426 human SLCs. Extrapolation from this analysis suggests that about 290 out of the 446 human SLCs could be electrogenic.*

Task 3.2 Assay development with GPCR-mediated label-free electrical impedance monitoring and second messenger mobilization (M6-M30). Partners involved: ULEI, Bayer. SLCs not predicted to be electrogenic or that fail to yield reliable assays in Task 3.1 will be tested for their suitability to be monitored by impedance changes using the xCELLigence system [31]. This approach has been successful with GPCRs [32] and relies on the principle that the equilibrative, non-electrogenic SLCs for a given metabolite might be seen by the concomitant presence of a GPCR for that metabolite (e.g. SLC29A1/adenosine receptor). The presence of the SLC dictates the extracellular amount of the endogenous ligand and hence the strength of the signal. When challenged with solutes, cells will be examined in real-time on gold microelectrodes in a multi-well plate format for cell proliferation, cell size/morphology, cell attachment quality, and cell invasion/migration, all leading to changes in cellular impedance. Similar to the electrical impedance monitoring, GPCR-mediated second-messenger mobilization approaches using FLIPR-based fluorescent and luminescent readouts (e.g. Ca^{2+} dyes and biosensors, cAMP biosensors) will be explored as a readout for GPCR-SLC interaction.

Task 3.3 Uptake assays with MRM mass spectrometry, radiolabelled or fluorescent compounds (M6-M33). Partners involved: CeMM. Uptake of specific compounds can be monitored by mass spectrometry using the multiple reaction monitoring assay or with classical assays based on radiolabelled or fluorescent compounds. Even if radiolabelled or fluorescent compounds have already been used to develop the transporter assay [33], the availability of the respective enabling chemicals is expected to limit this approach in general. When required, probe generation will be managed case by case by subcontracting the synthesis. Comparison of cell lines overexpressing or lacking an SLC (WP1) will allow us to determine the transport of molecules that are dependent on a specific transporter.

Task 3.4 Assay based on cellular thermal shift (M6-M36). Partners involved: CeMM. The cellular thermal shift assay (CETSA, [34]) has been adapted for integral membrane proteins (Fig. 8, also [35]). For chosen SLCs and candidate ligands, target engagement by CETSA can be monitored using antibodies, endogenously tagged genes (as in WP7) or, conveniently, employing the panel of tagged SLC cell lines (WP1). The stabilization effect can be indirect, but otherwise it can be very powerful to monitor SLC function in a 'native' membrane context.

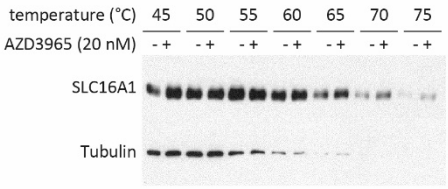

Figure 8: Cellular thermal shift assay on HEK293 cells treated with the SLC16A1 inhibitor AZD3965 (20nM). *Hashimoto, Superti-Furga et al., unpublished data.*

Task 3.5 Assays involving fluorescence (M6-56). Partners involved: MPIMR, Vifor, Novartis. We will develop new fluorescent probes for selected SLCs by derivatizing ligands of the SLC of interest, and a new class of fluorescent dyes that we developed previously and successfully used for live-cell imaging [36]. These probes will permit fluorescence imaging of individual SLCs and should become powerful tools for functional studies as well as screen applications. We will also develop/use fluorescent probes and environmentally-sensitive dyes to label SLC post-translationally, either using the developed SNAP-Tag or other orthogonal methods [37]. On occasion, it may be feasible to do single- molecule F/BRET by introducing two chemical tags or bioluminescent domains (Nano-Luc) within the same SLC or two subunits of heteromeric SLCs [38], or to develop assays based on fluorescence polarization (for example via novel Siliconrhodamine probes). To investigate cellular responses triggered by SLC-ligand interactions, fluorescence microscopy with high content imaging (HCI) analysis will also be applied using loss- and gain-of-function SLC cell lines and fluorescently labelled ligands generated in WP1 and WP3. Fluorescent ligands or SLC-specific antibodies, if available, will be used to detect the localization of SLCs and study, for instance their internalization from the plasma membrane. High-resolution fluorescence images from thousands of cells per well in multi-well plates (96 or 384 wells) will be acquired using automated-stage fluorescent microscopes (Operetta, Perkin Elmer or ScanR, Olympus). Image and data analysis will be performed by HCI software (Columbus, Perkin Elmer or ScanR Olympus) including algorithms for detection of cell regions (e.g. plasma membrane, cytoplasm, nuclei) and quantification of the associated fluorescence. Ligand-induced SLC internalization HCI assays might be further developed to high content screening platforms for identification of SLC inhibitors. Vifor has successfully applied HCI-based assays as drug-discovery platform for identification of inhibitors of one SLC family member [39]. Alternatively, if no fluorescent reagents for a particular SLC are available, changes in the cellular phenotype and function of isogenic cell clones, such as morphology, cell size and shape, apoptosis will be measured using automated image analysis applications. Finally, we will use fluorescent sensor proteins to directly measure the uptake of drugs and metabolites into cells, and measure changes in key metabolite concentrations in living cells. The metabolic state of cells as a function of SLCs will be measured by utilizing new fluorescent sensors, such as the one for NADPH/NADP+ as well as NAD+ [40].

Task 3.6 SSM-based electrophysiology (SURFE2R) (M6-M48). Partners involved: Sanofi. The SURFE2R is designed for activity measurement of electrogenic membrane transporters and pumps, which generate currents too low to resolve by patch clamp or which are not expressed in membranes accessible for patch clamp (e.g. bacterial, organelles). The technology enables cell, label- and radioactivity-free real time measurements with high time resolution. The SURFE2R 96SE is a fully parallel 96 well based system, generating 96 data points in about 5 minutes. The highly specialized software tools SURFControl and DataControl and a set of optimized workflow modules enable the automation of the experiment starting with

the preparation of the measuring sensor to export and data analysis. SSM-based electrophysiology is complementary to the electrogenic assays in Task 3.1.

Task 3.7 Use genetic interaction to engineer cell lines depending on specific SLCs for survival or readout (M6-M42). Partners involved: CeMM. The availability of synthetic lethality data from WP2 will be used to derive cell lines that depend on a specific transporter for survival by removing the second/other synthetically lethal gene(s). The resulting cell line will be dependent for fitness on a specific SLC that was not essential before, and can thus be screened for inhibitors. Importantly, this assay should work also with intracellular SLCs and SLCs that are full orphans. Hits can be validated using in-cell thermal shift assays or other orthogonal approaches.

Participation per partner

Partner number and short name	WP3 effort (%)
1 - CEMM	38.64
4 - AXXAM	4.15
5 - ULEI	15.45
6 - MPIMR	19.32
9 - Novartis	5.15
11 - Vifor	8.88
12 - Sanofi	5.79
13 - Bayer	2.57
Total	100

List of deliverables

Deliverable Number	Deliverable Title	Lead beneficiary	Type	Dissemination level	Due Date (in months)
D3.1	Assays for (electrogenic) transporters	4 - AXXAM	Report	Public	30
D3.2	Assays for SLCs based on fluorescent sensor proteins	6 - MPIMR	Report	Public	30
D3.3	Assays for SLCs based on mass spectrometry, thermal shift, radiolabeled or fluorescent compounds	1 - CEMM	Report	Public	36
D3.4	Assays for SLCs based on fluorescent ligands, fluorescence-labelling of SLCs or fluorogenic probes	6 - MPIMR	Report	Public	56
D3.5	Assays for SLCs based on SSM- electrophysiology	12 - Sanofi	Report	Public	48
D3.6	Assays for SLCs based on cell lines that couple growth to SLC function	1 - CEMM	Report	Public	42

Description of deliverables

D3.1 Assays for (electrogenic) transporters [30]. A report summarizing results and protocols obtained using fluorescent sensitive dyes and impedance measurements.

D3.2 Assays for SLCs based on fluorescent sensor proteins [30]. A report summarizing results and protocols on measuring SLC-dependent metabolic states through fluorescent sensor proteins.

D3.3 Assays for SLCs based on mass spectrometry, thermal shift, radiolabelled or fluorescent compounds [36]. A report summarizing results from classical transport assays.

D3.4 Assays for SLCs based on fluorescent ligands, fluorescence-labelling of SLCs or fluorogenic probes [56]. A report summarizing results from fluorescence-based assays.

D3.5 Assays for SLCs based on SSM-electrophysiology [48]. A report summarizing results and protocols obtained with the SURFE2R approach.

D3.6 Assays for SLCs based on cell lines that couple growth to SLC function [42]. A report summarizing results and protocols on assay based on genetic engineering of cell lines dependent on specific SLCs for survival.

Schedule of relevant milestones

Milestone number	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS5	Workflow implementation	12 - Sanofi	24	Implementation of a workflow for the identification of assays suitable for SLCs; Report to GA
MS6	Assays developed for >50% of human SLCs	12 - Sanofi	60	Assays developed for >50% of human SLCs; Report to GA

Work package number	4	Start Date or Starting Event	M1
Work package title	Selection of the 'SLC priority list' of targets Lead: ULEI, Co-lead: Sanofi		

Summary

The consortium will create a priority list of 72 SLCs by using a number of criteria, including SLC regulation, co-regulation, role in disease, availability of cell lines, etc., guided among others by the information in the database and repository of WP8. Each of the 6 EFPIA partners can select 12 SLCs for the priority list; the final choice is made in agreement with the Executive Board. It is anticipated that the priority list is dynamic and will be gradually built up over time, with the following schedule in mind:

- approx. 10 SLCs at month 0,
- approx. 36 SLCs at month 18,
- approx. 54 SLCs at month 30, and
- 72 SLCs at month 42.

To ensure that the consortium can start quickly, a small number of approx. 10 'accelerated target' SLCs, as the very first of the SLC priority list, will be selected, some of which already before the kick-off meeting. These targets will be analysed and studied from as many angles as possible during the first 18 months, in order to bring awareness and a reality check of the opportunities and challenges of these and other SLCs to all partners. Pursuing these targets will allow the pioneering of new assays and approaches and also enable the project teams to get to know each other better and start collaborating. For the 'accelerated targets' and the other SLCs on the priority list an action plan/research proposal will be generated, which will be tuned per target. This plan is based on overall tractability, i.e. i) the evidence/ likelihood of the SLCs being amenable to chemical engagement, ii) their specificity of expression profiles and disease association (disease database/genome-wide associations), iii) their structural folds, and iv) any new results generated in either WP1-3 and WP5-7 (e.g. availability and quality of reagents, genetic interaction, protein expression, subcellular localization, potential assay, etc.), or within Open PHACTS, eTOX and other EU based consortia, or as described in the scientific literature or public databases (WP8). It is anticipated that approx. 30% of the priority SLCs will be selected on the basis of results generated in WPs 1-3 and 5-8.

Since the research foreseen in RESOLUTE will apply an 'open-access ethos' and be precompetitive in nature, the consortium scientists agree that selection of SLCs for the priority list does not require an 'honest broker' to ensure confidentiality and prevent contamination. This is particularly true as IP regarding new chemical entities is not part of the program. Within the limit of a maximum of 72 SLC for the whole project, it is a common understanding that any consortium member can propose priority SLCs anytime until month 40, i.e. two months before the final list of 72 SLCs will have been conceived. To be eligible to the priority list, the proposed SLC will need at least one EFPIA champion and one champion from the other (non-EFPIA) partners in the consortium. If properly championed, the SLC is de facto added to the priority list, and a formal decision on inclusion will be made by the executive board based on the latest scientific knowledge and results. Thereafter, the list will be reviewed semi-annually by the GA for its timeliness and feasibility. To summarize:

- all partners (academic, SME and EFPIA) can propose targets for the priority list,
- each target on the priority list needs to be supported by an EFPIA champion,
- work on priority targets is done in collaboration with at least one other (non-EFPIA) partner, and
- taking a target off the priority list should be a joint decision by all partners actively working on the target with the main reason anticipated being overall feasibility.

Description of work and role of partners

Task 4.1 Define procedure to arrive at and to review priority list (M1-M12). Partners involved: All. At or before the kick-off meeting in year 1, consortium partners will identify approx. 10 'accelerated targets' to ensure timely testing of e.g. the deorphanisation engine and the new technologies. This will also allow EFPIA partners to invoke their in-kind contributions at an early stage of the consortium's lifetime. In close collaboration with WP8, a tractability analysis will be developed to generate the priority list of SLCs.

Task 4.2 Compile first version of priority list and action plan/research proposal for each prioritized SLC (M6-M12). Partners involved: All. The action plan will be produced collectively by the participating partners and revised on a regular basis.

Task 4.3 Confirm first priority list of 36 SLCs. (M12-M18). Partners involved: All. This will be done by the consortium (prospectively by the Executive Board, and retrospectively by the GA) and concluded with a detailed definition of the priority list.

Task 4.4 Reflect on and increase the priority list to 54 (M30) and 72 SLCs (M42), respectively (M12-60). Partners involved: All. This will be done by the Executive Board, and confirmed in the earliest General Assembly. Monitor progress for the remainder of the consortium's lifetime, which may bring some alterations to the priority list (M42-M60).

Participation per partner

Partner number and short name	WP4 effort (%)
1 - CEMM	4.93
2 - UOX	4.93
3 - ULIV	4.93
4 - AXXAM	4.93
5 - ULEI	12.17
6 - MPIMR	4.93
7 - UNIVIE	22.22
8 - Pfizer	4.93
9 - Novartis	6.17
10 - Boehringer	4.93
11 - Vifor	6.17
12 - Sanofi	14.81
13 - Bayer	6.17
Total	100

List of deliverables

Deliverable Number	Deliverable Title	Lead beneficiary	Type	Dissemination level	Due Date (in months)
D4.1	Protocol for the procedure to establish priority list	5 - ULEI	Report	Confidential	12
D4.2	Tractability analysis for SLCs on the priority list 1	5 - ULEI	Report	Confidential	18
D4.3	Tractability analysis for SLCs on the priority list 2	5 - ULEI	Report	Confidential	30
D4.4	Tractability analysis for SLCs on the priority list 3	5 - ULEI	Report	Confidential	42
D4.5	Consortium-approved SLC priority list 1Con	12 - Sanofi	Report	Confidential	18
D4.6	Consortium-approved SLC priority list 2	12 - Sanofi	Report	Confidential	30
D4.7	Consortium-approved SLC priority list 3	12 - Sanofi	Report	Confidential	42
D4.8	Updated versions of the SLC priority list 1	12 - Sanofi	Report	Confidential	48
D4.9	Updated versions of the SLC priority list 2	12 - Sanofi	Report	Confidential	54
D4.10	Updated versions of the SLC priority list 3	12 - Sanofi	Report	Confidential	60

Description of deliverables

D4.1 Protocol for the procedure to establish priority list [12]. The first endeavour is to compose a list of 10 'accelerated targets' already at or before kick-off, and thereafter work on a protocol leading to the very first version of the SLC priority list.

D4.2 Tractability analysis for SLCs on the priority list 1 [18]. Tractability is based on four criteria specified in the WP4 text. It is an ongoing endeavour with an accumulating number of SLCs on the priority list (36 SLCs at M12-18, 54 SLCs at M18-30 and 72 SLCs at M30-42).

D4.3 Tractability analysis for SLCs on the priority list 2 [30]. Tractability is based on four criteria specified in the WP4 text. It is an ongoing endeavour with an accumulating number of SLCs on the priority list (36 SLCs at M12-18, 54 SLCs at M18-30 and 72 SLCs at M30-42

D4.4 Tractability analysis for SLCs on the priority list 3 [42]. Tractability is based on four criteria specified in the WP4 text. It is an ongoing endeavour with an accumulating number of SLCs on the priority list (36 SLCs at M12-18, 54 SLCs at M18-30 and 72 SLCs at M30-42)

D4.5 Consortium-approved SLC priority list 1 [18]. The Executive Board prospectively approves the priority lists, after which this is done retrospectively by the GA

D4.6 Consortium-approved SLC priority list 2 [30]. The Executive Board prospectively approves the priority lists, after which this is done retrospectively by the GA

D4.7 Consortium-approved SLC priority list 3 [42]. The Executive Board prospectively approves the priority lists, after which this is done retrospectively by the GA

D4.8 Updated versions of the SLC priority list 1 [48]. Based on the latest scientific development, an updated list of priority SLC available on a half-yearly basis.

D4.9 Updated versions of the SLC priority list 2 [54]. Based on the latest scientific development, an updated list of priority SLC available on a half-yearly basis.

D4.10 Updated versions of the SLC priority list 3 [60]. Based on the latest scientific development, an updated list of priority SLC available on a half-yearly basis.

Schedule of relevant milestones

Milestone number	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS7	Priority list protocol	5 - ULEI	12	Protocol for the procedure to establish priority list; Approval by
MS8	Priority list	5 - ULEI	42	Full list of priority list SLCs available; Approval by EB

Work package number	5	Start Date or Starting Event	M6
Work package title	Generation of protein reagents for the SLC priority list Lead: UOX, Co-lead: Bayer		

Summary

While RESOLUTE expects to be able to establish recombinant protein expression for the vast majority of SLCs in WP1, the objective of WP5 is to dedicate special efforts to ensure that high-quality protein reagents are developed for the SLCs on the priority list. This involves optimizing expression, purification conditions, reconstitution into liposomes and nanodiscs, and the generation of selective, high affinity protein binders (i.e. antibodies, nanobodies, sybodies) for SLCs on the priority list (Fig 9). The aim is to generate the best possible reagents for experimentation and assay development for the majority of the SLCs on the priority list.

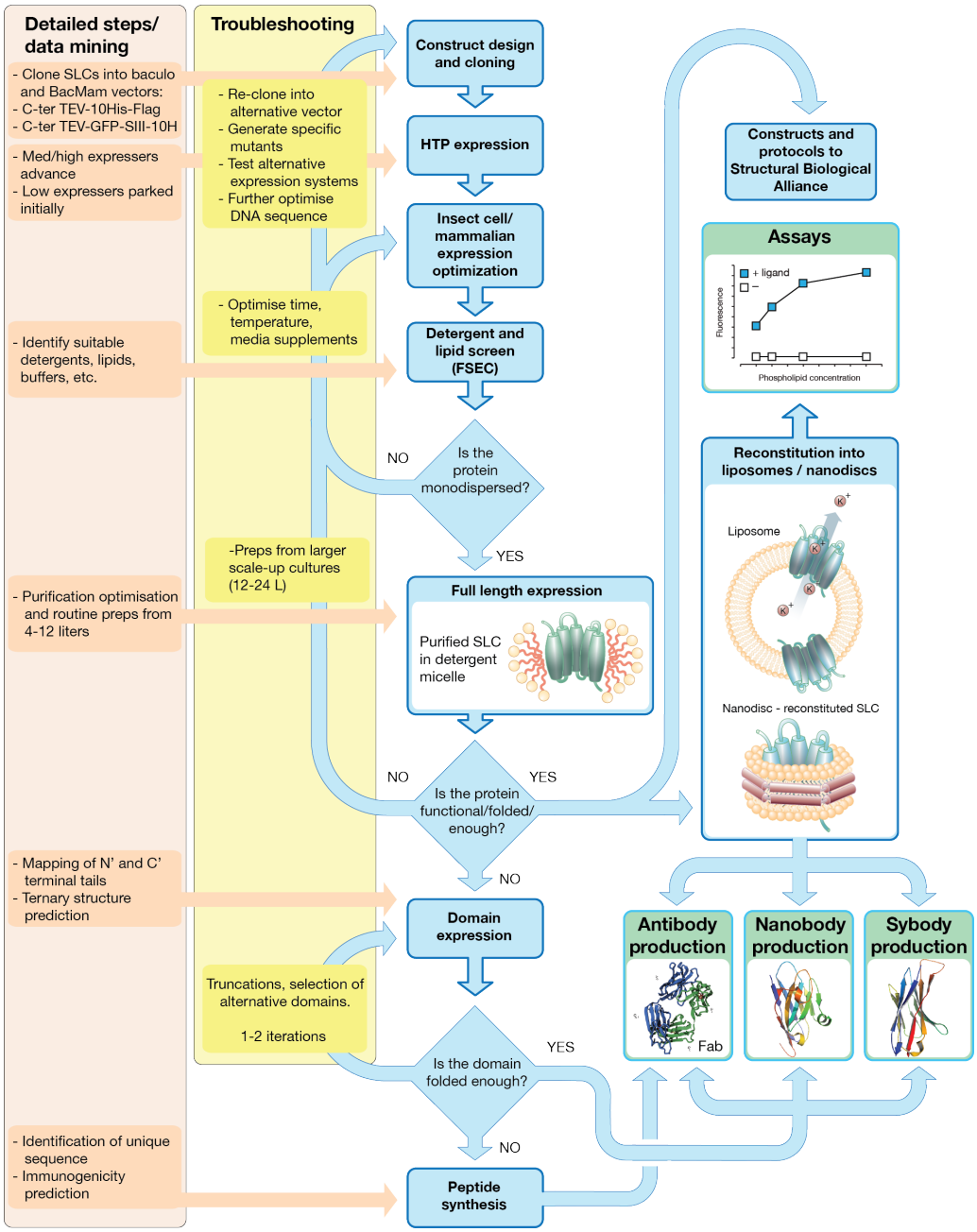

Figure 9: Protein production workflow. *Experimental workflow and decision tree from generation of the expression vectors to the various intended purposes and applications.*

Description of work and role of partners

Task 5.1 Optimization of SLC expression conditions, purification and stability in detergents (M6-M48). Partners involved: UOX, Pfizer, Bayer. In WP1 (Task 1.4) we described the small-scale expression and purification of GFP- tagged SLCs. WP5 is closely related to WP1 as it is based on the same pipeline for protein production. However, we will extend efforts to produce SLCs on the priority list at a larger scale and with significant troubleshooting of protein expression, purification and solubilization conditions (Fig. 9). For GFP-tagged priority SLCs, the material from the initial small-scale test purifications will be directly loaded onto an HPLC high-throughput size exclusion chromatography (SEC) system and analysed for monodispersity using a fluorescence detector (FSEC). Based on our previous studies of hundreds of membrane proteins, and the exploration of dozens of lipid combinations, we have arrived at a process that involves solubilization and purification of SLC proteins in dodecylmaltoside (DDM) supplemented with lipids and cholesteryl hemisuccinate, which mimics the native membrane environment in cells. Expression temperature, MOIs of viral transduction and expression time will be optimized to maximize expression levels in mammalian or insect cells. Depending on expression level/monodispersity, the constructs will be either moved forward to medium/large scale purification, or further tested in a number of different detergent/lipid/buffer conditions. While the major part of this work will be carried out by UOX, the EFPIA partner(s) (Bayer) will assist with the large-scale production of selected priority SLCs. Based on our experience and on current technology we expect to be successful for up to 30% of SLCs on the priority list. However, we expect that new developments will result from the synergies, the scale and the innovative approaches implemented within RESOLUTE and this may substantially increase the number of SLCs that can be successfully purified in high amounts. We therefore aim to identify optimal expression, purification and stability conditions for the majority of the priority SLCs during the RESOLUTE period.

Task 5.2 Reconstitution into proteoliposomes for transport assays and nanodiscs for antibody generation (M12-M48). Partners involved: UOX. Each high priority SLC that can be scaled up and purified in milligram quantities will be characterized in thermal shift assays to assess the overall stability, and reconstituted into proteoliposomes or nanodiscs. Proteoliposomes are not only invaluable tools to carry out in vitro transport assays (WP3, WP6) for confirming substrate candidates from deorphanisation experiments (WP2), but also have superior antigenic properties compared to purified protein in micelles or purified membranes. For optimal immunization results in animals, the proteoliposomes will be supplemented with Lipid A, a LPS derivative commonly used as adjuvant. For affinity reagent generation ex vivo (using phage/yeast or ribosome display libraries from the CROs, see Task 5.3), we will use nanodisc technology to stabilize SLCs in a more native-like environment. In addition to increasing the stability of the protein, nanodisc-incorporation also ensures exposure of both cytoplasmic and extracellular epitopes for effective screening of high affinity binders.

Task 5.3 Generation of high-affinity binders recognizing 3D epitopes or SLC termini (M15-M48). Partners involved: CeMM, UOX. Generation of high affinity antibodies recognizing SLCs is challenging because epitopes are often non-linear and consist of a combination of loops between transmembrane (TM) stretches. To retain correct folding and epitope formation we will use aforementioned proteoliposome and nanodisc technologies to prepare samples of SLCs encompassing the full transmembrane region for immunization or library screening. For priority SLCs, we will generate camelid nanobodies in collaboration with University of Zurich. Nanobodies are well-known for their exquisite affinity and specificity, but the immunization and selection processes require substantial amounts of purified protein reconstituted into proteoliposomes. RESOLUTE will provide University of Zurich with priority SLC antigens for camelid immunization including full-length SLCs in liposomes. Whenever high-level full-length expression cannot be achieved, large N-, C-terminal or inter-TM domains (>100 amino acids) will be produced by bacterial expression. Finally, for the most resistant cases, peptides predicted to have immunogenic properties will be synthetized and used for immunization. We therefore aim to obtain nanobodies for the entire set of priority SLCs. For the SLCs that are either part of the priority list, or are otherwise of strategic importance, we will also employ in vitro display technologies to generate high quality affinity reagents based on different protein scaffolds, like antigen-binding fragments (Fab), nanobody and sybody phage libraries [4]. The phage/yeast display and ribosome display screening platforms of the RESOLUTE Academic Expert Laboratories allow screening for binders to several different SLCs in parallel and require smaller amounts of purified material (0.5-1 mg). These synthetic

binders have additional advantages over classical antibody fragments: they are extremely stable single domain proteins that lack disulfide bonds, and can be produced in large quantities in E. coli or in the cytoplasm of eukaryotic cells. In the first 6-12 months of WP5, we will perform pilot experiments on a selected panel of SLCs to systematically compare the effectiveness of synthetic binders on SLCs, and test the resulting reagents in different in vivo and in vitro assays (see below). Based on this pilot study, we will choose the most productive platform to generate reagents for the SLCs on the priority list. As we generate more cell-based reagents for SLCs, we may also use SLC-expressing cells (and their corresponding knock-out controls) as antigens for in vitro selection [41].

Task 5.4 Validation of binders in cell lines and tissues (M15-M48). Partners involved: CeMM. The protein affinity reagents generated will be tested for reactivity and specificity using the k.o. and wild type cell line panels generated in WP1. Applications will include Western blotting, immunofluorescence, and immunoprecipitation [42]. Reagents that pass our stringent RESOLUTE validation will be made available for tissue staining, taking advantage of the large sample collection available within The Human Protein Atlas project (SciLifeLab). Obtaining high-affinity protein-binding reagents has been extraordinarily difficult for SLCs for a variety of technical challenges (high ratio of transmembrane region to total residues, heavy glycosylation, variable expression levels). Embarking now on a protein class-wide effort would require a reliable and proven strategy. Committing to a single approach for the five years would have to be justified by data. Our considerations are as follows:

- There is no leading technology to embrace as source of renewable high affinity reagents for SLCs,
- there is even a great uncertainty as if any of the high affinity technologies will work with SLCs,
- there is lack of consensus in our community as to which one to prioritize,
- while members of the consortium have experience with synthetic binders, we lack experience in using them for SLCs,
- we should remain flexible,
- we should test both approaches, and
- we should be entitled to adopt any superior third alternative that may become available.

We therefore will evaluate the merits of the synthetic binders during the first two years, submitting 5 SLCs to each of the two RESOLUTE Academic Expert Laboratories, with the overlap of 2. The evaluation, likely to be feasible at months 14-16 from inception, will dictate whether one or the other approach is workable and which may be superior. It is considered likely that we will keep engaging both laboratories and approaches. Possibly, one class of binders work better with a certain SLC fold and, even more likely, the throughput will be more efficiently secured accessing both laboratories simultaneously. In general, RESOLUTE considers being nimble and flexible a key to success. Too many academic full partners will lead to commitment of the resources to the point of impeding the ability to adapt to technical breakthroughs and innovations.

Task 5.5 Distribution of generated constructs, protocols and high-affinity binders (M24-M60). Partners involved: CeMM, UOX. The WP5 output will provide the RESOLUTE consortium members and the broader scientific community with the best-behaved expression plasmids and protocols for optimal expression and purification of SLCs on the priority list. The sequence of the validated binders will be made available to the consortium members and will be accessible to the scientific community *via* the RESOLUTE web portal.

Participation per partner

Partner number and short name	WP5 effort (%)
1 - CEMM	17.87
2 - UOX	48.25
8 - Pfizer	4.46
13 - Bayer	29.41
Total	100

Deliverable Number	Deliverable Title	Lead beneficiary	Type	Dissemination level	Due Date (in months)
D5.1	Constructs and protocols for priority SLCs	2 - UOX	Report	Public	48
D5.2	Protein production for the SLCs in the priority list	2 - UOX	Report	Public	48
D5.3	Reconstitution of SLCs in proteoliposomes and nanodiscs	2 - UOX	Report	Public	48
D5.4	Production of antigens and high-affinity binders	2 - UOX	Report	Public	48
D5.5	Production of validated SLC antibodies	1 - CEMM	Report	Public	60

Description of deliverables

D5.1 Constructs and protocols for priority SLCs [48]. Set of expression vectors and protein purification protocols for SLCs on the priority list.

D5.2 Protein production for the SLCs in the priority list [48]. Production of milligram quantities of highly pure (>90%) and stable protein samples for >30% of the priority SLCs.

D5.3 Reconstitution of SLCs in proteoliposomes and nanodiscs [48]. Nanodisc- and liposome-reconstituted SLCs for in vitro assays of successfully purified SLCs of the priority list.

D5.4 Production of antigens and high-affinity binders [48]. Antigens for animal immunization for priority SLCs, together with synthetic binders for priority SLCS and a panel of relevant SLCs.

D5.5 Production of validated SLC antibodies [60]. Large set of validated antibodies targeting SLCs, together with tissue localization data.

Schedule of relevant milestones

Milestone number	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS9	Pilot studies	2 - UOX	24	Pilot studies to assess affinity reagent platform suitability with a first set of 5 priority SLCs completed; Report to GA
MS10	First set binders / priority SLCs	2 - UOX	36	Validation of first set of binders completed and production of 15 additional priority SLCs initiated; Report to GA; addition to database

Work package number	6	Start Date or Starting Event	M2
Work package title	Generation of robust cell-based (high-throughput) and/or cell-free assay systems for all proteins on the SLC priority list Lead: Bayer, Co-lead: AXXAM		

Summary

Screening of large compound libraries is a key approach to identify bioactive modulators of the desired targets and pathways. Reproducible, standardized and robust assays, based on read out systems that are compatible with automated screening platforms, are key assets for modern drug discovery approaches. Thus, the overall aim of WP6 is to develop and adapt cellular and/or cell-free assay systems suitable for drug screening on industrial platforms as well as medium throughput biophysical assays for in depth characterization. Activities in WP6 will be instructed by the deorphanisation process and the development of novel assays that will take place in WP2 and WP3, will take advantage of tools generated in WP1 and WP2 (cDNA, cell lines, high-affinity ligands) and proteins purified in WP5. WP6 will also be largely influenced by the overall assay identification process depicted in Fig. 6 (WP3). The target selection will be an objective of WP4. Intense collaboration with EFPIA and academia partners is envisaged in WP6. EFPIA partners will contribute with tools, such as cDNA, constructs, recombinant cell lines, reference and tool compounds, proteins to be used in cell free systems, non-confidential datasets, and will advise on validation process and HTS assay development, based on their potential interest as candidates for drug discovery programs. EPFIA partners will also develop about 20 HTS assays in five years. Academic partners will contribute by providing novel tools and detections systems, such as new biosensors, which can allow to develop assays for challenging SLCs classes. They will also contribute to HTS assay development (~ 24 HTS assays in five years) and purify and reconstitute proteins for cell- free assay systems (WP5).

This tight collaboration will be advantageous for both of the two souls of the consortium: the EFPIA partners will benefit from the academic ones, since they will envisage the possibility to develop functional assays, suitable for screening campaign, for very challenging targets. The academic partners will have the confirmation of the validity of their new approaches, without investing additional time and at an industrial level.

The CRO, coordinating this WP6, will represent the *trait d'union* between the EFPIA partners and the academics, through the industrialization of lab-scale models and approaches.

The validated assays resulting from these activities will indeed be applicable for large screening campaigns, using industry's proprietary compound libraries, and for project translation toward drug discovery. Although not a core aspect of WP6, and currently not covered by the proposed budget, upon gaining access to robust high-throughput assays, in certain circumstances the consortium may elect, after internal consultation, to engage in chemical hit identification and chemical probe generation. The applicant consortium already includes validated high capabilities and expertise in screening technologies (i.e. AXXAM, ELF, etc.).

Description of work and role of partners

Task 6.1 Assay development (M2-M55). Partners involved: AXXAM, ULIV, UL, Pfizer, Novartis, Vifor, Sanofi, Bayer. The goal of this task is to designate and develop to industry standards the assay appropriate for each priority SLC. A total of 72 transporters will be nominated in WP4 as follows: 10 'accelerated targets' SLCs will be selected at the kick- off meeting, followed by approx. 36 SLCs at month 18, approx. 54 SLCs at month 30 to achieve the number of 72 SLCs at month 42. It is envisioned that the 72 nominated targets will undergo initial feasibility studies for assayability (also in WP3), taking into account high throughput and medium throughput methods. The final goal will be to demonstrate the possibility to develop high quality assays, for drug discovery programs, for SLCs belonging to different classes, using different detection technologies. In order to achieve the goal, out of the nominated 72 targets, 60-70% of them (about 50) will be selected based on assayability assessment and membership to different classes. Axxam will develop a range of 4 to 6 assays per year, depending on their complexity for a total of about 24 assays in 5 years. The development of the remaining assay number will be carried out by the EFPIA partners, the University of Leiden and the University of Liverpool. In detail, the following contributions are envisaged: Bayer 4-8 high throughput and 5-10 medium

throughput assays, Novartis 2-4 high throughput assays, Pfizer 4-8 high throughput assays, Sanofi 12-25 medium throughput assays, Vifor 2-4 medium throughput assays, Axxam 16-30 high throughput assays, University of Leiden 2-4 medium throughput assays and University of Liverpool 1-3 medium throughput assays. The generation of assays for these selected targets, based on their sub-class and on the applied experimental approach, will be fundamental to demonstrate a broader feasibility and to pioneer the way for the development of future high throughput and medium throughput assays, lying out of the scope of this project.

Assays successfully meeting parameters set for a functional assay will transfer to assay optimization, and those fulfilling acceptance criteria will finally enter assay adaptation. From WP2 and WP3, it is expected that for the majority of SLCs, we will already have identified a preferred path to a suitable assay. In fact, availability of reagents and viable indication of attractive functional assays is likely to affect, on top of disease association and numerous other parameters, eligibility to the priority list. However, not all transporters from the priority list may turn out to be compatible to high-throughput standards. It will also be evaluated if a non-HTS approach based on biophysical cell-free methods is a feasible approach.

The selection of the most suitable assay technology for each nominated transporter will be based on information and data assembled and integrated in the process and on the availability of the substrates upon the deorphanisation process, as resulting from WP2 and WP3. For the generation of a functional cell-based assay, different read out systems can be envisaged. On top of all mentioned in WP3, partners are also proficient in using 1) fluorescent calcium-sensitive dyes and the genetically encoded calcium sensors (e.g. GCaMP2.1 and GCaMP6) for transporters that induce calcium mobilization during transport; 2) fluorescent sodium-sensitive dyes for transporters that cause sodium influx during transport; 3) genetically encoded halide sensor eYFP for transporters that induce halide mobilization during transport; 4) luminescent assays for transporters that could be coupled to ATP detection; 5) patch-clamp recording for electrogenic transporters; 6) label-free, impedance based assays for cells expressing SLCs together with their respective G-protein coupled receptors; 7) Intellicyt HTP flow cytometry. The consortium will use substrates potentially identified in WP2 to stimulate transporter activity. If available different substrates/activators will be tested on the stable pools, in order to identify the most advantageous in the assay development and HTS tests. The best responding clones, showing pharmacology in line with WP1-3 results or literature, will be fully characterized and then optimized for their use in automated screening, in 384 well plate formats (microtiter plate, MTP). For the generation of a functional biophysical cell-free assay, different technologies can be envisaged. Partners are proficient in using assay technologies like thermal shift by differential scanning fluorometry (DSF) or nano-DSF, micro scale thermophoresis (MST), surface plasmon resonance (SPR) or fluorescence polarization (FP) binding assays. These technologies depend on the availability of purified SLCs generated in WP5, binding agents of sufficient affinity or fluorescent probes.

Task 6.2 Assay optimization (M8-M58). Partners involved: AXXAM, ULIV, ULEI, Pfizer, Novartis, Vifor, Sanofi, Bayer. This task deals with the optimization of the assays identified in Task 6.1. The following parameters will be analysed and optimized under semi-manual conditions, in order to prove the assay suitability for HTS: 1) cell growth conditions; 2) cell density in the 384 MTPs; 3) best seeding time for experiment; 4) reference substrate/inhibitor EC50/IC50 determination and reproducibility, if available; 5) stability of the signal over time, after different cell passages; 6) DMSO sensitivity; 7) robust Z' factor and intra and inter-plate standard deviation for both positive and negative modulators screening conditions, determined in a multi-plate test. For medium throughput biophysical assays, the following parameters will be analysed: 1) reference substrate/inhibitor Kd determination and reproducibility, if available; 2) stability of the signal over time; and 3) robust S/N ratio.

Task 6.3 Assay Adaptation to HTS (M10-M60). Partners involved: AXXAM, Pfizer, Novartis, Bayer. In order to determine the most suitable protocol for conducting an automated HTS, the following experiments will be carried out under fully automated conditions:

- 2-Day variability assessment: two independent experiments on three plates per day to evaluate max and min values and reference substrate pharmacology (EC50).

- Multi-plate test: 10 full-plates on a single day using the HTS automated protocol with controls and DMSO concentrations to be used in screening for determination of the robust Z' (RZ'), inter- and intra-plate variability and pharmacology.
- The following assay criteria have to be fulfilled by the assay in order to be eligible for HTS:
    - The 2-day EC50 for the reference substrate is within 5-fold of the predetermined reference value.
    - The multi-plate test shows a robust Z' ≥ 0.5 with intra and inter-plate CV ≤ 20%.

Participation per partner

Partner number and short name	WP6 effort (%)
3 - ULIV	1.28
4 - AXXAM	37.24
5 - ULEI	15.46
8 - Pfizer	3.22
9 - Novartis	7.15
11 - Vifor	8.89
12 - Sanofi	5.79
13 - Bayer	20.94
Total	100

List of deliverables

Deliverable Number	Deliverable Title	Lead beneficiary	Type	Dissemination level	Due Date (in months)
D6.1	HTS (High Throughput Screening) and MTS (Medium Throughput Screening) assay development-1	4 - AXXAM	Report	Confidential	15
D6.2	HTS and MTS assay development-2	13 - Bayer	Report	Confidential	27
D6.3	HTS and MTS assay development-3	4 - AXXAM	Report	Confidential	40
D6.4	HTS and MTS assay development-4	13 - Bayer	Report	Confidential	60

Description of deliverables

D6.1 HTS (High Throughput Screening) and MTS (Medium Throughput Screening) assay development-1 [15]. First report describing the experimental procedures to generate high throughput and medium throughput assays on the first group of SLCs in the priority list.

D6.2 HTS and MTS assay development-2 [27]. Second report describing the experimental procedures to generate high throughput and medium throughput assays on the second group of SLCs in the priority list.

D6.3 HTS and MTS assay development-3 [40]. Third report describing the experimental procedures to generate high throughput and medium throughput assays on the third group of SLCs in the priority list

D6.4 HTS and MTS assay development-4 [60]. Fourth report describing the experimental procedures to generate high throughput and medium throughput assays on the fourth group of SLCs in the priority list.

Schedule of relevant milestones

Milestone number	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS11	Assays development	13 - Bayer	36	Development of 50% of the expected assays; Report to GA

Work package number	7	Start Date or Starting Event	M6
Work package title	Interactome and regulome for priority SLCs Lead: Novartis, Co-lead: CeMM		

Summary

The early adoption by RESOLUTE of cell lines that express SLCs endogenously allows us to study the regulatory circuits in which these SLCs are embedded. The aim of WP7 is to gain deeper insight into these circuits by a combined transcriptomic, metabolomic, proteomic and bioinformatic analysis. RESOLUTE will achieve this by extending the general approaches described in WP1 (transcriptomics) and WP2 (metabolomics, proteomics) to include analysis with different stimuli and complementing it with proximity biotinylation methods [43] and the use of high-affinity protein binders. Integrative bioinformatic analysis of these datasets will allow us to identify core regulatory modules in which the targeted SLCs are involved, as well as their dynamics upon challenge by external stimuli.

We have harmonized this workflow and we will verify these interactions using several approaches (such as the dual use of AP-MS and BioID-like methods to exclude technical bias; data mining and filtering using existing tools, as well as the engineering of respective knockout and rescue cell models or the design of binary reporters), as is also listed in the specific tasks.

Description of work and role of partners

Task 7.1 The gene regulatory context of priority SLCs (M6-M48). Partners involved: CeMM, Boehringer. To uncover dynamic changes in regulatory mechanisms for SLCs, we will stimulate cells overexpressing priority SLCs with an average of 3 modulators for each individual SLC. This panel may include known substrates or inhibitors, compounds that excite orthogonal signalling pathways (e.g. growth factors, GPCR agonists or immune stimuli), as well as newly identified substrates and inhibitors. The final choice of modulators will be selected from those perturbations that produced optimal cell responses and are specific to the host cell system and the individual SLC studied. To model the relevant regulation circuits for selected SLC of each phase, the first-pass transcriptome and metabolome data obtained in WP1 and WP2 respectively, will be complemented with RNA-Seq and targeted metabolomics data after cell stimulation. These experiments will provide the transcriptome and metabolome in the presence or absence of stimuli. EFPIA partners (Novartis and Boehringer) will provide chemical agents and assist with the RNA-Seq experiments, while CeMM will perform targeted metabolomics experiments.

Task 7.2 The protein interactome of priority SLCs (M6-M48). Partners involved: CeMM, Novartis. For the quantitative proteomic analysis, CeMM will integrate and extend the interactome data obtained in WP2 by employing AP-MS to probe the dynamics of the complexes involving SLCs upon challenge with external stimuli (such as the aforementioned activators or inhibitors binding SLCs). The AP-MS approach will be extended to include large (>100 amino acids) loops or N- and C-terminal portions of the priority SLCs as baits, should full-length baits deliver disappointing results. We have found that the proximity-dependent biotin identification (BioID) approach delivers data that is congenially complementary to the AP-MS approach using normal affinity tags. The BirA tag fused to the SLC of interest is used to covalently bind exogenously added biotin to proteins that are in the vicinity of the bait protein within the cell, providing for a sampling of the native subcellular environment in a radius of ~10 nm [43]. BioID has been successfully employed to detect weaker and more transient interactions compared to the ones identified by AP-MS (Fig. 10). Alternatively, we may employ the engineered peroxidase APEX2 to tag bait SLCs [44]. The APEX2 approach offers faster kinetics than BioID and it may be more suited for experiments in presence of certain stimuli. SLC-specific high affinity protein reagents generated in WP5 will also be used to purify the endogenous targets and its interactors under conditions resembling a more physiologically relevant environment. Validation of SLC interactors will encompass CRISPR/Cas9 knock-outs of respective interactors and well-established methods to detect protein-protein interactions (PPIs), such as split-BioID, Nanobit, co-immunoprecipitation or mammalian-membrane two-hybrid assay (MaMTH). CeMM will generate N- and C- terminally tagged SLCs and cell lines overexpressing bait proteins, while Novartis will provide additional expertise on BioID, split-

BioID, APEX2 and general PPIs assays. Moreover, functional assays build up in WP3 will enable to confirm the physiological role of the identified interactome.

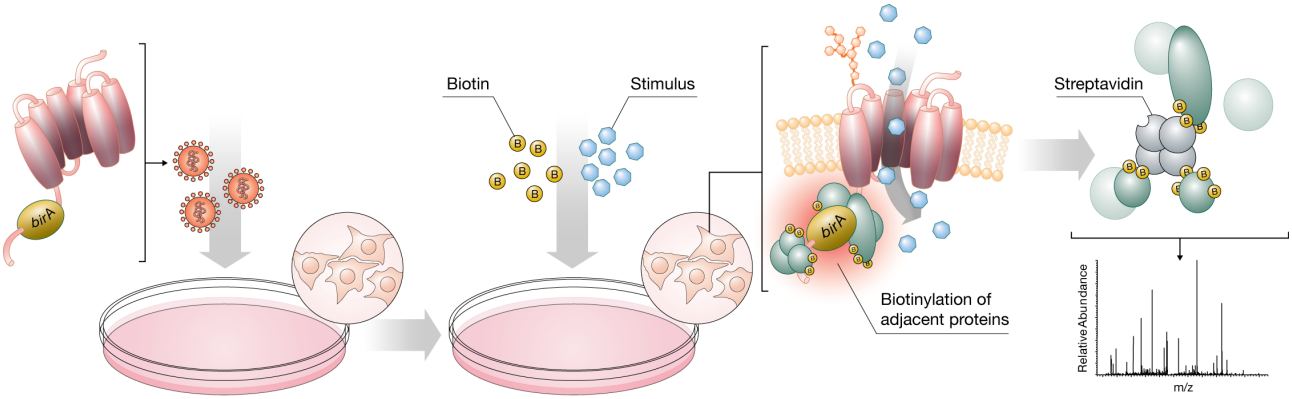

Figure 10: Workflow for the generation of proximity-dependent biotin ligation (BioID) proteomics experiments.

Task 7.3 Regulatory models for the priority SLCs (M9-M52). Partners involved: CeMM, Sanofi. Dynamic changes in the metabolomic and transcriptomic landscape of SLC mutant cells will be integrated with changes in SLC protein complex composition (AP-MS) and wider subcellular context (BioID, APEX2) to derive regulatory models including possible transcription factor networks and protein regulation. Data integration and analyses will combine efforts from CeMM and the EFPIA partner Sanofi. This analysis will provide us with robust protein interaction datasets, from which we will be able to extract true interactors, and construct interaction networks. Linking these networks to the RESOLUTE knowledgebase and application of network-based gene set enrichment analysis will reveal the presence of complexes and regulatory modules.

Participation per partner

Partner number and short name	WP7 effort (%)
1 - CEMM	84.35
9 - Novartis	4.39
10 - Boehringer	4.21
12 - Sanofi	7.03
Total	100

List of deliverables

Deliverable Number	Deliverable Title	Lead beneficiary	Type	Dissemination level	Due Date (in months)
D7.1	Transcriptome and metabolome for priority SLCs	1 - CEMM	Report	Public	48
D7.2	SLC Interactome for priority SLCs	1 - CEMM	Report	Public	48
D7.3	Physiological and physical network map for priority SLCs	1 - CEMM	Report	Public	52

Description of deliverables

D7.1 Transcriptome and metabolome for priority SLCs [48]. Quantitative differential transcriptome, metabolome data within a cell line that naturally expresses each SLC in the priority list in both basal and stimuli challenged states.

D7.2 SLC Interactome for priority SLCs [48]. Condition dependent protein interaction network data in response to on-target and orthogonal pathway stimuli modulating SLC function.

D7.3 Physiological and physical network map for priority SLCs [52]. Condition-dependent regulatory models integrating transcriptomics, metabolomics and proteomics data for priority SLCs.

Schedule of relevant milestones

Milestone number	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS12	Identification of efficient perturbations for Priority SLCs	9 - Novartis	48	Identification of efficient perturbations for Priority SLCs, Report to GA; addition to database
MS13	Identification of potential regulatory mechanisms and circuits	9 - Novartis	52	Identification of potential regulatory mechanisms and circuits by combining deliverables from metabolome, transcriptome and proteome underlying the function of priority SLCs; Report to GA; addition to database; publication in scientific journals

Work package number	8	Start Date or Starting Event	M1
Work package title	Knowledge integration and data management Lead: UNIVIE, Co-lead: Pfizer		

Summary

The aim of WP8 is to create a comprehensive and accurate resource of integrated state-of-the-art knowledge on the various SLC families, built upon available public as well as in-house (consortium) data sources. This knowledge will support deorphanisation strategies (WP2) and will provide input to the generation of the SLC priority list (WP4). Furthermore, a large-scale data repository will be set up to serve for deposition and sharing of raw data files, analyses, reports and electronic lab journals within the consortium. A plan detailing guidelines for data management and sharing will be developed that accounts for legal and operational concerns and the use of data by third parties during and after the project phase. Special attention will be paid to the definition of experiment annotation ontologies and domain specific quality control measures, as well-structured and high-quality data are essential to higher-level analyses. The RESOLUTE data warehouse will be the central entry point for data mining efforts to allow e.g. data-driven guidance to target selection in WP4. Code base, data structure and data formats will build on major public initiatives such as ChEMBL, Open PHACTS, ProteomeXchange, MetaboLights, or EBI's Sequence Read Archive to ensure compatibility and sustainability. With respect to the latter, CeMM commits to keep up the portal for additional 5 years and to find a suitable integration within public databases as e.g. those curated by the European Bioinformatics Institute.

Description of work and role of partners

Task 8.1 Data Management Plan (M1-M60) Partners involved: All. To ensure sound data management throughout the project, a data management plan (DMP) will be developed that implements the FAIR guidelines of the European Research Council's Horizon 2020 program for findable, accessible, interoperable and reusable scientific research data and publications. This document will be updated over the course of the project repeatedly.

Task 8.2 Integrated SLC knowledgebase (M1-M6) Partners involved: CeMM, ULIV, UNIVIE. Assessment and integration of publicly available data on the SLC family and associated drugs and ligands. Where possible, KNIME and Pipeline Pilot workflows will be developed to automatically retrieve and process data in a standardized and reproducible format. Data will be scored by quality and will contain information on provenance to assure traceability.

Task 8.3 Scalable data repository (M1-M12) Partners involved: CeMM. A scalable data warehouse will be set up to collect experimental results generated in the course of WP 1-3 and 5-7. The various data sources (genetic screens, protein interactome data, antibody information, protein structure data, assay readouts, etc.) will require a decision on domain specific, standardized data formats. The consortium will adopt or develop ontologies and controlled vocabularies for data annotation and domain specific quality control (QC) measures.

Task 8.4 Data sharing (M1-M18) Partners involved: All. Project internal guidelines including legal, operational and open innovation concerns (e.g. access to project data during and after the project) will be developed. Datasets fulfilling defined annotation and QC criteria will be shared within the consortium via the repository. An intuitive RESOLUTE web portal (both with internal and public access areas) will represent the interface of the consortium database and work and the outside world. It will also contain an interface to the RESOLUTE reagents portal, which includes recommendations (such as in Chemical Probes.org) to non-profit repositories (such as Addgene) and to high quality commercial operations in order to distribute all key RESOLUTE reagents (cell lines, vectors, binders, chemical probes). In addition, an SLC human genetics portal is pooling and assembling information on SLC variants, with a database and with links to rare disease organisations (through the Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases in Vienna) and human genetics.

Task 8.5 Data mining (M6-M60): Partners involved: CeMM, UNIVIE, ULIV. Chemo- and bioinformatic oriented data mining will be employed to allow SLC centric data integration and extraction. Where possible, automated quality assurance processes will be included, preferentially by developing respective workflows in KNIME and Pipeline Pilot. The results will provide essential support for the priority list generation (WP4) and throughout the project.

Participation per partner

Partner number and short name	WP8 effort (%)
1 - CEMM	54.99
2 - UOX	1.37
3 - ULIV	7.69
4 - AXXAM	1. 37
5 - ULEI	1. 37
6 - MPIMR	1. 37
7 - UNIVIE	20.34
8 - Pfizer	4.58
9 - Novartis	1. 37
10 - Boehringer	1. 37
11 - Vifor	1. 37
12 - Sanofi	1. 37
13 - Bayer	1. 37
Total	100

List of deliverables

Deliverable Number	Deliverable Title	Lead beneficiary	Type	Dissemination level	Due Date (in months)
D8.1	Data Management	7 - UNIVIE	Report	Public	6
D8.2	Data Management	7 - UNIVIE	Report	Public	30
D8.3	Data Management	7 - UNIVIE	Report	Public	60
D8.4	RESOLUTE knowledgebase	1 - CEMM	Report	Public	6
D8.5	RESOLUTE database	1 - CEMM	Websites, patents filling, etc.	Public	12
D8.6	RESOLUTE web portal	1 - CEMM	Websites, patents filling, etc.	Public	18
D8.7	Data Mining	7 - UNIVIE	Report	Public	12

Description of deliverables

D8.1 Data Management Plan 1 [6]. Initial version of DMP after 6 months, an updated version latest mid-term and a final version.

D8.2 Data Management Plan 2 [30]. Initial version of DMP after 6 months, an updated version latest mid-term and a final version

D8.3 Data Management Plan 3 [60]. Initial version of DMP after 6 months, an updated version latest mid-term and a final version

D8.4 RESOLUTE knowledgebase [6]. Concept for database of integrated publicly available knowledge and RESOLUTE web portal.

D8.5 RESOLUTE database [12]. Infrastructure and data warehouse for sharing annotated raw data files and analysis results; prototype ready after first year and annual releases of new versions.

D8.6 RESOLUTE web portal [18]. Data sharing and approval process, and prototype of interface for data access at public web portal up and running.

D8.7 Data Mining [12]. Data mining support for priority ranking of SLC family members for target selection in WP4.

Schedule of relevant milestones

Milestone number	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS14	RESOLUTE knowledgebase	7 - UNIVIE	6	Concept for RESOLUTE knowledgebase to integrate a significant part of publicly available knowledge on SLCs agreed; KNIME workflows to allow for informed prioritization of SLCs in place; Concept for knowledgebase agreed by all consortium members; first priority list of SLCs supported by KNIME workflows which mine public data
MS15	RESOLUTE web portal	7 - UNIVIE	18	RESOLUTE web portal is online and provides open access to released research data in the RESOLUTE database; Web portal accessible and used by the community

Work package number	9	Start Date or Starting Event	M1
Work package title	Project management and dissemination of results Lead: CeMM, Co-lead: Pfizer		

Summary

WP9 will provide professional project management in order to meet the requirements of the project with special attention to cross-linking of all work packages and managing trade-offs between time, quality and cost. A governance structure has to be installed, internal communication flow has to be defined and monitored, reporting to the IMI2 JU has to be organised, financial and legal issues have to be managed, quality control and risk management procedures have to be defined and gender and ethical issues have to be addressed and monitored.

A further objective of WP9 is to stimulate and facilitate potential adoption of project results within and outside the consortium: an integrated strategy to communicate, disseminate and exploit project outputs will be defined and implemented, regular updated information about the project and its results will be provided and the results and benefits of the project will be promoted to all stakeholders.

Description of work and role of partners

Task 9.1 Scientific coordination, quality and risk management (M1-M60). Partners involved: CeMM, Pfizer. The project coordinators (project leader EFPIA and academic coordinator) supported by the project management team will ensure strong scientific coordination and collaboration between the partners in order to support a target- oriented fulfilment of the work plan. Strategic orientation of scientific activities will be monitored and steered. By undertaking rigorous internal quality control in scientific work, the reproducibility of the research output shall be increased. Mechanisms of continuous quality control will be installed and risks as well as opportunities of the project will be identified, analysed and managed in collaboration with the work package leaders.

Task 9.2 Reporting, legal and financial management (M1-M60). Partners involved: CeMM, Pfizer. Formal coordination and monitoring will be performed by continuous evaluation of objectives and deliverables in order to ensure the accurate fulfilment of all contractual obligations (including gender and ethical issues) towards the IMI2 JU. Constantly, work plans and schedules will be monitored and corrective actions will be recommended if needed. The academic coordinator together with the project management team will execute all administrative activities including collection and distribution of internal reports, results and deliverables, preparation of reports together with work package leaders, and preparation and submission of annual and final reports to the IMI2 JU. Allocation and distribution of the IMI2 JU financial contribution among beneficiaries will be made in accordance with the grant agreement and the consortium agreement.

Task 9.3 Internal communication (M1-M60). Partners involved: CeMM, Pfizer. A continuous communication flow will be ensured and supported by extensive electronic resources for project communication, meeting organisation, etc. to reduce time and cost efforts for project management and traveling to a minimum and to optimize scientific progress. The project management team will organise meetings and teleconferences of the consortium on a regular basis during the duration of the project, in which progress will be reviewed, problems will be discussed, risks will be analysed and modifications of the overall project plan will be decided.

Task 9.4 Dissemination and Exploitation Plan (M1-M6). Partners involved: All. To ensure that project results will be optimally disseminated and exploited, the Dissemination and Exploitation Plan (DEP) outlined in section 2 (Impact) will be revised and adapted in an ongoing process throughout the project duration by the Project Management Team in collaboration with the Executive Board. All project partners will have a role in the execution of the DEP. The DEP describes in detail how RESOLUTE project results will be specifically communicated to key audiences, taking their role and needs into account. Furthermore, the DEP defines in detail how the RESOLUTE consortium will make optimal use of both newly developed and already available dissemination tools (see Task 9.5). It defines specific events, organized by the consortium to distribute project results and to increase awareness of the key audiences including (future) stakeholders. The DEP will be fine-

tuned to the specific needs of each type of key audience. Yearly updates will take into account developments and new results of the RESOLUTE project.

Task 9.5 Implementation of DEP/tools for dissemination of project results (M1-M60). Partners involved: All. An intuitive RESOLUTE web portal (both with internal and public access areas) will represent the interface of the consortium database and work to the outside world (see WP8). Beyond the RESOLUTE website, a range of dissemination tools will be made available and used by the RESOLUTE consortium, also in consultation with the IMI2 PR office, including: brochures, electronic newsletters, press releases (e.g. prepared by partner press offices), peer-reviewed scientific (primary and review) journals, social media platforms (e.g. LinkedIn group on SLCs), (inter)national scientific collaborative projects and professional networks (see below), national funding agencies, oral communications and poster presentations at workshops, conferences and seminars, networks of the members of the Scientific Advisory Board, training and education, e.g. via (available) summer schools, workshops organised by the EFPIA partners in conjunction with the yearly consortium meetings, etc. All relevant stakeholders will be invited to the final project meeting.

Nearly 190 SLC family members are mutated in human disease [45, 46], and for each SLC there are engaged disease and patient foundations. We will partner with ULTRA-DD and its patient program to identify those groups that are interested in funding or being involved in the research on the SLC of their concern. We expect that we will be able to form 3-5 alliances with disease and patient groups during the 5-year term.

Participation per partner

Partner number and short name	WP9 effort (%)
1 - CEMM	58.82
2 - UOX	1.96
3 - ULIV	1.96
4 - AXXAM	1.96
5 - ULEI	1.96
6 - MPIMR	1.96
7 - UNIVIE	1.96
8 - Pfizer	15.68
9 - Novartis	1.96
10 - Boehringer	1.96
11 - Vifor	1.96
12 - Sanofi	4.41
13 - Bayer	3.43
Total	100

List of deliverables

Deliverable Number	Deliverable Title	Lead beneficiary	Type	Dissemination level	Due Date (in months)
D9.3	Annual report #1	1 - CEMM	Report	Confidential	13
D9.4	Annual report #2	1 - CEMM	Report	Confidential	25
D9.5	Annual report #3	1 - CEMM	Report	Confidential	37
D9.6	Annual report #4	1 - CEMM	Report	Confidential	49
D9.7	DEP implemented (stepwise, up to M60)	8 - Pfizer	Report	Public	60
D9.8	Final report	1 - CEMM	Report	Confidential	60

Description of deliverables

D9.1 Project handbook [6]. Manual describing the project organisation and internal procedures of the project with regard to day-to-day communication and progress towards the timely delivery of the deliverables and within budget. It shall be used by all partners for all deliverables to the IMI2 JU and for deliverables between partners.

D9.2 DEP first revision accepted by the EB [6]. Document which summarises the beneficiaries' strategy and concrete actions related to the dissemination and exploitation of the project results.

D9.3 Annual report #1 [13]. Official report sent to IMI every year of the project, following the corresponding IMI2 rules and guidelines.

D9.4 Annual report #2 [25]. Official report sent to IMI every year of the project, following the corresponding IMI2 rules and guidelines.

D9.5 Annual report #3 [37]. Official report sent to IMI every year of the project, following the corresponding IMI2 rules and guidelines.

D9.6 Annual report #4 [49]. Official report sent to IMI every year of the project, following the corresponding IMI2 rules and guidelines.

D9.7 DEP implemented (stepwise, up to M60) [60]. DEP implemented (stepwise, up to M60)

D9.8 Final report [60]. Official report sent to IMI at the end of the project, following the corresponding IMI2 rules and guidelines.

Schedule of relevant milestones

Milestone number	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS16	Reports	1 - CEMM	60	All financial and technical reports submitted; Report to IMI2 JU

Work package number	10	Start Date or Starting Event	M1
Work package title	Ethics requirements Lead: CeMM		

Summary

The objective is to ensure compliance with the 'ethics requirements' set out in this work package.

Description of work and role of partners

WP10 - Ethics requirements [Months: 1-60] Partners involved: CEMM. This work package sets out the 'ethics requirements' that the project must comply with.

List of deliverables

Deliverable Number	Deliverable Title	Lead beneficiary	Type	Dissemination level	Due Date (in months)
D10.1	HCT - Requirement No. 1	1 - CEMM	Ethics	Confidential	2
D10.2	HCT - NEC -Requirement No. 2	1 - CEMM	Ethics	Confidential	6

Description of deliverables

The 'ethics requirements' that the project must comply with are included as deliverables in this work package.

D10.1 HCT - Requirement No. 1 [2]. Copies of relevant documents for using, producing or collecting human cells or tissues (e.g. import licence, accreditation/designation/authorisation/licensing) must be kept on file (to be specified in the grant agreement).

D10.2 HCT - NEC - Requirement No. 2 [6]. Copies of import/export authorisations, as required by national/EU legislation must be kept on file (to be specified in the grant agreement).

Table 3.1b: List of work packages

WP Number	WP Title	Lead beneficiary	Start month	End month
WP1	Generation of reagents and cell lines to allow system-wide study of the SLC family	1 – CEMM	1	60
WP2	SLC deorphanisation process	8 – Pfizer	1	60
WP3	Development of quantitative transporter assays	12 – Sanofi	6	56
WP4	Selection of the 'SLC priority list' of targets	5 – ULEI	1	60
WP5	Generation of protein reagents for the SLC priority list	2 – UOX	6	60
WP6	Generation of robust cell-based (high-throughput) and/or cell-free assay systems for all proteins on the SLC priority list	13 – Bayer	2	60
WP7	Interactome and regulome for priority SLCs	9 – Novartis	6	52
WP8	Knowledge integration and data management	7 – UNIVIE	1	60
WP9	Project management and dissemination of results	1 – CEMM	1	60
WP10	Ethics requirements	1 – CEMM	1	60

Table 3.1c: List of deliverables

Deliverable Number	Deliverable Title	WP number	Lead beneficiary	Due Date (in months)
D1.1	Vector generation	WP1	1 - CEMM	12
D1.2	SLC k.o. cell lines	WP1	1 - CEMM	36
D1.3	SLC k.o. in HEK293	WP1	9 - Novartis	36
D1.4	SLC overexpressing cell lines	WP1	1 - CEMM	36
D1.5	SLC overexpression in HEK293	WP1	9 - Novartis	36
D1.6	Protein expression screen	WP1	2 - UOX	60
D2.1	Signature metabolic profiles using targeted metabolomics	WP2	1 - CEMM	36
D2.2	Methodology for general detection of plasma metabolites and drug/dietary components	WP2	3 - ULIV	18
D2.3	Profile of plasma metabolite changes for each SLC cell line	WP2	3 - ULIV	60
D2.4	Informatic analysis of metabolomic data	WP2	8 - Pfizer	60
D2.5	SLC genetic interaction map	WP2	1 - CEMM	60
D2.6	SLC interactome	WP2	1 - CEMM	60
D2.7	Subcellular localization of target SLCs	WP2	1 - CEMM	56
D2.8	Determination of SLCs using ions as (co-) substrates	WP2	11 - Vifor	60
D3.1	Assays for (electrogenic) transporters	WP3	4 - AXXAM	30
D3.2	Assays for SLCs based on fluorescent sensor proteins	WP3	6 - MPIMR	30
D3.3	Assays for SLCs based on mass spectrometry, thermal shift, radiolabeled or fluorescent compounds	WP3	1 - CEMM	36
D3.4	Assays for SLCs based on fluorescent ligands, fluorescence-labelling of SLCs or fluorogenic probes	WP3	6 - MPIMR	56
D3.5	Assays for SLCs based on SSM- electrophysiology	WP3	12 - Sanofi	48
D3.6	Assays for SLCs based on cell lines that couple growth to SLC function	WP3	1 - CEMM	42
D4.1	Protocol for the procedure to establish priority list	WP4	5 - ULEI	12
D4.2	Tractability analysis for SLCs on the priority list 1	WP4	5 - ULEI	18
D4.3	Tractability analysis for SLCs on the priority list 2	WP4	5 - ULEI	30
D4.4	Tractability analysis for SLCs on the priority list 3	WP4	5 - ULEI	42
D4.5	Consortium-approved SLC priority list 1	WP4	12 - Sanofi	18
D4.6	Consortium-approved SLC priority list 2	WP4	12 - Sanofi	30
D4.7	Consortium-approved SLC priority list 3	WP4	12 - Sanofi	42
D4.8	Updated versions of the SLC priority list 1	WP4	12 - Sanofi	48
D4.9	Updated versions of the SLC priority list 2	WP4	12 - Sanofi	54
D4.10	Updated versions of the SLC priority list 3	WP4	12 - Sanofi	60
D5.1	Constructs and protocols for priority SLCs	WP5	2 - UOX	48
D5.2	Protein production for the SLCs in the priority list	WP5	2 - UOX	48
D5.3	Reconstitution of SLCs in proteoliposomes and nanodiscs	WP5	2 - UOX	48
D5.4	Production of antigens and high-affinity binders	WP5	2 - UOX	48
D5.5	Production of validated SLC antibodies	WP5	1 - CEMM	60
D6.1	HTS (High Throughput Screening) and MTS (Medium Throughput Screening) assay development-1	WP6	4 - AXXAM	15
D6.2	HTS and MTS assay development-2	WP6	13 - Bayer	27

Deliverable Number	Deliverable Title	WP number	Lead beneficiary	Due Date (in months)
D6.3	HTS and MTS assay development-3	WP6	4 - AXXAM	40
D6.4	HTS and MTS assay development-4	WP6	13 - Bayer	60
D7.1	Transcriptome and metabolome for priority SLCs	WP7	1 - CEMM	48
D7.2	SLC Interactome for priority SLCs	WP7	1 - CEMM	48
D7.3	Physiological and physical network map for priority SLCs	WP7	1 - CEMM	52
D8.1	Data Management Plan 1	WP8	7 - UNIVIE	6
D8.2	Data Management Plan 2	WP8	7 - UNIVIE	30
D8.3	Data Management Plan 3	WP8	7 - UNIVIE	60
D8.4	RESOLUTE knowledgebase	WP8	1 - CEMM	6
D8.5	RESOLUTE database	WP8	1 - CEMM	12
D8.6	RESOLUTE web portal	WP8	1 - CEMM	18
D8.7	Data Mining	WP8	7 - UNIVIE	12
D9.1	Project handbook	WP9	1 - CEMM	6
D9.2	DEP first revision accepted by the EB	WP9	8 - Pfizer	6
D9.3	Annual report #1	WP9	1 - CEMM	13
D9.4	Annual report #2	WP9	1 - CEMM	25
D9.5	Annual report #3	WP9	1 - CEMM	37
D9.6	Annual report #4	WP9	1 - CEMM	49
D9.7	DEP implemented (stepwise, up to M60)	WP9	8 - Pfizer	60
D9.8	Final report	WP9	1 - CEMM	60
D10.1	HCT - Requirement No. 1	WP10	1 - CEMM	2
D10.2	HCT - NEC -Requirement No. 2	WP10	1 - CEMM	6

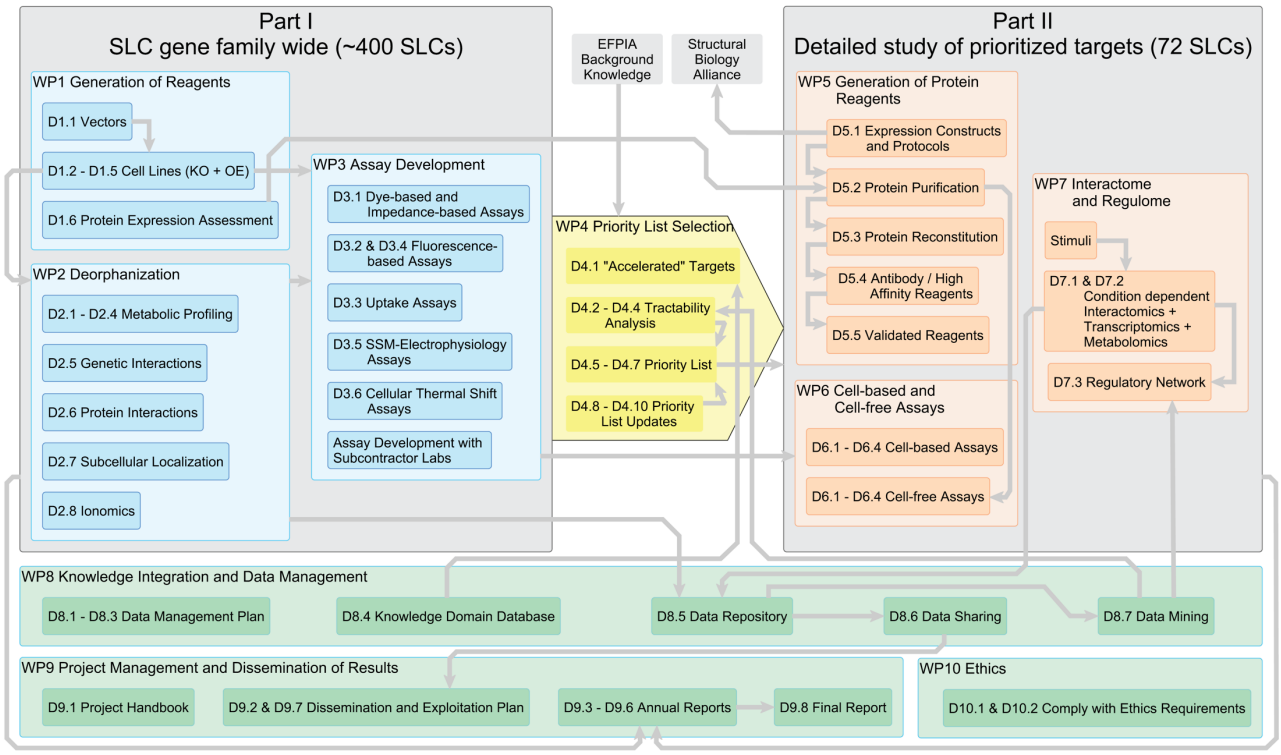

Figure 11: PERT chart of deliverables and their interrelations. *RESOLUTE takes a bold but pragmatic approach in tackling all tractable SLCs from the beginning for protein expression and purification, basic interactome and genetic studies and metabolic deorphanisation. It is expected that data obtained on one SLC may shed light on close members of the family or SLCs that share properties (such as functionally interacting by genetics). Deliverables have been simplified.*

3.2 Management structure and procedures

The RESOLUTE consortium includes universities, research institutes, SMEs and EFPIA members. Efficient management structures and procedures are essential to ensure the smooth realization of the project deliverables. This includes facilitating collaboration among partners and optimising the organization and timing of activities and resources, so that both scientific and strategic goals can be fully attained. The project management structure has been introduced before and the work to be performed has been detailed in WP9. Here, the background on the management bodies is provided (Fig. 12).

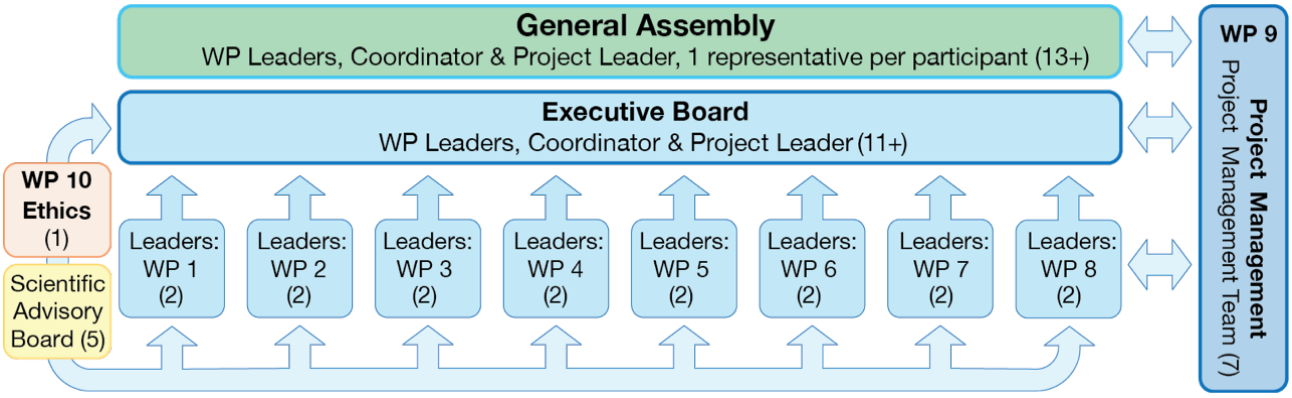

Figure 12: Governance structure of the RESOLUTE consortium.

The composition of the boards to be implemented will ensure that the interests of all partners will be represented equally and will allow sustainable decision making. This structure will be further supported by extensive electronic resources (namely communication tools such as Skype or WebEx, SharePoint solutions, etc.) for project communication, result dissemination, etc. The precise governance structure of the consortium, including operational rules and decision-making procedures, as well as rights and responsibilities, will be laid down in detail in the final consortium agreement. We foresee the following tasks for the various bodies:

Overall scientific progress will be the responsibility of the **project leader** (EFPIA member) assisted by the academic coordinator. To manage this, the project leader will give WP leaders key performance indicators and timelines, progress toward which will be communicated every two months. If progress slips, the project leader may implement changes her-/himself, or may call for a meeting of the EB.

Project management team (PMT): The day-to-day management will be addressed by the project management team (PMT). It will consist of two project coordinators (EFPIA project leader and academic coordinator), a scientific secretary and a director of education. The PMT is supported by dedicated project manager(s) with access to legal and secretarial support. Additionally, two legal officers (one representing the EFPIA partners and one representing the applicant consortium) will support the PMT, seeking consultation with IMI2 JU when needed.

Work package leaders (WPLs): Each WP will be led by one WP leader from EFPIA and one from the applicant consortium to ensure efficient cross-talk. The WPL will be responsible for day-to-day coordination of their respective WP. Tasks include assuring the quality of the work, managing of deliverables, screening of project results on IP potential prior to publication and liaising with the EB on exploitation/dissemination and ensuring smooth communication inside and outside WPs. All WPLs have a seat in the EB to ensure direct communication with the PMT.

Executive Board (EB): The executive board (EB) will consist of the project coordinators (PCs: project leader EFPIA and academic coordinator) and the WP leaders and will be responsible for the scientific progress and quality of the project. The participation of the PCs in both management bodies will ensure short communication lines. Tasks include: 1) Coordination of the integration of the WPs; 2) Monitoring and evaluation of overall progress and timely delivery of the deliverables; 3) Taking corrective action in order to ensure project progress and quality (major changes need approval by the GA and, where appropriate, by the IMI2 JU project officer); 4) Reporting (2x/year) to, and preparing decisions for the GA; 5) Liaising with the

Scientific and Ethics Advisory Board; 6) Reviewing and proposing budget reallocations to the parties (to be approved by the GA); 7) Ensuring adherence to ethical regulations, 8) validating the priority list of SLC and prepare further review with the GA.

In order for an EB meeting to be quorate, 75% of its members as well as the representatives of the project leader and the coordinator need to attend. Decisions will be taken by a simple majority (>50%). Project meetings with the EB will be organized four times a year, through two face-to-face and two teleconference meetings. One face-to-face meeting coincides with the yearly workshops organized by and for the RESOLUTE consortium.

General Assembly (GA): The general assembly (GA) is the main strategic decision-making body of the consortium. The GA will be chaired by the PCs, and will comprise the WPL and a representative of each of the partners who do not provide a WPL. The GA will work on consensus decisions as much as possible, and will resort to voting only if this is unavoidable.

In order for a GA meeting to be quorate there shall be present no fewer than 75% of the GA representatives. Each beneficiary will, through its representative, have one vote in the GA. Decisions will be taken by a simple majority (>50%), except where a decision necessitates a major change to the allocated work or a change to the allocation of any funding. In either of those cases, any decision must be taken by a majority of 75%. The parties agree to abide by all decisions of the GA. This does not prevent the parties from submitting a dispute for resolution. The GA will meet once per year. *Ad hoc* meetings, e.g. in the form of teleconferences, can be arranged by the PMT or the EB, if issues occur that require GA approval.

The General Assembly will for example undertake, and decide on, the following matters, provided such matters and their implementation are in compliance with the terms of the Grant Agreement:

- supporting the Project Leader and Coordinator in fulfilling their obligations towards the IMI 2 JU,
- reviewing the project progress,
- deciding on strategic direction, changes to the scope and project direction, proposal to expand or extend the project, etc.,
- deciding on principles for effective communication,
- agreeing on procedures and policies in accordance with the Grant Agreement for dissemination of results,
- agreeing on adequate management procedures, quality standards and quality for project completion, and
- agreeing on entries of new beneficiaries and departures of existing beneficiaries.

The competences of the GA will be listed in more detail in the Consortium Agreement.

Scientific Advisory Board (SAB): An external scientific advisory board consisting of 3-5 renowned experts in the field will be nominated by and report to the Executive Board. The members of the SAB will critically monitor the progress and overall performance of the project. SAB members will give independent advice to the project, participate in risk assessment, and foster active communication and exchange of best practice. Moreover, they will advise on external developments relevant to achieving progress towards the impacts of the call and act as ambassadors of the consortium. The SAB will be invited to all project meetings, but may participate in WP meetings too. The important role of self-assessment of the consortium will be pursued by yearly meetings of the SAB with the EB and GA during the project meetings of the consortium. The following world experts have accepted in writing to become members of the provisional SAB:

- Kathy Giacomini (UCSF): Genetics, genomics,
- Rajini Rao (John Hopkins University School of Medicine): Physiology, medicine,
- Avner Schlessinger (Icahn School of Medicine at Mount Sinai): Biocomputing, modelling and chemoinformatics
- Nieng Yan (Princeton University): Structure, biochemistry and
- Christiane Druml (University of Vienna): Ethics.

Ethics Advisor (EA): RESOLUTE will appoint Professor Dr. Christiane Druml as Ethics advisor. She will 1) review all planned and new activities of RESOLUTE and 2) be available for advice throughout and to all partners and individuals (including students and technical staff). Moreover, the Ethics Advisor will:

- Be a member of the RESOLUTE SAB,
- have regular meetings with the Executive Board and review progress and new aspects to enable appropriate action, if required,
- have access to all proceedings and be able to attend all RESOLUTE meetings, and
- decide if/when, a full-fledged Ethics Board may become necessary.

Table 3.2a: List of milestones

Milestone number	Milestone title	WP number	Lead beneficiary	Due Date (in months)	Means of verification
MS1	Collections of SLC k.o. and SLC overexpressing cell lines covering >80% of the family members	WP1	1 - CEMM	36	Availability of quality-controlled cell lines to members of consortium; report to GA
MS2	Assessment of SLC protein expression levels	WP1	1 - CEMM	60	Family-wide assessment of SLC protein expression levels; Report to GA
MS3	Deorphanisation strategy	WP2	8 - Pfizer	24	Development and validation of an integrated multi-approach deorphanisation strategy broadly applicable to the SLC family; Report to GA
MS4	Identification of potential substrates for orphan SLCs	WP2	8 - Pfizer	60	Identification of potential substrates for orphan SLCs; Report to GA; addition to database; publication in scientific journals
MS5	Workflow implementation	WP3	12 - Sanofi	24	Implementation of a workflow for the identification of assays suitable for SLCs; Report to GA
MS6	Assays developed for >50% of human SLCs	WP3	12 - Sanofi	60	Assays developed for >50% of human SLCs; Report to GA
MS7	Priority list protocol	WP4	5 - ULEI	12	Protocol for the procedure to establish priority list; Approval by EB
MS8	Priority list	WP4	5 - ULEI	42	Full list of priority list SLCs available; Approval by EB
MS9	Pilot studies	WP5	2 - UOX	24	Pilot studies to assess affinity reagent platform suitability with a first set of 5 priority SLCs completed; Report to GA
MS10	First set binders / priority SLCs	WP5	2 - UOX	36	Validation of first set of binders completed and production of 15 additional priority SLCs initiated; Report to GA; addition to database
MS11	Assays development	WP6	13 - Bayer	36	Development of 50% of the expected assays; Report to GA
MS12	Identification of efficient perturbations for Priority SLCs	WP7	9 - Novartis	48	Identification of efficient perturbations for Priority SLCs, Report to GA; addition to database

Milestone number	Milestone title	WP number	Lead beneficiary	Due Date (in months)	Means of verification
MS13	Identification of potential regulatory mechanisms and circuits	WP7	9 - Novartis	52	Identification of potential regulatory mechanisms and circuits by combining deliverables from metabolome, transcriptome and proteome underlying the function of priority SLCs; Report to GA; addition to database; publication in scientific journals
MS14	RESOLUTE knowledgebase	WP8	7 - UNIVIE	6	Concept for RESOLUTE knowledgebase to integrate a significant part of publicly available knowledge on SLCs agreed; KNIME workflows to allow for informed prioritization of SLCs in place; Concept for knowledgebase agreed by all consortium members; first priority list of SLCs supported by KNIME workflows which mine public data
MS15	RESOLUTE web portal	WP8	7 - UNIVIE	18	RESOLUTE web portal is online and provides open access to released research data in the RESOLUTE database; Web portal accessible and used by the community
MS16	Reports	WP9	1 - CEMM	60	All financial and technical reports submitted; Report to IMI2 JU

Risk management

The RESOLUTE consortium will conduct risk management, including continuous risk identification, evaluation and monitoring activities, on an ongoing basis, and will provide the necessary tools for timely detection and control of any significant risks.

Risks will be identified by the project management in close collaboration with all WP leaders and assessed by the EB considering both impact and probability, each of them measured in a scale ranging from low to high in order that the consortium can focus on the most relevant ones. Essential characteristics of each risk will be defined and mitigation plans as well as contingency plans will be elaborated for priority risks. A brief description of the currently perceived top risks is offered hereunder.

Table 3.2b: Critical implementation risks and mitigation actions

Risk number	Description of risk	WP Number	Proposed risk-mitigation measures
1	Cell toxicity due to functional overexpression and the IRES co-expression strategy of a live cell marker, which could not work well in all cells.	WP1, WP2, WP7	We will employ inducible expression of the gene of interest using HekR4 Tet-On system, or alternatively applying BacMam approach for transient transfection described in Task 1.6.
2	Methods are based on C18 columns, and the most polar drugs will not be retained on such column.	WP2	If the drugs that turn out to be of most interest in certain cases fall into this category, we shall use/ add the appropriately polar HILIC columns, with ion-pairing reagents as necessary. In addition, simple direct injection MS will be able to target specific drugs without chromatographic separation.
3	The relatively high degree of redundancy expected among SLCs might reduce the number of synthetic lethality interactions identified.	WP2	We expect that the large scale of this effort, likely to result in overlapping hits, together with the possibility to use higher complexity or genome- wide libraries, will contribute to overcome some of the effects of functional redundancy among SLCs.
4	Difficulty in proving the electrogenic properties of the selected SLCs.	WP3	Further cell lines and/or different substrate sets might be tested.
5	Availability of ligands (in particular for orphan SLCs) or design/preparation of labeled radio or fluorescent ligands might be a limiting factor.	WP3	Probe generation will be managed case by case by subcontracting the synthesis.
6	The consortium's desire to strive for open access in the selection process may be challenged and limited.	WP4	Mitigation is in calling upon an 'honest broker' as a back-up plan.
7	Decision making by the Executive Board may be questioned by partners.	WP4	This is mitigated through the final assessment in the GA of the consortium.

Risk number	Description of risk	WP Number	Proposed risk-mitigation measures
8	Some of the priority SLCs might be unstable (i.e. partial unfolding over time) despite sufficiently high expression levels, making them less effective antigens for affinity reagent generation.	WP5	Detergent-free extraction, reconstitution into membrane-mimetics (e.g. nanodiscs) and protein engineering (introduction of thermostabilising mutations) will be used to increase the stability of the purified protein.
9	For some of the priority list SLCs it might not be possible to purify sufficient quantities for liposome reconstitution and affinity reagent generation.	WP5	Affinity reagents will be generated against a domain or peptide fragments of the respective SLC. Moreover, new priority SLCs can be nominated by the EFPIA to replace the previous ones.
10	The development of some challenging assays might require more effort than expected and therefore the final number of developed assays may be lower.	WP6	The development of challenging assays for novel promising targets can justify a final lower number of assays, since the added value will be very high in any case.
11	Some of the assays for the selected targets might not me adaptable to HTS.	WP6	Such assays could be optimized in semi-manual mode, to allow low throughput screening. Nonetheless, they may still be valuable for the identification of novel molecules acting on the tested SLC. Alternatively, new targets may be nominated to replace the previous ones.
12	Lack of specificity by selected stimuli.	WP7	Extend the number of previously identified substrates, tool compounds and chemical probes for individual SLCs. SLCs with analogue responses (e.g. family members) will be knocked out by CRISPR/Cas9.
13	Although global interaction maps can be validated down to binary interaction confirmations, the number of false positives could be challenging.	WP7	Public and EFPIA protein-protein interactions and methodology databases will help to stratify these hits. In addition, the identified protein partners can be assayed to determine their functional and structural role, which will include knock down thereof.
14	Licensing of desired public resources could prohibit integration into SLC knowledgebase.	WP8	Check respective licenses and identify potential alternatives if necessary; however, experience gained in Open PHACTS confirm that major public data depositories such as ChEMBL allow integration and reuse.

Risk number	Description of risk	WP Number	Proposed risk-mitigation measures
15	Partners might not be able to interact with the data repository due to internal guidelines and requirements or technical limitations.	WP8	We nominate one individual per partner as interface to Data Management to identify and resolve this kind of issues early on.
16	Legal concerns raised by project partners could constrain data release.	WP8	Legal aspects to be solved upfront on the consortium agreement
17	Controlling for pleiotropic effects during focused targeted metabolomics.	WP2	Varying the substrate as independent variable.
18	Software analysis of lyophilised intra- and extracellular extracts.	WP2	Use of commercial Progenesis Qi software that also works with high-resolution mass spectra.
19	Proteomics studies: Low expression of tagged constructs in chosen cell lines.	WP2	Additional engineering/modification of the constructs or the use of alternative cell lines.
20	Subcellular localization studies: Low expression of tagged constructs.	WP2	Additional engineering/modification of the constructs. Whenever available, use SLC-specific antibodies.

Sustainability actions beyond the end of the grant agreement

As the intent of RESOLUTE is to deliver new open access research tools, techniques, reagents and knowledge to the biomedical research community, RESOLUTE will devise an appropriate plan to enable this critical element. Initially, the RESOLUTE web portal, containing techniques, protocols, research tools and SLC databases, will be maintained for an additional 5 years. The RESOLUTE sustainability plan will be focused on identifying a suitable, long term integration of knowledge within public databases as e.g. those curated by the European Bioinformatics Institute and long-term repository for accessibility to reagents. The RESOLUTE sustainability plan will be aligned with the overarching mission of RESOLUTE to trigger an escalation in the appreciation and intensity of SLC research. This integrated SLC family-wide knowledge base and state-of-the-art tools and technologies will be highly enabling for both target identification and drug discovery efforts. The sustainability plan should ensure that advances in SLC research continue on the trajectory initiated by the RESOLUTE consortium and result in long lasting impact on SLC research. The impact of RESOLUTE during its funding period and beyond will be to 'unlock' the SLC family to enable drug discovery efforts to be conducted 'at will' across the whole family of SLC transporters.

3.3 Consortium as a whole

The academic applicants' part of the RESOLUTE project combines 6 academic partners and one SME (see '4.1 Participants'), all of them have shown:

- participation in **networks of recognized experts** in sectors relevant for the outlined project;
- **experience in successfully collaborating in networks with industry** (IMI, IMI2, Lead Factory, several individual collaborations with industry, foundation of companies) thus combining industrial know-how with academic translation and research excellence;
- a unique expertise in successful **high-impact large-scale studies** and an equally unique expertise in **systematic analysis** of SLCs as target classes**;**
- experience in a series of **studies of physiologically and therapeutically relevant proteins** (including SLCs) and in achieving high-impact outcomes catalysing research in **pioneer target areas of drug discovery**, thus forming the scientific basis for unlocking the therapeutic potential within the SLC gene family.

A criterion of the consortium is **high complementarity** with few but highly specialized and experienced partners:

- The experience of all partners in participating in / managing **large-scale EU-funded projects** (e.g. IMI, IMI2, Lead Factory, ERC grants) including finance and IP will ensure the achievement of results on time and within budget.
- The world-leading expertise of the UOX/SGC in the **large-scale expression, purification and characterization of human proteins**, leading to an extraordinary output of new 3D structures and matching chemical probes but also **in production and characterization of recombinant protein binding tools** such as antibodies, synthetic binders as well as in **expression, characterization and structure determination of integral membrane proteins** at large scale (e.g. single-particle cryo-electron microscopy, X-ray crystallography) which is particularly important for protein expression and purification in WP1 and for generation of protein reagents, protein expression and purification for proteins in WP5. Furthermore, UOX has proven expertise in building relevant biophysical, biochemical, and cellular/phenotypic assays.
- The pioneering competence of the ULIV in **metabolomics** (co-invention of the name and the approach) and of CeMM in regulatory principles in molecular networks.
- Specific experience of CeMM in **genetic screens** (incl. human haploid cell culture, CRISPR, experience with the SLC family) and the ability for **next generation sequencing** as well as expertise of CeMM, UOX and ULIV in **applying mass spectrometry**, such as enrichment and evaluation of membrane proteins at scale, methods suitable for the detection of protein-protein interactions (e.g. AP-MS, BirA-mediated BioID), methods to detect, identify and quantify metabolites and other small molecules and to assess changes in these molecules (e.g. targeted metabolomics, LC/GC-MS, library of metabolites), bioinformatics capabilities necessary to analyse systems-level data, also at the metabolite level. This will be essential for the generation of cell lines, deorphanisation, assay development and the generation of robust cell-based and cell-free assay systems.
- Experience in developing **cell-free *in vitro* target engagement assays** (CeMM, UOX, AXXAM, MPIMR, ULEI) will contribute to the development of specific assays and assay methodologies (including high-throughput screening assays) for studying a focused set of SLCs.
- CeMM, UOX and ULEI with their proven **know-how in quality control metrics** will contribute in setting up a quality control monitoring system in order to ensure high quality data and results.
- CeMM, UOX, AXXAM, ULEI, ULIV and UNIVIE show experience with relevant **public databases** (The Human Protein Atlas, UniProt/neXtProt, Bioparadigms SLC Tables, IUPHAR Guide to Pharmacology, TCDB, Pfam, Yeastnet, etc.) which is essential for data management and open access use of research output by the research community. UNIVIE has particular expertise in generation of data mining workflows as well as integration and mining of open data sources for answering complex drug-discovery related questions.

The EFPIA partners represent formidable research powerhouses who have contributed to research on membrane associated targets for more than half a century. Advancement in knowledge relevant to

RESOLUTE through the EFPIA partners ranges from basic research to clinical development and includes several marketed drugs. The RESOLUTE EFPIA members encompasses companies who have already successfully contributed to IMI2 projects. The 6 pharmaceutical companies involved represent the majority of the European pharmaceutical industry and therefore provide an outstanding critical mass and expertise for the RESOLUTE project. The EFPIA contributions will largely be in the form of *in vitro* biology resources of all kinds, as well as expertise and reagents that enable and support all WPs. Furthermore, contributions to data, data-mining techniques and bioinformatic analysis will also be provided. The fact that the industry consortium also includes companies with an R&D focus in the US can be considered as a proof of the global ambition of the project.

Each work package public leadership team is matched with industry leadership, which has been assigned considering the companies' particular interest and expertise for the WP topic.

Moreover, a partner **network of key experts** has been established. The **Structural Biology Alliance (SBA)** (see Figure 14) will link the RESOLUTE consortium to existing relevant initiatives and act as independent advisers for issues on protein expression, purification as well as SLC-class specific biochemical assays. The SBA will be in constant contact by receiving protocols and constructs. The SBA will provide services and expertise free of charge.

The **Binder Partner** (see Figure 14) provide services free of charge for tissue profiling of RESOLUTE validated binders (Human Protein Atlas, SciLifeLabs). Quality control and feedback from the Human Protein Atlas on discovery operation and data will be accessed at an early stage. Annotation jamborees will occur as satellite meetings to the yearly workshops and divided in families and modules, where appointed scoutmasters assemble scoreboards with the relevant information.

The **RESOLUTE Academic Expert Laboratories** (see Figure 14) are laboratories specialized in a given technology, assay or SLC family member and represent a rich source of expertise that may be called upon at different stages. RESOLUTE Academic Expert Laboratories will be contacted for specific research services and will provide in-kind contributions against payment (at cost, without profit). The decision of involving a given RESOLUTE Academic Expert Laboratory will be based upon a discussion within the consortium. All of these RESOLUTE Academic Expert Laboratories share the excitement of unlocking SLCs and adhere to the RESOLUTE vision and principles and have expressed their written intention to cooperate with the project, detailing their field of contribution. Apart from the contribution of expertise regarding specific SLCs (see Fig. 14), the RESOLUTE Academic Expert Laboratories might be involved for example for specific assay development (WP3), if the assays described in our workflow (Fig. 6) do not yield the wished-for results, or for the generation of protein-affinity reagents (such as nanobodies or sybodies; see also WP5). Admittedly, the expedient of listing a number of RESOLUTE Academic Expert Laboratories bears a few uncertainties and managerial challenges. Yet we deliberated that these would be, altogether, better manageable than the coordination of a very large consortium of laboratories. Of the currently listed RESOLUTE Academic Expert Laboratories, it is perhaps helpful to state that we do not envisage to engage more than five or six in total, making an average of 1 engagement per year.

In the case, where a specific technical expertise or a specific technology/reagent is necessary for the success of the RESOLUTE consortium in unlocking the biological role of specific SLCs, and this expertise or technology is not available within the consortium or the larger network of RESOLUTE Academic Expert Laboratories, the RESOLUTE consortium might additionally involve **Subcontractors**. Subcontractors will be engaged after a discussion within the General Assembly (GA), doing a proper market search and ensure best value for money at the point of assignment.

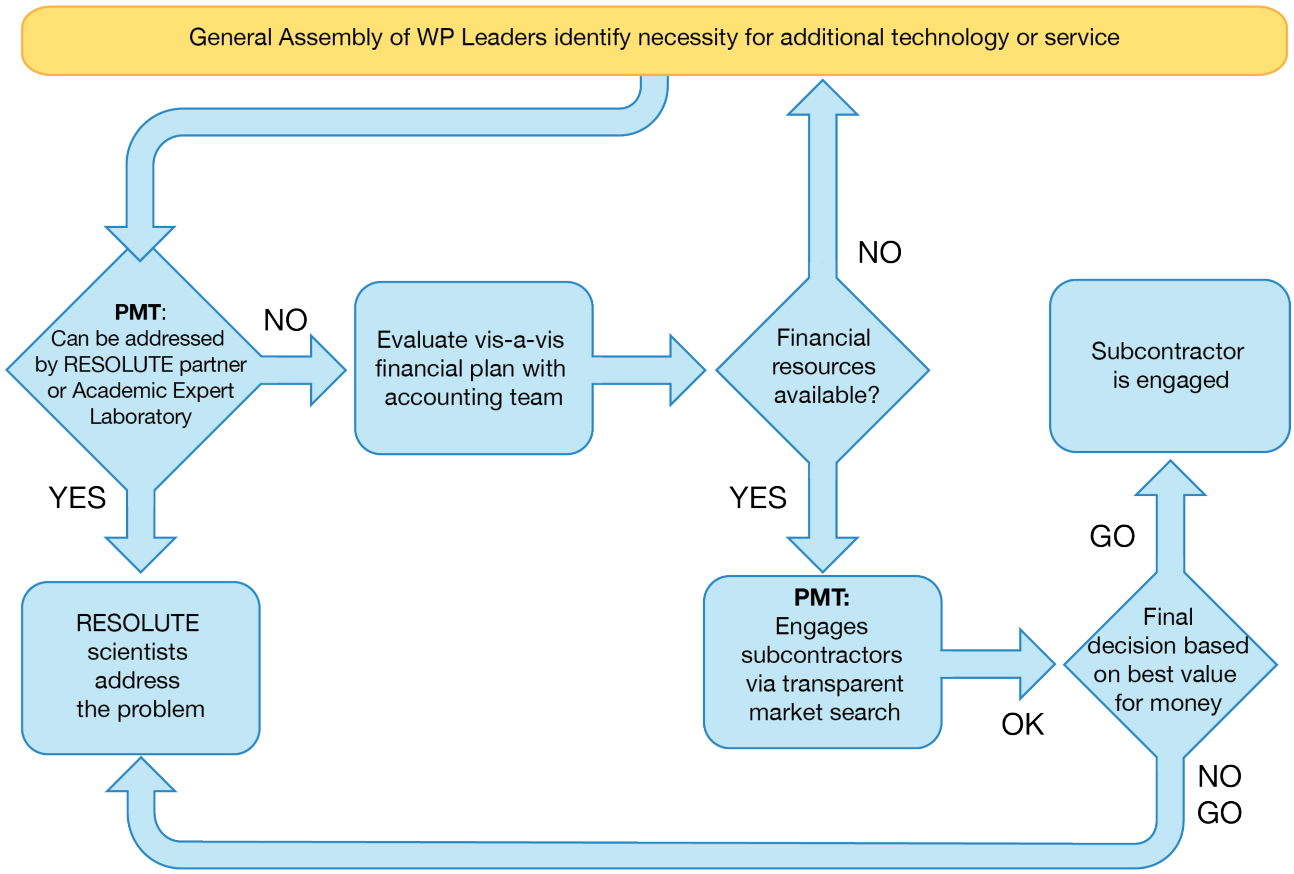

Figure 13: Subcontractor decision tree for the RESOLUTE consortium.

RESOLUTE ACADEMIC EXPERT LABORATORIES

name	organisation	cc	special expertise	SLC family
P ARTUSSON	Univ Uppsala	SE	mass spec of SLCs, liver and intestine cells	all
M FREISSMUTH	Med Uni Vienna	AT	allostery, regulation, SAR	SLC6
B GASNIER	Univ Paris Descartes	FR	TEVC current in Xenopus oocytes/ radiolabelled flux/ TFEB fluorescence microscopy assay	SLC17, 36, 38
J GERTSCH	Univ Berne	CH	radiometric, TLC uptake assays	all
U GETHER	Univ Copenhagen	DK	fluorescent techniques	SLC6
E GEERTSMA	Goethe Uni	DE	sybody development	all
C INDIVERI	Univ Calabria	IT	proteoliposomes, transport assays	all
P KRISTENSEN	Aarhus Uni	DK	Cell-based Fab phage libraries	all
H MICHEL	MPI Biophysics	DE	human/insect cell production, purification, transport assays	SLC4, 6, 9, 16, 23, 26
A NIES	IKO	DE	LC-MS/MS analysis xenobiotics and endogenous substrates	SLC16, O, 22
G NOVARINO	IST Austria	AT	stem cell-derived neuronal cultures, cerebral organoids	SLC7, 3
R OWENS	Univ Oxford	UK	Nanobody phage libraries	all
SF PEDERSEN	Univ Copenhagen	DK	acid/base transport assays	SLC4, 9
S STEFANIC	Univ Zurich	CH	Nanobody generation	all
J-L REYMOND	Univ Berne	CH	chemoinformatics visualization	all
M SCHWAB	IKP	DE	fruit fly models	all
M SELBACH	Max Delbrück Center	DE	endogenous tagging, SLC proteomics	SLC2
H SITTE	Med Uni Vienna	AT	tracer flux assays, FRET	SLC6, O
G SZAKACS	Med Uni Vienna	AT	fluorescent assays	SLCO (OATP)
M TRAUNER	Med Uni Vienna	AT	pathophysiology	SLC10, O

STRUCTURAL BIOLOGY ALLIANCE

name	organisation	cc
A CAMERON	Univ Warwick	UK
D DREW	Stockholm Univ	SE
H MICHEL	Max Planck Institute Biophysics	DE
S NEWSTEAD	Univ Oxford	UK
P NISSEN *	Univ Aarhus, EMBL Nordic Alliance	DK
M PALACIN	IRB Barcelona	ES
C PAULINO	Univ Groningen	NL
B PEDERSEN	Univ Aarhus	DK
N REYES	Pasteur Institute	FR
C TATE	LMB MRC Cambridge	UK
S WEYAND	Univ Cambridge	UK
C ZIEGLER	Univ Regensburg	DE

* chair

BINDER PARTNER

organisation	The Human Protein Atlas
name	M UHLÉN
function	tissue profiling

Figure 14: Overview of the RESOLUTE partner network. Some 5% of the total budget (incl. EFPIA contribution) has been allocated to the overall coordinator but is reserved to access the services of the partner network when necessary. RESOLUTE Academic Expert Laboratories will provide services and expertise in kind against payment, the Structural Biology Alliance is involved free of charge, and if needed subcontractors will be engaged on a best value for money basis.

3.4 Resources Committed

The RESOLUTE consortium's budget

The RESOLUTE consortium was awarded an Innovative Medicine Initiative grant in the context of the H2020 Programme of the European Union. The project costs are covered by a €12 million grant under the auspices of the IMI joint undertaking as well as in-kind contributions from industry partners for €11.85 million. The IMI (https://www.imi.europa.eu/) is a partnership between the European Union and the European pharmaceutical industry. Since 2008, IMI has facilitated open collaboration in research to advance the development of personalized medicines for the general health and well-being, especially in areas of unmet medical needs.

Structural Biology Alliance, RESOLUTE Academic Expert Laboratories, Subcontractors

The consortium is aware that this ambitious project requires a certain amount of flexibility to overcome the scientific challenges of changing project needs. Thus, it was decided to establish two partner networks (RESOLUTE Academic Expert Laboratories and Structural Biology Alliance, both described in more detail earlier on), whose knowledge and expertise can be drawn upon if necessary. Not making those partners full members of the consortium from the beginning highlights RESOLUTE's intention to remain dynamic and flexible and to seek for each challenge the best scientific answer available. If, however, during the project phase the significance of a potential partner becomes apparent both in terms of scientific contribution as well as costs involved, RESOLUTE plans on also providing the possibility of making this expert a full member of the consortium by amending the original grant agreement. This may be subject to IMI approval.

To engage RESOLUTE Academic Expert Laboratories (in kind against payment) and when needed subcontractors (on best value for money basis), the consortium allocated ~5% of the total budget to CeMM, who as project coordinator will supervise the distribution of these funds to those project partners, who are faced with the need to engage experts in order to be able to fulfil their project tasks as set out in this Description of Action (DoA).

Other subcontracting

Some of the EFPIA partners are planning to use subcontractors for sequencing services. The total budget for other subcontracting in this regard corresponds to ~2% of total budget, of which ~1% of total budget is planned as non-EU.

Overheads

For calculation of overheads, the IMI2 25% flat rate of the direct costs (excluding subcontracting and in-kind contributions against payments not used in the premise of the beneficiaries) was used. This resulted in a total of 10% of the total budget.

EFPIA contribution

The €12 million financial contribution from the IMI JU funded beneficiaries is complemented by a total EFPIA in-kind contribution amounting to €11.85 million. Non-EU in-kind contributions by Pfizer and Novartis (in part) are needed due to their unique scientific contributions to RESOLUTE that can only occur in their dedicated laboratories. The scientific contribution of Pfizer will come from the Discovery Sciences group (Groton, Connecticut, USA) as well as the Medicinal Design group and the Computational Sciences group (both in Cambridge, Massachusetts, USA). The non-EU in-kind from Novartis will occur due to the involvement of their location in the USA (Novartis Institutes for BioMedical Research, Cambridge, Massachusetts, USA). We are aware that the overall non-EU in-kind contributions must be justified. For a detailed description of the infrastructure and unique contributions for Pfizer and Novartis see also section 4.1.

4. MEMBERS OF THE CONSORTIUM

4.1 Participants (applicants)

1 – Forschungszentrum für Molekulare Medizin / Research Center for Molecular Medicine (CeMM)

Description of the legal entity

The mission of the **Research Center for Molecular Medicine** of the Austrian Academy of Sciences (Vienna), founded 10 years ago, is to strengthen biomedical research in Austria and to achieve maximum scientific innovation in molecular medicine to improve healthcare. Strategically located in a new purpose-built research building in the heart of the General Hospital in Vienna (the largest single-site hospital in Europe with 2,000 MDs), a team of 130 scientists and medical doctors from 37 nations pursues free-minded life science research in a large and vibrant hospital environment. Its research is based on post-genomic technologies and focuses on immune disorders, infections, cancer and metabolic disorders. Composed of 12 independent research groups, CeMM operates in a unique mode of super-cooperation, connecting biology with medicine, experiments with computation, discovery with translation, and science with society and the arts. CeMM is one of the founding members of EU-Life, an alliance of 13 top European research centers established to exchange best practices in life sciences, and has also been voted Europe's best place to work in academia in a 2012 survey by *The Scientist*. CeMM's goal is to pioneer the science that nurtures the precise, personalized, and preventive medicine of the future.

Key personnel

Giulio Superti-Furga (M | Academic Coordinator, Lead WP1, WP7, WP9) Scientific Director and CEO of CeMM, has >20 years of experience in the use of chemical biology and omics techniques to understand drug action. He performed his studies in molecular biology at the University of Zurich, Genentech/San Francisco and the IMP in Vienna. He was a postdoctoral fellow at the EMBL/Heidelberg becoming team leader in 1995. He co-founded the biotech companies Cellzome, Haplogen and Allcyte. He was responsible for several large-scale projects such as genome-wide characterization of the yeast protein complexes, the entire NF-κB pathway, the viral interactome and organization of the human lipidome. He contributed to the first human essentialome and the generation of a large human collection of isogenic mutant haploid cells. His work now focuses on molecular networks and the mechanism of action of drugs. After characterizing SLC transporters required for drugs to enter cells and others required to couple nutrient availability to mTOR activity, he became an advocate of more research on SLC transporters. His whole genome is online as part of the genomic educational and scientific programme 'Genom Austria'. He is a member of five scientific academies, has been awarded 4 ERC grants and since 2017 is a member of the Scientific Council of the ERC.

André C. Müller (M | Head of the Proteomics and Metabolomics Facility) obtained his Master's degree in biochemistry at the University of Cologne, Germany. In 2007 he joined the proteomics research group of Dr. Keiryn Bennett at CeMM, Vienna. Since then André has gained more than 10 years of expertise in LCMS-based proteomics being involved in several bio-medical projects which resulted in more than 37 publications. In 2016 he joined the European Application Support Specialist team of Thermo Fisher Scientific before returning to CeMM as head of the Proteomics and Metabolomics Facility in June 2017.

Kristaps Klavins (M | Deputy Head for Metabolomics Facility) is a trained analytical chemist and holds a PhD in biotechnologies with focus on metabolomics. He has profound expertise in the different mass spectrometry and separation techniques, including the applications in targeted metabolomics. In the past, he has been involved in the development of the novel MS-based kits for targeted metabolomics analysis as well as applying these tools for identification of novel biomarkers in neurodegenerative and oncological diseases. Currently, he is responsible for setting up state-of-the-art metabolomics and lipidomics workflows at CeMM.

Enrico Girardi (M) obtained a PhD from King's College London (UK) working on protein expression and crystallography, followed by postdoctoral studies at the La Jolla Institute for Allergy and Immunology in San Diego, USA. In 2014 he joined the group of Giulio Superti-Furga where he leads a small team focusing on the systematic characterization of drug-transporter interactions by genetic and biochemical methods.

Gernot Wolf (M | Co-Lead WP1) received his Ph.D. in Molecular Biology from Aarhus University in the laboratory of Dr. Finn Skou Pedersen. Afterwards he joined the lab of Dr. Todd Macfarlan at the NIH, where he has been mainly working on KRAB zinc finger proteins (KRAB-ZFPs). During his postdoc he mastered the art of cell line engineering using CRISPR/Cas9 and he will now apply his expertise to the generation of cell lines for RESOLUTE.

Ulrich Goldmann (M | Data Scientist, Co-Lead WP8) obtained his PhD at ETH Zürich, Switzerland, on the bioinformatic analysis of the cell surface proteome by integrative analysis of genomics, transcriptomics and proteomics data. As Data Scientist at CeMM, his expertise is large-scale data analysis, integration and visualization as well as applied statistics, machine learning and web development.

Álvaro Inglés-Prieto (M | Communication and Business Development Manager, former Co-Lead WP7) is a Biochemist and Ph.D. in Chemistry. During his Ph.D., he gained knowledge in protein characterization in terms of structure and stability. During his first postdoctoral stage, he used synthetic biology approaches to develop light controlled membrane receptors. He has proven expertise in protein engineering and synthetic biology, which includes the generation of tagged membrane proteins, cell lines overexpressing modified proteins and biochemical and cellular assays. He joined RESOLUTE in 2017 as postdoctoral scientists for proteomics at CeMM. In December 2019, he became Communication and business development manager for RESOLUTE.

Vitaly Sedlyarov (M | Data Scientist, Dissemination Officer) studied Bioengineering and Bioinformatics in Lomovosov Moscow States University, Russia and Leiden University Medical Center, The Netherlands (MSc). Obtained PhD in molecular biology and immunology from University of Vienna, Austria for work on systems biology of posttranscriptional regulation of inflammation resolution. Joined the CeMM in 2016 as a bioinformatics postdoctoral fellow with the main focus on regulation of immune homeostasis and metabolism by membrane transporters. Since 2019 he is working as data scientist on the RESOLUTE project.

Tabea Wiedmer (F | RESOLUTE Scientific Project Manager) studied Pharmaceutical Sciences at the University of Basel (Switzerland) and University of Copenhagen (Denmark). During her MSc, she studied an antagonist binding site on glutamate receptors. After an internship in the oncology biology department at a Swiss biotech company, she moved to the University of Bern (Switzerland), where she obtained her PhD in cell biology, centered on autophagy and therapeutic options for pancreatic neuroendocrine tumors. She joined RESOLUTE in 2018 as postdoctoral scientist, focusing on metabolomics and assay development at CeMM. In December 2019, she became the new Scientific Project Manager for RESOLUTE.

Daniel Lackner (M | First RESOLUTE Scientific Project Manager) did his graduate work at the Wellcome Trust Sanger Institute in Hinxton, UK. After obtaining his PhD from the University of Cambridge, he joined The Salk Institute for Biological Studies in San Diego, USA, for his post-doctoral work. There, he studied telomere biology and aging with a focus on large-scale approaches. Afterwards he worked for a small biotech company in Vienna, Austria, where he led a team to generate human knock-out cell lines using CRISPR/Cas9. In 2018, he joined CeMM as scientific project manager. Daniel passed away on 31 August 2019, at the age of 41, after severe illness.

Relevant publications

César-Razquin, A., et al., *A call for systematic research on solute carriers.* Cell, 2015. **30**(162): p. 478-487. A recent Leading Edge perspective and review highlighting how SLCs are an extremely promising but highly understudied protein superfamily and calling for large, systematic efforts to deorphanise and study transporters.

Winter, GE., et al., *The solute carrier SLC35F2 enables YM155-mediated DNA damage toxicity*. Nat Chem Biol, 2014. **10**(9): p. 768-773. An example of the striking and exquisite requirement for a SLC protein for the cellular uptake of a cytotoxic drug.

Rebsamen, M., et al., *SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1.* Nature, 2015. **519**(7544): p. 477-481. The paper describes the identification of a novel nutrient sensing mechanism linked to the mTOR pathway. By combining proteomics, biochemical and subcellular localization studies with transport assays the authors were able to deorphanise this SLC and suggest it acts as a transceptor (a transporter/substrate able to induce signalling within a cell).

Huber, KV., et al., *Stereospecific targeting of MTH1 by (S)-crizotinib as an anticancer strategy.* Nature, 2014. **508**(7495): p. 222-227. The identification of a stereochemically specific inhibition of an important enzyme by chemical biology methods.

Köberlin, MS., et al., *A conserved circular network of coregulated lipids modulates innate immune responses*. Cell, 2015. **162**(1): p. 170-183. Systems-level analysis of the cell lipidome by combining genetic perturbations and lipidomics approaches.

Relevant previous projects or activities

ERC AdG 2016 - *Solute carrier proteins as gates managing chemical access to cells* (GameofGates). An ERC advanced grant to study SLCs at the systems level in a model haploid human cell line combining genetic engineering, proteomics and metabolomics approaches.

FWF 2016 - *Viral transportome: solute carriers proteins at interface of the viral life cycle* (ViTra). This grant supports a project aiming at understanding the role of SLCs in viral infection and viral cycle by genetic and proteomics approaches.

MSCA-IF 2015 - *Solute carrier proteins and the uptake of cytotoxic approved drugs* (DrugsUp). This fellowship supported a postdoctoral fellow in determining SLC/drug associations by a CRISPR/Cas9 genetic screening approach.

ERC PoC 2012 - *Acute inflammation resolution by soluble human inhibitory protein* (AIRSHIP). This grant supported a project aiming at determining the therapeutic potential of a novel, secreted, soluble enzyme as a negative regulator of pro-inflammatory immunity receptors.

ERC AdG 2009 - *Interferon Innate Immunity Interactome and Inhibitome* (iFIVE). An ERC advanced grant supporting the identification of innate immune response regulators by proteomics means.

Relevant infrastructure and/or other achievements

CeMM offers a truly supportive environment that gives access to the latest molecular technologies and fosters cutting-edge research. There are laboratories for organic synthesis, a proteomics and metabolomics facility (including Thermo Scientific Orbitrap Fusion Lumos and Agilent 6470A Triple Quadrupole LC/MS instruments), a chemical biology screening platform connected to a high-content imaging system and a formatted compound library of around 100,000 small molecules, as well as a biomedical sequencing facility (using Illumina HiSeq 3000/4000, HiSeq 2000, and MiSeq instruments) offering also bioinformatic data processing. Access to animal research facilities can be provided, and CeMM's administration and scientific support team is dedicated to take organizational burden from researchers (e.g. central purchase, wash and media kitchen, lab manager taking care of safety, and the maintaining and operation of equipment).

2 – University of Oxford (UOX)

Description of the legal entity

The **University of Oxford** is a world-leading centre of learning, teaching and research and the oldest university in the English-speaking world. Oxford's research activity involves 70+ departments, 1,600+ academic staff, 4,100+ research & research support staff, and 5,500+ graduate research students. The Medical Sciences Division is an internationally recognized centre of excellence for biomedical and clinical research.

The **Structural Genomics Consortium (SGC)** is a not-for-profit public-private partnership to accelerate drug discovery through open access research by 250+ scientists, located at 6 sites across the globe. The SGC Oxford over the past 13 years has become a research centre leading in human protein structure and chemical biology, with the structure determination of over 500 novel human soluble proteins, 30 protein-protein complexes and 10 human integral membrane proteins (IMPs). It has strong and expanding capabilities in assay development and chemical screening, in particular in the context of its impactful chemical probe programme.

These successful developments and the SGC Oxford international contribution have been sustained through active and innovative method and technology development. The SGC is renowned for the quality and

reproducibility of the generated data and reagents, as well as for the robust methodologies developed. It has been a training ground for many scientists worldwide.

SGC Oxford through its research, technological and methodological developments and reagents has naturally become over the years an epicenter for collaborations, both with academia and with industry. It has built as international collaborative network spanning 9 pharmaceutical partners, over 300 leading academic labs and several biotech companies.

The SGC is one of the major champions for open source science and drug discovery. The reagents and data produced by the SGC are delivered in an open manner framework to facilitate collaboration, reduce wastage, catalyse academic and industrial science. The SGC has produced over 400 papers (2.5 per week in 2013-2014), co-authored with over 1,300 different authors from 35 academic institutions and 20 companies. Membrane protein structural biology is exclusively carried out at the largest site located in Oxford, with two group leaders (Prof. Liz Carpenter and Dr. Katharina Duerr) managing a total of more than 20 scientists dedicated to production of membrane proteins for structural studies by cryo-EM and X-ray crystallography. Supported by the biotech group of Dr. Nicola Burgess-Brown, two IMP pipelines have been established, enabling high throughput expression and purification of hundreds of human IMPs in insect and mammalian cells, respectively.

Key personnel

Katharina L. Duerr (F | Lead WP5), group leader at the SGC since 2016, has been studying the structure and function of membrane proteins for 15 years. She performed her graduate studies at the Max-Planck Institute of Biophysics Frankfurt and at the Technical University Berlin. She has been a postdoctoral fellow with Dr. Eric Gouaux at OHSU (Portland), determining X-ray and cryo-EM structures of ionotropic glutamate receptors. At the SGC Oxford, she has established a mammalian pipeline for high-throughput characterization of human membrane proteins by fluorescence-detection size exclusion chromatography. It has enabled the rapid screening of sub-microgram quantities of highly pure protein samples from >1,000 constructs of 150+ human membrane proteins, including ion channels, transporters, enzymes and tetraspanins. In less than a year, her group has moved more than 20 human membrane proteins from this pipeline into crystallization trials or studies by single-particle cryo-electron microscopy. In addition, the group has established expression and purification protocols for robust recombinant production of >15 different antibody fragments against a variety of human integral membrane protein targets.

Relevant publications

Dürr, KL., et al., *Structure and Dynamics of AMPA Receptor GluA2 in Resting, Pre-Open, and Desensitized States.* Cell, 2014. **158**(4): p. 778–792. The paper describes the structure of the ionotropic GluA2 AMPA receptor in several states and defines mechanistic principles for activation and desensitization in AMPA iGluRs.

Savitsky, P., et al., *High-throughput production of human proteins for crystallization: the SGC experience.* J Struct Biol, 2010. **172**(1): p. 3-13. This paper describes the methods used to produce recombinant human proteins at the SGC, many of which will be applied in RESOLUTE.

Mahajan, P., et al., *Medium-throughput production of recombinant human proteins: protein production in insect cells.* Methods Mol Biol, 2014. **1091:** p. 95-121. This paper describes the methods used at SGC to express recombinant human proteins in insect cells, similar to the approach proposed within RESOLUTE.

Dürr, KL., et al., *Measuring cation transport by Na,K- and H,K-ATPase in Xenopus oocytes by atomic absorption spectrophotometry: an alternative to radioisotope assays*. J Vis Exp, 2013. **72**. This paper describes the use of atomic absorption spectrophotometry to track Rb(+) or Li(+) transport by ATPases in single cells.

Dürr, KL., et al., *A D-pathway mutation decouples the Paracoccus denitrificans cytochrome c oxidase by altering the side-chain orientation of a distant conserved glutamate*. J Mol Biol, 2008. **384:** p. 865–877. The manuscript describes the crystal structures of an oxidase variant and the implication of this mutation for proton routing through the D-pathway of cytochrome C oxidase.

Relevant previous projects or activities

X-ray crystallographic, cryo-EM and electrophysiological studies of glutamate receptors. This project required extensive optimization of the expression and purification conditions of glutamate receptors which are large integral membrane proteins of fundamental importance to excitatory synaptic neurotransmission. Many of the techniques that were key for the success of the project are also applicable to SLCs.

Atomic absorption spectroscopy as a tool to study the transport of ion pumps in non-radioactive flux assays using Xenopus oocytes. The project was aimed at the functional characterization of another family of transporters, the P-type ATPase family of primary active transporters. To enable Rb^+ (a K^+ analogue) uptake measurements in Xenopus oocytes, this non-radioactive assay was developed. It was also used to confirm inhibition of transport inhibitors ouabain (of the sodium pump) and omeprazole (for the gastric H,K-ATPase) and could be therefore further developed as a HTP assay to screen for novel inhibitors of these two potassium-transporting ion pumps. Similar assays can be established for compound screening of K^+-transporting SLCs, such as members of the SLC12 family.

H+ transport assays of proteoliposome-reconstituted membrane proteins. This work was focused on studying mutants of cytochrome c oxidase. To that end, 10-30 mg of functionally active protein was purified from a recombinant Paracoccus denitrificans strains and used for reconstitution into proteoliposomes. The proteoliposomes were then used in a stopped-flow spectrometric approach to measure proton and electron transport across the liposome membrane, using a fluorescent proton-sensitive dye. Similar *in vitro* assay can be developed to study SLCs that co- or countertransport protons in addition to another ion or organic substrate.

Relevant infrastructure and/or other achievements

The SGC Oxford offers state-of-the-art facilities for high-throughput cloning, allowing the generation and screening of fifty 96-well plates a year, equivalent to 4800 expression constructs. It also has excellent tissue culture facilities for large-scale expression of membrane proteins in insect and mammalian cells, with typical growth cultures exceeding 500 L each month. In addition to several large-scale purification platforms, the labs are equipped with a gel filtration system with fluorescence detector and autosampler for high-throughput monodispersity screening of nanogram quantities of membrane proteins. The Molecular Biophysics group of the SGC Oxford manages a variety of instruments that can be used for biophysical characterization of purified membrane proteins, including microscale thermophoresis, nanoDSF, ITC and SPR. For crystallization, SGC has liquid-handling systems for preparation of custom 24 and 96-well screens, several crystallization robots capable of dispensing membrane proteins in lipidic cubic phase (LCP) and crystal plate imaging systems with UV and fluorescence detectors. SGC structural biologists have frequent access to microfocus beamlines at Diamond Lightsource, as well as to several microscopes suitable to perform screening and data collection for single-particle cryo-electron microscopy. This includes multiple low- and medium-end microscopes at the Dunn school of Pathology and the Oxford Particle Imaging Centre (T12, F30 and Talos Arctica), and two high-end 300 kV microscopes (Titan Krios) which are each installed at these EM facilities. In addition, the two IMP groups are part of a bloc-allocation group headed by Dr. Juha Huiskonen, with regular access to four additional Titan Krios microscopes at the Electron Bio-Imaging centre (eBIC) at Diamond. Each of these six high-end microscopes is equipped with a direct detector integrated into FEI's automated data acquisition software, enabling rapid acquisition of sufficient particles for 3D reconstruction and structure determination. The Oxford site works closely with the recombinant antibodies group at SGC Karolinska, headed by Dr. Susanne Gräslund. The facility has already generated high affinity antibodies against 24 soluble targets by phage-display and is now expanding the technology to antibody production against human membrane proteins.

3 – The University of Liverpool (ULIV)

Description of the legal entity

The **University of Liverpool**, represented by the **Institute of Systems, Molecular and Integrative Biology** (formerly the Institute of Integrative Biology (IIB, www.liverpool.ac.uk/integrative-biology) is one of four research-intensive Institutes within the Faculty of Health and Life Sciences located at the heart of a thriving

biomedical and environmental science campus in Liverpool. Their academic staff contribute to biological, biomedical and veterinary science undergraduate curriculums through close links with the Faculty's undergraduate teaching institutes (Institute of Life and Human Sciences; Institute of Clinical Sciences; Institute of Veterinary Science). Their engagement across a range of scientific areas supports the delivery of research-driven teaching to our undergraduate and postgraduate communities. The institute has 68 academic colleagues in the Institute, including 31 Professors, 9 independent research fellows, ~100 staff on permanent and fixed-term research contracts, ~100 postgraduate students and 28 technical staff. They are administered by 18 professional services staff providing management, financial, research and clerical support. With a research income of ~£9.2m annually, IIB scientists deliver ground-breaking research which spans the complete range of biological scales from genes and genetic regulation through proteins, whole organisms, populations and ecosystems.

Key personnel

Douglas B. Kell (M | Lead WP2), Research Professor in Bioanalytical Science, is a pioneer of metabolomics (and co-invented the term 'metabolome' 18 years ago). He has developed a variety of methods for long-term experimental mass-spectrometry-based metabolomics, both GC-MS and LC-MS, together with drift correction, QC and statistical and machine learning methods. The latter will be used to develop the QSAR models that are at the heart of WP2. He has pioneered in the development of cheminformatics methods (used by RESOLUTE), and his 2008 review in Nature Reviews in Drug Discovery has revised substantially our general view on the role of SLCs in pharmaceutical drug transport. He was part of the consortium responsible for the human metabolic network reconstruction Recon2. From 2008-13 he was also seconded (0.8 FTE) as CEO of the UK Biotechnology and Biological Sciences Research Council. He is a Fellow of the Learned Society of Wales, and, unusually for someone from the UK, he is also a Fellow of the American Association for the Advancement of Science. His work is widely cited, with in excess of 30,000 citations in the Web of Knowledge (H-index=88) and over 50,000 in Google Scholar (H-index=112). His full set of publications is listed on his group's personal website, at http://dbkgroup.org/publlications/.

Relevant publications

Wright Muelas M, Mughal F, O'Hagan S, Day PJ, Kell DB: The role and robustness of the Gini coefficient as an unbiased tool for the selection of Gini genes for normalising expression profiling data Sci Rep 2019, 9:17960. An update including many more datasets of the paper below, with the same conclusion.

O'Hagan S, Wright Muelas M, Day PJ, Lundberg E, Kell DB: GeneGini: assessment via the Gini coefficient of reference ''housekeeping'' genes and diverse human transporter expression profiles Cell Syst 2018, 6:230-244. Showing transporters are among the most divergently expressed proteins in a large dataset of human cell lines and tissues.

Kell, DB., et al., *The metabolome 18 years on: a concept comes of age.* Metabolomics, 2016. **12**(9): p. 148. This paper summarises development since the PI and colleagues coined the term 'metabolome' in 1998.

Kell DB, Oliver SG: How drugs get into cells: tested and testable predictions to help discriminate between transporter-mediated uptake and lipoidal bilayer diffusion. Front Pharmacol 2014, 5:231. A review of the evidence that drug entry into cells via trans-phospholipid bilayer transport is functionally negligible.

Dunn, WB., et al., *Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry.* Nat Protoc, 2011. **6**: p. 1060-1083. This paper summarises all the analytical methods that were developed during the so-called Husermet (Human Serum Metabolome in Health and Disease) project, whereby the PI amongst other things learned to deal with LC/GC-MS drift over periods of years while analysing approximately 3,000 serum samples for their metabolome.

Zelena, E., et al., *Development of a robust and repeatable UPLC-MS method for the long-term metabolomic study of human serum.* Anal Chem, 2009. **81:** p. 1357-1364. Methods in the previous paper included UPLC-MS, which will be the standard method here. This paper is the core paper that describes these methods in more detail.

O'Hagan, S., et al., *A 'rule of 0.5' for the metabolite-likeness of approved pharmaceutical drugs.* Metabolomics, 2015. **11**: p. 323-339. The first of many cheminformatics papers the PI has done comparing

the structures of endogenous human metabolites and marketed pharmaceutical drugs. (The latest is O'Hagan S, Kell DB: Consensus rank orderings of molecular fingerprints illustrate the 'most genuine' similarities between marketed drugs and small endogenous human metabolites, but highlight exogenous natural products as the most important 'natural' drug transporter substrates. ADMET & DMPK 2017; 5:85-125.)

Dobson, PD., & Kell, DB., *Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule?* Nat Rev Drug Disc, 2008. **7**: p. 205-220. The first of the PIs many reviews setting out the evidence that any 'background' flux of transmembrane cellular uptake of drugs through the bilayer is actually negligible, with all of the transmembrane uptake being via SLCs (and efflux via ABCs).

Relevant previous projects or activities

BBSRC 2017 - SWATH strategies for determining solute transporter substrates. This project is based on the recognition that if SLC X is a transporter for substrate Y there should be a co-variation in the levels of X and Y when compared via the natural co-variation between existing cell lines. It is very complementary to RESOLUTE, but (i) no cloning or knockouts are involved, and (ii) it considers only drugs and not metabolites.

BBSRC 2014- Centre for Synthetic Biology of Fine and Speciality Chemicals. This is a very large grant (£10M) to the UoM to develop the methods of synthetic biology for enhancing biotechnological fluxes. The link to SLCs (or more accurately transporters generally) comes from the recognition that in many cases we need to enhance or modify their activity for substrate uptake and product efflux in productive microbial processes.

BBSRC LINK 2006-11 (GSK) Pharmaceutical drug uptake. This was the project that first allowed us to understand that indeed 'drugs need transporters', and developed a series of computational and experimental methods in yeast for assessing this. Although it is long finished, the thinking on which it is based underpins our current strategies.

BBSRC LINK 2004-2011 (GSK & AZ). The human serum metabolome in health and disease. This is the Husermet project mentioned above, where (with collaborators) we collected some 3,000 serum samples from healthy volunteers as a vehicle for understanding the extent of variation of the human serum metabolome in 'normal' individuals.

BBSRC 2005-11 The Manchester Centre for Integrative Systems Biology. This was another large project grant (£6M) led by the PI before his secondment to BBSRC. Inter alia, it pioneered the consensus ('jamboree'-based) development of principled biochemical network models that used modern methods of cheminformatics to describe small molecule structures. It developed all methods first in baker's yeast before translating them to humans. Our participation in the human metabolic network reconstruction Recon2 has given us the 'parts list' of endogenous metabolites whose similarities we have compared with marketed drugs.

Relevant infrastructure and/or other achievements

IIB houses state-of-the-art facilities in genomics, proteomics, metabolomics and computational biology and expertise in applying these techniques to meet societal challenges in healthcare, bioscience, agriculture and environmental science. A recent acquisition by the PI is an Intellicyt iQue Plus flow cytometric screening system, which can effect flow cytometry at 35,000 cells.s^{-1}, taking just 5min for a 96-well plate (whereupon it can start again or take another plate via a robotic arm). This instrument will be ideal for assessing the kinetics of uptake of drugs that are either fluorescent or that competes for uptake with a molecule that is.

4 – Axxam Spa (AXXAM)

Description of the legal entity

Axxam Spa is a privately-owned contract research and discovery company with a team of 70 highly skilled employees; most of them experienced scientists, located at OpenZone Science Park, Bresso (Milan, Italy). Since its inception in 2001, as a spin-out from the Bayer Group, AXXAM has built up an integrated discovery platform called 'Lead Engine', which consists of 'assay development', 'high throughput screening', 'hit follow-up' and 'medicinal chemistry' modules. This streamlined industrial platform is used for the discovery of novel bioactive compounds and is provided by AXXAM services' unit. The Company has developed a multi-year proven track record as a third-party research and discovery services' provider for the life science industry.

AXXAM has been collaborating with more than 50 international partners, ranging from small Biotech to large pharmaceutical companies and agrochemical companies, and a growing network of research institutions. In the last few years, AXXAM has expanded its business model, by establishing its own suite of internal discovery programs against attractive biomedical targets, which are then out-licensed at the lead candidate stage to a collaborative/licensing partner.

Key personnel

Lia Scarabottolo (F | Lead WP6) is Director of Discovery Services at AXXAM since January 2007, having the following responsibilities: Direction of the units belonging to the Discovery Services Department (Cell Biology, Biochemistry, Screening Technologies, Electrophysiology and Technical Services), covering activities like gene cloning and expression analysis, cell based and biochemical assay configuration, high throughput screening and related data analysis, chemical compound collection management, compound profiling and Hit-to-Lead processes, manual and automated patch clamp, liability and cardiac safety platforms implementation, enabling technologies for novel assay development approaches; She's program director for many of the collaborations in place with national and international companies; she's also principal investigator for projects granted by the Italian government, the European community and international organisms; Member of the executive team and the management team.

Michela Stucchi (F) is Head of Screening Technologies at AXXAM. Since 2007, she has been in charge of the management and coordination of the scientific activities of the Screening Technologies group, including HTS, compound profiling, hit to lead, data analysis, automation and compound management. She is program manager of several industrial collaborations for the discovery of new bioactive molecules and serves as principal investigator of proprietary drug discovery programs supported by grants awarded by international agencies. In 2001 Michela joined AXXAM as senior scientist and established the FLIPR platform for the development of cell-based assays for ion channels, electrogenic transporters and GPCRs. She gained her background and expertise on ion channels and GPCRs physiology and assay development at the Bayer Research Center in Milano. Michela received a degree in Biological Science specializing in pharmacology from the University of Milan where she also worked as an undergraduate student in the Laboratory of Applied Pharmacology.

Loredana Redaelli (F) leads the Cell Biology group at AXXAM; she coordinates the research activities of the Cell Biology group in the field of development and validation of miniaturized, functional cell-based assays for high-throughput screening. She joined AXXAM in 2001 and worked in the Biochemistry group. From 1995 to 2001, as a research scientist at the Bayer Research Centre in Milan, she generated cell-based high-throughput screening assays for various pharmacological targets involved in cardiovascular diseases. Loredana holds a degree in Biological Sciences from University of Pavia and a Master in Biotechnology from the University of Milan.

Claudia Caserini (F) is head of Technical Services, she is responsible and coordinates the activities and resources of this group. Claudia has long lasting experience in this role and in the field of large scale and standard procedures of cell culturing and molecular biology technologies. Particular expertise in automated and standardized procedures necessary for supporting automated and high throughput platforms.

Relevant publications

Di Silvio, A., et al., *Identification of State-Dependent Blockers for Voltage-Gated Calcium Channels Using a FLIPR-Based Assay.* Methods Mol Biol, 2016. **1439**: p. 197-206. This publication highlights the potential of the FLIPR-based approach for compound screening. Indeed, by setting ad-hoc protocols, it is possible to run compound screening with specific biophysical statuses of the electrogenic protein, which resembles the pathological conditions.

Mistretta, FA., et al., DFL23448, *A Novel Transient Receptor Potential Melastin 8-Selective Ion Channel Antagonist, Modifies Bladder Function and Reduces Bladder Overactivity in Awake Rats*. J Pharmacol Exp. Ther, 2016. **356**(1): p. 200-211. This publication shows Axxam capabilities in developing robust, sensitive and HTS grade assays for the Transient Receptor Potential Channel class.

Corazza, S., *All in one with chAMPion: a generic luminescence assay platform for any GPCR signaling type*. Assay Drug Dev Technol, 2009. **7**(3): p. 304-307. chAMPion technology is an acronym for a cell line developed

at Axxam suitable to analyse the functionality of the different classes of GPCRs. The tool takes advantage from the coexpression of a Ca2+ sensitive luminescent protein and a CNG channel in CHO. This cell line could be used to study the functionality of transporters correlated to cAMP or Ca2+ intracellular pathways.

Bovolenta, S., et al., *Development of a Ca(2+)-activated photoprotein, Photina, and its application to high-throughput screening.* J Biomol Screen, 2007. **12**(5): p. 694-704. This publication describes the development, of a Ca2+ sensitive photoprotein, of Axxam property, having characteristics of high Ca2+ affinity and brightness. This biosensor can be used to study the functionality of transporters, which transport Ca2+ ions inside the cells.

Corazza, S., et al., *An innovative cell-based assay for the detection of modulators of soluble guanylate cyclase.* Assay Drug Dev Technol, 2006. **4**(2): p. 165-103. This publication describes the development of a cell line suitable to identify modulators of soluble guanylate cyclase. This tool could be used to establish assays for transporters, which might be indirectly correlated with guanylate cyclase activity.

Relevant previous projects or activities

PHAGO - IMI2 (01/11/2016 – 31/10/2021): *Targeting TREM2 and CD33 of phagocytes for treatment of AD*. The project addresses microglia activation in neurodegenerative processes; a strong synergy and sharing is expected between the PHAGO and NGN-PET; functional screening assays and hiPSCs-derived microglia will be developed during PHAGO (WP5-6).

OPTEL - EuroTransBio Programme (01/12/2015 – 30/11/2017): *A novel optogenetic electrophysiology platform for ion channel and transporter screening*. The goal of this project is the generation of a new platform, developed by combining powerful optogenetics technologies to fluorescence based screening and automated patch clamp devices, at HTS scale, and to the use of induced pluripotent stem cell derived cardiomyocytes. These integrated technologies will allow testing the activity of a large number of compounds on electrogenic proteins, improving the early identification of specific hits and/or toxic compounds.

Neurosafe - EuroTransBio Programme (01/11/2012 - 31/10/2014): *Development of a human integrated in vitro Neurotoxicity Safety Platform*. Goal of the project was the development of a human integrated *in vitro* Neurotoxicity Safety platform, which allows to quickly assessing potential neurotoxicity effects of chemical hits, at a very early stage of the drug discovery.

NCX1 blockers - FastForward LLC & Merck KGaA. (01/05/2011 – 31/10/2012): *Small molecule NCX1 blockers for axonal survival in MS*. The overall aim of this project was the identification and early development of drug-like molecules which selectively block the Reverse Mode (RM) activity of Na+/Ca2+ exchanger 1 (NCX1) and therefore have the potential of halting neurodegeneration in Multiple Sclerosis (MS) by specifically dampening disease-associated NCX1-RM activity in demyelinating neurons.

Framework Programme VI – CONCO (01/02/2007 – 31/01/2012): *Applied venomics of the cone snail species Conus consors for the accelerated, cheaper, safer and more ethical production of innovative biomedical drugs*. This project aimed both at developing an existing drug lead compound from the cone snail species *Conus consors*, namely XEP-018, whose biological activity proof of concept has been established and also at identifying and caracterizing new drug candidates from the same species.

Relevant infrastructure and/or other achievements

AXXAM's discovery platform refers to a fully integrated drug discovery process ranging from target identification to generation of 'qualified hits'. The approach is centered on assay development platform, high throughput screening, smart compound library design, hit follow-up platform including hit confirmation and orthogonal, proof of concept and liability assays. The company has setup an industrialized process for assay development which has produced high throughput screening assays for over 250 key drug targets. Recently AXXAM has also successfully implemented the innovative technologies of gene editing nucleases and optogenetics tools, for the development of smarter 'next generation' cellular assays. AXXAM operates with two highly customized state-of-the-art high throughput screening stations designed to run biochemical and cell-based assays. The screening stations are able to generate more than 50,000 test points per day. In addition to these systems, AXXAM is equipped with several automated compound handling and compound management instruments. AXXAM also has an electrophysiology unit equipped with both manual and

automated patch-clamp devices and has recently created a team focused on the chemistry part in the Lead generation phase for the proprietary programs.

AXXAM laboratories offer in-house equipment for molecular biology, cell handling and for cell based and biochemical assay development. Recently AXXAM has acquired an automated compound storage (Brooks US600L), able to store up to 14,000 plates in 96 or 384 format. AXXAM has also implemented the GeneData software for HTS analysis and BIOVIA Pipeline Pilot for chemoinformatic analysis and has created a proprietary database for data storage and mining. The medicinal chemistry laboratories, fully dedicated to internal research projects, are equipped with all the instrumentation and software needed for modern synthetic organic chemistry and analytical chemistry.

The HCS microscopy platform comprises: two Opera® QEHS (PerkinElmer) confocal fluorescence microscopes with integrated dispensers. One Opera is integrated in the Robocon HTS system allowing fully automated HCS operations, under BSL2 conditions. Live cell imaging and single cell analysis can be performed, also without fixing the cells. Several images per well can be acquired and the integrated dispenser enables kinetic experiments. The instruments can process 384 and 1536-well/microtiter plates. The instruments can process 384 and 1536-well/microtiter plates.

Since 2014, Axxam has been allowed to perform radioactive activities in order to internally set up biological radioactive assays and radioactive screening of compounds. The dedicated laboratory includes a Tri-Carb 2900Tr Packard-TopCount liquid scintillation counter and MicroBeta® TriLux Perkin Elmer 1450 LSC multi-detector instrument designed for liquid scintillation or luminescence detection of samples in microplates, tubes or on filters. According to local fire department and Italian institution, Axxam has obtained authorization to acquire, manage and waste beta-isotopes such as S35, P32, P33, C14 and 3H.

5 – Universiteit Leiden / University of Leiden (ULEI)

Description of the legal entity

Leiden University (The Netherlands) is one of Europe's leading research universities with 7 faculties in Leiden and The Hague. ULEI has 6,500+ staff members and 26,900 students. ULEI has been an international university from its founding in 1575. The motto of the University is 'Praesidium Libertatis' – Bastion of Freedom. In the first decades of its existence, the university was host to many international students, from Europe and beyond. Since those early days, it has developed into a truly international university, annually hosting more than 3,000 international and exchange students in its Bachelor and Master programs, almost 1,000 international PhD students and more than 800 international academic staff. Leiden University continuously ranks in the top 100 of universities worldwide in all relevant rankings. The university is member of several international university networks. Membership in the League of European Research Universities is of particular strategic importance for Leiden University.

The Leiden Academic Centre for Drug Research of ULEI is a first-class environment for innovation in drug research as judged by an international peer review committee in 2016. Its research divisions encompass all disciplines relevant for advanced drug research, including pharmacology, toxicology, medicinal chemistry and analytical biosciences. The Centre is continuously positioned in the top-20 of the QS World University Rankings.

Key personnel

Ad P. IJzerman (M | Lead WP4) obtained his PhD degree from VU Amsterdam and landed at Leiden University thereafter where he became full professor of medicinal chemistry at LACDR in 2000. His focus has always been on new pharmacological concepts for future drugs, including inverse agonism and allosteric modulation in the research domain of G protein-coupled receptors. He has obtained national and international funding (both EU and USA) for his research and led many national and international public-private partnerships over the years. In this way he has gained ample expertise in leading collectives of scientists of vary different backgrounds and ages, including IMI's K4DD consortium. He has also formalized international education programs in these consortia to provide young research fellows with both technical and soft-skills training. This expertise will be made available to RESOLUTE. He is the (co)author of >350 publications and inventor on a dozen patents. He has chaired a number of professional organizations in the pharmaceutical sciences in

The Netherlands, including FIGON, the federation of innovative drug research, and the Medicinal Chemistry Society.

Laura H. Heitman (F) is associate professor of molecular pharmacology at LACDR/ULEI, where she obtained her PhD degree in 2009. Her interests are to establish and develop new technologies to better interrogate drug targets. She has pioneered the use of so-called label-free technologies to sensitively measure cellular changes induced by drug-target interactions. She has been awarded a personal excellence Veni grant by the Dutch Research Council NWO. She is the (co)author on >60 publications.

Relevant publications

Zheng, Y., et al., *Structure of CC chemokine receptor 2 with orthosteric and allosteric antagonists.* Nature, 2016. **540**(7633): p. 458-461. This paper for the first time shows the simultaneous presence of two antagonists in the crystal structure of the chemokine CCR2 receptor, a typical G protein-coupled receptor. It provides evidence that drug targets such as SLCs can be occupied by multiple ligands, turning them into 'allosteric machines'.

Guo, D., et al., *Kinetic Aspects of the Interaction between Ligand and G Protein-Coupled Receptor: The Case of the Adenosine Receptors.* Chem Rev, 2017. **117**(1): p. 38-66. This paper is a critical review of the importance of structure-kinetics relationships next to more traditional structure-activity relationships. It emphasizes that for any drug target, including SLCs, binding kinetics are of eminent relevance and serve as an additional triage criterion in the selection of drug candidates in the early phases of drug discovery.

Hillger, JM., et al., *Whole-cell biosensor for label-free detection of GPCR-mediated drug responses in personal cell lines.* Biosens Bioelectron, 2015 **74**: p. 233-42. This paper reflects our first endeavors to use label-free technologies for cell-based pharmacology. It shows that drug action can be very sensitively monitored in individual cell lines, providing convincing evidence that personalized medicine is within reach indeed. This is the technology that will be ULEI's contribution to RESOLUTE.

De Bruyn, T., et al., *Structure-based identification of OATP1B1/3 inhibitors.* Mol. Pharmacol, 2013. **83**(6): p. 1257-1267. This paper is a collaborative effort to allow successful screening of new inhibitors of transport proteins such as SLCs.

Liu, W., et al., *Structural basis for allosteric regulation of GPCRs by sodium ions.* Science, 2012. **337**(6091): p. 232-236. This paper provides a very high resolution crystal structure of the adenosine A2A receptor, showing an intriguing network of water molecules inside the transmembrane protein. It also provides evidence for the importance of explicit water molecules in the ligand binding site to accommodate ligands of various chemical nature. Such explicit water molecules may also dictate the behavior of transport proteins such as SLCs.

Relevant previous projects or activities

EU-IMI K4DD – public-private partnership (2012-2017); this 20 partner consortium studied the relevance of target binding kinetics for drug discovery.

TI Pharma (the Netherlands): the GPCR forum – public private partnership (2007-2012); this 10 partner consortium studied novel pharmacological concepts for G protein-coupled receptors (GPCR).

TI Pharma (the Netherlands): Binding Kinetics – public private partnership (2009-2014); this 3 partner consortium studies studied the importance of target binding kinetics for the chemokine CCR2 receptor.

NWO-TOP grant (Dutch Research Council, IJzerman) – personal excellence grant (2011-2017); in this project allosteric modulation of GPCRs was the leading theme, following the structure elucidation of the adenosine A2A receptor.

NWO-Veni grant (Dutch Research Council, Heitman) – personal excellence grant (2010-2015); this grant allowed the setting-up of label-free technologies as a new means to interrogate drug-target interactions.

Relevant infrastructure and/or other achievements

Radioligand binding facilities, label-free technologies, medicinal chemistry infrastructure, chem- and bioinformatics software suite.

6 – Max Planck Gesellschaft Zur Förderung der Wissenschaften / Max-Planck Institute for Medical Research (MPIMR)

Description of the legal entity

The **Max-Planck Institute for Medical Research** (Heidelberg) focuses its research on the interface between biology, chemistry, physics and materials science. A specific goal is to discover and develop novel chemical and physical approaches that analyze biomolecular mechanisms, modulate and control cellular physiological function, and image molecular interactions at the nanometer level, non-invasively and in real-time. These approaches should provide a quantitative understanding of the (patho)physiology of cells at the molecular level. Our laboratory has introduced a number of important and widely used research tools and used these tools to make important biological discoveries. These past achievements give us confidence that we will be able to achieve the ambitious goals of the present research application.

Key personnel

Kai Johnsson (M | Lead WP3) has >20 years of experience in chemical biology and his major scientific achievements were in the following three areas:

Protein-based tools for research in biology and medicine. One of the main achievements of our lab is the development of a new class of protein tags, i.e. SNAP-tag, CLIP-tag and ACP-tag, which can be labeled with synthetic probes in living cells. These tags have become popular research tools as they enable scientists to approach problems that cannot be resolved with conventional techniques. SNAP-, CLIP- and ACP-tag are commercialized through New England Biolabs and our papers introducing the tags have been citied 710, 305 and 210 times, respectively. In 2014 we introduced a conceptually new class of bioluminescent biosensors, published in Nature Chemical Biology, that are ideally suited for point-of-care therapeutic drug monitoring. The technology is the basis of new a start-up from our lab (Lucentix) that aims at its further development for point-of-care applications.

New fluorescent probes. One of the key strengths of our laboratory is our expertise in synthetic chemistry. This has allowed us to design and synthesize various novel fluorescent probes to visualize biochemical activities. For example, we have generated probes for superresolution imaging of the cytoskeleton in living cells. Work describing these probes has been published in Nature Methods and Nature Chemistry in the last two years. These probes lead to a start-up from our lab (Spirochrome) and a new product for protein labeling from New England Biolabs, underscoring the utility of these reagents.

Drug mechanism of action. Our group has a longstanding interest in studying the mechanism of action of clinically used drugs or drug candidates. We have developed a new yeast three-hybrid method for the identification of the protein targets of bioactive molecules, published in Nature Chemical Biology in 2011. Using this approach, we were able to identify the target of the important anti-inflammatory drug sulfasalazine, whose mechanism of action has remained obscure since its introduction in 1942! These findings also suggested a new therapeutic use for sulfasalazine, namely for the treatment of neuropathic pain. A clinical trial to evaluate the potential of sulfasalazine for the treatment of neuropathic pain is currently conducted by the group of Clifford Woolf at Boston Children's Hospital. We have furthermore identified an off-target for anti-bacterial sulfa drugs that provides a rational for the CNS side effects of this important class of drugs. This work, which was published in Science in 2013, should also translate into an improved medical use of sulfa drugs. Finally, we have elucidated the unusual mechanism of action of a new class of anti-tuberculosis drug candidates that are in preclinical development. This work, which was published in JACS in 2012, represents an important step in the development of these molecules towards clinical use.

In summary, our laboratory has introduced a number of important and widely used research tools and used these tools to make important biological discoveries. These past achievements give us confidence that we will be able to achieve the ambitious goals of the present research application.

His research focuses on development of chemical tools to study protein function and drug mechanisms incl. the introduction of approaches to specifically label proteins in living cells (e.g. SNAP-tag, CLIP-tag, ACP-tag). Johnsson developed various new fluorophores for live-cell imaging such as the SiR probes SiR-tubulin, SiR-actin etc. Current work focuses on the development of fluorescent sensors for measuring metabolite and drug concentrations in live cells, development of fluorescent and bioluminescent probes and methods to

identify and characterize drug-protein interactions. He is a new director at Max Planck Institute for Medical Research in Heidelberg and a reviewing editor for *Science.*

Philipp Leippe (M | Co-lead WP3) obtained his PhD in Organic Chemistry at the Ludwig-Maximilians-University in Munich, Germany. During his PhD, he combined synthetic chemistry and protein engineering to light-control ion channels, GPCRs and receptor tyrosine kinases. In October 2018, he joined RESOLUTE as a postdoc to develop genetically encoded biosensors for quantitative transporter assays.

Relevant publications

Lukinavicius, G., et al., *Fluorogenic probes for live-cell imaging of the cytoskeleton.* Nat Methods, 2014. 11(7): p. 731-733. This paper describes novel far-red, fluorogenic probes that, coupled with stimulated emission depletion microscopy, allow for unprecedented resolution for imaging intracellular structures.

Lukinavisius, G., et al., *A near-infrared fluorophore for live-cell super-resolution microscopy of cellular proteins.* Nat Chem, 2013. **5**(2): p. 132-139. The paper describes a silicon-rhodamine probe that can be coupled specifically to proteins using different labelling techniques. A similar approach will be applied to SLCs within RESOLUTE.

Haruki, H., et al., *Tetrahydrobiopterin biosynthesis as an off-target of sulfa drugs.* Science, 2013. **340**(6135): p. 987-991. It reveals an unexpected aspect of the pharmacology of sulfa drugs, providing a mechanist explanation of some of the side effects of these drugs through inhibition of tetrahydrobiopterin biosynthesis.

Masharina, A., et al., *A Fluorescent Sensor for GABA and Synthetic GABA(B) Receptor Ligands.* J Am Chem Soc*,* 2012. **134**(46): p. 19026-19034. This paper describes the GABA-Snifit fluorescent sensors, which allow for measurement of intracellular concentrations of GABA.

Chidley, C., et al., *A yeast-based screen reveals that sulfasalazine inhibits tetrahydrobiopterin biosynthesis.* Nat Chem Biol, 2011**. 7**(6): p. 375-383. We describe a yeast three-hybrid screen to detect drug-protein interactions, providing insights in the mechanism of clinically approved drugs.

Relevant previous projects or activities

Swiss National Science Foundation (SNSF), *Determining the intracellular concentration of therapeutic peptides with sub-cellular resolution* (2013-2015). Novel fluorescent sensors to measure drug uptake were introduced.

Swiss National Science Foundation (SNSF), *Identifying the mechanism of action of biguanides through chemical biology* (2014-2017). This project aims at uncovering direct protein targets of metformin and phenformin.

Swiss National Science Foundation (SNSF), *New Sensors and Fluorogenic probes for biology* (2013-2016). New sensors for measuring cofactor and metabolite concentrations in live cells were developed.

Swiss National Science Foundation (SNSF), *Identifying and characterizing drug-protein interactions* (2013-2016). Strategies to identify the binding partners of bioactive compounds were developed.

Marie-Curie ITN Sphingonet, *Shingolipid Homeostasis: Basic Biology and Applications* (2012-2016). New sensors for sphingolipids were developed.

Relevant infrastructure and/or other achievements

MPG offers state-of-the-art infrastructure for research in the field of chemical biology incl. the infrastructure for organic synthesis, biochemistry, cell biology as well as imaging.

7 – Universität Wien / University of Vienna (UNIVIE)

Description of the legal entity

The **University of Vienna** encompasses a broad spectrum of scientific disciplines, ranging from theology, jurisprudence, economics, computer science, to the humanities and to the social and natural sciences (https://www.univie.ac.at). The Pharmacoinformatics Research Group (https://pharminfo.univie.ac.at),

headed by G. Ecker, is part of the Division of Drug Design and Medicinal Chemistry at the Department of Pharmaceutical Chemistry, Faculty of Life Sciences. The groups' expertise lies on structural modelling of proteins, structure-based drug design, chemometric and *in silico* chemogenomic methods, statistical modelling and machine learning approaches for development of predictive computational systems with focus on transporters. Furthermore, the group has ample experience in integration and mining of open data sources for answering complex drug-discovery related research questions. Especially the generation of data mining workflows as well as knowledge on transporter modeling will be of special use for RESOLUTE.

Key personnel

Gerhard Ecker (M | Lead WP8) is an expert in transporter informatics with focus on transporters expressed in the liver and at the blood-brain barrier. Methodologies provided comprise all areas of computational drug design, ranging from 2D- and 3D-QSAR studies on modulators of P-glycoprotein, machine learning based classification and prediction of substrates and non-substrates for several ABC-transporters and the hERG potassium channel, as well as structure-based modelling approaches for transmembrane transporters. The group is also engaged in the development of similarity-based descriptors and the application of artificial neural networks and deep learning. With the coordination of the Open PHACTS project, G. Ecker got massively involved in semantic data integration and mining of large scale integrated life science data. Special emphasis is put on the development of workflows for targeting complex research questions. Gerhard served as President of the European Federation for Medicinal Chemistry 2009-2011, and is currently one of the Directors of the Open PHACTS Foundation.

Daniela Digles (F | Senior Lecturer) has strong expertise in semantic data integration and development of workflows for complex queries across multiple data domains. Daniela developed KNIME workflows for mining the Open PHACTS Discovery Platform in order to pursue read across and transporter selectivity profiling. She is also involved in the technical user support of the Open PHACTS Discovery Platform.

Relevant publications

Digles, D., et al., *Open PHACTS Computational Protocols for in silico Target Validation of Cellular Phenotypic Screens: Knowing the Knowns.* Med Chem Comm, 2016. **7**: p. 1237-44. This paper describes the application of KNIME workflows to mine the OPEN PHACTS Discovery Platform for analysis of phenotypic screening data. Analogous approaches will be used for data mining in RESOLUTE WP8.

Azzaoui, K., et al., *Scientific competency questions as the basis for semantically enriched open pharmacological space development.* Drug Discov Today, 2013. **18**: p. 843-52. This paper outlines the process how the Open PHACTS project prioritized data sources. An analogous approach will be used for prioritizing public data sources for the RESOLUTE SCL Knowledge Hub.

Kotsampasakou, E., Ecker, GF*., Predicting drug-induced cholestasis with the help of hepatic transporters – an in silico modeling approach.* J Chem Inform Mod, 2017. **57**: p. 608-615. This paper outlines the combination of transporter modeling with data mining and statistical modeling to develop predictive computational models for DILI. Analogous statistical techniques will be used for QSAR modeling in RESOLUTE WP2.

Ratnam, J., et al., *The application of the Open Pharmacological Concepts Triple Store (Open PHACTS) to support Drug Discovery Research.* PLoS One, 2014. **9**:e115460. This paper describes the massive use of KNIME workflows for analyzing integrated open data and thus exemplifies the experience of UNIVIE in developing and applying KNIME workflows.

Goldmann, D., et al., *Empowering pharmacoinformatics by linked life science data.* J Comp Aided Mol Des, 2017. **31**: p. 319-28. This paper provides an overview of the various modeling techniques UNIVIE uses for transporter modeling.

Relevant previous projects or activities

IMI Open PHACTS - *The Open Pharmacological Concepts Triple Store* (03/2011 – 02/2016); the Open PHACTS project developed a semantically integrated life science data warehouse, which comprises data on compounds, targets, pathways, disease, and patents and is open to the public. UNIVIE served as academic coordinator of this 20 Mio € project and was mainly engaged in use case development, platform testing, and data mining.

FWF (Austrian) SFB35 - *Transmembrane transporter in health and disease* (01/2008 – 12/2017); this special research cluster focuses on a set of SLC- and ABC-transporter involved in neurological disorders and drug resistance; the Ecker lab is running sub project 2 - The molecular basis of drug-transporter interaction. This comprises ligand- and structure-based modeling of SERT/DAT/NET, GAT1-3, BGT1, LAT1, ABCB1, B4, B11, G2, and C2. Furthermore, UniVie is setting up a transporter informatics workspace to ensure sustainability of the data created.

H2020 EuToxRisk - *An Integrated European 'Flagship' Program Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st Century* (11/2015 – 10/2021); UniVie is engaged in *in silico* assessment of all study case compounds, which includes mining of the Open PHACTS Discovery Platform and other public databases, as well as building predictive *in silico* models for selected toxicity endpoints. The data compiled will also support the establishment of new Adverse Outcome Pathways (AOPs) for toxicity endpoints. Finally, UniVie provides biological assays for measuring the interaction of compounds with a set of ABC-transporter.

IMI eTOX - *Integrating bioinformatics and chemoinformatics approaches for the development of expert systems allowing the in silico prediction of toxicities* (01/2010 – 12/2016); UniVie created a set of *in silico* models for prediction of compound-transporter interaction and implemented them in eTOXsys as well as in the Vienna livertox workspace, which is open to the public. Furthermore, models for predicting cholestasis, DILI, and hyperbilirubinemia were developed and implemented.

IMI K4DD - *Kinetics for drug discovery* (11/2012 – 10/2017); K4DD focuses on all aspects of ligand binding kinetics and its importance in drug discovery and development. UniVie built and hosts the consortium internal database of all data created during the lifetime of the project (db.k4dd.eu). This includes also respective loading and analysis tools. At the end of the project, all data will be transferred to the public domain via donation to ChEMBL (hosted by EBI). UniVie also developed a set of QSKR models for Hsp90 ligands.

Relevant infrastructure and/or other achievements

The group works with several small computer clusters and has access to the Vienna Scientific Cluster. In the framework of the IMI K4Dd project, the Ecker lab built and hosts the K4DD database (db.k4dd.eu). The group also runs a web service for prediction of compound-transporter interaction and liver toxicity (livertox.univie.ac.at), which is steadily expanded towards other transporter and toxicological end points.

8 – Pfizer Ltd. (Pfizer)

Description of the legal entity

At **Pfizer**, we apply science and our global resources to bring therapies to people that extend and significantly improve their lives. We strive to set the standard for quality, safety and value in the discovery, development and manufacture of health care products. Our global portfolio includes medicines and vaccines as well as many of the world's best-known consumer health care products. Every day, Pfizer colleagues work across developed and emerging markets to advance wellness, prevention, treatments and cures that challenge the most feared diseases of our time. Consistent with our responsibility as one of the world's premier innovative biopharmaceutical companies, we collaborate with health care providers, governments and local communities to support and expand access to reliable, affordable health care around the world. For more than 150 years, Pfizer has worked to make a difference for all who rely on us.

The Medicine Design group within Pfizer will contribute to the Unlock SLC/RESOLUTE project with contributors located in the United States. The contributors have backgrounds in medicinal chemistry, pharmacology, metabolomics, bioinformatics, data analysis and laboratory based SLC research.

Key personnel

Claire Steppan, PhD (F | EFPIA Co-lead overall), an Associate Research Fellow in Pfizer's Primary Pharmacology, has played an integral role in establishing Pfizer's enabling SLC platform. Claire is an accomplished pharmacologist with scientific leadership spanning from early discovery through clinical development in obesity, diabetes and neurodegenerative diseases. Within Pfizer, she has been the research project leader for multi-disciplinary teams that produced two FIH starts and one Phase 2 start for the

treatment of Type 2 diabetes. She has a long-standing interest in SLC transporters beginning with Pfizer's SGLT2 inhibitor in which her group provided all the pharmacology data for the PhIII clinical candidate to the current global efforts within Pfizer with Dr. David Hepworth on enabling SLC transporters as therapeutic targets. She has presented at international conferences on transporters and recently organized a New York Academy of Sciences symposia on SLC transporters.

David Hepworth, D. Phil (M | EFPIA Co-lead overall), is Head of Medicinal Chemistry for Pfizer's Inflammation and Immunology group leading a team of around 60 Pfizer scientists across research sites in Cambridge Massachusetts and Groton, Connecticut discovering new small molecule drug candidates for the treatment of inflammatory, autoimmune and fibrotic diseases. Previously he has worked across a number of disease areas and Pfizer sites. He has a long-standing interest in membrane proteins and SLC transporters in particular, having worked in the area since his first Pfizer project in 1999 focusing on the serotonin transporter, through the initiation of Pfizer's SGLT2 program for diabetes treatment (leading to a molecule currently in PhIII clinical development) to current research interests. In recent years, David has co-led, together with Dr Claire Steppan, a Pfizer team building research capabilities in SLC transporters. He has given numerous presentations at international conferences on transporters, and organized several conferences on this topic.

Matthew Crawford, PhD (M | Computational Biologist), is a biologist who has spent the last ten years supporting target selection and prioritization, primarily by developing web-based tools for integrating structured and unstructured content. He is representing the expertise of the Computational Sciences group of Pfizer, which specializes in scientific tool development, bio-and-chemoinformatics, and experimental results analysis.

Relevant publications

César-Razquin, A., et al., *A call for systematic research on solute carriers.* Cell, 2015 **30**(162): p. 478-87. A recent review highlighting how SLCs as drug targets offer huge potential but are understudied thereby requiring large, systematic efforts to deorphanise and study transporters and unlock their potential.

Vincent, F., et al., *Developing predictive assays: the phenotypic screening "rule of 3".* Sci Transl Med, 2015. **7**(293): p. 293ps15. This commentary addresses critical elements for optimal assay design and execution for phenotypic screens.

Huard, K., et al., *Discovery and characterization of novel inhibitors of the sodium-coupled citrate transporter (NaCT or SLC13A5).* Sci Rep, 2015. **5**: p. 517391. These studies demonstrate of *in vitro* and *in vivo* efficacy of a small molecule inhibitor of SLC13A5.

Mascitti, V., et al., *Discovery of a clinical candidate from the structurally unique dioxa- bicyclo[3.2.1]octane class of sodium-dependent glucose cotransporter 2 inhibitors.* J Med Chem, 2011. **54**(8): p. 2952. This study presents the design, synthesis, preclinical *in vitro* and *in vivo* pharmacology of PF-04971729. Ertugloflozin is awaiting FDA approval for the treatment of type 2 diabetes.

Robinson, RP., et al., *C-Aryl glycoside inhibitors of SGLT2: Exploration of sugar modifications including C-5 spirocyclization.* Bioorg Med Chem Let, 2010. **20**: p. 1569-1572. Modifications to the sugar portion of C-aryl glycoside sodium glucose transporter 2 (SGLT2) inhibitors were evaluated preclinically with *in vitro* and *in vivo* efficacy.

Relevant previous projects or activities

Leader/Manager and/or contributor to several proprietary Pfizer programs targeting SLC transporters and Pfizer's Global SLC enabling platform.

Leader in establishing Pfizer's metabolomics platform to aid in SLC deorphanising.

Co-organizers of international symposium Unlocking SLCs for Effective Therapies, at the New York Academy of Sciences, April 2016; Organizing committee member for Bioparadigms Biomedical Transporters Conferences, Lugano (2015); Lausanne (2017).

Computational tools generated within Pfizer: GeneFamilyTool, a visual interface for querying over 3,000 structural, genetic, phenotypic, indications-specific traits, and expression profiles to assist in the prioritization

of targets; Coronte, an automated suite of tools for integrating ontologies using supervised machine learning based on lexical and semantic word meanings.

Relevant infrastructure and/or other achievements

Pfizer offers access to Pfizer's Research capabilities including assay development and optimization for high throughput screening, leading edge metabolomics platform, protein expression and purification expertise for integral membrane proteins, and computational sciences group. This includes access to high throughput and high content screening systems (FLIPR, Envision, InCell6000, Opera, Flow cytometry), our structural biology and biophysics department, and Computational Sciences group of Pfizer, which specializes in scientific tool development, bio-and-chemoinformatics, and experimental results analysis. Our metabolomics capabilities include untargeted metabolomics using ion-pairing LC HRMS for both positive and negative polarities, untargeted lipidomics capable of detecting all major lipid subclasses and integrated omics for data processing. Dedicated metabolomics instruments are Q-exactive plus and Q-exactive high field both equipped with Agilent 1290 UPLC pumps.

9 – Novartis Pharma AG (Novartis)

Description of the legal entity

Novartis Pharma, the Novartis Institute for biomedical research (NIBR) Basel, Switzerland. Co-located with our corporate world headquarters, NIBR Basel is an integral part of the Bio Valley, Europe's biotechnology hub. Drug discovery here focuses on autoimmunity, transplantation, and inflammation, as well as musculoskeletal diseases, neuroscience, and oncology. The Chemical Biology and Therapeutics unit (CBT), is an early pipeline target and lead-finding engine built on the principle of collaboration and partnership. Our focus is on the integration of target discovery, emerging technologies and expanded lead generation methods to drive innovation in identifying novel therapeutics. Our approach to Chemical Biology creates new types of small molecules – and biomolecules – to illuminate human biology. We apply chemical tools and technologies to interrogate biological processes and targets. These efforts help in identifying innovative ways of understanding disease and mechanisms, to drug targets and processes, while also delivering new therapeutic modalities. Our interdependent Foundational Areas work alongside disease-area scientists in integrated teams and as part of a scientific network within our internal and external community.

Key personnel

Klaus Seuwen (M | Lead WP 1) is Executive Director at NIBR in Basel. He currently leads target discovery efforts supporting several therapeutic areas.

Juergen Reinhardt (M | Lead WP7) is Senior Investigator II at the Novartis Institute of Biomedical Research; Chemical Biology and Therapeutics. He has > 15 years' experience in industrial cellular lead finding which includes Transporters and Ion channels related to RESOLUTE'S focus, target identification and project team head function.

Relevant publications

Yamada, K., et al., *Discovery and Characterization of Allosteric WNK Kinase Inhibitors.* ACS Chem Biol, 2016. **11**(12): p. 3338-3346. Lead finding and pharmacological modulation of WNK1 kinases regulating the NKCC transporter.

Qiu, Z., et al., *SWELL1, a plasma membrane protein, is an essential component of volume-regulated anion channel.* Cell, 2014. **157**(2): p. 447-58. Identification of a missing link in cell volume regulation, a hallmark of ion channel and transporter function.

Kümmel, A., et al., *Differentiation and visualization of diverse cellular phenotypic responses in primary high-content screening.* J Biomol Screen, 2012. **17**(6): p. 843-9. Differentiation of phenotypic readouts for lead finding, an element in WP1,3 and 6.

Lerner M et al. *An extracellular loop of the human nongastric H,K ATPase alpha subunit is involved in apical plasma membrane polarization.* AJP 2006. **18**(1-3): p. 75-84. Spatial distribution and regulation of ion transporting secondary active ATPases.

Reinhardt, J., et al., *Stimulation of protein kinase C pathway mediates endocytosis of human nongastric H, K ATPase, ATP1AL1.* AJP, 2002. **283**(2): p. F335-43. Spatial distribution and regulation of ion transporting secondary active ATPases.

Relevant previous projects or activities

The Novartis team brings significant experience with the target family of G protein-coupled receptors and associated signaling pathways.

Relevant infrastructure and/or other achievements

Novartis offers access to global Novartis Research lead finding platforms and all relevant core facilities, to Novartis compound libraries and academic collaboration libraries, to molecular tool boxes, proteins, antibodies, cell line repository, cDNA and sgRNA and siRNA libraries, high throughput and high content screening systems, high throughput cell engineering platform, genetic screening facilities as well as a protein science unit including structural biology and biophysics department.

10 – Boehringer-Ingelheim (Boehringer)

Description of the legal entity

Boehringer Ingelheim group is one of the world's 20 leading pharmaceutical companies. Headquartered in Ingelheim, Germany, it operates globally with 145 affiliates and more than 42,000 employees. Since it was founded in 1885, the family-owned company has been committed to researching, developing, manufacturing and marketing novel products of high therapeutic value for human and veterinary medicine.

Key personnel

Charles Whitehurst (M) is the Senior Associate Director of the Drug Concept Discovery, Inflammation & Respiratory Diseases Department at Boehringer Ingelheim.

Martin Lenter (M) is the Director of Target Validation Technologies at Boehringer Ingelheim.

Bradford S. Hamilton (M | principal scientist) earned his Ph.D. in Medical Science, and has 20 years of experience in the pharmaceutical industry with Boehringer Ingelheim in metabolic diseases research.

Holger Klein (M) is the Head of Computational Biology Expert Function at Boehringer Ingelheim.

Relevant publications

Yi, G., et al., *Structural and functional attributes of the Interleukin-36 receptor.* J Biol Chem, 2016. **291**(32): p. 16597-16609. *Demonstrates expertise in Cell Biology and Biochemistry.*

Cortez, A., et al., *Incorporation of Phosphonate into Benzonaphthyridine Toll-like Receptor 7 Agonists for Adsorption to Aluminum Hydroxide.* J Med Chem, 2016. **59**(12): p. 5868-5878. Demonstrates experience in small molecule drug discovery.

Saha, SS., et al., *Signal transduction and intracellular trafficking by the interleukin 36 receptor.* J Biol Chem, 2015. **290**(39): p. 23997-24006. Demonstrates experience in signal transduction and molecular immunology.

Wu, TY., et al., *Rational design of small molecules as vaccine adjuvants.* Science Transl Med, 2014. **6**(263): p. 263ra160. Demonstrates experience in small molecule drug discovery.

Birzele, F., et al., *Analysis of the transcriptome of differentiating and non-differentiating preadipocytes from rats and humans by next generation sequencing.* Mol Cell Biochem., 2012. **369**: p. 175-181. Demonstrates experience with RNAseq analysis.

Relevant previous projects or activities

Boehringer has been actively involved in previous IMI projects, e.g. BT-Cure, Cancer ID, COMPACT and DIRECT all aimed at addressing unmet medical needs and delivery value to patients.

Proprietary projects involving SLCs including development of SGLT2 inhibitors.

Relevant infrastructure and/or other achievements

Access to state-of-the-art sequencing core facility. Demonstrated expertise in developing SLC modulators as therapeutics.

11 –Vifor (International) AG (Vifor)

Description of the legal entity

Vifor (International) AG, is a global specialty pharmaceuticals company that researches, develops, produces and markets its own pharmaceutical products and is the partner of choice for innovative, patient-focused solutions. Vifor Pharma Group has a long history in the research and development of pharmaceutical products for treating iron deficiency and is continually evaluating and optimising its nephrology and cardio-renal therapies. The pre-clinical research of **Vifor (International) Ltd.** as an entity of Vifor Pharma Group is focusing on development of novel medicines targeting molecules involved in iron metabolism, kidney disease and cardio-renal indications. As an example, an oral inhibitor of Ferroportin (SLC40A1) for treatment of iron overload conditions has been discovered and is presently in development for testing in humans. The expertise of Vifor's teams in analytics of inorganic ions and development of transporter cellular assays will be employed to support the RESOLUTE project.

Key personnel

Maria Wilhelm (F |Co-lead of WP2) is Head of Analytical Development, Chemical and Pre-clinical R&D, Vifor (International) Ltd., St. Gallen. She performed her studies and PhD in biology/ecology at the Leopold-Franzens-University of Innsbruck, Austria. From 2001 until present she is engaged in the development of new analytical methods for the characterization of drug substances, for routine and stability testing of drug substances and products, the establishment of state-of-the-art techniques, e.g. Inductively Coupled Plasma - Mass Spectrometry, Liquid chromatography with Mass spectrometer (LC-MS), Quantitative Chemometric Models for Near Infrared Spectroscopy (NIR) as well as in the validation of analytical methods for drug substances/products. Furthermore, she has been supporting Regulatory Affairs with respect to dossiers, questions from Health Authorities.

Vania Manolova (F |Co-lead of WP3 and WP6) is Head of Biology in the Chemical and Pre-clinical R&D, Vifor (International) Ltd., Zurich. She performed her PhD at the University of Basel investigating dendritic cell biology and immunology aspects of presentation of self and bacterial antigens to T cells. From 2002-2010 Ms. Manolova was engaged in pre-clinical development of virus like particles-based vaccines targeting cancer, asthma and autoimmune diseases at Cytos Biotechnology, Zurich. From 2011 until present she deals with the discovery and pre-clinical development of drugs in hematology and nephrology therapeutic areas.

Relevant publications

Neiser, S., et al., *Physico-chemical properties of the new generation IV iron preparations ferumoxytol, iron isomaltoside 1000 and ferric carboxymaltose.* Biology of Metals, 2015. **28**(4): p. 615-635. This paper is a critical comparison of the latest IV iron preparations to the older IV iron preparations. The presented data are a step forward in the characterization of these non-biological complex drugs, which is a prerequisite to understand their cellular uptake mechanisms and the relationship between the structure and physiological safety as well as efficacy of these complexes.

Wilhelm, M., et al., *The iron-based phosphate binder PA21 has potent phosphate binding capacity and minimal iron release across a physiological pH range in vitro.* Clinical nephrology, 2014. **81**(4): p. 251-258. This paper for the first time shows the mode-of-action of the iron-based phosphate binder PA21. It provides evidence that PA21 works successfully in the physiological pH-range.

Dürrenberger, F., et al., *Functional characterization of fluorescent hepcidin.* Bioconjug Chem, 2013. **24**(9): p. 1527-32. In this paper the synthesis of fluorescent hepcidin and the development of cell-based assays for quantification of ferroportin (SLC40a1) internalization have been described. These cell-based assays have been used in a pre-clinical program at Vifor to identify inhibitors of ferroportin. Similar approaches might help to develop medium throughput assays and characterize some prioritized SLCs in WP3 and WP6.

Manolova, V., et al., *Ferroportin inhibitors improve ineffective erythropoiesis and prevent iron loading in a beta-thalassemia disease model. Seventh Congress of the International BioIron Society (IBIS) Biennial World Meeting (BioIron 2017) May 7 – 11, 2017.* Am J Hematol, 2017. **92**(8). The data presented summarized the development of a novel small molecule ferroportin inhibitor and its efficacy in disease models of hemochromatosis and thalassemia. The gain of knowledge in the biology of ferroportin as a member of the SLC protein family will certainly aid the research of Vifor's team in the RESOLUTE project.

Relevant previous projects or activities

Vifor's Biology group has worked on several drug discovery projects aiming to develop inhibitors targeting SLC molecules. Among them DMT1 (SLC11A2) and ferroportin (SLC40A1) are involved in the dietary absorption of iron and are implicated in pathology of iron loading anemias and hereditary hemochromatosis. Vifor's Biology team aimed to discover inhibitors of DMT1 and ferroportin as disease modifying medication in these disorders. Orally bioavailable ferroportin inhibitors with low nanomolar potency and good efficacy in disease models and favorable safety profile have been identified and currently forwarded to clinical development. Another protein from the SLC family that attracted the attention of Vifor's research team is NaPi2b (SLC34A2) which transports inorganic phosphate from the intestinal lumen into epithelial cells. The biology group of Vifor identified inhibitors of NaPi2b (SLC34A2), which blocked efficiently the transport of inorganic phosphate in disease models. However, this project has been discontinued because of lack of clinical efficacy of competitor's NaPi2b inhibitor.

Relevant infrastructure and/or other achievements

Analytical laboratories equipped with instruments and infrastructure allowing experimental work in the field of ionomics and analytical chemistry. Major technical equipment includes inductively Coupled Plasma – Optical Emission Spectrometer, high resolution – Inductively Coupled Plasma – Mass Spectrometer, (Ultra) Liquid chromatographs equipped with Refractive Index detectors, Photodiode array detectors, electrochemical detectors, mass spectrometer (Q-Time of flight, Triple Quadropol, Single Quadropol), conductivity detector, Gas chromatograph with Headspace Sampler, Flame Ionization detector, Mass spectrometer, Fourier transform - Near infrared spectrometer, Fourier transform – Infrared spectrometer, Elemental analyzer (CHNS), Spectrophotometers, titration and Karl Fisher titration instruments, pH meters, centrifuges, lab shakers, mixer, drying oven, halogen moisture analyzer, etc.

Biology laboratories equipped with instruments and infrastructure allowing experimental work in the field of cell biology (P2 Biosafety level) and biochemistry. Major technical equipment includes 2 fluorescent microscopes with automated stage and medium throughput imaging capacity, Flow cytometer with automated stage and medium throughput sample acquisition capacity, q-RT-PRC cycler with medium throughput capacity, plate readers for detection of fluorescence emission and polarization, luminescence and absorption, automated liquid handling instruments (dispensers and washers) with medium throughput pipetting capacity and ultracentrifuge.

12 – Sanofi Recherche et Développement (Sanofi)

Description of the legal entity

Sanofi is a global life sciences company committed to improving access to healthcare and supporting the people we serve throughout the continuum of care. From prevention to treatment, Sanofi transforms scientific innovation into healthcare solutions, in human vaccines, rare diseases, multiple sclerosis, oncology, immunology, infectious diseases, diabetes and cardiovascular solutions and consumer healthcare. More than 100,000 people at Sanofi are dedicated to make a difference on patients' daily life, wherever they live and enable them to enjoy a healthier life. With a presence in more than 100 countries and industrial sites in more than 40 countries, our industrial network, know-how and teams deliver health solutions to millions of individuals with high quality and maximum safety, regardless wherever they are manufactured. We concentrate our research efforts where the most pressing medical needs and public health issues are, bringing solutions to patients and contributing to a healthier society worldwide.

Sanofi Aventis Research and Development is a legal entity of Sanofi. The Chilly Mazarin Site in France (Paris Area) hosts a large part of the R&D value chain, ensuring continuum between R & D and innovation in various

fields such as cardiovascular disorders, fibrosis, and neurodegenerative disorders. It brings together the skills and talents for the discovery and development of innovative therapeutic solutions, medicines addressing major public health issues and the needs of patients. The co-location of our Research and Development teams, in conjunction with hospital and academic centers of excellence, particularly in the Paris region, is stimulating our approach to Translational Medicine. Integrated within a fertile biomedical ecosystem Sanofi is connected to the global network of innovation in the field of health.

Sanofi Aventis Research and Development will be a strong contributor of the RESOLUTE consortium.

Key personnel

Bruno Biton (M | Lead WP4) received his Master's degree in 'Structure and functions of integrated biologic systems' in 1986 in Orsay, France as well as a PhD in Pharmacy. He has been a research scientist in *in vitro* cardiac electrophysiology and has contributed to several projects targeting ion channels or transporters having reached clinical phases and more particularly his team was involved in the functional characterization/profiling of Glyt-1, Glyt-2, ASC-1 inhibitors (see below). He is currently leading a team in the translational science unit at Sanofi. His main activities include target identification and validation, focusing on ion channels, expressed in their native environment, as well as mutation-induced phenotype characterization in IPSC-derived neurons and cardiomyocytes.

Frédéric Puech (M | Lead WP3) received his PhD in 1988 in Organic Chemistry at the University of Montpellier (synthesis of nucleotidic and nucleosidic analogs as potential anticancer or antiviral agents). From 1988-1992 he performed research in the virology department for the development of nucleotidic prodrugs of anti-viral agents. From 1993-2009, he has been leading various research projects in the CNS domain, projects targeting enzymes, receptors, transporters, ion channels, or based on phenotypic screening for the identification of candidates for Preclinical Development. Since 2010, he has been involved in the development of innovative internal or collaborative drug delivery projects and for the conception of probes for medical diagnosis or clinical studies.

Relevant publications

The three following publications show how in a recent past, Sanofi R&D has been involved the identification and synthesis of compounds able to interact with the glycine transporter Glyt-1 (SLC6A9):

Boulay, D., et al. *Characterization of SSR103800, a selective inhibitor of the glycine transporter-1 in models predictive of therapeutic activity in schizophrenia.* Pharmacol Biochem Behav, 2008. **91**: p. 47-58.

Depoortere, R et al. *Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic.* Neuropsychopharmacology 2005. **30**: p. 1963-1985.

Ponce, J et al. *Transmembrane domain III plays an important role in ion binding and permeation in the glycine transporter GLYT2*. J Biol Chem*,* 2000. **275**: p. 13856-13862.

The 2 following papers illustrate our expertise in the field of ion channel drug discovery and our capability to develop high throughput screening assays to tackle these targets. As electrogenic SLC transporters share many common features with ion channels, these expertises should be very useful to the project:

Bertrand, D et al. *Functional Studies of Sodium Channels: From Target to Compound Identification.* Curr Protoc Pharmacol, 2016. **75**: p. 9211-92135.

Biton, B., et al., *SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (1) binding and functional profile.* Neuropsychopharmacology, 2007. **32**(1): p. 1-16

Relevant previous projects or activities

Active member of project teams dedicated to Glyt-1 (SLC6A9), Glyt-2 (SLC6A5) and ASC-1(SLC7A10) antagonist identification. Characterization of those compounds using either patch-clamp in rat spinal cord slices (Glyt-1) or multi-array electrode/long term potentiation recordings in rat or mouse hippocampal slices (ASC-1). One compound (Glyt-1) has reached Phase I.

Scientific contribution to various SLC transporters still considered as targets in Sanofi.

Relevant infrastructure and/or other achievements

Access to utilisation of voltage-sensitive dies (µCell/FDSS), patch-clamp in routine lab work.

13 – Bayer AG (Bayer)

Description of the legal entity

Bayer AG is a global enterprise with core competencies in the Life Science fields of health care and agriculture. Its products and services are designed to benefit people and improve their quality of life. At the same time, the group aims to create value through innovation, growth and high earning power. Bayer is committed to the principles of sustainable development and to its social and ethical responsibilities as a corporate citizen. In fiscal 2016, the Group employed around 115,200 people and had sales of EUR 46.8 billion. Capital expenditures amounted to EUR 2.6 billion, R&D expenses to EUR 4.7 billion. These figures include those for the high-tech polymers business, which was floated on the stock market as an independent company named Covestro on October 6, 2015.

Bayer Lead Discovery and Drug Development have substantial experience in exploiting SLC transporters as drug targets. Despite being a druggable target class, there is a lack of broadly applicable methods to reveal physiological and molecular function of a majority of SLC transporters. Bayer regards RESOLUTE as a unique opportunity to address these shortcomings and contribute to the investigation of this underexplored target class.

Key personnel

Alexander Ehrmann (M | Lead WP6) holds a PhD in Biochemistry and is working at Bayer since 2010 as a Lab Head on the development and realization of cell-based assays for identification of new lead structures by ultra-high throughput screening.

Andreas Becker (M | Lead WP5) holds a PhD in Biochemistry and is working at Bayer since 1990 as Lab Head responsible for protein purification and characterization (1990-1998), Group Leader Proteomics (1999-2001), Director Research Informatics (2002-2007) and Director Protein Technologies since 2007.

Relevant publications

Kapoor, K., et al., *Mechanism of inhibition of human glucose transporter GLUT1 is conserved between cytochalasin B and phenylalanine amides.* Proc Natl Acad Sci USA, 2016. **113(**17): p. 4711-4716. Co-crystallization studies on human glucose transporter GLUT1 revealing the basis for subtype selectivity for different inhibitor classes.

Siebeneicher, H., et al., *Identification and Optimization of the First Highly Selective GLUT1 Inhibitor BAY-876.* ChemMedChem, 2016. **11**(20): p. 2261-2271. Description of HTS approaches and MedChem optimization to identify N-(1H-pyrazol-4-yl)quinoline-4-carboxamides with single-digit nanomolar activity on GLUT1 and selectivity vs. GLUT2, GLUT3 and GLUT4 suitable for *in vivo* studies.

Siebeneicher, H., et al., *Identification of novel GLUT inhibitors*. Bioorg Med Chem Lett, 2016. **26**(7): p. 1732-1737. Description of HTS approaches and MedChem optimization to identify 1H-pyrazolo[3,4-d]pyrimidines with single-digit nanomolar activity on GLUT1 and selectivity vs. GLUT2 suitable for *in vivo* studies.

Gottwald, M., et al., *Public-Private Partnerships in Lead Discovery - Overview and Case Studies.* Arch. Pharm. Chem. Life Sci, 2016. **349**: p. 692-697. Overview on different collaboration models with a clear focus on public-private partnerships. Learnings and key success factors for work in large consortia are outlined.

Arrowsmith, CA., et al., *The promise and peril of chemical probes.* Nat Chem Biol, 2015. **11**: p. 536–541. Publication on quality criteria and the need for high quality research tools for elucidating target biology.

Relevant previous projects or activities

ULTRA-DD - *Unrestricted Leveraging of Tools for Research Advancement and Drug Discovery*: Development of Chemical Probes; Bayer contributions: Protein expression and purification, structural biology, assay development, compound characterization, medicinal chemistry.

K4DD - *Kinetics for Drug Discovery*: Improving our understanding of the impact of binding kinetics; Bayer contributions: Protein expression and purification, structural biology, assay development, compound characterization.

ELF - *European Lead Factory*: collaborative public-private partnership aiming to deliver innovative drug discovery starting points; Bayer contributions: generation of a joint SMOL library, assay development and screening.

General achievements: Development of homogenous assays and successful realization of uHTS campaigns for various SLC transporters

Relevant infrastructure and/or other achievements

Bayer offers access to a state-of-the-art platform for expression, purification, analytics and biophysics of membrane proteins as well as a state-of-the-art assay development platform focused on homogenous cell-based assays using various luminescent and fluorescent readouts (e.g. membrane potential dyes, sodium and potassium sensors/dyes, biosensors for various metabolites).

4.2 Third parties involved in the project (including use of third party resources)

All RESOLUTE consortium members not listed in the tables below do not plan to involve any third parties in the course of the project. Please note that this might be subject to change with regards to the RESOLUTE Academic Expert Laboratories and Structural Biology Alliance as outlined in section 3.4.

1/CeMM

Does the participant plan to subcontract certain tasks?	Yes
For details on subcontracting planned by CeMM please refer to section 3.4.	
Does the participant envisage that part of its work is performed by linked third parties?	No
Does the participant envisage the use of contributions in kind provided by third parties?	Yes

2/UOX

Does the participant plan to subcontract certain tasks?	Yes
For details on subcontracting planned by UOX please refer to WP5.	
Does the participant envisage that part of its work is performed by linked third parties?	No
Does the participant envisage the use of contributions in kind provided by third parties?	No

8/Pfizer

Does the participant plan to subcontract certain tasks?	Yes
In delivery of Task 2.2 of WP2, Pfizer will work with Elucidata for access to their proprietary algorithm, Combi-T for data analysis. As metabolomics data processing and analysis is a major bottleneck to providing deconvoluted metabolomics data. Elucidata's cloud-based platform eliminates computational bottlenecks and significantly reduces the time needed to derive enriched network maps. The net result of Pfizer utilizing Elucidata will be delivery of enriched metabolic network maps in a faster timeframe to enable SLC deorphanising effort.	
Does the participant envisage that part of its work is performed by linked third parties?	No
Does the participant envisage the use of contributions in kind provided by third parties?	No

9/Novartis

Does the participant plan to subcontract certain tasks?	Yes
Sequencing efforts for cell line characterization.	
Does the participant envisage that part of its work is performed by linked third parties?	No
Does the participant envisage the use of contributions in kind provided by third parties?	No

10/Boehringer

Does the participant plan to subcontract certain tasks?	Yes
Cell line sequencing and general quality control will be outsourced to CRO.	
Does the participant envisage that part of its work is performed by linked third parties?	No
Does the participant envisage the use of contributions in kind provided by third parties?	No

12/Sanofi

Does the participant plan to subcontract certain tasks?	Yes
QC, targetetd RNA seq forcasted in WP1 as subcontracting cost in case of overload of assignment in a short period of time.	
Does the participant envisage that part of its work is performed by linked third parties?	No
Does the participant envisage the use of contributions in kind provided by third parties?	No

5. ETHICS

Human cells or tissues (Section 3 of Horizon 2020 ethics self-assessment form)

- We plan to use the established cancer cell lines 1321N1, HuH-7, SK-MEL-28, MDA-MB-468, LS 180, DMS 79 and HEK-293. These are available commercially through repositories such as ECACC (https://www.phe-culturecollections.org.uk/collections/ecacc.aspx), ATCC (https://www.lgcstandards-atcc.org/) or JCRB (http://cellbank.nibiohn.go.jp/english/). No human tissue is used in the grant.
- In the current research plan, all cell lines generated within the project and to be used elsewhere in the project originate at CeMM. We will update the cell line inventory throughout the project if new cell lines enter the consortium through any institution.

Non-EU countries (Section 6 of Horizon 2020 ethics self-assessment form):

Some of the research in the project will be conducted at EFPIA company laboratories in the USA and Switzerland. In order to conduct this research, small quantities of laboratory reagents will shipped to-from these countries and the EU. Typical research materials are small quantities of

- engineered cell lines,
- cell extracts and lysates,
- research grade chemical, antibody and protein reagents, and
- low-grade radioactive biological reagents.

All shipping of materials will be conducted according to approved import and export procedures relevant to the nature of the material to be shipped. Copies of import and export licenses will be kept on file.

Each member of the consortium will be responsible for their own actions and performing laboratory based work as per their standard operating procedures and trainings.

Environment, health & safety (Section 7 of Horizon 2020 ethics self-assessment form):

The research in this project will use laboratory materials such as

- engineered cell lines,
- cell extracts and lysates,
- research grade chemical, antibody and protein reagents, and
- low-grade radioactive biological reagents.

All materials will be handled by research staff trained to conduct the relevant work, and after having consulted all relevant research risk assessment materials (Material Safety Data Sheets, COSHH documents, etc). All work will be carried out in laboratories approved to conduct the relevant work. Laboratory waste will be disposed of according to approved local procedures and regulations. The safety classifications of each laboratory conducting the research will be kept on file.

Each member of the consortium will be responsible for their own actions and performing laboratory based work as per their standard operating procedures and trainings.

CRISPR/Cas9: ethical significance within the RESOLUTE project

The use of CRISPR/Cas9 has revolutionized modern biology in recent years, especially concerning human genetics. Consequently, in a project of this scale and with the clear aim to have an impact on human health, RESOLUTE will make use of this technique to achieve its goals.

As clearly described in the grant DoA, CRISPR/Cas9 will be the method of choice to generate the SLC knock-out cell lines, which will be at the core of all further downstream analysis (Work package number 1, Task 1.1 and Task 1.2). To put it simply, without CRISPR/Cas9 technology it would be impossible to generate these cell lines. Furthermore, CRISPR/Cas9 will be used to conduct genetic screens for SLC gene interactions (Work package 2, Task 2.5) as well as for the generation of double knock-out cell lines to confirm such genetic interactions (Work package 3, Task 3.7; Work package 7, Task 7.2).

We are highly aware of the current discussion regarding the safe and ethical use of CRISPR/Cas9. For the RESOLUTE project we do not foresee any problems in this regard, due to the following reasons:

- The nature of the human cell lines used in our project: we will only use established human somatic cell lines.
- No primary human cell lines, patient samples or animal models will ever be used within the RESOLUTE project.
- CRISPR/Cas9 technology and the derived cell lines will exclusively be used in a tightly regulated, controlled environment, with no risk for genetic drift.
- There is mounting body of evidence suggesting the very specific and exact nature of genetic alterations that can be introduced using CRISPR/Cas9.

The cell lines that will be used are either cancer cell lines (1321N1, MDA-MB-468, HuH-7, DMS 79, LS 180, SK-MEL-28) or transformed cell lines (HEK-293), which are all highly described and are per definition already "highly altered" in their genetic material as these have already acquired many mutations (hence, these are cancer cell lines). Additional mutations (the knocking-out of genes) will be introduced in a highly specific manner. In no instance will we use primary human cell lines or animal models. Furthermore, all CRISPR/Cas9 experiments will be conducted in a highly controlled environment (in petri dishes within laminar flow hoods) that prohibits any "exchange" of genetic material with the outside environment. After conducting the corresponding experiments, these cell lines can easily be disposed of – without posing any threat to human health.

Genetic interaction screen using CRISPR/Cas9 (Work package 2, Task 2.5) will be conducted in the exact same controlled environment in established human somatic cell models without any risk of genetic drift.

Off-target editing is another issue that is often raised regarding the safe use of CRISPR/Cas9 (i.e. the alteration of DNA at an unwanted site other than the intended target region). Several recent studies have addressed off-target effects of CRISPR/Cas9 (e.g. Cencic et al., PLoS Genetics 2014; Tsai et al., Nature Biotechnology 2015) and the emerging picture is the following: While off-target editing can occur in some cases, it can be minimized or even almost completely avoided when using the latest bioinformatics tools that allow for the identification of the most specific reagents. Furthermore, we would like to point out that the main focus of the studies of off-target editing relates to its potential use in a therapeutic setting in the clinic, which is not the case for the RESOLUTE project. For our intended use, the generation of model cell lines with defined genetic mutations, the CRSIPR/Cas9 technology is the only technology available to date that allows for such precise genome editing.

We are also highly aware of the ongoing legal battle regarding the patent for the use of CRISPR/Cas9 in eukaryotic cells, between "opposing" teams headed by scientists from the University of Berkeley and the Broad Institute. The RESOLUTE consortium will of course react to any changes, should the outcome of this ongoing patent dispute affect the licensing situation of the technology.

We want to emphasize again that we are not intending to use CRISPR/Cas9 in any way other than described in the RESOLUTE DoA: in established human somatic cell lines and within a tightly regulated, controlled environment. Should this change through any circumstances, we will only proceed after consulting Professor Dr. Christiane Druml, who will serve as Ethics Advisor to RESOLUTE, as well as the EC. However, such a change in research plan regarding the use of CRISPR/Cas9 is highly unlikely.

To summarise: CRISPR/Cas9 is an essential technology that will enable the successful implementation of the RESOLUTE grant. We are aware of the ethical significance of its use in mammalian cells and prerequisites for its safe use and we do not foresee any problems.

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7. ABBREVIATIONS

ADME: Absorption, distribution, metabolism and excretion; **AP-MS**: Affinity purification mass spectrometry; **B/FRET**: Bioluminescence/Fluorescence resonance energy transfer; **BioID**: Proximity-dependent biotin identification; **Cas9**: CRISPR-associated protein-9 nuclease; **cDNA**: Complementary DNA; **CETSA**: Cellular thermal shift assay; **CRISPR**: Clustered regularly interspaced short palindromic repeats; **CRO**: contract research organization; **DEP**: Dissemination and exploitation plan; **DMP**: Data Management Plan; **DOI**: Digital object identifier; **EB**: Executive board; **EBI**: The European bioinformatics institute; **EFPIA**: European federation of pharmaceutical industries and associations; **EMA**: European medicines agency; **ES+/-**: Positive / Negative electrospray ionization; **FDA**: US Food and Drug Administration; **FLIPR**: Fluorescence imaging plate reader; **Fab**: Antigen-binding fragment; **FSEC**: Fluorescence size exclusion chromatography; **GA**: General assembly; **GFP**: Green fluorescent protein; **GPCR**: G-protein coupled receptor; **HA**: hemagglutinin; **HCI**: High content imaging; **HILIC**: Hydrophilic interaction chromatography; **HPLC**: High performance liquid chromatography; **HTS**: High Throughput Screening; **ICP-MS**: Inductively coupled plasma mass spectroscopy; **ICP-OES**: Inductively coupled plasma optical emission spectrometry; **IMI2 JU**: Innovative medicines initiative 2 joint undertaking; **IP**: Intellectual property; **IRES**: Internal ribosome entry site; **KNIME**: Konstanz information miner; **K.O.**: Knock-out; **LC/GC-MS**: Liquid/gas chromatography coupled to mass spectrometry; **LCP**: Lipid cubic phase; **LPS:** Lipopolysaccharide; **mAB**: monoclonal antibody; **MOI**: Multiplicity of infection; **MRM**: Multiple reaction monitoring; **MSP**: membrane scaffolding protein; **MTP**: microtiter plate; **MTS**: Medium throughput screening; **NMR**: Nuclear magnetic resonance; **ORCID**: Open researcher and contributor ID; **PBS**: Phosphate buffered saline; **PLS**: Partial least squares; **PMML**: Predictive model markup language; **PMT**: Project management team; **p.p.b.**: Parts per billion; **PPI**: Protein-Protein interaction; **p.p.m.**: Parts per million; **QC**: Quality control; **QSAR**: Quantitative structure-activity relationship; **RA**: Risk assessment; **R&D**: Research and development; **SAB**: Scientific Advisory Board; **SEC**: Size exclusion chromatography; **sgRNA**: Single guide RNA; **SLC**: Solute Carrier; **SMALP**: Styrene maleic acid lipid particles; **SMEs**: Small and medium enterprises; **S/N**: signal-to-noise; **SPR**: Surface plasmon resonance; **SSM**: Solid supported membrane; **SSRI**: Selective serotonin reuptake inhibitor; **TM**: transmembrane; **UPLC-ESMS**: Ultra-performance liquid chromatography-electrospray mass spectrometry; **URL**: Uniform resource locator; **WP**: Work package; **WPL**: Work package leader; **WT**: Wild type.


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human
table
meeting
report
activity
chembl
condition
operate
KNIME
within
able
known
budget
used
allow
level
provide
assess
APMS
substrate
total
issue
academic
deliverable
expert
even
annual
agreement
majority
order
affinity
page
source
possible
name
carrier
communication
right
given
might
open
core
discovery
study
ULIV
good
change
database
including
short
approx
procedure
impact
public
community
mean
map
develop
deliver
atlas
metabolomic
made
expertise
group
range
start
outlined
phase
account
central
accessible
ethos
darpins
kind