06/2024 | CARE Biannual Newsletter

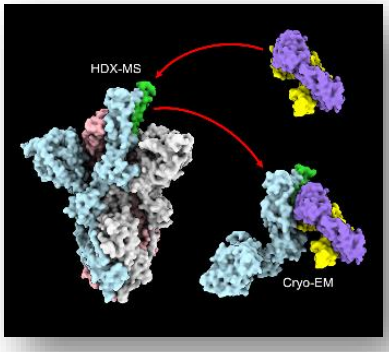
C A R E
CORONA ACCELERATED R&D IN EUROPE

In this issue we talk about **cryo-EM** and how this exciting new technology is benefiting our scientific endeavours across CARE. We also take a closer look at three more of our partners: **Utrecht University, the Institute for Virology and Immunology, and Nuvisan**

A picture paints a thousand words: Utrecht University expertise in cryo-EM technology continues to bring benefit to CARE's small molecule and antibody development work.

Dr. Daniel Hurdiss, Assistant Professor, Utrecht University

Cryogenic electron microscopy (cryo-EM) capability has been made available to CARE through the expertise of CARE partner, Utrecht University (UU). Virologists from UU's Biomolecular Health Sciences department use cryo-EM as one of their core techniques to study viruses. These structural studies are led by Dr. Daniel Hurdiss who has used this method to study virus structures over the last 10 years.



Antibody TPP-30431 binds to a conserved epitope (green) on the SARS-CoV-2 spike receptor binding domain. Using HDX-MS and cryo-EM, CARE researchers identified the antibody binding site and demonstrated that antibody binding disrupts the prefusion structure of the spike protein.

Why cryo-EM is so important for CARE's research

The importance of cryo-EM for coronavirus pandemic preparedness and responsiveness cannot be overstated. The first human coronavirus spike structure was obtained using this technique in 2016, and this served as a roadmap for the development of pre-fusion stabilized vaccines. Similarly, this technique was essential to visualize other coronavirus proteins for the first time, such as the RNA-dependent RNA polymerase (RdRp) complex and membrane protein.

A picture paints a thousand words (Cont'd)

Within CARE, all these proteins, and more, have been investigated as targets for pharmacological intervention. As such, cryo-EM plays an essential role in understanding the molecular mechanism of candidate antiviral molecules, as well as facilitating their optimization.

About cryo-EM

Cryo-EM, or cryogenic electron microscopy, is a powerful imaging technique used to study the structures of biological molecules at high resolution. It involves rapidly freezing samples to preserve them in their native state, followed by imaging with an electron microscope, and subsequent computational analysis to determine a three-dimensional structure

Cryo-EM and small molecule development

In Work Package 2 (Target-based drug discovery and design), cryo-EM was used to study how a macrocyclic peptide inhibitor, developed by UU, binds to the SARS-CoV-2 spike protein. This three-dimensional information is now being used to guide the rational improvement of this molecule.

Cryo-EM was also essential for determining the mechanism of action of a first-in-class SARS-CoV-2 membrane protein-targeting compound, developed by CARE partner KU Leuven. This data can now be used to further improve the potency and breath of these molecules.

"Cryo-EM has had an important contribution to the development of our novel small-molecule antiviral. Thanks to cryo-EM performed by UU, we've been able to confirm that our lead series is binding to the membrane protein of SARS-CoV-2, which will potentially help us to further optimize potency."
Patrick Chaltin, KU Leuven

In the same vein, cryo-EM was key in determining the mechanism of action of Bemnofosbuvir, a nucleotide analogue prodrug initially developed against the Hepatitis C virus and repositioned in 2020 against SARS-CoV-2. The drug binds to two independent sites in nsp12, namely the RNA dependent RNA polymerase and the NiRAN domain, opening avenues to further improve the design of novel inhibitors.

Cryo-EM and antibody development

Cryo-EM structural analysis is a powerful tool for epitope mapping of antibodies targeting the viral spike protein and inhibiting SARS-CoV-2 cell entry. In Work Package 4 (Antibody development), many cryo-EM structures of spike-antibody complexes were determined by UU and CHUV. Defining the different epitopes of neutralizing antibodies allows us to understand their mechanism of action and to increase our knowledge of how antibodies can confer protective immunity against SARS-CoV-2 infection. Furthermore, precise knowledge of an antibody's epitope allows a scientist to design antibody combinations that effectively target the viral

A picture paints a thousand words (Cont'd)

spike protein without the antibodies interfering with each other, and to select antibodies that bind to more mutationally silent regions of the spike that are less susceptible to mutations that could confer viral resistance to the neutralizing antibodies. Computational antibody design is another emerging field that leverages structural data to generate therapeutic antibodies with improved potency and neutralizing breadth against the emerging variants of concern.

Precise knowledge of an antibody's epitope allows a scientist to design antibody combinations that effectively target the viral spike protein without the antibodies interfering with each other.
Daniel Hurdiss, UU
Craig Fenwick, CHUV

How might it be used in the future?

Given how important the knowledge derived from structural biology has been prior to, and during, the COVID-19 pandemic, it is safe to assume

that this method will continue to be used to provide fundamental insights into the structure and function of viral proteins and facilitate the development of vaccines and antiviral molecules. Conceivably, with the ongoing improvements in AI for small molecule and antibody design, these methods can be used synergistically to design and validate future antiviral therapies. Thinking even further ahead, developments in cryo-EM which allow viral proteins to be visualised inside the infected cell may reveal new druggable targets that can be exploited in the future.

What added value has cryo-EM brought to CARE?

Beyond the valuable biological insights cryo-EM contributes to drug discovery efforts within CARE, these data also provide a wonderful instrument for communicating scientific results and concepts to a wider audience. After all, a picture paints a thousand words.

Introducing Utrecht University – a CARE academic organisation

Utrecht University

Established in 1636, Utrecht University has a long history and is rich in tradition. Utrecht University was formed by the provincial government in 1636. It is the top Dutch university (Shanghai Ranking) and offers 45 undergraduate and 167 graduate programs, has some 30,000 students served by 7,500 academic staff.

Today the university has seven faculties in Veterinary Medicine, Medicine, Science, Geosciences, Social and Behavioural Sciences, Humanities and Law, Economics and Governance.

The main CARE contributor from the university is the Virology section at the Faculty of Veterinary Medicine, studying various viruses (coronavirus, influenza, picornavirus) to develop novel innovative antiviral drugs, therapeutic antibodies and vaccine approaches. It has longstanding and extensive experience in coronavirus research, specifically in

1. Receptor identification and virus-receptor interactions
2. Structure-function studies of the viral Spike protein, which is the protein involved in binding receptor(s) and mediating cellular entry and the main target for humoral immune responses, and as such a key viral protein for development of intervention strategies
3. Determinants of coronavirus inter-species transmissibility
4. Development of intervention strategies (antibodies, vaccines, antiviral drugs) and diagnostics for (emerging) coronaviruses

It has about 45 members including researchers from all seniority levels and support staff, rising to up to 60 researchers when housing students performing their MSc internships.

Why did Utrecht University choose to get involved in CARE?

The Virology Section has 50+ years of experience in coronavirus biology and broad expertise in human and animal coronaviruses, particularly in virus structure, virus-receptor interactions, virus entry mechanisms, genome replication and virus-host interactions.

Introducing UU (Cont'd)

The CARE project enabled us to quickly contribute our knowledge with many other research groups, whilst also benefitting from increased collaboration from the CARE partners.

What has Utrecht University delivered for CARE?

UU's achievements span the innovations and findings in Work Packages (WPs) 1, 2, 4 and 5. Overall, their efforts aim to identify potential antiviral drugs, peptides, nanobodies and antibodies, to understand their mechanisms of action, and to explore host factors involved in coronavirus infection, which could inform the development of effective therapies against SARS-CoV-2 and other related coronaviruses.

WP1: Evaluation of a large number of small molecules for antiviral activity against SARS-CoV-2 and other coronaviruses, characterized antiviral effects and performed Mechanism of Action studies to reveal their molecular mechanisms.

WP2: Development of macrocyclic peptide inhibitors and nanobodies targeting the Spike protein of SARS-CoV-2. The team studied the site in Spike that is targeted by the macrocyclic peptide using cryo-Electron Microscopy (cryo-EM), which revealed that the peptide binds to a conserved region of the SARS-CoV-2 spike protein that has hitherto not been exploited by antibodies or small molecules and has not been mutated in any of the variants of concern.

WP4: Establishing assays and reagents for the assessment and characterization of human neutralizing antibodies (nAbs) against SARS-CoV-2 and variants. The team generated bispecific Abs to enhance potency and breadth of nAbs against SARS-CoV-2 and related coronaviruses. They also developed broad-spectrum nAbs against porcine deltacoronavirus (PDCoV), an emerging coronavirus with pandemic potential. Finally, they isolated and characterized human Abs that target host receptor aminopeptidase N (APN). These Abs are highly resistant to viral escape and can be employed for coronaviruses that share the same receptor for entry, increasing our pandemic preparedness against newly emerging coronaviruses.

WP5: Determining dynamic changes in the metabolome in coronavirus-infected cells and identification of essential host factors for coronavirus infection via genetic CRISPR-Cas9 screens. In follow-up experiments, we conducted Mechanism of Action studies, and tested inhibitors interfering with intracellular signalling or targeting essential host factors for their antiviral effects.

For more information about the different work packages, please click here.

Introducing UU (Cont'd)

Who is working in the CARE team at Utrecht University?

The Utrecht University team is led by two Principal Investigators: Professor Dr Frank van Kuppeveld who studies the interaction between viruses and their host; and Dr Berend Jan Bosch (Associate Professor) who studies virus-receptor interactions and cell entry mechanism of membrane-enveloped viruses, particularly coronaviruses. During the CARE project, Dr Daniel Hurdiss, a structural virologist, was promoted from a post-doctoral researcher to Assistant Professor. He is a structural virologist who, among others, uses cryo-EM to study the 3D structure of viral proteins and understand how this relates to their function.

Prof. Dr Frank van Kuppeveld (WPs 1, 2, 5)

Dr Berend Jan Bosch (Associate Professor) (WPs 2 & 4)

Dr Daniel Hurdiss (Assistant Professor) (WPs 2 & 4)

Many of UU's virologists have been involved in CARE from the start, all of whom aim to translate their knowledge into intervention strategies targeting either viral entry and/or replication: Frank Buitenwerf, Marianthi Chatziandreou, Oliver Debski Antoniak, Tim Donselaar, Wenjuan Du, Preeti Hooda, Ruben Hulswit, Yifei Lang, Joline van der Lee, Rutger Luteijn, Wendy Meijer, Vera Nijman, Lonneke Nouwen, Collins Owino, Judith Oymans, Itziar Serna Martin, Jill Ver Eecke, Marleen Zwaagstra.

What benefits have Utrecht University experienced through being part of CARE?

- Access to a broader range of expertise, technologies, and facilities
- Opportunities to work alongside industry partners, other academic institutions, and governmental agencies which fosters knowledge exchange, interdisciplinary research, and the pooling of diverse perspectives and skills
- Academic researchers' opportunities for networking, professional development, and establishing collaborations with leading experts and institutions in the field
- Support to conduct cutting-edge research, accelerate progress towards the development of effective therapies, and make meaningful contributions to the global effort to combat infectious diseases

Click here to learn more about Utrecht University

The COVID-19 pandemic is over so what is the value of CARE? We asked CARE Principal Investigators to share their views on why CARE is so important for future pandemic preparedness. The words they shared through LinkedIn are below and highlight that there is so much more to be done to put the world in a better place of readiness for when the next pandemic strikes.

"With the evolution of the virus and the emergence of new variants, the number of reinfections is high despite the vaccines. Many studies describe long-term complications (long COVID) associated with reinfection and co-infection, with persistent respiratory, cardiac and neurological symptoms. **The need to develop new drugs to block infection, mostly in fragile populations, is thus still crucial.** Understanding the physiopathology of long COVID or long-term complications is one of the main objectives of the CARE Work Package 5". ***Yves Lévy, Inserm/VRI***

"**The drug discovery effort within CARE is still of prime importance** to tackle re-emerging COVID cases and new variants of concern, especially with direct acting antivirals which could as well be repurposed when the time comes, to closely related RNA viruses for preparedness purposes". ***Cyril Dousson, Ai-biopharma***

"The COVID-19 pandemic has transitioned towards an ongoing health issue. Knowledge of SARS-CoV-2 and related viruses is of continued importance to be prepared to tackle new variants of concern and future zoonotic threats by other (corona)viruses. **Public-private research partnerships, like CARE, are indispensable for enhancing future pandemic preparedness, leveraging the collective strength of academia, industry, and other (external) partners.** These collaborations foster rapid knowledge exchange, innovative solutions, and efficient resource mobilization, creating a robust network to tackle emerging health crises on a global scale". ***Frank van Kuppeveld, Utrecht University***

"CARE has enabled a multi-disciplinary team of investigators to develop invaluable tools, reagents and therapeutics to treat COVID-19 patients. **The highly productive and collaborative nature of this consortium is a model for future pandemic preparedness programs".** ***Rob Jordan, Bill & Melinda Gates Foundation***

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Introducing IVI – a CARE academic organisation

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Federal Department of Home Affairs FDHA
Institute of Virology and Immunology IVI

In 1917, Swiss laws on epizootics were drawn up to combat foot-and-mouth disease effectively. In response, the Swiss Confederation built the Federal Vaccine Institution in 1942.

In 1992, the institute was renamed to Institute of Virology and Immunoprophylaxis and moved to a new facility with a high containment laboratory in Mittelhäusern, approximately 15km from Bern. Since 2014, a merger with the Institute of Veterinary Virology at the Vetsuisse faculty led to the current Institute of Virology and Immunology (IVI).

The IVI is Switzerland's reference laboratory for the diagnosis and research of highly infectious epizootic diseases, including viral zoonoses. The IVI employs approximately 120 staff at its sites in Bern and Mittelhäusern. These include scientists, students and support staff (engineers and technical staff, animal caretakers, administration and biosafety).

Two divisions of IVI contribute to CARE. The team of Prof. Volker Thiel specialise in respiratory and enteric viral infections and zoonoses. The team of Prof. Charaf Benarafa are experts in molecular, cellular and in vivo pathways of innate immune responses in infectious and inflammatory disease.

Prof. Volker Thiel, Head Virology, IVI

Prof. Charaf Benarafa, Associate Professor of Immunology, IVI

Why did IVI choose to get involved in CARE?

IVI decided to get involved with CARE primarily through the involvement in the SCORE project of which IVI were one of the core eight partners. All 8 partners from SCORE are also in the CARE consortium. Secondly, the Thiel team has more than 25 years of experience of working with coronaviruses, so the opportunity to combine efforts with the Benarafa team was a possibility that could not be missed due to their considerable expertise in dissecting the immune response using infectious disease models.

What has IVI delivered for CARE?

IVI's achievements in CARE include the production of fluorescent viruses in Work Package 1 (WP1) which were pivotal in the development of a high-throughput dual-reporter screening assay for antiviral drug discovery.

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Introducing IVI (Cont'd)

This was possible due to the close collaboration with the team of Pieter Leyssen at KU Leuven (DOI: 10.1016/j.antiviral.2022.105506).

Second, the IVI team also made a series of mutant viruses incorporating changes implicated in drug-resistance. This was done with the team of Dirk Jochmans from KU Leuven also within the context of WP1 (DOI: 10.1016/j.antiviral.2022.105506).

In WP6, the IVI has contributed considerably by developing animal models for testing the efficacy of antivirals developed in the drug pipeline of the CARE project.

What benefits has IVI enjoyed through participating in CARE?

Both the Thiel and Benarafa teams have been able to broaden their networks, which have led to the instigation of a new EU-funded project to develop broad acting antivirals against RNA viruses with pandemic potential (PanViPrep), which began at the start of 2024. Other collaborations have also started with CARE colleagues working on other coronaviruses (including Feline Infectious Peritonitis). Undoubtedly, these opportunities have been facilitated through our participation in the CARE project.

In addition to Volker and Charaf, the IVI team includes

Melanie Brügger (Postdoctoral Research Scientist, WP6)

Nadine Ebert (Senior Research Scientist, WP1)

Lisa Thomann (Project Manager WP1, WP6)

And Bettina Trüeb (Postdoctoral Research Scientist, WP1)

For more information about the different work packages, please click here.

Want to know more about IVI?

https://www.ivi.admin.ch/ivi/en/home.html

https://www.ivi.unibe.ch/index_eng.html

Want to know more about our fantastic CARE Work Package teams?

CARE has eight Work Packages but what do they all do and how do they join up?

Following the three CARE fundamentals infographics which were published earlier in 2024, we are now publishing a series of infographics about each of our Work Packages.

The first of these provide insights into Work Packages 1 and 2 which are responsible for drug discovery in phenotypic virus cell-based assays, and Target-based drug discovery and design respectively.

The infographic provides an at-a-glance view on the team's objectives, collaborations, breakthrough moments and some impressive stats! For a closer look, click here: WP1 and WP2.

To see more of these, ensure you are following us on LinkedIn so that they appear in your feed as we publish the rest of the series over the coming months. The Work Package 3 infographic will be published in July.

Please feel free to like/comment/repost our infographic posts when you see them.

Introducing Nuvisan – a CARE EFPIA organisation

Founded in Europe in 1989 as an independent, family-owned contract research organization (CRO), NUVISAN was set up with a clear purpose: to leverage science to foster positive change and enhance the lives of patients.

Initially providing bioanalytical laboratory services and PK/PD studies, the company later expanded to other areas, such as early discovery, safety testing, clinical trial management, and regulatory support. It has grown significantly since its founding and has expanded its services and capabilities to meet the changing needs of its clients.

NUVISAN *"The Science CRO. We know how to discover,* develop*, and bring the next generation of medicines to market – all under one roof!"*

Operating under the name NUVISAN since 2010, today the company has more than 1,000 employees and operates in multiple locations in Europe and the United States. It is a fully integrated contract research and development and manufacturing organization (CRO/CDMO) that offers unique, high-quality, and tailored integrated solutions along the drug discovery and development value chain. These include:

- Target Identification and Validation
- Target-to-Lead
- Lead-to-Candidate
- Preclinical
- Clinical

Why did NUVISAN choose to get involved in CARE?

NUVISAN chose to get involved in order to support the development of therapeutics in addressing the current or future coronavirus outbreaks. The CARE consortium offered the exciting opportunity for NUVISAN to team up with renowned research organisations jointly discovering and developing new treatment options to treat the current and future coronavirus outbreaks.

Introducing Nuvisan (Cont'd)

What has NUVISAN delivered for CARE?

In addition to our initiator role for the CARE project and bringing different expertise in medicinal chemistry (Work Package 3) and *in vitro* ADMET compound profiling (Work Package 6) into the team, NUVISAN is a project lead for a joint Hit-2-Lead project together with Servier (high throughput screening), Merck and Takeda (synthesis), Iktos (AI, design), KU Leuven and LUMC (antiviral assays), Université d'Aix-Marseille (biochemical & biophysical assays), Helmholtz-Zentrum Für Infektionsforschung Gmbh and The Institute of Virology and Immunology (*in vivo* compound profiling).

These collaborative efforts have now achieved an *in vivo* proof-of-concept and the project is moved into lead optimization phase.

For more information about the different work packages, please click here.

What benefits has NUVISAN enjoyed through participating in CARE?

Nico Bräuer, NUVISAN PI for CARE, comments: "We are delighted to be engaging with so many globally renowned research institutions and pharmaceutical companies in this impactful and exciting project. While collaborating on this joint endeavour, we continue to discover new ideas and unlock scientific innovation through extensive knowledge exchange and discussions with other members. Together, we can accelerate the development and utilization of effective therapies for patients around the world."

Nico Bräuer
Senior Principal Research Scientist, WP3

In addition to Nico, the NUVISAN team in CARE includes

Christian Lechner (Head of Transporter Profiling, WP6)

Luis Bering (Scientist, WP3)

Want to know more about NUVISAN? click here.

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All about CARE

CARE is one of 8 IMI EC funded consortia playing a role in supporting efforts targeting coronavirus. It was launched in April 2020 and is Europe's largest scientific research initiative committed to tackling COVID-19.

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Its dual goals were firstly to find solutions to address the arising emergency; and secondly for future pandemic preparedness, exploring small molecule and antibody options.

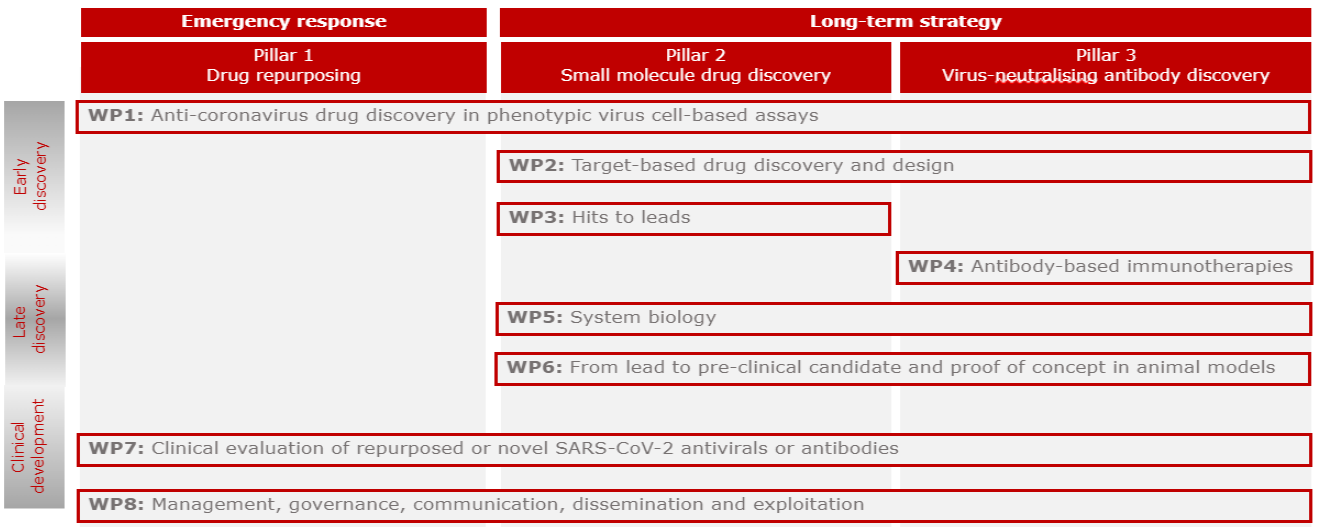
CARE comprises 38 highly respected partners from around the globe, bringing together the relevant academic and industry expertise, with a budget of 76 million euro split between contributing EFPIA partners and matched by the European Commission. It is led by Marnix Van Loock of Johnson & Johnson, with Kumar Singh Saikatendu of Takeda as co-lead, and Professor Yves Lévy of VRI-Inserm as the project co-ordinator.

Project Coordinator: Professor Yves Lévy, Professor of Clinical Immunology and Executive Director, VRI-Inserm

Project Lead: Marnix Van Loock, Senior Scientific Director and R&D Lead of Emerging Pathogens, Communicable Diseases, J&J

Project Co-lead: Kumar Singh Saikatendu, Senior Director Research Public-Private Partnerships, Takeda

The consortium comprises three research pillars, addressed by eight work packages working independently and collaboratively towards our goals.



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Initial efforts in the emergency space did not yield results, but progress has steadily been made in the preparedness space in both small molecules and antibodies, with teams continually taking account of the evolving context as the virus yielded new variants.

The consortium is steadily building a pipeline of potential small molecule assets to move forward, with promising signs of differentiation from current standard of care. On the antibody front, two very promising candidates were developed with good breadth and potency across all currently known variants of concern, which are now being developed in the clinic, outside of CARE.

CARE is committed to serve society through science and collaboration. Its partners are dedicated to undertaking efforts to make potential new treatments accessible for broad populations including in low and lower-middle income countries at an affordable price. Naturally, the consortium is keen to focus its remaining resources towards the most promising candidates that will bring new benefit to patients.

More information: Go to the CARE website for more information about

The CARE project overview	The CARE consortium partners	The CARE Clinical Trial Platform
CARE news	CARE publications	CARE resources

About this Newsletter

Having passed the project half way point, with many new discoveries and achievements under our belt, we will be sharing our progress each June and December via the newsletter; as well as more frequent posts being shared on LinkedIn.

All CARE partners will automatically receive a link to this newsletter. If you would like to be added to the distribution list please e-mail the CARE Project Management Office.

Reminders

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 101005077. The JU receives support from the European Union's Horizon 2020 research and innovation programme, EFPIA, BILL & MELINDA GATES FOUNDATION, GLOBAL HEALTH DRUG DISCOVERY INSTITUTE and UNIVERSITY OF DUNDEE.

The content of this publication only reflects the author's view and the JU is not responsible for any use that may be made of the information it contains.

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- www.IMI-CARE.eu
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Contact

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