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Sharing lessons learnt across European cardiovascular research consortia

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Research consortia in Europe often compete with each other for skills, human and technical resources and, eventually, recognition of the scientific impact of their work. In response to the same EU Horizon2020 call, we received funding for our research project proposals to identify and validate novel drug targets for cardiovascular disease treatment. Each consortium followed a unique and independent research strategy. However, as coordinators of these consortia we envisioned we could increase impact, outcomes and efficiency by intensifying our interaction. At an agreed stage during our projects we chose to share our knowledge, vision and ideas. In this paper we present what we learned, in the hope that future consortia will see the benefits of this approach.

Introduction

It is almost impossible to predict whether new targets for pharmaceutical intervention will be a success in the clinic. Identifying 'druggable' targets and developing a novel drug are extremely challenging tasks. On average, across various therapeutic areas, only one in ten candidate drugs that enter the clinical-testing-in-patients stage is successful in reaching the market. The major reason for such a rate of attrition is the proof-of-concept phase, during which the attrition rate can be very high owing to lack of efficacy and/or adverse effects.

Cardiovascular disease (CVD) is a key global medical health problem, predicted to cause 23.6 million deaths in 2030. It is the largest cause of death in the European Union (EU), accounting for ~40% of deaths, circa 2 million people per year. CVD affects the

heart and its blood vessels, and comprises events such as myocardial infarction, heart failure and stroke. The silent development (over decades) of this multifactorial disease – factors such as gender, nutrition, smoking, exercise and genes – and the limited availability of clinical samples and translational models, complicates the identification of drug targets and development of efficient therapies.

Identifying novel targets

This prompted the European Commission to publish, within the FP7 2013 Health Innovation Framework, a call for proposals focusing on 'discovery research to reveal novel targets for CVD treatment'. Research organisations across EU were invited to submit cutting-edge research projects to 'further explore available and emerging molecular, genomic and other omics data, from large-scale population studies, in order to allow the identification,

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characterisation and validation of *in vitro* and *in vivo* models of novel therapeutically relevant targets'. In other words, to deliver a validated drug target that would appear so promising that pharmaceutical companies would consider developing a drug specifically for that target, and new tools to support the development process. Research organisations were invited to take a multidisciplinary approach, using advanced biotechnology tools and investing at least 30% of their own capital (€2 million from ~€6 million) and to involve small–medium enterprises (SMEs) in the research.

The EU funding was awarded to three consortium proposals: CarTarDis, with TNO (The Netherlands) in the lead; TransCard, coordinated by the University Medical Center Groningen (UMCG, The Netherlands); and CVgenes@target, coordinated by the Deutsches Herzzentrum München (DHM, Germany). All three consortia met the call criteria but with different approaches: CarTarDis took a pharmaceutical industry approach to discover and validate novel targets; TransCard focused on targets linked to disturbed lipid metabolism; and CVgenes@target used drug targets revealed by population genetics to validate a predefined set of candidate genes involved in the development of cardiac disease and stroke.

CarTarDis consortium: a 'virtual' pharma company

The CarTarDis consortium consisted of the following partners: TNO (The Netherlands, coordinator), Helmholtz Zentrum München (Germany), Karolinska Institutet (Sweden), AstraZeneca

(Sweden), Icelandic Heart Association (Iceland), Leiden University Medical Center (The Netherlands), Quorics (The Netherlands), Polygene (Switzerland), Bioneer (Denmark), Imabiotec (France), Morphisto (Germany), Umeå University (Sweden) and Molecular Profiling Consulting (UK).

CarTarDis adopted a nonacademic approach and formed, with 13 consortium partners, a 'virtual' pharmaceutical company that mirrored the R&D processes the industry would use (Fig. 1). The aim was not only to identify and validate novel CVD drug targets and develop new tools but also to increase the knowledge and competitive position of the participating partners – especially the SMEs – in drug discovery. TNO brought together partners from its network with expertise in different aspects of pharmaceutical target discovery, supplemented with new partners offering complementary specialisms. The research partners used cardiovascular cohorts, preclinical models, clinical biobanks and innovative molecular imaging methods. Based on the pharmaceutical expertise available at TNO and particular key partners, an approach was designed that precisely mirrored that of the pharmaceutical industry, adopting its best practices from the start.

First, available and emerging molecular data, from three large independent cardiovascular cohorts, were mined for statistical association with CVD phenotypes with a preference for genetic associations [1]. Then a database and target-selection workflow were designed to select and prioritise candidate CVD drug targets. The selection filters were inspired by the 5R strategy of AstraZeneca

FIGURE 1

CarTarDis workflow as applied within the project. After selection and prioritisation in WP1, each target was assigned a target champion. This target validation director led a small multidisciplinary team that designed and performed the target validation experiments in WP2 and WP3, following which all results were integrated in WP4. The target director was also in the lead of the dissemination activities for the target.

and included genetics, omics, druggability, novelty, feasibility and pathways [2].

Target champions

Across the project work-packages, for each preselected target, a target champion was appointed. Each champion led a multidisciplinary team of consortium scientists in defining key experiments to validate that particular target. All sub-teams were guided and supported by experienced drug hunters and clinical cardiovascular experts from the CarTarDis consortium, paralleling the project structures of pharmaceutical companies. It was essential to ensure that the teams communicated their activities and priorities clearly, particularly because the individual consortium partners contributed their expertise to most, if not all, target-validation teams. The full CarTarDis consortium met twice a year, hosted by the partners, often with visiting local scientists who presented their work in seminars. In addition, the scientists in each target-validation team had multiple interactions as needed. In the final 18 months of the project, progress was closely monitored via monthly conference calls with the target champions.

Validation of the candidate targets used two simultaneous approaches. For mechanistic validation, novel *in vitro* (cellular) and *in vivo* (rodent) preclinical models were used in which the candidate targets were studied regarding mechanism of action and roles in particular aspects of cardiovascular processes [3,4]. Clinical validation took place via two independent and complementary clinical CVD biobanks: Socrates (LUMC) and BiKE (Karolinska Institutet), and several high-resolution molecular methods for investigation of target-related pathways at RNA, protein and small-molecule levels using *in situ* hybridisation, immunohistochemistry and mass spectrometry imaging, respectively [5,6]. The SMEs in the consortium contributed strongly in these two validation activities, bringing in their own technological expertise. An example of this multidisciplinary approach is provided by mechanistic analysis of the lipid phosphate phosphohydrolase 3 pathway that demonstrated its contribution to atherosclerosis through endothelial cell activation [7]. Other mechanistic insights are being published [8].

The CarTarDis project delivered a unique interactive database that allows the partners to summarise key features of preselected, candidate drug targets. Selection criteria can easily be adapted to yield a mixed portfolio of drug targets varying in novelty, druggability, omics evidence and scientific value. Within our project, of the 380 preselected candidate targets, seven targets were ultimately prioritised, assigned a target champion and evaluated. At the end of the project, three targets appeared promising enough for further validation. The resulting database and the tools found applications in other (new) projects developed by the consortium partners and will be reported soon.

TransCard consortium: disturbed lipid metabolism

The TransCard consortium consisted of the following partners: UMCG (The Netherlands, coordinator), Entelechon/Polyquant (Germany), Nebion (Switzerland), University of Copenhagen (Denmark), Academic Medical Center of the University of Amsterdam (The Netherlands), Universität Zürich (Switzerland) and Novamen (France).

TransCard focused on one discrete aspect of CVD: disturbed lipid metabolism, because this aspect has, essentially, been the key to the most-effective drugs on the market to date. Making use of a former partnership in the FP-6 Framework, the UMCG formed a consortium in which the partners brought excellence in the basic science of lipid and lipoprotein biology, in experimental animal studies, in patient-related research and in prospective cohort association studies. Two SMEs were attracted: one specialised in quantitative proteomics and the other in mapping RNA data. With six partners, TransCard chose for the identification of new targets through studies *in silico*, *in vitro*, in mouse models and in humans. With complementing expertise and technical and human resources in the consortium, the key targets were studied in cell culture systems, in experimental animal studies, in patients and at the genetic epidemiological level.

Identification

The lead SME explored *in silico* methods via an extremely large, curated compendium of (public) transcriptomics data related to CVD. Making use of existing knowledge on lipid biology, TransCard explored transcriptomic changes associated with those in key genes in lipid metabolism; this led to the identification of potentially valuable leads. One of the academic partners conducted an unbiased, genome-wide, image-based RNA interference screening for genes that limit the uptake of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) by hepatocytes. A second academic partner identified a series of novel genes involved in the transport of hepatic lipoprotein receptors (affecting plasma cholesterol levels) in mouse models.

Characterisation and validation

For all preselected target genes, experimental animal models were generated and characterised. At the same time, targeted next-generation sequencing studies were conducted to investigate the sequences of these target genes. Common and rare variations in the given genomic loci were studied in a large number of cases for effects on plasma lipid levels and brain and ischaemic heart disease. This helped to prioritise TransCard research efforts.

In TransCard (see Fig. 2 for project overview), each of the partners had their own work package that allowed them to focus on their own strengths. The whole consortium gathered face-to-face once each year; the coordinator planned regular site visits (during the first three years) to ensure synergy between the partners. The TransCard results significantly increased our understanding of intracellular lipoprotein receptor trafficking [9–12]. Using a whole-genome knockdown approach, the consortium also identified a series of new genes that regulate plasma lipid levels through affecting hepatic lipoprotein uptake [13,14].

Ensuring synergy

For future consortia, the consortium leader would probably change the approach, so that every partner would appoint a dedicated post doc for the duration of the project. This would result in an increase of each partner's focus and enable a more intensified collaboration or interaction between the partners, concerning outcomes, goals, needs and expectations, and would further optimise synergy within the consortium.

FIGURE 2

TransCard project workflow as applied within the project. TransCard was chosen for the identification of new targets through studies *in silico*, *in vitro*, in mouse models and in humans. Each of the partners had their own work package, allowing them to focus on their own strengths.

CVgenes@target consortium: genetic variants and mechanisms

The CVgenes@target consortium consisted of the following partners: Deutsches Herzzentrum München (Germany, coordinator), University of Leicester (UK), Universität München (Germany), University of Oxford (UK), Universität zu Lübeck (Germany), University Medical Center Utrecht (The Netherlands), Bioceros (The Netherlands), European Screening Port (Germany), Clinical Gene Network (Sweden), 4SC Discovery (Germany), Genedata (Switzerland) and Horizon Discovery (UK).

CVgenes@target was dedicated to identifying the genetic variants and mechanisms involved in the development of cardiac disease and stroke. Many genes and genetic mechanisms appear to be involved in the development of CVD, but scientists are only certain about a few. The members of the consortium – a merging of groups in Germany, The Netherlands and the UK who had previously collaborated in other consortia – have all invested heavily, in recent years, in the detection of CVD genetic variants; their joint ambition was to take the science in this field to the next level.

The CVgenes@target consortium encompassed all necessary skills to successfully complete the programme (Fig. 3). The blocks demonstrate the different types of expertise of the participants. The arrows reflect the ideal workflow from target discovery to application. (i) Targets discovered in clinical research underwent verification in in-house clinical biobanks. (ii) Pathway analyses and informatics tools were applied to unravel additional targets and revealed pathways of interest. (iii) *In vitro* and animal experiments were executed to study mechanisms of disease and support the decision for subsequent drug screening and development. (iv) After drug screening and development the effect was tested in diseased animal models. (v) A clinical application is being considered. The participants are all well recognised for their publication

track records and have often presented at invited lectures and plenary sessions.

List of genetic variants

The consortium partners are content with the project outcomes: they found new drug targets and have doubled the number of known genetic variants involved in coronary artery disease. They were involved in the discovery of genetic mutations that led to loss of function in several genes and discovered that some mutations actually protect against this disease [15–18].

Network analyses

A surprising finding of the group was that many of the genetic variants causing CVD act by mechanisms that are, to date, not targeted by any medical treatment. To specify how such treatment could work, novel methods were developed and applied to functionally characterise the new genetic variants, such as network analyses to study interactions between genes and gene expression. Fundamental to the research was identifying whether specific variants might impact gene transcription. These variants were associated with gene expressions that were disease-condition relevant: atherosclerosis, for example [19–21].

The scientists of the CVgenes@target consortium also demonstrated that transcriptional effects might even occur at a trans-tissue level: for example, gene expressions in the liver might affect gene expressions in visceral abdominal fat tissue and vice versa [22]. One can assume that such cross-tissue networks interact by endocrine communication, which could be a fruitful target for medical treatment. The same approach was used to study interactions between gene variants and drug efficacy. Some drugs on the market, such as cyclooxygenase-2 (COX-2) inhibitors (nonsteroidal anti-inflammatory drugs) and glatiramer acetate (an immu-

FIGURE 3

CVgenes@Target workflow as applied within the project. The arrows reflect the ideal workflow from target discovery to application. (i) Targets discovered in clinical research underwent verification in in-house clinical biobanks. (ii) Pathway analyses and informatics tools were applied to unravel additional targets and revealed pathways of interest. (iii) *In vitro* and animal experiments were executed to study mechanisms of disease and support the decision for subsequent drug screening and development. (iv) After drug screening and development the effect was tested in diseased animal models. (v) A clinical application is being considered. The participants are all well recognised for their publication track records and have often presented at invited lectures and plenary sessions.

nomodulator medication currently used to treat multiple sclerosis), increase the risk of stroke or heart attack. The scientists now know which variants in the genes are directly affected by these drugs. Key to success of the CVgenes@target consortium was extensive communication between the project partners: the whole consortium met, for several days, twice a year. 'Collaboration bears the best fruit when you know each other well', was their motto.

Sharing lessons learnt

Once all three projects were ongoing, we decided to organise a joint symposium. We knew that our approaches were very different yet could be complementary. The timing of the event was crucial, because the consortia needed to have sufficient results to share but also sufficient remaining time to use information exchanged at the event. In September 2016, during the third year of the projects, all the CarTarDis, TransCard and CVgenes@target partners – 73 scientists in total – gathered at the Dutch coastal town of Zandvoort to share insights, ambitions, goals and ideas. The opening speech was given by the EU commission project officer of CarTarDis, indicating the relevance of CVD research and this event to the EU. The first day was dedicated to presenting the goals of the three projects and highlights of the results gained thus far. The second day featured pitches by individual partners from each of the consortia, presenting their unique technologies, databases and biobanks.

It became an inspiring, effective and, in a way, a very common-sense meeting: partners of the different consortia realised that the

same objective could be approached from very different perspectives and learned much about the methods used by the others. For one partner, applying molecular technologies like mass spectrometry imaging to analyse CVD biobanked samples was novel. For another, it was the different genetic cohorts and their vast molecular data, or the biobanks and their collections of samples that were of most interest. The SMEs had a unique opportunity to present their unique assets and added value for future consortia on CVD research. The symposium enthusiastically identified that cross-consortium synergy is possible and laid the foundations for future collaborations. As example, CarTarDis and TransCard scientists secured new national funding for comprehensive mass-spectrometry-based analysis on biological samples to improve insight in lipoprotein and cellular biology.

Concluding remarks

We believe that, if we want to make significant steps in our translational scientific community towards elucidating disease mechanisms and developing novel diagnostics and drug interventions, we need to work together, preferably from the very start. This is particularly the case with complex health issues such as cardiovascular disease. Despite impressive European funding that complements national programmes, we – as coordinators of multi-partner research consortia – are rarely stimulated to join forces and often operate in relative isolation. However, if we want to be globally competitive and develop innovative, effective treatments for heart

disease, long-term (>5 years) pan-European collaboration is our only real option. We have taken steps towards inter-consortium collaboration; we propose that the EU follows our example and actively promotes interaction and knowledge transfer between research consortia to lift European research expertise to a higher level.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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