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INSPIRE: A European training network to foster research and training in cardiovascular safety pharmacology

Pieter-Jan D. Guns[a,*], Brian D. Guth[b], Stefan Braam[c], Georgios Kosmidis[c], Elena Matsa[c], Annie Delaunois[d], Vitalina Gryshkova[d], Sylvain Bernasconi[e], Harm J. Knot[f], Yair Shemesh[g], Alon Chen[g], Michael Markert[b], Miguel A. Fernández[h], Damiano Lombardi[h], Céline Grandmont[h], Berta Cillero-Pastor[i], Ron M.A. Heeren[i], Wim Martinet[a], Jeanette Woolard[j], Matt Skinner[k], Vincent F.M. Segers[a,l], Constantijn Franssen[l,m], Emeline M. Van Craenenbroeck[l,m], Paul G.A. Volders[n], Thomas Pauwelyn[o], Dries Braeken[o], Paz Yanez[p], Krystle Correll[q], Xi Yang[r], Helen Prior[s], Gábor Kismihók[t,u], Guido R.Y. De Meyer[a], Jean-Pierre Valentin[d]

[a] *Laboratory of Physiopharmacology, University of Antwerp, Antwerp, Belgium*
[b] *Boehringer Ingelheim Pharma GmbH & Co KG, Drug Discovery Sciences, Biberach an der Riss, Germany*
[c] *Ncardia B.V, Leiden, the Netherlands*
[d] *UCB Biopharma SRL, Early Solutions, Development Science, Non-Clinical Safety Evaluation, Braine-l'Alleud, Belgium*
[e] *NOTOCORD, an Instem company, Le Pecq, France*
[f] *TSE Systems GmbH, Bad Homburg, Germany*
[g] *Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel*
[h] *Inria, Sorbonne Université & CNRS, Paris, France*
[i] *The Maastricht MultiModal Molecular Imaging Institute (M4I), Division of Imaging Mass Spectrometry, Maastricht University, Maastricht, the Netherlands*
[j] *Division of Physiology, Pharmacology and Neuroscience, Centre of Membrane Proteins and Receptors (COMPARE), School of Life Sciences, University of Nottingham, United Kingdom*
[k] *Vivonics Preclinical Ltd, BioCity, Nottingham, United Kingdom*
[l] *Department of Cardiology, Antwerp University Hospital, Antwerp, Belgium*
[m] *Cardiovascular Diseases, GENCOR, University of Antwerp, Antwerp, Belgium*
[n] *Department of Cardiology, CARIM, Maastricht University Medical Center+, Maastricht, the Netherlands*
[o] *Life Science Technologies, imec, Leuven, Belgium*
[p] *Department of Research Affairs & Innovation, University of Antwerp, Antwerp, Belgium*
[q] *Safety Pharmacology Society, Reston, Virginia, United States*
[r] *Division of Cardiovascular and Renal Products, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD, United States*
[s] *National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), London, UK*
[t] *Leibniz Information Centre for Science and Technology, Hannover, Germany*
[u] *Marie Curie Alumni Association, Brussels, Belgium*

Abbreviations: ADRs, adverse drug reactions; AOP, Adverse Outcome Pathways; CiPA, Comprehensive *in vitro* Proarrhythmia Assay; CPI, FDA's Critical Path Initiative; CSPS, Chinese Safety Pharmacology Society; CSRC, Cardiac Safety Research Consortium; CVOT, CardioVascular Outcome Trials; DSP, Diplomate in Safety Pharmacology; EFPIA, European Federation of Pharmaceutical Industries and Associations; EMA, European Medicine Agency; ESR(s), early stage researcher(s); ETN, European Training Network; FDA, US Food and Drug Administration; FIH, first-in-human; HESI, Health and Environmental Sciences Institute; HESI-CSTC, HESI Cardiac Safety Technical Committee; hiPSC, human induced Pluripotent Stem Cell; ICH, International Council on Harmonization; IMI, Innovative Medicines Initiatives; ICT, Information Communications Technologies; IQ Consortium, International Consortium for Innovation and Quality in Pharmaceutical Development; IWG, Implementation Working Group; J-ICET, Japan activity for Improvement of Cardiovascular Evaluation by Telemetry; J-SPS, Japan Safety Pharmacology Society; JiCSA, Japan iPS Cardiac Safety Assessment; MRI, Magnetic Resonance Imaging; MSCA, Marie Skłodowska-Curie Actions; MSCA-ITN, Marie Skłodowska-Curie Actions – Innovative Training Networks; MSI, Mass Spectrometry Imaging; NC3Rs, National Centre for the Replacement, Refinement and Reduction of Animals in Research; NCE(s), new chemical entity; PET, Positron Emission Tomography; PKPD, pharmacokinetic-pharmacodynamic; PPP, Public-Private Partnerships; PSTC, Predictive Safety Testing Consortium; PWV, Pulse Wave Velocity; QIVIVE, Quantitative *In Vitro* to *In Vivo* Extrapolation; SPS, Safety Pharmacology Society; SPiT, Safety Pharmacology endpoints into Toxicology studies; TdP, Torsades de Pointes; TKI, tyrosine kinase inhibitors; TQT, Thorough QT study; VEGF, vascular endothelial growth factor

* Corresponding author at: University of Antwerp, Campus Drie Eiken, Building T2.30, Universiteitsplein 1, B-2610 Wilrijk, Belgium.
E-mail address: pieter-jan.guns@uantwerpen.be (P.-J.D. Guns).

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ABSTRACT

Safety pharmacology is an essential part of drug development aiming to identify, evaluate and investigate undesirable pharmacodynamic properties of a drug primarily prior to clinical trials. In particular, cardiovascular adverse drug reactions (ADR) have halted many drug development programs. Safety pharmacology has successfully implemented a screening strategy to detect cardiovascular liabilities, but there is room for further refinement. In this setting, we present the INSPIRE project, a European Training Network in safety pharmacology for Early Stage Researchers (ESRs), funded by the European Commission's H2020-MSCA-ITN programme. INSPIRE has recruited 15 ESR fellows that will conduct an individual PhD-research project for a period of 36 months. INSPIRE aims to be complementary to ongoing research initiatives. With this as a goal, an inventory of collaborative research initiatives in safety pharmacology was created and the ESR projects have been designed to be complementary to this roadmap. Overall, INSPIRE aims to improve cardiovascular safety evaluation, either by investigating technological innovations or by adding mechanistic insight in emerging safety concerns, as observed in the field of cardio-oncology. Finally, in addition to its hands-on research pillar, INSPIRE will organize a number of summer schools and workshops that will be open to the wider community as well. In summary, INSPIRE aims to foster both research and training in safety pharmacology and hopes to inspire the future generation of safety scientists.

1. Introduction

Suboptimal non-clinical and clinical safety has been a major cause of drug attrition, discontinuation, withdrawal and severe adverse events. In particular, cardiovascular adverse drug reactions (ADRs) have halted many drug development programs (Bhatt, Northcott, Wisialowski, Li, & Steidl-Nichols, 2019; Laverty et al., 2011; Weaver & Valentin, 2019). Safety pharmacology studies aim to detect, evaluate and investigate potential safety-related liabilities of a drug mainly prior to entering clinical development. ICH S7A "*Safety Pharmacology studies for human pharmaceuticals" (*Anon., 2001*)* is the central document describing the guiding principles of safety pharmacology evaluation of new chemical entities (NCEs). For the cardiovascular system, an *in vivo* telemetry-based study in a non-rodent species is recommended to assess drug-induced hemodynamic effects. Further, ICH S7B *"The non-clinical evaluation of potentially delayed ventricular repolarization" (*Anon., 2005*)* defines a regulatory strategy to identify the risk of drug-induced Torsades de Pointes (TdP) arrhythmias, based on a combination of *in vitro* (hERG inhibition) and *in vivo* (QT-prolongation) assays. Almost 15 years later, the suggested preclinical strategy to detect cardiovascular ADRs, such as delay of cardiac repolarization, has shown acceptable, although not absolute, predictive value to humans (Authier et al., 2017; Bhatt et al., 2019; Komatsu et al., 2019). *In vitro* and more recently *in silico* hERG screening has enabled researchers to engineer out hERG and consequently QT liability from NCEs, possibly at the cost of losing potentially promising new drug candidates. Further, Phase-I clinical trials are very safe from a cardiovascular perspective, possibly reflecting effective preclinical testing and elimination of major cardiovascular safety liabilities prior to entering clinical development (Butler et al., 2017). In contrast, extrapolating the clinical relevance of a drug-related cardiovascular safety liability detected in a non-clinical study towards a large population of diseased patients on a chronic dosing regimen remains challenging. Hypothesis-driven mechanistic non-clinical studies, possibly in disease models, or Cardiovascular Outcome Trials (CVOT) in large patient populations may help to address possible long-term safety concerns. For antidiabetic drugs, CVOT have become the standard to evaluate overall long-term benefit and safety (Menon & Lincoff, 2014). Inclusion of Safety Pharmacology endpoints in repeat-dose Toxicology (SPiT) studies is another promising approach to monitor long-term cardiovascular safety (Authier, Vargas, Curtis, Holbrook, & Pugsley, 2013; Milliken et al., 2020). Novel technologies, such as non-invasive (or minimally-invasive) jacketed telemetry or ultrasound imaging have enabled more integrated innovative trial design, thereby potentially reducing the number of animals required, thereby contributing to the implementation of the 3R-principles (*i.e.*, refinement, reduction and replacement of *in vivo* animal studies)(Berridge, Schultze, Heyen, Searfoss, & Sarazan, 2016). Along this line, safety pharmacology is increasingly using social group housing of laboratory animals as this improves animal welfare thereby reducing stress (Prior et al., 2016; Skinner, Ceuppens, White, & Prior, 2019). Additionally, recent developments in large-scale production of human induced Pluripotent Stem Cell (hiPSC)-derived cardiomyocytes combined with innovative assay technologies hold promise to, at least partly, reduce some early *in vivo* screens (Pang et al., 2019). For instance, the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative has provided proof-of-concept of integrated proarrhythmia risk evaluation based on *in vitro* assays utilizing hiPSC-derived cardiomyocytes supplemented with *in silico* modelling (Wallis et al., 2018). In summary, the screening strategy described in the ICH guidelines have proven quite effective to identify major acute (cardiovascular) safety liabilities, but meanwhile safety pharmacology has not refrained from exploring new ways to further refine the non-clinical safety evaluation of new drugs, in particular long-term and subtle effects. Indeed, safety pharmacology has evolved into a dynamic, multidisciplinary discipline that actively engages in technology exploration, characterization, validation and implementation. Moreover, pro-active exploration of technological innovations for safety evaluation is encouraged by regulatory authorities as well (*e.g.* European Medicine Agency (EMA)'s Mission Statement and US Food and Drug Administration (FDA)'s Predictive Toxicology Roadmap, Anon., 2017).

2. Opportunities and challenges associated with exploring, validating and implementing novel technologies for safety evaluation

A wealth of new technologies has emerged with the potential to refine, or even drastically transform, safety evaluation of new drug candidates. Examples of possible breakthrough technologies include hiPSC-based humanized *in vitro* assays, organ-on-chip platforms, omics-based biomarkers, molecular and functional imaging capabilities (*i.e.*, Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI) and ultrasound imaging), systems biology and *in silico* modelling approaches, big data and artificial Intelligence. However, introducing novel methodologies in safety pharmacology is a major challenge, since thorough validation of the technology, robust confidence in the biology of the test system and its predictive value for human biology is required. As such, the threshold for introducing new technologies and associated read-out in safety pharmacology is high. Consequently,

many promising technologies may never translate into useful applications, unless a coordinated and collaborative approach is adopted. Moreover, exploring, validating and eventually implementing novel technologies comes at a significant economic cost, requires time and represents an uncertain return on investment. Hence, a collaborative strategy is preferable, thereby sharing the investment cost. Public-Private Partnerships (PPP) are a proven strategy to identify and address commonly faced challenges that are too big for the individual stakeholders (Maxfield, Buckman-Garner, & Parekh, 2017; Pierson et al., 2013). In the context of safety pharmacology, PPP offer the opportunity for regulatory agencies to join the consortium and to provide input during the validation process to define carefully the context of use of the novel assays. Along with this, the introduction of novel technologies should be accompanied with proper education within the field. This is an important aspect, since the hype associated with a new technology may include the danger of *"throwing away the baby out with the bathwater"* as regards to established animal models and the knowledge we have gained from them. Conversely, there may be reluctance from safety pharmacologists to move away from well-established, characterized and validated (*in vivo*) assays. Hence, a coordinated effort is required to address such, often unconscious, intrinsic bias originating from one's *in vivo, in vitro* or *in silico* background. In this regard, PPP often represent a multidisciplinary environment where scientists from different backgrounds collaborate and share knowledge. As such, PPP constitute a hands-on training setting and the participating scientists may serve as appropriate ambassadors educating the field on the opportunities and limitations offered by a novel technology platform. In this review, we will present the INSPIRE project, a European Training Network (ETN), that aims to foster multidisciplinary research and training in safety pharmacology, with active involvement of industry, academia and relevant stakeholders. Since INSPIRE aims to be complementary to ongoing research, we initially started by creating an inventory of existing collaborative initiatives addressing commonly faced issues in the cardiovascular safety evaluation (Table 1). We acknowledge that this list is incomplete and welcome any further initiatives to explore potential synergies with the INSPIRE programme.

3. Overview of ongoing collaborative research initiatives in safety pharmacology

Safety pharmacology has been shaped by intensive interactions between the pharmaceutical industry and regulatory authorities under the auspices of ICH, eventually leading to a set of guidance documents, such as ICH S7A, ICH S7B, ICH E14, ICH S6, ICH S9 and ICH M3. Further, the *Safety Pharmacology Society* (SPS) has been an important driver to promote collaboration and provide education in safety pharmacology (Pugsley et al., 2018). SPS's Annual Meeting is a key forum for sharing best practices and discussing future directions of safety pharmacology. SPS provides an educational programme for newcomers and a continuous education track for experienced safety scientists. Moreover, SPS has established the *Diplomate in Safety Pharmacology* (DSP) programme that certifies the knowledge and skills of qualified safety pharmacologist (Authier et al., 2015). Similarly, the *Society of Toxicology* (SOT) has been promoting collaboration among toxicologists and safety pharmacologists. Further, a range of initiatives has motivated the field to refine existing assays and to explore new technologies. Probably the best known organization involved with such activities is the *Health and Environmental Sciences Institute* (HESI) that supports pre-competitive collaborative research involving major safety themes. In particular, *HESI's Cardiac Safety Technical Committee* (HESI-CSTC) has been a vivid forum for identifying issues commonly faced in the evaluation of cardiovascular safety. HESI-CSTC has started a number of collaborative research projects, such as "*The Comprehensive in vitro Proarrhythmia Assay*" (CiPA) Initiative. CiPA aims to develop an integrated, mechanism-based approach to proarrhythmia evaluation. While a single hERG channel block by a NCE mostly correlates with QT prolongation in experimental models, it has limited performance for predicting overall clinical proarrhythmia risk, especially when a NCE interferes with multiple cardiac ion channels (Gintant, Sager, & Stockbridge, 2016; Sager, Gintant, Turner, Pettit, & Stockbridge, 2014). To overcome this, CiPA proposed to evaluate NCE effects on multiple ion channels beyond hERG (*e.g.*, I_{Ks}, I_{Na}, $I_{Ca,L}$) that are then used for *in silico* reconstruction of cellular cardiac electrophysiological activity. Next, the *in silico* predictions are compared to experimental data obtained from hiPSC-derived cardiomyocytes - human cells were specifically chosen to exclude potential species-related differences in cardiac electrophysiology (Fermini et al., 2016). CiPA aims to transform the assessment of drug-related proarrhythmia risk, including, but not limited to TdP risk. While initial results from CiPA are promising, further cross-validation against both clinical and preclinical datasets is needed (Wallis et al., 2018). In the validation process, CiPA has gathered data on the robustness of high-throughput methods for cardiac ion channel screening and has included a validation of hiPSC-derived cardiomyocytes assays. Meanwhile, discussions have been initiated with the ICH S7B/E14 Implementation Working Group (IWG) to revise these guidelines through a Questions & Answers process taking into account the latest scientific and technological developments. Other focus areas of HESI-CSTC include the exploration of hiPSC-based assays to assess functional and structural cardiotoxicity, including the identification of translational biomarkers (Pierson et al., 2013). Complementary to this programme, the *Japan iPS Cardiac Safety Assessment* (JiCSA) (Kanda, Yamazaki, Osada, Yoshinaga, & Sawada, 2018) has evaluated the utility of hiPSC cardiomyocytes assays for cardiac safety evaluation. Another initiative of the *Japan Safety Pharmacology Society* (J-SPS) is the *Japan activity for Improvement of Cardiovascular Evaluation by Telemetry system* (J-ICET) (Komatsu et al., 2019). Similarly, HESI-CSTC is engaged in a multi-site comparison and cross-validation of the sensitivity and reproducibility of minimally invasive telemetry methods for measuring hemodynamic parameters, including cardiac contractility. The latter has become increasingly important, since some pharmacological mechanisms (*e.g.*, tyrosine kinase inhibitors (TKIs)) may negatively affect cardiac contractility and may predispose patients to heart failure. The

Table 1
A non-exhaustive overview of Public-Private Partnership (PPP) and Scientific Societies fostering collaborative and coordinated research in (cardiovascular) safety pharmacology and toxicology.

PPP or scientific societies abbreviations	Website for further information
ICH	https://ich.org
SPS	https://www.safetypharmacology.org
J-SPS	http://www.j-sps.org
CSPS	No website available as yet
SOT	https://www.toxicology.org
HESI	https://hesiglobal.org
HESI-CSTC	https://hesiglobal.org/cardiac-safety-technical-committee
CiPA	*https://cipaproject.org*
PSTC	https://c-path.org/programs/pstc
CSRC	https://cardiac-safety.org
CSAHi	http://csahi.org/en
JiCSA	Japan iPS Cardiac Safety Assessment (Kanda et al., 2018) (Ando et al., 2017)
J-ICET	Japan activity for Improvement of Cardiovascular Evaluation by Telemetry (Komatsu et al., 2019)
IQ-consortium	https://iqconsortium.org
IMI-2	Preclinical Safety Leadership Group (DruSafe); https://www.imi.europa.eu
TransQST	http://transqst.org
EBiSC2	https://ebisc.org
eTOX	http://www.e-tox.net
TransBioLine	https://transbioline.com
SAFE-T	http://www.imi-safe-t.eu
TRISTAN	https://www.imi-tristan.eu
NC3Rs	https://www.nc3rs.org.uk

Cardiac Safety Research Consortium (CSRC) is another initiative worth mentioning in the setting of cardiovascular ADRs. The CSRC, coordinated by Duke University, was established in 2006 as part of FDA's Critical Path Initiative (CPI) and organizes annual Think Thank meetings and regularly publishes white papers to stimulate research in specific areas of cardiovascular safety. This way, CSRC actively contributed to the design of CiPA. Another important project of CSRC involves the set-up of a data warehouse of ECG signals that can be used for training of automated ECG analysis algorithms. Another CPI consortium is the *Predictive Safety Testing Consortium* (PSTC) aiming to validate clinical safety biomarkers, especially for drug-related kidney and liver injury. Further, the *International Consortium for Innovation and Quality in Pharmaceutical Development* (IQ consortium) is worth mentioning. Its *Working Group on Non-Clinical to Clinical Translational Safety* has established a database for benchmarking various assays used in toxicology and safety evaluation (Monticello et al., 2017). The *Innovative Medicines Initiatives 2* (IMI-2) programme also has a number of projects in its portfolio related to toxicology and safety evaluation. IMI-2 is a PPP, funded by the European Commission and equally matched by in kind contribution from the pharma industry, aimed to develop pre-competitive capabilities to accelerate drug development. IMI launches competitive calls on a regular basis. The topics of these calls are defined by members of the European Federation of Pharmaceutical Industries and Associations (EFPIA). Since its conception, IMI has funded over 148 projects, providing 5.3 billion € in funding. IMI-projects cover topics such as improving safety by adopting a systems toxicology approach (*e.g.*, eTOX, TransQST), developing safety biomarkers (*e.g.*, TransBioLine), exploring imaging markers (*e.g.*, SAFE-T, TRISTAN) or enabling hiPSC-derived assays (*e.g.*, EBiSC2). The United Kingdom's *National Centre for the Replacement, Reduction and Refinement of animals in research* (NC3Rs) is also actively engaging the safety pharmacology community to promote and support research on best practices with respect to implementation of the 3Rs (Jackson et al., 2019; Redfern et al., 2017). Given the complexity of the cardiovascular system, the use of animal models in safety pharmacology evaluation is still considered crucial to assess cardiovascular function. Accordingly, the S7A guideline requires *in vivo* animal experimentation to fulfil regulatory recommendations before first-in-human (FIH) trials. Nevertheless, there are still opportunities to minimize, replace or refine the use of animals or to improve animal welfare during the experimental procedures. Examples of cardiovascular research supported by NC3Rs include *in silico* modelling of cardiac electrophysiology (Passini et al., 2017), systems biology approaches aimed to develop effective Adverse Outcome Pathways (AOP) for cardiotoxicity (Margiotta-Casaluci, Dusza, Moreira, Winter, & Prior, 2019) and social housing of laboratory animals during telemetry studies to better reflect normal behavioral environments and reduce stress levels of animals during the test procedures (Prior et al., 2016; Skinner et al., 2019).

4. INSPIRE – a European Training Network in safety pharmacology, part of the MSCA-ITN programme

INSPIRE is a European Training Network (ETN) in the field of safety pharmacology. INSPIRE has started in January 2020 and will run until December 2023 and is funded through the European Commission's Horizon 2020 Marie Skłodowska-Curie Actions (MSCA) Innovative Training Networks (ITN) programme. The MSCA-ITN programme does not have predefined priority research areas, but relies on scientific proposals submitted, bottom-up, by consortia. The primary focus of ETN projects is to offer a stimulating, multidisciplinary research and training environment for Early Stage Researcher (ESR; *i.e.*, PhD students) fellows, which will enable them to develop a scientific career in industry or academia. ETN consortia are selected by a very competitive evaluation process (success rate $< 10\%$). Selected projects receive a budget to recruit ESR fellows for a period of 36 months. The budget covers the salary of the recruited ESR fellows, as well as a contribution to expenses related to research and training activities. The total budget is proportional to the number of ESR fellows with a maximum of 15 ESR fellows per ETN; this can be up to approximately 4 million € of funding. Matching internal funding may extend the length of the individual ESR projects to increase the likelihood of the ESR fellow to obtain a high quality PhD-degree. ESR fellows have mandatory secondments within the network to access new methodologies and foster knowledge sharing. Furthermore, complementary to the hands-on research programme, INSPIRE includes a training programme tailored to the educational needs of the ESR fellows, consisting of summer schools and

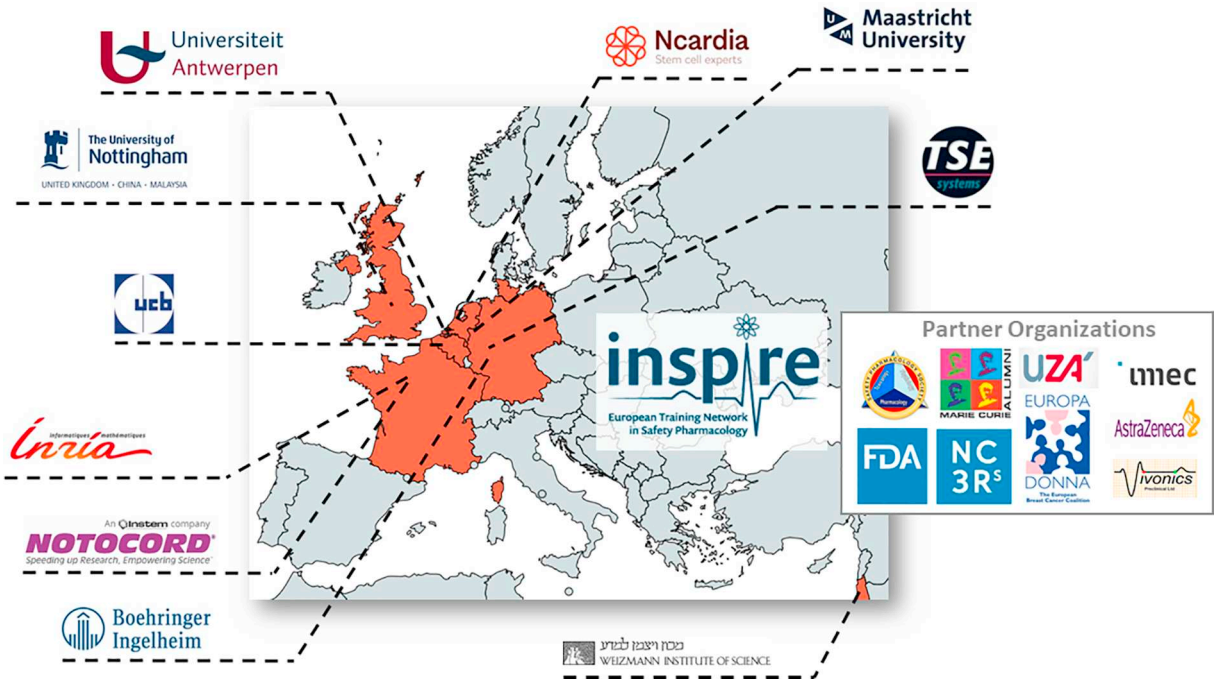

Fig. 1. Overview of the INSPIRE consortium. INSPIRE consists of 10 beneficiaries, supplemented with 9 strategic partner institutions supporting the training network.

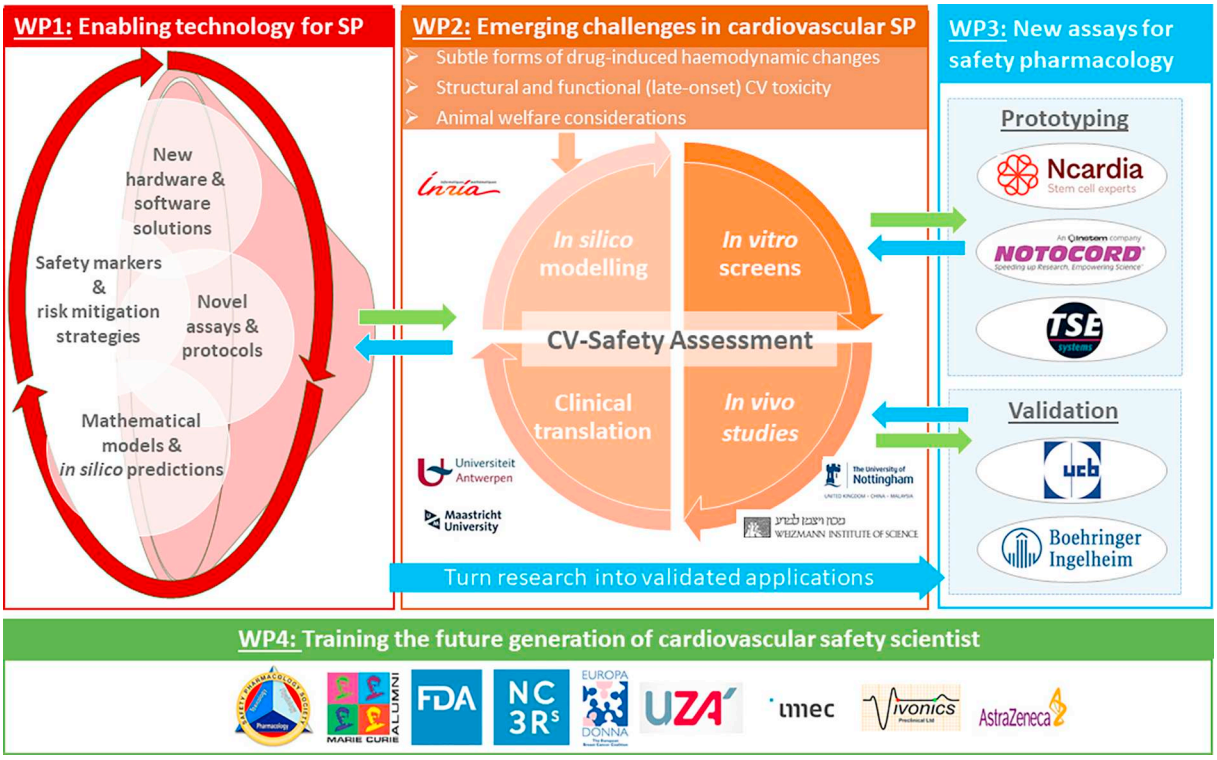

Fig. 2. Overview of INSPIRE's strategic objectives and its collaborative multidisciplinary approach.

workshops, that are open for the wider community. More information on INSPIRE is available through the website (http//www.inspire-safety-pharmacology.eu).

The INSPIRE project is built upon 10 beneficiaries (hosting 15 ESR fellows) supplemented by 9 partner organizations (supporting training activities) (Figs. 1 and 2). Given the fact INSPIRE is primarily a PhD-training network, its scope is less focused compared to challenge-driven research consortia, such as the CiPA initiative. To achieve a multi-disciplinary training environment, the network deliberately explores a variety of technology-platforms and experimental models to refine the safety evaluation. Moreover, the project architecture is designed such that individual ESR projects are not inter-dependent, whereas opportunities for synergy are maximally exploited. The central theme of INSPIRE is the exploration of novel technologies, often requiring multi-disciplinary collaboration, that are intended to improve cardiovascular safety evaluation with the hope to find acceptance from both pharmaceutical industry and regulatory agencies. Importantly, INSPIRE has been designed to be complementary to ongoing research initiatives (*cf.* Section 3 and Table 1), while avoiding duplication of efforts.

5. Research programme of INSPIRE

The core of INSPIRE are 15 individual ESR research projects (Table 2), each of them exploring an approach for refining cardiovascular safety evaluation, either by investigating novel technological capabilities or by utilizing experimental models to increase mechanistic insight. The ESR projects are clustered around 5 focus areas that were defined upon an analysis of ongoing research initiatives and the respective knowledge gaps. Therefore, the topics of the ESR projects are necessarily aligned with the expertise, capabilities and infrastructure of the participating institutions. In the next section, the different scientific focus areas of INSPIRE and the associated ESR projects are presented.

5.1. *Refining human cardiomyocytes assays for safety evaluation*

While the CiPA, JiCSA and other initiatives have demonstrated the

Table 2
Overview of the 15 ESR research projects of INSPIRE.

ESR n°	Recruiting participant	Description/topic
1.	Ncardia Services	Development and validation of improved hiPSC cardiomyocytes assays to study cardiac safety.
2.	UCB Biopharma	hiPSC cardiomyocytes model as a predictive assay to assess functional and structural cardiac liabilities.
3.	NOTOCORD	Empowering predictivity and speed of hiPSC cardiomyocytes assays by machine learning approach.
4.	TSE systems	Development of novel telemetry implants with added 3D micro-GPS functionality.
5.	NOTOCORD	Extending NOTOCORD-Sense™ with behavioral analyzers in a cloud-based architecture.
6.	Weizmann Institute of Science	Development of a software to analyze and quantify social interactions and behavior.
7.	Boehringer Ingelheim	Validation and use of novel telemetry implants with added 3D micro-GPS functionality.
8.	INRIA	An *in silico* approach to monitor and predict hemodynamics during safety pharmacology studies.
9.	Maastricht University	Development of MSI tools to study drug distribution and associated tissue-specific effects.
10.	University of Antwerp	Measuring arterial stiffness at different scales: a new toolbox for safety pharmacology?
11.	University of Nottingham	New preclinical screen in safety pharmacology assessment: detection of cardiovascular effects in “failed” NCE.
12.	University of Nottingham	Assessing the cardiovascular safety liabilities of growth factor inhibition.
13.	University of Antwerp	Optimize risk analysis and preventive measures to mitigate cardiovascular adverse effects.
14.	University of Antwerp	Chemotherapy-induced functional myocardial alterations: is heart failure with preserved ejection fraction (HFpEF) preceding HFrEF?
15.	Maastricht University	Personalized safety pharmacology against drug-evoked proarrhythmia.

potential utility of hiPSC cardiomyocytes assays (also coupled to *in silico* modelling for some purposes) for detecting drug-induced electrophysiology defects, the full potential of hiPSC assays has not been realized yet (Pang et al., 2019). Current challenges in using hiPSC cardiomyocytes for safety evaluation include their immature phenotype as compared to adult cardiomyocytes (Branco et al., 2019; Karbassi et al., 2020), variability of the results generated with different cell models and laboratories (Blinova et al., 2018) and lack of a widely accepted cut-off criteria for *in vitro* cardiotoxicity evaluation. (Magdy, Schuldt, Wu, Bernstein, & Burridge, 2018; Satsuka & Kanda, 2019).

ESR1 will focus on standardization of culture and differentiation protocols, and improvements in maturation of hiPSC cardiomyocytes in culture. For instance, the possible benefits of 3D culture conditions and co-cultures will be investigated (Branco et al., 2019). Simultaneously, ESR1 will focus on optimization of existing assays for contractility, mitochondrial function, and structural damage, for use in chronic safety pharmacology assessment.

ESR2 will exploit hiPSC cardiomyocytes assays, including microphysiological systems, to obtain early, selective and sensitive biomarkers of *in vitro* cardiac injury. The translatability of these biomarkers will be assessed by analyzing hiPSC derived cardiomyocytes cultures from healthy subjects and patients with observed drug-induced cardiotoxicities (Burridge et al., 2016). Assays based on contractility, electrophysiology and structural toxicity will be applied to develop the most predictive models able to detect/discriminate different types of cardiac toxicity. Their predictive capacity and human relevance will be assessed using a set of well-validated reference compounds. It is expected that these next-generation hiPSC cardiomyocytes assays can be used to monitor drug effects and toxicity over a longer period of time, thereby replicating late- or slow-onset effects as is sometimes observed in patients (Magdy et al., 2018).

ESR3 will address the bottleneck of handling, sharing and analyzing large datasets produced by hiPSC-derived cardiomyocytes assays. Innovative approaches for data handling will be designed and integrated into the cloud-based NOTOCORD-Sense™ architecture for fast access and improved computational performance. Further, an *in-silico* assay will be developed using a machine learning (*e.g.* deep learning, reinforcement, *etc.*) approach combined with mathematical modelling (bidomain equations) and numerical simulation (Raphel et al., 2018). One objective is to extract the effects of a compound on cardiac ion channels conductance by using an extensive dictionary of biomarkers automatically selected and ranked by the model during training. This automated process will reduce the manual selection of biomarkers and the time-consuming manual tagging of hundreds of signals. The end goal is to let the experts focus on interpreting the results and make informed decisions based on highlighted potential cardiac risks. Ultimately, an integrated dashboard is envisaged, delivering a complete cardiotoxicity profile of test compounds based on multiple endpoints, that will aid safety scientists in their decision-making.

ESR15 aims to employ patient-derived hiPSC cardiomyocytes assays to investigate patient's genotype-phenotype correlation with respect to electro-mechanical coupling. Previously, proof-of-concept of genotype-phenotype correlation was demonstrated for acute doxorubicin-induced cardiotoxicity (Burridge et al., 2016).

5.2. Prototyping a telemetry system for cardiovascular and behavioral monitoring of group housed animals

In vivo cardiovascular safety studies using telemetry-based data acquisition (as mentioned in ICH S7A) have been performed in the past on single-housed animals for technical reasons, despite social deprivation being known as a stress factor (Olsson & Westlund, 2007). From an animal welfare perspective, group housing is preferable, since the species used for these studies generally live in social groups and maintenance of the normal environment reduces stress and may thereby positively affect the quality of the data collected. More than that, individual variation between group members (such as hierarchy) that may affect different cardiovascular parameters might be displayed only in a group context. INSPIRE has the ambitious plan to deliver an unprecedented telemetry platform enabling simultaneous analysis of both behavioral traits and cardiovascular parameters obtained from socially interacting, group-housed animals in semi-natural environment. This approach will reduce non-relevant stressors, holds potential for more accurate cardiovascular evaluations and may in the long-term partly reduce the need for independent neuro-behavioral animal studies.

ESR4 will add micro-GPS functionality to the existing Stellar™ telemetry platform (Markert et al., 2018). In brief, the exact location of each animal (with mm accuracy) will be calculated based on a triangulation algorithm by virtue of vectorized transmission field strength signals coming from the implant to three strategically placed antennas.

ESR5 will develop software components to collect information on location and posture of an animal, generated by multiple sources in a cloud-based platform as well as to visualize and analyze them in real time. Next, the analysis of behavioral traits will start from video-tracking or micro-GPS telemetry input provided by ESR4 and ESR6. These algorithms will be further implemented in NOTOCORD-Sense™, thereby enabling real-time combination of behavioral analyzers with already existing cardiovascular analyzers. ESR5 will also address technical challenges associated with providing remote and fast access to the video streams and behavioral analysis results while respecting FDA/EMA requirements.

ESR6 will incorporate advanced video-tracking tools that utilize machine-learning approaches for pose detection in groups of mice in semi-natural setups and test a micro-GPS telemetry system that can potentially increase the accuracy of the pose detection. Further, ESR6 will develop algorithms for detection and quantification of complex social interactions and readouts. ESR6 will also develop behavioral paradigms that measure individual differences between group members at the physiological and behavioral levels at basal and stressoric conditions (Forkosh et al., 2019). The performance of the refined algorithms for behavioral analysis will be benchmarked against datasets of substances having known behavioral effects previously obtained from TSE's PhenoWorld system.

ESR7 will investigate the influence of social interaction during safety pharmacology studies with reference compounds. More specifically, it will be explored whether understanding of social traits, such as social hierarchy, dominance and other behaviors (Forkosh et al., 2019) can be exploited to reduce data variance.

5.3. Refining hemodynamic assessment

ICH S7A recommends conducting a hemodynamic assessment (*e.g.*, heart rate and blood pressure) in conscious animals. While hemodynamic effects observed in *in vivo* telemetry studies show acceptable translation to humans (Bhatt et al., 2019) they provide limited mechanistic insight, making it sometimes difficult to develop an integrated clinical risk assessment and associated mitigation and management plan. For instance, torcetrapib was halted during Phase 3 clinical development due to increased cardiovascular related morbidity and mortality. In non-clinical studies, a small rise in blood pressure by torcetrapib was detected, but it was deemed to not be clinically relevant at that time (Joy & Hegele, 2008). Nevertheless, chronic subtle deviations of systolic blood pressure in the larger population have been shown to correlate with the risk of cardiovascular disease, such as myocardial infarction and stroke (Bundy et al., 2017; Ettehad et al., 2016). While safety pharmacology conventionally focuses on the evaluation of acute ADRs, the discipline may need to become more engaged in the long-term (cardiovascular) risk assessment as well (Bass, Pugsley, Sannajust, Yoshinaga, & Valentin, 2019). A valuable initiative in this regard is the inclusion of Safety Pharmacology endpoints into Toxicology (SPiT) studies to extend the time horizon of cardiovascular

safety evaluation (Authier et al., 2013). Furthermore, mechanistic studies using multi-parametric readouts, possibly utilizing imaging technology or experimental disease models may be helpful to contextualize an observed hemodynamic ADR. Additionally, innovative mathematical approaches may help to extract hidden information from hemodynamic datasets. For instance, the attractor analysis enables quantification of temporal changes in pressure waveform morphology (Aston, Christie, Huang, & Nandi, 2018). Further, integrating experimentally obtained parameters in an *in silico* model of vascular biomechanics and hemodynamic regulation may help to understand hemodynamic changes during drug toxicity studies.

ESR8 will develop a mathematical model of a portion of the arterial tree, based on a previously described model (Formaggia, Quarteroni, & Veneziani, 2009). The major role of the model, besides providing a quantitative insight into the investigated phenomena, is to make it possible to relate measurements with predictions, which are usually quantities that cannot be reliably measured. In this respect, a particular focus will be put on the drug-induced effects on the hemodynamic function (change in blood flow, pressure and wall stiffness). Several experimental data will be used in order to validate the model, such as regional flow rate (ESR11), artery wall displacements obtained by non-invasive ultrasound imaging (ESR10) and local blood pressure simultaneously assessed by intravascular catheters.

ESR10 will focus on drug-induced effects on arterial stiffness. Arterial stiffness has not been widely considered in safety pharmacology, although it is a blood pressure-independent prognostic factor to predict the risk for cardiovascular disease. The effect of a number of well-characterized drugs on regional and local pulse wave velocity (PWV) will be determined, by tonometry and high frequency ultrasound respectively (Leloup et al., 2014). Additionally, drugs affecting arterial stiffness will be evaluated *ex vivo* in a proprietary organ set-up (*i.e.*, the Rodent Oscillatory Tension Set-up to study Arterial Compliance, ROTSAC) that enables determination of intrinsic arterial stiffness, independent of confounding factors, such as blood pressure or heart rate (Leloup et al., 2016). The collected dataset will allow for evaluating the added value of arterial stiffness on top of blood pressure assessment.

ESR11 will focus on blood flow, which is physiologically a highly relevant determinant, but usually not assessed in safety pharmacology studies. Failed clinical candidates, such as torcetrapib, bardoxelone, *etc.* will be evaluated in a rat Doppler ultrasound model, which entails the measurement of regional vascular conductance simultaneously in 3 different vascular beds (renal, mesenteric and hindquarters) in addition to heart rate and arterial blood pressure, in conscious animals with all reflex systems intact (Carter, Fretwell, & Woolard, 2017). The data generated in the Doppler ultrasound model will also be used to investigate whether novel markers can be derived from the arterial pressure waveform that correlate to drug-induced effects at the vascular level as previously reported (Aston et al., 2018). Such markers could also be assessed in conventional *in vivo* telemetry-based cardiovascular safety pharmacology studies. Furthermore, as mentioned, the experimental dataset of ESR10 and ESR11 will be used to refine and iterate the *in silico* numerical model.

5.4. Mass Spectroscopy Imaging (MSI) for quantifying tissue exposure and biomarker discovery

Commonly, plasma concentrations or are used to relate drug exposure to physiological or toxic effects. In this respect, pharmacokinetic-pharmacodynamic (PKPD) analysis is a powerful approach to improve the sensitivity and predictive value of *in vivo* safety studies (Komatsu et al., 2019). PKPD modelling is increasingly adopted by regulatory authorities as well. The ICH E14 Q&A document recognizes the utility of PKPD modelling and a well-designed *concentration-QTc* (c-QTc) trial (Garnett et al., 2018) may waive the necessity for a designated *Thorough QT* (TQT) trial. Further, suitable PKBD modelling is an essential step towards *in silico* modelling to support *Quantitative In Vitro to In Vivo Extrapolation* (QIVIVE). For instance, the TransQST consortium has demonstrated proof-of-principle of QIVIVE for predicting drug-induced liver injury (Albrecht et al., 2019). *In situ* tissue drug concentrations, however, could be more relevant than bulk plasma concentrations, especially when a drug accumulates in a specific organ having an effect. The gold standard for determining tissue distribution is whole-body autoradiography, but this approach is not compatible with standard safety pharmacology studies.

ESR9 will optimize Mass Spectrometry Imaging (MSI) protocols and methods (Chughtai & Heeren, 2010) to analyze drug distribution and its collateral effects. MSI is a label-free quantitative detection method with unique molecular and spatial specificity. Moreover, it is able to study a wide range of analytes, from low molecular weight drugs to proteins. Hence, MSI offers the exciting possibility of performing experiments that monitor both distribution (parent compound and metabolites) and pharmacological-toxicological mechanisms involved in ADRs in parallel (Karlsson & Hanrieder, 2017). Furthermore, the molecular images can be co-registered with other imaging techniques, such as immunohistochemistry based optical images.

5.5. Cardio-oncology: extending and challenging traditional safety pharmacology studies

Paradoxically, improved survival of cancer patients has led to new concerns of the deleterious cardiac and vascular effects of oncology therapies, resulting in the establishment of the field of cardio-oncology. Numerous anti-cancer drugs have been associated with left ventricular dysfunction, heart failure, hypertension, proarrhythmia risk, thromboembolic risk and exacerbating existing cardiovascular disease (Zamorano et al., 2016). Chemotherapy-related ADR may relate to direct cytotoxicity (*e.g.*, anthracyclines) or may be due to interference with signaling pathways regulating cardiovascular homeostasis, such as ErbB2/4 receptors (*e.g.*, trastuzumab), vascular endothelial growth factor (VEGF) receptors (bevacizumab), tyrosine kinases pathways (sunitinib and many others), *etc.* Moreover, ADRs due to cancer therapy sometimes have a late-onset and may only be recognized in cancer survivors. The challenges associated with the preclinical evaluation of anti-cancer drugs are comprehensively reviewed elsewhere (Seltzer et al., 2019). Both *in vitro* and *in vivo* studies may be helpful to identify and investigate mechanisms of cardiovascular toxicity. In addition, prospective studies exploring novel biomarkers or imaging techniques for cardiovascular monitoring will help to identify patients at risk and, where feasible, to manage and mitigate the risk by implementing cardio-protective strategies (*e.g.*, personalized medicine). In this regard, a more intense collaboration between scientists in safety pharmacology and cardio-oncology may lead to novel insights and concepts for safety evaluation.

ESR12 will investigate experimentally the cardiovascular consequences of TKI targeting growth factor signaling. TKI have been associated with hypertension and myocardial dysfunction (Lengel et al., 2015; Skinner et al., 2014). Patients treated with anti-VEGF therapies often develop dose-limiting hypertension and many patients subsequently become resistant to further therapy. Although hypertension related to inhibition of VEGF signaling can be reproduced in animal models (Carter et al., 2017), the mechanisms involved are not fully understood and warrant further in-depth investigation (Lengel et al., 2015). ESR12 will utilize a Doppler ultrasound rat model (Carter et al., 2017), enabling quantification of blood flow in different vascular beds, to obtain comprehensive insight in both systemic and local changes in hemodynamics.

ESR13 will test selected drugs, that are potentially cardiotoxic, in experimental models of heart failure to predict which forms of pre-existing cardiac dysfunction increase toxicity of the drugs. In a second part, the preventive effects of specific molecules such as neuregulin-1 (De Keulenaer et al., 2019), which is an agonist of the ERBB4 tyrosine-

kinase receptor, will be examined. The ultimate goal is to develop novel cardioprotective therapies to protect patients at risk for drug-induced cardiotoxicity.

ESR14 will perform translational research aiming to develop novel strategies for identifying patients at risk of developing chronic cardiotoxicity upon anthracycline treatment. Anthracycline causes dose-dependent cardiotoxicity, but there is considerable variability in susceptibility among patients, as well as in the timing of cardiotoxicity (*i.e.*, acute *versus* late-onset) (Zamorano et al., 2016). ESR14 will investigate functional (echocardiography) and molecular (microRNAs and MSI-profiling of endomyocardial biopsies) markers, both in experimental models and anthracycline-treated patients. More specifically, myocardial stiffness and impaired diastolic function will be assessed as a possible first step in the development of cardiotoxicity and may serve as an early marker for patient stratification and follow-up (*e.g.*, personalized medicine).

6. Training programme of INSPIRE

Safety pharmacology as a scientific discipline faces significant challenges in training and certifying investigators in integrative approaches. An impending mass retirement of experienced safety pharmacologists is expected and these need to be replaced to maintain the knowledge pool and develop future leaders in the discipline. Safety pharmacology calls for scientists with a broad range of knowledge and skills. They should have the ability to integrate knowledge of Physiology, Pharmacology and Toxicology (Pugsley et al., 2018) for translating drug effects at the cellular level to human whole-body physiology with intact compensatory response mechanisms. Further, they should have an understanding of the basic principles of a variety of *in silico, in vitro* and *in vivo* methodologies and their respective context of use. The explosion of new molecular techniques has caused a decline in training in integrative biomedical sciences, resulting in a growing deficiency in qualified individuals with the knowledge and skills required to conceptualize biomedical hypotheses and experiments at the level of the intact animal. This latter is partly addressed by SPS's educational programme and DSP certification scheme (Authier et al., 2015). Another challenge is the fact that the discipline is becoming increasingly multidisciplinary with introduction of novel methodologies, calling for specific technical expertise such as *in silico* modelling. Hence, safety pharmacology calls for individuals with both specific and holistic scientific knowledge. Further, safety pharmacologists should have strong communicative skills, a collaborative attitude, a technology-exploring mind-set and the ability to adapt to a dynamic and ever-changing industry.

The INSPIRE project will contribute to the training of the future generation of safety scientists in two ways. First, INSPIRE offers a stimulating and multidisciplinary PhD-research training environment to its ESR fellows. Depending on the scope of each individual ESR project, the recruited fellows will have a different educational background (*i.e.*, training in Physiology, Pharmacology, Cardiology, Biomedical Sciences, Engineering, Mathematics, Information Communications Technologies (ICT), Data Sciences, *etc.*). The supervisors and host institutions will add complementary insights and knowhow from different sectors, such as the pharmaceutical industry, technology- and service providers, academics, regulatory agencies, scientific societies, animal welfare and patient organizations. The obligatory exchange (*i.e.*, secondments) programme will enable sharing of knowledge between ESRs and supervisors. Secondments will offer the opportunity for specific hands-on training, or to have access to state-of-the-art research infrastructure. This *training through research* track aims to deliver 15 ESR with the potential to become future leaders in the field.

Secondly, INSPIRE will organize a number of network-wide training events, which will be open for the wider research community. The educational programme of INSPIRE will include 3 summer schools, respectively in Antwerp (2020), Paris (2021) and Maastricht (2022). Besides theoretical courses covering various aspects of safety pharmacology, there will be emphasis on transversal themes, such as research integrity, open-science policy (*e.g.*, open data), equality and diversity in science, and building sustainable research careers. Further, a workshop with regulatory agencies is planned. Another workshop will provide hands-on training in MSI. We refer the reader to the INSPIRE website (http//www.inspire-safety-pharmacology.eu) for a detailed and up-to-date overview of educational activities. Overall, INSPIRE aims to contribute to the education of a new generation of creative, open-minded, and innovation-oriented scientists with a broad background in safety pharmacology.

7. Conclusion

The current article presents the INSPIRE project, which is a European Training Network supporting 15 ESR fellows and their research projects. INSPIRE aims to foster multidisciplinary research and training in cardiovascular safety pharmacology. While INSPIRE recognizes the merit of established assays and ongoing research, we believe there is unrealized potential to refine cardiovascular safety evaluation, mainly through exploration of novel technological capabilities. Additionally, INSPIRE focuses on challenges in cardio-oncology, such as ADRs with unknown mechanisms, lack of disease modelling and long-term effects without available screening technology. In line with INSPIRE's inclusive open-science policy, we believe it is good practice to share the strategic goals of this substantial project at the start to foster collaboration. Further, describing the current state-of-the-art will enable monitoring of tangible project outcomes over the years (*e.g.* publications, patents, product applications and awarding PhD degrees to ESRs). Ultimately, INSPIRE hopes to have a legacy that outlasts the project duration by educating future leaders in safety pharmacology that will inspire the field in the coming decades.

Author listing and contributions

1st author: Pieter-Jan Guns (Project Coordinator, PJG drafted the manuscript); 2nd author: Brian Guth (BG significantly revised the text); positions 3rd-31st: listing of beneficiaries and partners according to the order in the original research proposal (all authors have contributed to and approved the final version); 32nd and 33rd position: Guido De Meyer and Jean-Pierre Valentin (Directors of Training); 33rd& last author: Jean-Pierre Valentin (JPV significantly revised the text).

Declaration of Competing Interest

The views expressed in this publication are those of the authors and do not necessarily represent the decisions, policy or views of their respective institutions.

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