Brussels, 17 May 2024

COST 069/24

DECISION

Subject: Memorandum of Understanding for the implementation of the COST Action "European Network for Sigma-1 Receptor as a Therapeutic Opportunity" (SIGMA-1EUROPE) CA23156

The COST Member Countries will find attached the Memorandum of Understanding for the COST Action European Network for Sigma-1 Receptor as a Therapeutic Opportunity approved by the Committee of Senior Officials through written procedure on 17 May 2024.

MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

COST Action CA23156
EUROPEAN NETWORK FOR SIGMA-1 RECEPTOR AS A THERAPEUTIC OPPORTUNITY (SIGMA-1EUROPE)

The COST Members through the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action, referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any document amending or replacing them.

The main aim and objective of the Action is to address open questions on the pharmacology of sigma-1 receptors as a pluripotent physiological modulator, and to provide innovative chemical tools, research procedures and protocols, validation of novel molecular pathways, and clinical developments for sigma-1 therapeutics through tight interaction with members from industry. This will be achieved through the specific objectives detailed in the Technical Annex.

The present MoU enters into force on the date of the approval of the COST Action by the CSO.

OVERVIEW

Summary
The sigma-1 receptor (S1R) is a ligand-regulated endoplasmic reticulum chaperone protein and a target for innovative compounds for the treatment of neurodegenerative and inflammatory diseases, cancers and pain diseases. The SIGMA-1 EUROPE network will bring together disciplines and expertises across Europe to advance the exploration and identification of the role of S1R in physiology and pathologies, to design innovative S1R ligands for cellular biology and medicine, and ultimately to train Young Reserachers and Innovators to revise current views of the diseases, to think out-of-the-box and explore novel and innovative S1R-based therapeutic opportunities.

Areas of Expertise Relevant for the Action	**Keywords**
● Basic medicine: Pharmacology, pharmacogenomics, drug discovery and design, drug therapy ● Health Sciences: Public and environmental health ● Chemical sciences: Molecular chemistry ● Chemical sciences: Chemical reactions: mechanisms, dynamics, kinetics and catalytic reactions ● Basic medicine: Molecular and cellular neuroscience	● Pharmacology ● Drug development ● Translational training ● Cytoprotection ● Pain

Specific Objectives
To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

Research Coordination
● Develop novel sigma-1 receptor-based diagnostics and molecular probes for the pharmacology and cellular biology
● Establish a strategic consensus for the clinical exploitation of sigma-1 receptors
● Disseminate sigma-1 receptor knowledge

Capacity Building
● To promote and foster active and multidisciplinary collaborations on each aspect of sigma-1 receptor research with the aim of advancing global, fundamental knowledge, addressing actual unsolved questions, and accelerate major discoveries in S1R research, to ultimately benefit European health and society.
● To create synergies between complementary groups. Such synergies appear today as the only effective means to address, particularly, the criteria of efficacy and activity of sigma-1 receptor ligands, alone and in combination, in the main pathological indications highlighted above.

TECHNICAL ANNEX

1. S&T EXCELLENCE

1.1. SOUNDNESS OF THE CHALLENGE

1.1.1. DESCRIPTION OF THE STATE OF THE ART

Neurodegenerative diseases, pain and cancer are three human diseases heavily afflicting society. The number of people living with dementia in the European Union is estimated to be 7.9 million and it will almost double by 2050 increasing to 14.3 million people.[1] The cost per single dementia-suffering patient has been estimated around 22,000 €/year, thereby causing considerable economic burden for the European Health System. Chronic pain affects around 20% of the adult population in Europe.[2] Yet it remains poorly managed and under-treated, affecting not only the patients, but the whole society as a whole. The estimated annual healthcare cost and loss in productivity associated with chronic pain equal 3% of the European gross domestic product (GDP). With more than 3 million new cases and 1.7 million deaths each year, cancer represents the second most important cause of death and morbidity in Europe.[3] On a global scale, Europe comprises only 1/8 of the world population but has around 1/4 of the global total of cancer cases with >4 million new patients in 2020. Cancer cost was 199 billion € in 2018, with healthcare accounting for 40%.[4] These pathologies appear to be uncorrelated, since they affect different cell types and pathological processes. However, they are all more prevalent in the elderly and share a common intracellular protein among their underlying molecular actors, the **sigma-1 receptor (S1R) protein**. The value of S1R as a therapeutic target appears of major importance in all these diseases since: (i) its expression is maintained during ageing, (ii) it can be targeted by small molecules acting as agonists or antagonists, and (iii) it participates in major cytoprotective pathways.

A ligand-operated cellular chaperone. The S1R has been associated with many diseases including stroke, addiction, pain, cancer and neuropsychiatric and neurodegenerative diseases.[5] In the brain, for instance, S1R is expressed in neurons and glial cells (including astroglia and microglia). At the cellular level, S1R is mainly located at the endoplasmic reticulum (ER) membrane and concentrated in regions in contact with mitochondria, known as mitochondria-associated-ER membranes (MAMs).[6] In resting conditions, S1R resides in ceramide- and cholesterol-rich lipid microdomains associated with the ER-resident chaperone GRP78 (BiP). Under cellular stress leading to ER injury, S1R dissociates from BiP and binds IP_3 receptors and other partners to enhance cell survival, through the control of calcium signalling between ER and mitochondria.[6] S1R then translocates to other cell compartments and binds G protein-coupled receptors, ion channels and other partners (e.g., ranGAP), inducing adaptive cellular responses. Through protein-protein interactions, S1R activity directly affects numerous intracellular pathways, including NMDA receptor activity, K^+ channel activity, phospholipase C and protein kinase C activities, the Rac1-GTP pathway, and trophic factors (BDNF, NGF) signalling and autophagy.[7] Stimulation with S1R agonists mimics stress-induced S1R dissociation from BiP and S1R delocalisation, while S1R ligands classified as antagonists impede this process. Altogether, these results have led to a model in which S1R is "silent" in physiological conditions, whereas in pathological cases it behaves as a chaperone, regulating client proteins to the benefit of cell survival.

S1R in neurological and neurodegenerative diseases. The S1R functions have been assessed in established *in vitro* and *in vivo* models of CNS pathologies, *i.e.*, stroke recovery, traumatic brain injury, epilepsy, motor neuron diseases, multiple sclerosis, depression, Alzheimer, Parkinson's and Huntington's and rare diseases.[8] These studies included pharmacological strategies to unravel the dynamics of pathophysiological mechanisms, taking advantage of appropriate pharmacological models and transgenic rodent models. The efficacy of selective S1R agonists has shown that the receptor is neuroprotective and neurorestorative by enhancing recovery of neurological function and maintaining proteostasis following experimental stroke, in neurodegenerative and rare genetic diseases.[9] It is of note that some neurodegenerative conditions like motor neuron disease are caused by mutations in the S1R gene, highlighting the prominent role of S1R in the pathogenesis.[10]

S1R in pain. Inadequate response to drug therapy currently constitutes a substantial unmet need in patients with **neuropathic pain**. S1R antagonists inhibit pain symptoms and both peripheral and central mechanisms that underlie neuropathic pain development and maintenance.[11] Therefore, the S1R is now considered as a new drug target for neuropathic pain treatment. Indeed, a very selective S1R antagonist (coded S1RA), developed by a European pharmaceutical company, is currently undergoing phase II clinical studies in neuropathic pain patients.[12] However, additional S1R antagonists with anti-

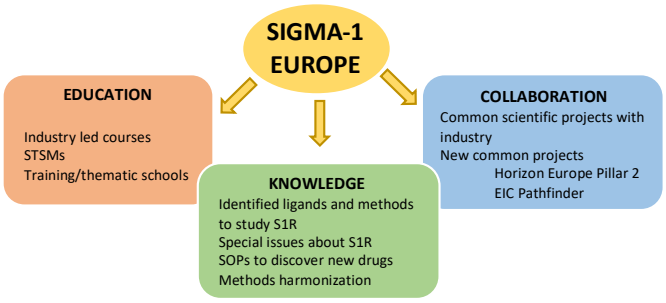
neuropathic activity are necessary. **Inflammatory pain** treatment is mainly based on non-steroidal anti-inflammatory drugs but, despite their common use, their side effects are frequent and can be serious, particularly when they are used chronically. Therefore, the identification of new targets is a relevant clinical need in inflammatory pain. Recently, several S1R antagonists have been reported to be analgesic, suggesting a class effect.[13] However, only some of them inhibit inflammation, suggesting a dual mode of action for some S1R antagonists that might be useful in inflammatory diseases. Moreover, several dual-acting S1R compounds with improved analgesic effects have been developed. This successful strategy can be extended to many targets to identify analgesics active on several types of pain with better therapeutic/adverse effect ratio than current standards.

S1R in cancer. The S1R has been detected in many tumours (glioblastoma, pancreatic cancer cells,...) and S1R ligands have been proposed for cancer cells labelling. At the functional level, some S1R agonists reduce cancer cell growth while antagonists stimulate apoptosis *in vitro* and *in vivo*, suggesting that S1R interferes with cancer cell development.[14] On the other hand, S1R expression stimulates the trafficking and/or the function of several ion channels involved in angiogenesis, metastatic spreading and apoptosis resistance.[15] Accordingly, it has been recently shown that in colorectal cancer and myeloid leukaemia, S1R promotes the formation of membrane signalling platforms, which associate K^+ channels and microenvironment receptors, leading to enhanced *in vivo* invasiveness. These data suggest that the pro-survival functions of S1R observed in neuronal disorders might be adapted by cancer cells to stimulate their development. This new concept may represent a fundamental basis to develop therapeutic strategies targeting S1R in cancer. Therefore, it is crucial to define the role of S1R in cancer tissues and the underlying molecular mechanisms.[16]

1.1.2. DESCRIPTION OF THE CHALLENGE (MAIN AIM)

Research on the mechanism of action and pharmacological value of S1R, as a pluripotent physiological modulator, is very promising and particularly active in Europe. Numerous compounds with high S1R selectivity and affinity have been identified, several mechanisms by which the protein exerts its cellular modulation have been delineated, and the impacts of S1R agonists or antagonists on several indications have been demonstrated and tested clinically for some of them.

At the same time, the crystallographic model of the S1R 3D-structure did not allow for reconciliation with previous biochemical structural information. A unifying activity test pertinent to the complex mechanism of action of the protein is still a matter of debate. Moreover, the unequivocal determination of the agonist/antagonist/ modulator nature of each receptor ligand remains to be accepted. Moreover, due to its pleiotropic action, the primary role of S1R in the different indications has yet to be clarified. Several common determinants have been identified, relying on calcium homeostasis, regulation of membrane receptor expression, cellular localisation and activation by ligands or physiological signals, but the global scheme of S1R activity in different biological settings remains to be fully understood.



Based on the involvement of the numerous and very active European academic and industry teams investigating S1R in aspects related to medicinal chemistry, biochemistry, molecular biology, pharmacology, and clinic, SIGMA-1 EUROPE Action will build an unprecedented network of experts with different and complementary backgrounds that together can foster collaborations, train young scientists, reinforce the knowledge and fill the gaps that exist in the field. This Action will be the lever to joint efforts and collaborative projects focused on S1R. It will directly address open questions from a comprehensive and multidisciplinary perspective and particularly provides: (i) innovative chemical tools to improve knowledge of the biological, physiological and pharmacological roles of S1R; (ii) a fundamental contribution in removing the critical hurdles hindering the understanding of S1R-ligands pharmacological profile, to foster the development of second generation S1R-targeting ligands and of customised S1R-based theragnostics; (iii) the dissemination of the most updated research procedures/standardized preclinical protocols; (iv) validation of novel molecular pathways modulated by S1R as target for therapy; (v) the definition of rationally-conceived clinical developments for S1R therapeutics; and (vi) tight interaction with members from industry to foster the path to clinical development. This interdisciplinary research network will be an unique opportunity to bring researchers and innovators together to investigate in this field.

1.2. PROGRESS BEYOND THE STATE-OF-THE-ART

1.2.1. APPROACH TO THE CHALLENGE AND PROGRESS BEYOND THE STATE OF THE ART

This Action aims at consolidating knowledge and transversal validity of targeting S1R in the different indications. First, pharmacological understanding of the S1R cellular nature and role remains to be obtained. To date, the endogenous S1R ligands have not been conclusively identified yet, nor has the role of all S1R ligands been unequivocally established, in terms of their agonistic or antagonistic receptor activity. The protein has been crystallized and the remarkable plasticity of the ligand recognition site is making the drug discovery of new selective chemical entities challenging. The binding affinity of the S1R ligands discovered so far is measured by radioligand displacement assays that, albeit adopted as a routine test for K_i determination, are affected by several caveats and requires dealing with radioactive compounds and dedicated instrumentation, with consequent environmental and safety issues. Moreover, a consensus activity test to unambiguously define agonistic and antagonistic ligands remains to be identified by the community. The signalling pathways of the S1R ligand are still a subject of intensive investigation, and novel protein partners, which are activated/deactivated/contacted by S1Rs, are continuously discovered. Second, the definition of S1R-based diagnostic tools or assays would likely address several pathologies and the precise and pertinent mechanisms of action of S1R-acting drugs in the different pathological condition, mostly in neurological disorders, pain and cancer, have to be identified. Third, in each indication of interest, developing optimized lead compounds with high specificity and efficacy in humans or multitarget candidates designed with unexplored combination of S1R and other targets, would pave the road to ambitious candidate drugs for clinical applications. Fourth, innovative methodological approaches must be more systematically developed in the field, including transcriptomics or lipidomics, to get a complete information on the physiopathological impacts of S1R activation, or artificial intelligence-assisted analyses for imaging, behavioural pharmacology or big data processing, Several current projects on S1R involves these approaches. By reassembling complementary expertise from the drug design to the clinical development and including cellular biology, pharmacology and genetic approaches, the networking activities will permit fundamental steps ahead in all these aspects and the coordinated activities of the Working Groups (WGs) will permit decisive developments in the field from the bench to the patient bedside.

1.2.2. OBJECTIVES

1.2.2.1. Research Coordination Objectives

The main objectives of the SIGMA-1 EUROPE are to place the European research on sigma-1 receptors at a new level in the international context; to connect and promote knowledge transfer between researchers, companies, regulatory authorities; to organize research in an intersectoral and inter-country manner; and to bring together nationally-funded projects and initiatives in a collaborative pan-European activity. The Action will be committed to leveraging current S1R research to achieve the following objectives:

RCO1. Develop novel S1R-based diagnostics and molecular probes for the pharmacology and cellular biology

Identified steps:

a) molecular dynamics simulations and determination of S1R structure in complex with selected molecules (*e.g.*, allosteric modulators, BiP) by X-ray crystallography will aid the understanding of S1R function and modulation by ligands;
b) synthesis of second generation S1R agonists and antagonists;
c) synthesis of fluorescent probes as powerful tools to replace the radiative binding assays and study the subcellular pathways of S1R;
d) synthesis of photo switchable ligands;
e) development of nanoparticles and formulations for drug-targeted delivery and solve ADME issues;
f) PET studies to gain insights into the S1R occupancy by drugs and drug candidates and to produce powerful diagnostics of S1R-involving diseases;
g) synthesis of multi-target-directed ligands (MTDLs) for a polypharmacology approach to the multifaceted pathologies that involve the S1R.

RCO2. Establish a strategic consensus for the clinical exploitation of S1R

Identified steps:

a) identification of molecular mechanisms and signalling pathways connected with S1R in different pathologies, from neurological and neurodegenerative diseases to pain and cancer;
b) analysis of the potential of S1R activation/inhibition in combined therapies;
c) analysis of the potential of repurposing drugs with S1R agonist/antagonist activity (as suggested for fluvoxamine, for instance) in particular indications;

d) establishment of S1R potential as a prognostic/diagnostic biomarker in diseases;
e) collaboration with academic drug discovery labs to perform lead optimization for future clinical development;
f) establish collaboration with academic laboratories to create a “Task force” dedicated to identify and validate S1R-target engagement biomarkers;
g) structure-based design of peptides and peptide-mimetic compounds able to inhibit S1R quaternary assemblies or interaction with protein partners, thereby mimicking S1R ligands activities (peptides and peptide-mimetics will be purchased from external companies).
h) virtual screening-aided procedures to: 1) identify drugs approved for different indications that are likely to act as S1R ligands (drug repurposing); and 2) identify receptors that are likely to be off targets for S1R ligands (side effect prediction);
i) biochemical assays to directly measure S1R binding to small molecules, peptides/peptide-mimetic compounds or partner proteins like BiP (e.g., by fluorescence titration and surface plasmon resonance); and to determine S1R aggregation state in presence of ligands (e.g., by mass spectrophotometry)

RCO3. Disseminate S1R knowledge

SIGMA-1 EUROPE will implement the communication skills of all Action members. Dissemination will develop over 3 tracks: towards the scientific community, the medical community, and the general public. SIGMA-1 EUROPE members will generate consensus protocols and review papers for dissemination to the scientific community and build up a network of highly qualified researchers using state-of-the-art technologies in the S1R field. Dissemination of S1R knowledge in the scientific community will be achieved through regular Symposia, Training schools, Short-Term Scientific Missions (STSMs), and Industrial Stages. The medical community will be specifically targeted through professional media, symposia and webinars. SIGMA-1 EUROPE will be implementing communication skills of all Action members. The Action will be fostering dissemination on S1R knowledge and value as a potent target in indications with an unmet therapeutic need to the general audience by setting up social media accounts and a YouTube channel, lecturing at the Universities participating in the Action, participating in initiatives oriented to the general public such as science festivals, science-in-the-pub, University-of-the-third-Age, and meeting sponsored by charities and non-profit health care and patient associations.

1.2.2.2. Capacity-building Objectives

To face these ambitious challenges, the Action will gather together European scientists involved in S1R-research. By promoting scientific exchanges, technological workshops and strategic innovation, by proposing standardized and consensual research protocols, and by clarifying the specific requirements for optimal drug development towards the clinic for the most innovative S1R compounds, the Action intends to accelerate the discovery of therapeutic solutions based on this unique target in different indications. The Action will first involve PhD students and Young Researchers and Innovators (YRIs) and rely on the experience of senior scientists. It will rationalize and timely focus research efforts in Europe by dealing with knowledge-based treatment progress in major and societal-burdening human pathologies.

Research on S1R inherently requires the contribution of a multidisciplinary team of excellent scientists. Europe is in the prominent position for assembling the required, highly qualified expertise residing in its member states. Therefore, this Action will gather these scientists together with the aim of advancing the knowledge on S1R and its transferability from bench to bedside. Accordingly, the capacity-building objectives are:

CBO1. To promote and foster active and multidisciplinary collaborations on each aspect of S1R research with the aim of advancing global, fundamental knowledge, addressing actual unsolved questions, and accelerate major discoveries in S1R research, to ultimately benefit European health and society.

CBO2. To create synergies between complementary groups. Such synergies appear today as the only effective means to address, particularly, the criteria of efficacy and activity of S1R ligands, alone and in combination, in the main pathological indications highlighted above.

2. NETWORKING EXCELLENCE

2.1. ADDED VALUE OF NETWORKING IN S&T EXCELLENCE

2.1.1. ADDED VALUE IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

Although research on S1R is historically active in Northern America, Japan and Europe, no initiative comparable to the SIGMA-1 EUROPE Action was ever undertaken either in Europe or other countries. The S1R community only gathers at one-time events such as satellite symposia or special sessions in international meetings (such as during the *AD/PD* meeting in Nice in 2015 or the *Monitoring Molecules in Neurosciences* meeting in Gothenburg in 2016). Noteworthily, a European symposium "*Physiology of sigma-1 receptors*" has been regularly organized, in Nice (2015), Barcelona (2017), Riga (2019), Bari (2021) and Montpellier (2023). The symposia gathered 30-65 participants. These meetings however focused on the broader field of S1R, from cellular biology to medicinal chemistry and clinical applications, and seeded the necessity for more ambitious and inclusive actions. In the past, collaborative research and training activities in selected areas of S1R pharmacology have been funded by national or cross-border agencies (e.g., DFG, ANR, MUR, Agencia Estatal de Investigación...), but an extensive effort focused on S1R was never undertaken. The Action's ambitious goal is to build up a broad, qualified, and a motivated European task force to bring new, safe, inexpensive, and efficacious drugs based on S1R targeting to the clinics. The Action will cover all steps needed to fulfil this aim by funnelling and rationalizing the joint efforts of structural and cellular biologists, bio and medicinal chemists, epidemiologists, pharma and biotech industry researchers, clinical pathologists, and physicians dedicated to patient's care. The Action will be an inestimable added value for the European scientific community and European health care and will lay the foundations for future Horizon initiatives and innovative international projects. Moreover, several synergies with other COST Actions have already been identified including ERNEST CA18133 on *GPCRs*, Nano2Clinic CA17140 on *cancer nanomedicine*, or STRATAGEM CA17104 on *New diagnostic and therapeutic tools against multidrug-resistant tumours*. The gain of SIGMA-1 EUROPE will be to provide a broader expertise within these complementary Actions, to address highly specifically a precise biological object, from chemistry and drug development to complex biological issues.
The Action will organize Training Schools covering various aspects of S1R from basic mechanisms of action to potential clinical outcomes. They will particularly target Ph.D. students and YRIs allowing formation in all these aspects of S1R research by senior researchers from the different fields covered by the Action and in a friendly environment.

2.2. ADDED VALUE OF NETWORKING IN IMPACT

2.2.1. SECURING THE CRITICAL MASS, EXPERTISE AND GEOGRAPHICAL BALANCE WITHIN THE COST MEMBERS AND BEYOND

The SIGMA-1 EUROPE network will rely on the participation of participants from different European countries. Academic members' scientific expertise will embrace all fields of current S1R research from medicinal chemistry to pathology, neuroscience, basic and clinical medicine. Participants that will be involved in the network will be perfectly suited to dissect the role of S1R at the cell, organ, and whole-body levels, exploring basic pharmacology, intracellular pathways, molecular biology and genetics, as well as behavioural and symptomatic consequences of S1R activity modulation. Several member teams will create and optimise molecular probes including fluorescent ligands, biased ligands and photoswitchable ligands to address complex cellular biological problems *in vitro* and *in vivo*, in pertinent animal models such as zebrafish larvae, which is particularly relevant in that it complies with 3R recommendations. SIGMA-1 EUROPE members will be skilled in obtaining *in vivo* preclinical models of pathological conditions such as neurodegenerative and metabolic diseases, pain, or cancer. Action members will share their knowledge on the pathogenesis of these diseases to clarify the involvement of S1R and select the best therapeutic strategies (small molecule vs peptide-mimetic, agonist vs antagonist, positive vs negative allosteric modulator, selective vs multitarget ligand or hybrid molecules) to pursue in clinical phases. Collaborations through the Action network will set the standards for appropriate preclinical experimentations and translation of S1R-targeting drugs towards the clinic. To this end, and based on the participation of clinicians, pharmacologists, medicinal chemists, biochemists, bioinformaticians, geneticists, and industrial partners in the network, dedicated WGs will outline and standardise as much as possible the specificities of S1R drug development and help select the most suitable subsets of patients to target, with the most appropriate chemical formulations and administration routes, thus fostering the development of new drugs for indications uncovered at present. These tasks will be fundamental to reach one of the Action's main goals, which is to accelerate the deployment of

S1R-based therapies in human medicine. Indeed, the network includes investigators from pharma and biotech companies who are already involved in late stage clinical trials or have recently reached the market with drugs partly acting through S1R. Industrial partners will have the chance to access unpublished data emerging from the Action and an opportunity to firsthand access diagnostic kits and molecular tools generated in the Action. Specific IP and MT agreements and revenue distribution will be carefully pre-arranged (in WG5).
The network will provide the opportunity to access resources including hospital patients and collections of biological samples through academic collaborations, and will foster the establishment of new scientific collaborations in a multidisciplinary and multicultural environment. The broad expertise and geographical distribution of Action members will benefit PhD students and YRIs, who will have access to advanced ground-breaking technologies and knowledge from all over Europe, will be exposed to a stimulating multicultural environment and will gain visibility and autonomy in their creative research. SIGMA-1 EUROPE will be strengthened by establishing long-term collaborations among the participants and prompting the establishment of novel collaborations among scientists with diverse backgrounds and expertise, thus stirring cross-fertilization across scientific, cultural, and disciplinary boundaries. SIGMA-1 EUROPE network will disseminate novel awareness on research and health issues among stakeholders and the public.

2.2.2. INVOLVEMENT OF STAKEHOLDERS

The stakeholders that will be involved in the SIGMA-1 EUROPE Action include young and established European scientists, graduate and undergraduate students, academic and research institutions in the different participating countries, national health systems, scientific societies, pharma and biotech companies, private and public funding bodies and charities, and patients' associations. The Action plans to involve them at all steps of the networking as listed below.
1. **Scientists** will participate in staff exchanges, conferences and meetings organized in the Action. They will directly benefit from networking activities and take an active part in discussing upcoming scientific questions arising in the field. They will be involved in writing position, methods, and dissemination papers; share reagents and expertise; take advantage of standardized procedures; establish new collaborations; and be involved in the clinical development of drugs generated in the Action. They will participate in common European and local funding opportunities, and, at another scale, be involved in the dialogue with different national and European science and health authorities.
2. **PhD students and YRIs** will be given the opportunity to participate in the activities as they will be the main subjects involved in Short-Term Scientific Missions and training schools. Participation in these activities, Working Groups (WGs) and Management Committee (MC) will increase their technical and managing skills and give them a chance to collaborate with the most expert researchers in the field from all over Europe, thus expanding their professional network and facilitating their grants applications and independent research establishment and career opportunities throughout Europe.
3. The **European and international scientific community**. The SIGMA-1 EUROPE Action will generate discoveries, reagents, and standardised experimental and diagnostic procedures that will benefit the European and international scientific community. Recruitment of new participants in the network will be implemented continuously throughout the duration of the Action. Advertisements of the Action activities will be advertised through a dedicated Action website and social networks (YouTube, Twitter). Scholars from the laboratories and universities concerned by the Action and related fields will be invited to participate in conferences, seminars, webinars, and schools organized throughout the Action duration. The MC Members and WG Leaders will have a major role in promoting efficient information flow within and outside the SIGMA-1 EUROPE network to ensure a maximal and timely audience.
4. **Universities** from countries participating in the Action will benefit from the conferences and training schools organized by the Action and opened to undergraduate and graduate students, PhDs, and MDs. Public funding bodies and research institutions will benefit from the revenues generated by patenting and licensing of transferable deliverables generated by the Action. The major benefit will also be facilitating the participation in other transnational networking activities such as the Erasmus exchange and European research programs for the academic members of the Action. Indeed, one crucial lever will be to promote PhD student mobility and facilitate co-tutorship and exchange of PhD students. PhD Schools and training across universities will be implemented during the Action.
5. **Biotech and Pharma companies**. The SIGMA-1 EUROPE network will include several industrial partners who are developing drugs in the field. A specific WG will be devoted to the transfer of generated data and protocols to the industry and the Action will promote the generation of spin-off targeting therapies, diagnosis or services through all WGs. The exchange with industry will include the flow of knowledge on druggable targets and mutual provision of experimental tools and molecules, following

appropriate agreements. Industrial Young Researchers will be encouraged to take part in the training schools and meetings associated with the Action.
6. **Charities and Patients Organizations**. National or international charities and Foundations will be involved as funding bodies in applications for research grants but also as partners through regular contacts with Action members, as a levers to disseminate new findings emerging from the WGs through communication with the patients and general audience. The Action will organise regular meetings with patient and their relatives in all indications targeted by S1R drugs and particularly in parallel with the progression of innovative drugs in the clinic. The Action will also provide targeted expertise and plans to better involve patients and caregivers in preclinical and clinical research, to be more receptive to healthcare choices.
7. **European citizens and the general audience**. Classic channels will be used to disseminate major data and initiatives from the SIGMA-1 EUROPE Action to European citizens and the general audience, including the Action and COST websites, social media, YouTube, Twitter, press and media releases.

3. IMPACT

3.1. IMPACT TO SCIENCE, SOCIETY AND COMPETITIVENESS, AND POTENTIAL FOR INNOVATION/BREAKTHROUGHS

3.1.1. SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS

The SIGMA-1 EUROPE Action aims at accelerating the clinical use of S1R-based medicines by simultaneously widening the field of possible indications, strengthening the basic knowledge on S1R biological roles and pharmacological mechanisms of action and by proposing standardized assays and protocols to address the specificity of this chaperon-like, receptor-like protein involved in numerous cellular signalling pathways.
In the short-term, the SIGMA-1 EUROPE Action will stimulate collaborations between the multidisciplinary academic groups working in the field, which will develop novel collaborative approaches to address the innovative research strategy through SIGMA-1 EUROPE. Collaborations between academia and industry will be critical and included within the actual course of the Action itself. Collaborations with ITCs will be encouraged. These collaborations will generate high profile, multidisciplinary publications and stimulate research means by acquisition of grant money from EU, national and local funding bodies. Exchange of knowledge and technological knowhow will be supported through symposia, STSMs, and training schools, and will result in an increase of collaborations, more publications, novel transborder grant applications, and promotion of YRIs. The Action will stimulate mobility and exchange throughout Europe and between academia and industry by networking at the meetings and advertising of open positions on the Action website.
In the long-term, the Action will promote novel concepts on therapeutic drug development, far beyond the updated one disease-one target-one drug view and draw a precise roadmap to develop the second and third generation of S1R ligands in numerous major therapeutic indications in terms of social cost. Economic activity in Europe, exemplified by the pharmaceutical companies involved in the Action, will be stimulated from these resources to optimize the development of drugs for the market. S1R targeting therapies are expected to have a significant and positive socioeconomic impact by improving human health in Europe and worldwide.

3.2. MEASURES TO MAXIMISE IMPACT

3.2.1. KNOWLEDGE CREATION, TRANSFER OF KNOWLEDGE AND CAREER DEVELOPMENT

SIGMA-1 EUROPE Action is meant to increase trans-European networking among academia/industry/public. The Action will concentrate on the multidisciplinary training of YRIs, as they will drive the field in the future. STSMs and training/thematic schools will offer them career perspectives and opportunities. Efficacy will be measured by attendee numbers of events, online counting of views, number of published documents, results of implementation questionnaires at the beginning and the end of the project. Regular assessment of the measures will be done during the Action meetings.

Knowledge creation	● increase the awareness of the importance of S1R in chemistry, neurological, inflammatory, cardiological and oncological disorders ● Selection of best methods to synthesize and study S1R ligands ● Cooperation between the leading SIGMA-1 EUROPE experts

Transfer of knowledge	● Formation of the network in this Action ● Establishment of the platform for internal and external communication ● Regular WG meetings (twice a year in person and monthly virtual meetings) ● STSMs (with a duration of one week up to three months) ● Three training schools will be organized to enable a more intensive exchange among young and senior researchers: (i) chemistry; (ii) *in vitro* and *in vivo* methods; (iii) translation from academic bench to industry ● Workshops, seminars, the final conference, and public awareness events will be used to disseminate the awareness of the importance of S1R, data and documents of SIGMA-1 EUROPE to external stakeholders and the public (by public panel discussions, radio/TV interviews, online videos and own platform) ● ITCs will host several of these events to achieve a significant impact by attracting additional young researchers from the host countries ● Identification and evaluation of tech transfer project and spin-off creation
Career development	● Building up Young Researchers and Innovators (YRIs) own network, to secure their future career advancement ● YRIs will take on tasks that will allow them to develop their soft skills and gain experience in management positions ● YRIs will be involved in the Working Groups and decision-making groups within the network (*e.g.*, scientific representative, STSM Coordinator, Science Communication Coordinator)

3.2.2. PLAN FOR DISSEMINATION AND/OR EXPLOITATION AND DIALOGUE WITH THE GENERAL PUBLIC OR POLICY

The following dissemination tools will be used:

- A website will be created and regularly updated, both for the participants and the general public. It will be managed by the MC and organised in sections according to the WGs. A restricted area will be accessible only to participants for internal documents exchange and dissemination such as MC meeting reports, WG activity progress, STSM reports and mailing lists.
- A conference will be organized annually, open to all Action participants and external collaborators. Participation in other international conferences relevant to the specific indications and of interdisciplinary character out of the Action will be supported.
- Joint scientific publications among participants in peer-reviewed journals and books will be strongly encouraged by the MC. This will constitute an excellent opportunity to promote the Action and disseminate the main results and progress within one or more WGs or arising out of STSMs.
- Training schools and workshops on focused topics will be organized aiming at know-how and expertise transfer and exchange to all participants, especially to YRIs.
- MC and WG will use periodic group and conference calls to enhance communication, update tasks, and discuss scientific or technical issues.
- A report will be published at the beginning and the end of the Action. It will include a summary of the project, the achieved objectives, the main expected/potential impact, and network presentation.
- An exploitation plan will be elaborated in the last year to enhance the translation of results and discoveries. Indications for the preparation of clinical trials in defined diseases will also be elaborated.

Specific actions aimed towards the general public:

- Dissemination to the public will be achieved by news in media based on relevant research papers published during the Action. This will be performed through the Communication Offices from the participating institutions.
- Action website and social networks. A dedicated website will be active to promote scientific discussion for the specialists and to raise public awareness, with appropriate access for experts and non-experts. The website will show the structure of the WGs, provide links to the participants' biosketches and/or websites and related organizations, announce events (thematic schools, workshops...), list participants' publications and meeting proceedings, and contain audio-visual production and material from the didactic activities (abstract books, conference, webinar, workshop programmes). There will be a private and a public access to the site. The content will be regularly updated. Moreover, all participants will be encouraged to share social media tools such as LinkedIn, ResearchGate, Facebook or Twitter/X, with the general public.
- Participation in Brain Awareness weeks in all countries contributing to the Action (through science museums close to our institutions or hosted by Universities/Research Centers) and in the European Researchers' Night, which is an opportunity to engage with citizens of all ages to raise awareness about our research; seminars to target audience.
- Webinars and YouTube video clips. Webinars will be organized every six months to present key

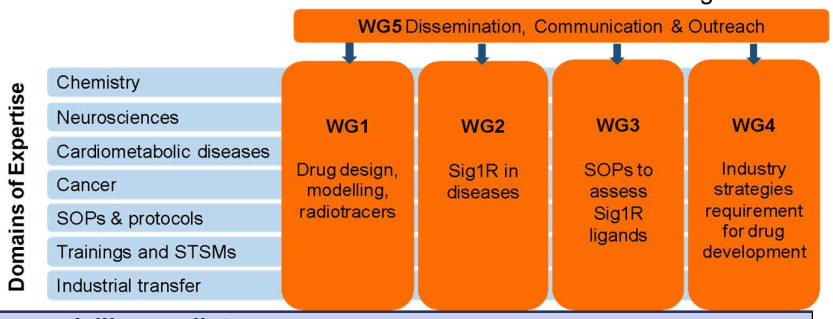
achievements and discuss specific topics through Teams/Zoom platforms. The video clips will be shared between the Action website and YouTube to maximise their outreach to audiences.

4. IMPLEMENTATION

4.1. COHERENCE AND EFFECTIVENESS OF THE WORK PLAN

4.1.1. DESCRIPTION OF WORKING GROUPS, TASKS AND ACTIVITIES

The SIGMA-1 EUROPE network will achieve its intended goals by mobilizing five Working Groups (WG): WG1- WG4 will focus on establishing the role of the S1R on diseases development, identifying the best-suited S1R drugs and will focus on the developing protocols and Standard Operating Procedures (SOPs); WG5 will manage and oversee the dissemination and communication initiatives and guarantee the outreach with stakeholders and policymakers outside of the Action. Each WG is divided into focused aims and tasks. All WGs start at month 1 of the Action and all work in parallel, closely linked to each other.



WG1	Drug design, modelling, radiotracers

The WG1 will involve medicinal chemistry teams that will act in close association, will provide insights into the conformational and, therefore, pharmacological changes that are responsible for the activity of S1R ligands and drugs in clinical development that act by interaction with the S1R. Very small structural changes may unpredictably switch an agonist into an antagonist. However, functional assays are not harmonized and an agreement has not been reached on which assay best measures ligands properties. WG1 will work to fill this gap with tools and methods that will profoundly implement the necessary knowledge about the receptor, both at the preclinical and clinical levels.

MAIN ACTIVITIES

Task 1.1. Structural studies on S1R. To understand what are the various conformational changes of S1R that occur upon binding of agonists, antagonists or allosteric modulators, the following experiments will be performed: (1) **Crystallization** of S1R in complex with different ligands to obtain their X-ray structures; (2) **Comparative Molecular Dynamics** (MD) simulations of S1R in the presence of S1R agonists, antagonists or drugs in clinical development and of S1R carrying mutations responsible for different pathologies; (3) **Small Angle X-ray scattering** (SAXS) and **Cryo-EM experiments** will be attempted in order to gain insights into the effect of agonists/antagonist on the S1R polymerization/depolymerization mechanisms. This Task will provide knowledge: (i) On the functional conformational changes and the differences between wild type and mutated S1R by comparing their different folding; (ii) For the design of a focused library of S1R modulators (agonists, antagonists or allosteric modulators) with drug-like properties to be studied in WG2; (iii) The analysis of the S1R surface to design peptides targeting the S1R BiP interaction. In parallel, based on the available S1R 3D structures, a series of about 15 peptides/peptide-mimetics will be designed that cover the entire surface of the soluble domain of S1R, some of which will be able to hamper S1R oligomerization and other interaction with BiP and/or other partner proteins. These peptides will be useful both to better understand the mechanism of action of S1R ligands and as potential drugs and drug precursors.

Task 1.2. Designed and directed S1R ligands and peptides will be synthesized, based on the structural models from Task 1.1. Synthetic feasibility considerations will be included early in the design process, and evaluated case-by-case, to avoid the selection of candidates not easily chemically accessible, thus minimizing the risks of unaffordable compounds.

Task 1.3. Fluorescent small S1R ligands will be designed and synthesized to be used as powerful imaging tools in *in vitro* fluorescent cell-based assays and in *in vi*vo investigations (optical imaging in zebrafish/mouse models). These compounds will also be validated to perform S1R binding assays in live cells to replace the classical radioligand binding assays with greener and safer binding assays avoiding the manipulation and waste of the typical radioligand ([^{3}H]-(+)-pentazocine). The development of specific and well-performing fluorescent ligands is additionally

recommended to overcome some limitations of the S1R antibodies in use. Characterization of the developed fluorescent tools will need effective cooperation among WGs.

Task 1.4. Development of photoswitchable ligands will be pursued. Such ligand alter their chemical structure upon irradiation and concomitantly their pharmacological properties. Such ligands have not yet been described for the S1R. They offer the possibility for unprecedented spatio-temporal control of S1R in cellular settings and therefore offer the chance for other WGs to investigate different receptor populations and their time-course of activation depending on the tissue and even *in vivo* in zebrafish.

Task 1.5. Development of appropriate nanoparticles or drug-conjugates for the targeted delivery to CNS or cancer cells will be pursued to improve the release of the drug candidates to the target organs, by-passing potential delivery issues. The size, nature and surface functionalization of the delivery systems will be adjusted according to the specific target organ, or cells and according to the nature of the drug. Also, drug formulations will be adjusted in case of solubility issues. Characterization of these novel systems requires a strict cooperation with the other WGs, while the medicinal chemists in the WG1 will cooperate to produce drug(s) to be delivered in the appropriate amount for in vitro and in vivo studies.

Task 1.6. PET study. [^{18}F]Fluspidine, the S1R targeted brain-penetrant radioligand, will be employed to calculate *in vivo* the target engagement and the receptor occupancy (RO) of the S1R ligands under study *in vivo* and to validate the S1R as the interactor responsible of the drug action. The radiochemists will take advantage of the already known synthesis of this ready-to-use radiotracer to resynthesize it in the right amount for the *in vivo* studies, to determine the above important parameters for the drug development process. In the meantime, [^{18}F]fluspidine will be further investigated as a diagnostic for CNS diseases that involve S1R, since (pre)clinical and experimental data indicate the suitability of fluspidine for neuroimaging. In the meantime, other potential S1R PET agents will be studied.

Task 1.7. Identification of repurposed ligands and natural ligands. In the different indications of interest, screening assays will allow the investigation of drug libraries, based on the well-known result that some approved clinical drugs act partly through S1R (*e.g.*, fluvoxamine, haloperidol, donepezil). Therefore, molecule libraries will be screened both virtually and physically to identify mixed S1R ligands susceptible to be rapidly used in clinical trials. Similarly, active principles from phytotherapy and natural products libraries will be explored for their S1R activity.

Task 1.8. Synthesis of MTDLs. Based on the positive clinical outcome of Blarcamesine in treating AD and other CNS diseases, the most promising S1R ligands may be structurally modified to obtain multi-target-directed agents. These agents can engage other disease-related targets in addition to S1R and exert an additive/synergistic effect by simultaneously acting on multiple targets, thus increasing their efficacy. Complex diseases such as CNS diseases and cancer are rarely treatable with one drug only, and need a polypharmacological approach.Ideally, this may be obtained with one drug acting on more-than-one target, thereby reducing pharmacokinetics issues and better matching patients' compliance.

All these tasks (T1.1-T1.8) will be based on well-connected collaborations, among researchers belonging to the same WG1 that do not usually cooperate with each other but collaborate with teams involved in WG2 or industries (WG4). Interaction with different WGs is fundamental for most tasks, which will be developed in an iterative process, while industries will be fundamental to exploit the technology transfer potentials of the outcome of these activities. For the design of novel ligands innovative approaches based on artificial intelligence (*de novo* design) will be employed which today is conceivable due to the availability of dataset containing large collection of active compounds (*i.e.*, CHEMBL).

Many of the tasks will be accelerated by the mutual visits of YRIs to laboratories of different WGs to learn innovative technologies and broaden their expertise.

WG2	**S1R in neurodegenerative, inflammatory, cancer and cardiometabolic diseases**

S1R is a multi-faceted protein that is involved in numerous physiopathological pathways. The main aim of this WG will be to identify the mechanistic links between the pathogenesis of neurodegenerative, inflammatory, cancer and cardiometabolic diseases and the modulation of S1R activity by ligands. With the aid of the molecules/probes developed in WG1, the Action will particularly address the exact molecular mechanisms and their contribution to complex neuroprotective and neurorestorative processes; develop approaches for the treatment of neuropathic or inflammatory pain; and aim at a clear understanding of S1R function in tumour cell growth and/or apoptosis.

MAIN ACTIVITIES

Task 2.1. Identification of molecular mechanisms and signalling pathways connected with S1R in the different pathologies, from neurological and neurodegenerative diseases to pain, cancer and cardiometabolic diseases. Analysis of transcriptomic and proteomic data from control and S1R-silenced cells or cells expressing mutant S1R associated to pathologies (*e.g.*, ALS) will provide crucial information on how the presence of S1R alters signalling maps and metabolic pathways. In parallel, S1R interactome in pathological versus normal cells will be established. The modality of interaction between S1R and its partners and the functional implications will be evaluated by a wide range of techniques available in the consortium such as FRET, single molecule imagery, patch clamp, 3D culture, organoids, cytotoxic assays, ER stress analysis, calcium signalling... The effects of S1R on proteostasis (including autophagy), the identification of S1R-GPCR complexes, the investigation of S1R interactome and the deciphering of S1R/ion channel interactions in pathologies will be explored. Changes in regional S1R density associated with neurological pathologies and therapies and other important parameters (activation of microglia, neuronal integrity, neuronal density, regional energy metabolism, etc) will be addressed.

Task 2.2. Identification of S1R role in cytoprotection and maintenance and restoration of brain function in different CNS pathologies. Effects of S1R on protein aggregation will be elucidated. S1R-GPCR complexes will be identified by investigating the S1R interactome. S1R/ion channel and S1R/autophagy-related protein interactions in neurological pathologies will be characterised. Changes of regional S1R density associated with neurological pathologies and the impact of therapies on regional S1R density and other important parameters in the human brain (neuroinflammation, neuronal integrity, neuronal density, regional energy metabolism, etc) will be assessed. Fluorescent and PET probes as well as photoswitchable ligands created by WG1 will be particularly useful for the two above tasks.

Task 2.3. Attainment of new analgesics targeting S1R for pain treatment. The analgesic efficacy of the new S1R ligands developed in WG1 will be evaluated in several pain models with different aetiologies. The activity of S1R ligands acting as antagonists (analgesics) or agonists (proalgesics) and the possible advantages of these compounds for the treatment of several types of pain will be assessed.

Task 2.4. Identification of the mechanisms involved in anti-tumour activities of S1R ligands. Different experimental approaches (electrophysiology, neurochemistry, immunohistochemistry, molecular biology) will be used to identify the mechanisms underlying the therapeutic activity of S1R ligands (either already known or newly developed by WG1). Moreover, exploiting the *in vitro* and *in vivo* models of cancer available in the consortium, including KO and Tg mouse models, the consequences of S1R silencing on cancer cell hallmarks and tumour development will be assessed. The results will allow deciphering how S1R presence impacts cancer development, which is a prerequisite to the exploitation of S1R as a therapeutic target.

Task 2.5. Analysis of the potential of S1R activation/inhibition in combination therapies, and design of proof of principle studies to test if novel pharmacological entities (WG1) bind S1R at therapeutically relevant doses in the human brain and are effective in treating pathologies, notably regarding neurodegenerative diseases and pain. The effect of nanoparticles (WG1) will also be studied to determine whether an increase in delivery is achieved. Using mice or zebrafish tumour xenograft models, spontaneous mice cancer models, the impact of treatments with S1R ligands on tumour growth and metastatic spreading in addition to standard chemotherapies will be studied, to explore S1R ligands potential as adjuvant drugs in cancer. These results will foster the development of focused MTDLs.

Task 2.6. Evaluation of the possibility to target S1R as a prognostic/diagnostic marker in diseases. Methods to quantify S1R expression in large cohorts of patients in relation to disease stage will be established (all techniques and biological resources being available within the consortium). S1R expression and distribution in the organs of primary tumours and metastases will be evaluated by PET in Humans and murine models available in the consortium. PET probes by WG1 will be fundamental for the two above tasks.

WG3	**Establishment of SOPs for in vitro and in vivo models to assess S1R ligands**
WG3 aims are to design and implement SOPs in order to ensure compliance of SIGMA-1 EUROPE procedures and processes with applicable national and EU regulations and international standards, as well as to meet ethical and customer requirements. This will ensure traceability and continuous improvement of quality, efficiency, performance and reputation of SIGMA-1 EUROPE. In addition, STSMs will be used to harmonise the protocols between participants and train a new generation of researchers to build new skills.	
MAIN ACTIVITIES	

Task.3.1. Identification of a model for the development of SR ligands. Results from WG1 (molecular dynamics and crystallography) will identify a structural model for S1R ligands that act as agonists, antagonists or modulators of the receptor, thus guiding the identification of the most appropriate *in vitro* and *in vivo* methods to test S1R ligands functional profile.

Task 3.2. Identification of the most appropriate *in vitro* and *in vivo* methods to test S1R ligands. If successful fluorescent ligands are developed in WG1 the classical protocols to define the affinity of novel ligands will be substituted: the appropriate fluo-ligand in the appropriate cell cultures will likely replace the use of the radioligand binding in animal tissues, in agreement with the 3Rs. Results from WG2 will guide to the most appropriate methods to define the functional profile (agonist, antagonist, modulator) and the mechanism of action behind the potential activity of S1R ligands related to molecular/cellular mechanisms and outcome in disease models.

The PET radioligand from WG1 will at the same time be selected as the tool for the *in vivo* animal and human imaging studies. Altogether, the Action will build a consensus on SOPs among scientists.

Task 3.3. Identification of optimal safety profile for S1R drug development. Adverse side-effects of investigational drugs are related to the chemical scaffold, the cellular and physiological effects of the target and the physiopathological conditions. For instance, conformational similarities between the hERG and S1R pharmacophores proposed by Kratz[17] and Glennon[18] are coherent with a lack of selectivity for several chemical series and hERG therefore appears to be a primary off-target.

Task 3.4. Organise STSMs for both young and established investigators at academic and industrial facilities; 1) to learn novel techniques and experimental systems to investigate neurodegenerative, cancer and cardiometabolic diseases, and drug design and 2) to harmonize SOPs.

Task 3.5. Organise a workshops and training schools in advanced techniques (such as Leica STELLARIS 8 STED system for confocal and TauSTED (STED/FLIM) imaging, Ai-assisted imaging analysis) to investigate signal transduction in *in vitro* cell and organotypic cultures/organoids and in animal models of diseases (such as advanced video tracking systems, high-definition and AI-assisted behavioural phenotyping system for mice).

WG4	**Industry strategies requirement for drug development**

The goal of WG4 is to establish close industry-academia collaborations to develop a novel class of safe and efficacious S1R ligands for the treatment of neurodegenerative and neurodevelop-mental, cardiometabolic diseases, pain disorder and cancer. Industry partners will establish close ties with academic partners, to introduce academia to the requirements that are necessary for a successful drug development program. Also, industry partners will provide various courses that will enable academic partners to acquire knowledge to meet industry needs.

MAIN ACTIVITIES

Task 4.1. Collaboration with academic laboratories to define a process for characterization and optimization of compounds for future clinical development.

- Screening of compounds meeting pre-defined criteria for affinity and selectivity for the S1R (screen binding against other CNS targets, including S2R).
- Define a panel of assays to assess functionality on the S1R.
  - *In vitro* assays to assess agonistic/antagonistic activity (*e.g.*, BDNF secretion, BiP-S1R association, S1R oligomerization).
  - Panel of *in vivo* assays (*e.g.*, depression and anxiety, cognition, pain).
  - Compare activity against predefined, selected S1R ligands which will serve as benchmark compounds (*e.g.*, pridopidine, PRE-084).
- Define a dose response in *in vitro* and *in vivo* experiments.
- Define a panel of assays to evaluate ADME properties including solubility, metabolic stability, cell permeability properties, appropriate protein binding,
- Define panel of assays to evaluate initial safety pharmacology/toxicology assays including hERG inhibition activity, genotoxicity, and standard safety panel, such as the IRWIN battery of tests.

Task 4.2. Collaboration with academic laboratories to create a "Taskforce" dedicated to developing S1R-specific biomarkers. Reliable biomarkers are critical for successful clinical development. Currently no S1R-specific biomarkers exist. The Taskforce will work towards developing standardised biomarker assays, which can be used in preclinical assays and clinical trials.

- Identify potential biomarkers for S1R activation.

Task 4.3. Identification of S1R gene variants that have the potential to moderate the effects of S1R activation. These variants would be those that occur at frequencies greater than 5% in the general population. Once identified, they will be assessed *in vitro* to see their effect on various S1R biomarkers. These findings could influence S1R ligands efficacy in different populations.

Task 4.4. Establish collaborations for joint experimental projects to evaluate novel CNS-acting, S1R-targeting drugs developed by the Action members and/or the members from Industries.
Task 4.5. **Implementation of a training plan on Technology Transfer and IP issues**.
Task 4.6. Organisation of visits to Industry partners and innovation clusters.

WG5	Dissemination, Communication and Outreach

WG5 objective is to ensure awareness about SIGMA-1 EUROPE Action among the academic community, state institutions, society and industry on the European level.

MAIN ACTIVITIES

Task 5.1. Dissemination via web and social media. Set up an Action website for a specialist and non-specialist audience to increase awareness of S1R in the pathophysiology of diseases. Establishment of SIGMA-1 EUROPE accounts on LinkedIn, Facebook, Twitter, and Instagram. These social media platforms will help disseminate significant findings from Action's meetings and advertise calls for STSMs, publications generated in the Action, work positions. SIGMA-1 EUROPE will identify a referent for each country involved in the Action who will translate the main contents into each language, thus facilitating contacts with the general public and the press.

Task 5.2. Expansion of the SIGMA-1 EUROPE Action to increase the number of affiliated Countries, **especially ITCs, and Young Researchers and Innovators' participation** via active advertisement around Europe and the World, Universities, Hospitals, and scientific societies in which Action members take part. Special attention will also be paid to increase the number of industry members involved in the Action.

Task 5.3. Organization of Special issues about S1R in open access high-impact journals (in line with MC decisions). At least one Special issue in the field of S1R will be organized. A special issue provides an excellent opportunity to review a particular theme, examine previously unaddressed aspects, propose and develop novel approaches, exchange perspectives and encourage new lines of research, thus increasing the recognition of the SIGMA-1 EUROPE Action not only in Europe, but also in the whole world. Support to the joint publication of open access scientific papers will be provided.

Task 5.4. Promotion of the participation of Action members in dissemination initiatives such as Science days, science-in-the-pub meetings, and exhibitions. Contacts with major scientific societies in the field will be organised.

Task 5.5. Dissemination of novel results and concepts to the medical community. By encouraging the publication of review articles in clinical journals, the organisation of webinars to European networks of medical specialists, the participation to European medical congress, and by an efficient relay from the clinicians participating in the Action, a constant and up-to-date information will be provided to the physicians and clinical specialists about the progresses in the field.

4.1.2. DESCRIPTION OF DELIVERABLES AND TIMEFRAME

WGs	DELIVERABLES	Delivery time/ MONTHS
WG1 Drug design, modeling, radiotracers	D1.1 Comparative molecular dynamics. Definition of a model for a directed development of S1R agonist vs antagonist.	18
	D1.2 Development of both high affinity S1R agonists and antagonists to be studied as disease modifying agents.	18, 30
	D1.3 Development of fluorescent small S1R ligands	24, 36
	D1.4 Development of high affinity S1R photoswitchable probes for S1R agonist or antagonist activities	30, 42
	D1.5 Development of appropriate nanoparticles or drug-conjugates	48
	D1.6 A diagnostic PET agent for specific CNS disease(s)	48
	D1.7 Identification of repurposing or natural ligands with S1R activity	24, 36
	D1.8 Development of MTDL candidates in complex pathologies with the potential of becoming disease modifying agents	30, 42
WG2 S1R in diseases	D2.1 Finding out S1R molecular mechanisms and signalling pathways	18, 30, 42
	D2.2 Identification of S1R as diagnostic/prognosis marker	24, 36
	D2.3 Obtention of new analgesics for pain treatment targeting the S1R	24, 36
	D2.4 Identification of the mechanisms involved in anti-tumour activities of S1R ligands	45
	D2.5 To analyze the potential of S1R activation/inhibition in combination therapies (articles)	30, 48
	D2.6 Finding out whether S1R can be proposed as a prognostic/diagnosis marker in diseases	42
WG3 Establishment of SOPs	D3.1 Identification of the most appropriate in vitro and in vivo methods to test S1R ligands	12
	D3.2 Implementation of SOPs and harmonized protocols	12,24,36
	D3.3 Identification of optimal safety profile for S1R drug development	24
WG4 Industry strategies requirement	D4.1 Definition of a process for characterization and optimization of compounds	6
	D4.2 Identify variants in S1R gene that potentially have influence on moderating the effects of S1R activation	45
	D4.3 Development of training plans and implamentation on Technology Transfer, IP issues and industry needs from academics	6,18,30,46
WG5 Dissemination Communicatio n Outreach	D5.1 Action website and promotional materials/leaflet	6
	D5.2 Dissemination via Web and social media	12, 24, 36, 48
	D5.3 Reports and communication to the medical community	12, 24, 36, 48

4.1.3. RISK ANALYSIS AND CONTINGENCY PLANS

The risk management aims at ensuring that risks with a potential impact on the Action are anticipated and mitigated in time to increase the probability of success. The Table below summarizes a preliminary identification of such risks, with their evaluation and the mitigation and contingency action.

Risk description and evaluation	Risk mitigation and contingency measures
Task implemented with delay *Probability high; impact low.*	Weaker development load in the second of the Action. Buffer times are reserved at the end. If some members are underperforming, the Management Committee and WGs Leaders will evaluate which action to take, including the possibility to reallocate tasks and resources.
Capacity/ poor engagement in WG from different countries *Probability medium; impact medium.*	Redundancy of expertise allowing back-up plans. Constantly promote Action to recruit new members.
WG members do not reach a consensus on relevant preclinical in vitro/ animal models and approaches *Probability low; impact low.*	Well-defined management structures and conflict resolution approaches will minimize the risk. In case of difficulties the WG leaders will interact with the WG members trying to identify blocking situations and shortcomings that have led to results which are not

	shared by the WG members so as to address any weakness and thus improve the overall quality.
Failure to outreach outside the network Probability – low; impact – medium.	Members of the Action network will be part of several scientific international programs. Development of Dissemination plan.
In person training & meetings not possible (e.g., COVID). *Probability high; impact high.*	Promote webinars. Consider delaying physical training/ meetings where possible.
Diverse public opinion and different national approaches in animal welfare and animal use in research *Probability medium; impact medium.*	Ensure that animal welfare is always a topic for discussion at networking and MC meetings and as part of communication and dissemination. Promote elaborated *in vitro* models such as organoids.

4.1.4. GANTT DIAGRAM

The GANTT diagram below displays the general planning and timing of the COST Action SIGMA-1 EUROPE. The detailed timing will depend on the possibilities of the co-location with appropriate conferences and workshops, to achieve the maximum dissemination impact.

WG	Task		YEAR 1				YEAR 2				YEAR 3				YEAR 4			
			Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
WG1		**Drug design, modelling, radiotracers**		W		W		W		W		W		W		W		W
	1.1	Comparative Molecular Dynamics (articles)						D1.1										
	1.2	Designed and directed S1R ligands (articles, ligands)						D1.2				D1.2						
	1.3	Fluorescent small S1R ligands (articles, ligands)								D1.3				D1.3				
	1.4	Development of photoswitchable ligands (articles, ligands)										D1.4				D1.4		
	1.5	Development of nanoparticles or drug-conjugates (articles, ligands)																D1.5
	1.6	A diagnostic PET agent for specific CNS disease(s) (articles, ligands)																D1.6
	1.7	Identification of repurposing and natural ligands (articles, ligands)								D1.7				D1.7				
	1.8	Synthesis of MTDLs (articles, ligands)										D1.8				D1.8		
WG2		**S1R in neurodegenerative, inflammatory, cancer and cardiometabolic diseases**		W		W		W		W		W		W		W		W
	2.1	Finding out S1R molecular mechanisms and signalling pathways (articles)						D2.1				D2.1				D2.1		
	2.2	Identification of S1R as diagnostic/prognosis marker (reports, articles)								D2.2				D2.2				
	2.3	Obtention of new analgesics for pain treatment targeting the S1R (ligands, articles)								D2.3				D2.3				
	2.4	Identification of the mechanisms involved in anti-tumour activities of S1R ligands (articles)															D2.4	
	2.5	To analyze the potential of S1R activation/inhibition in combination therapies (articles)										D2.5						D2.5
	2.6	Finding out whether S1R can be proposed as a prognostic/diagnosis marker in diseases (SOPs)														D2.6		
WG3		**Establishment of SOPs for in vitro and in vivo models to assess Sig1R ligands**		W		W		W		W		W		W		W		W
	3.1	Identification of in the most appropriate *in vitro* and *in vivo* methods to test Sig1R ligands (SOPs)				D3.1.												
	3.2	Implementation of SOPs for in vitro and in vivo methods to study Sig1R ligands.				D3.2				D3.2				D3.2				
	3.3	Identification of optimal safety profile for S1R drug development (SOPs)								D3.3								
WG4		**Industry strategies requirement for drug development**		W		W		W		W		W		W		W		W
	4.1	Definition of a process for characterization and optimization of compounds		D4.1														
	4.2	Identify variants in S1R gene that potentially have influence on moderating the effects of S1R activation														D4.2		
	4.3	Development of training plans and implamentation on Technology Transfer, IP issues and industry needs from academics		D4.3				D4.3				D4.3						D4.3
WG5		**Dissemination, Communication and Outreach**		W		W		W		W		W		W		W		W
	5.1	Action website and promotional materials/leaflet		D5.1														
	5.2	Dissemination via web and social media				D5.2				D5.2				D5.2				D5.2
	5.3	Reports and communication to the medical community				D5.3				D5.3				D5.3				D5.3

K : Kick-off meeting M : MC meeting W : WG meeting

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