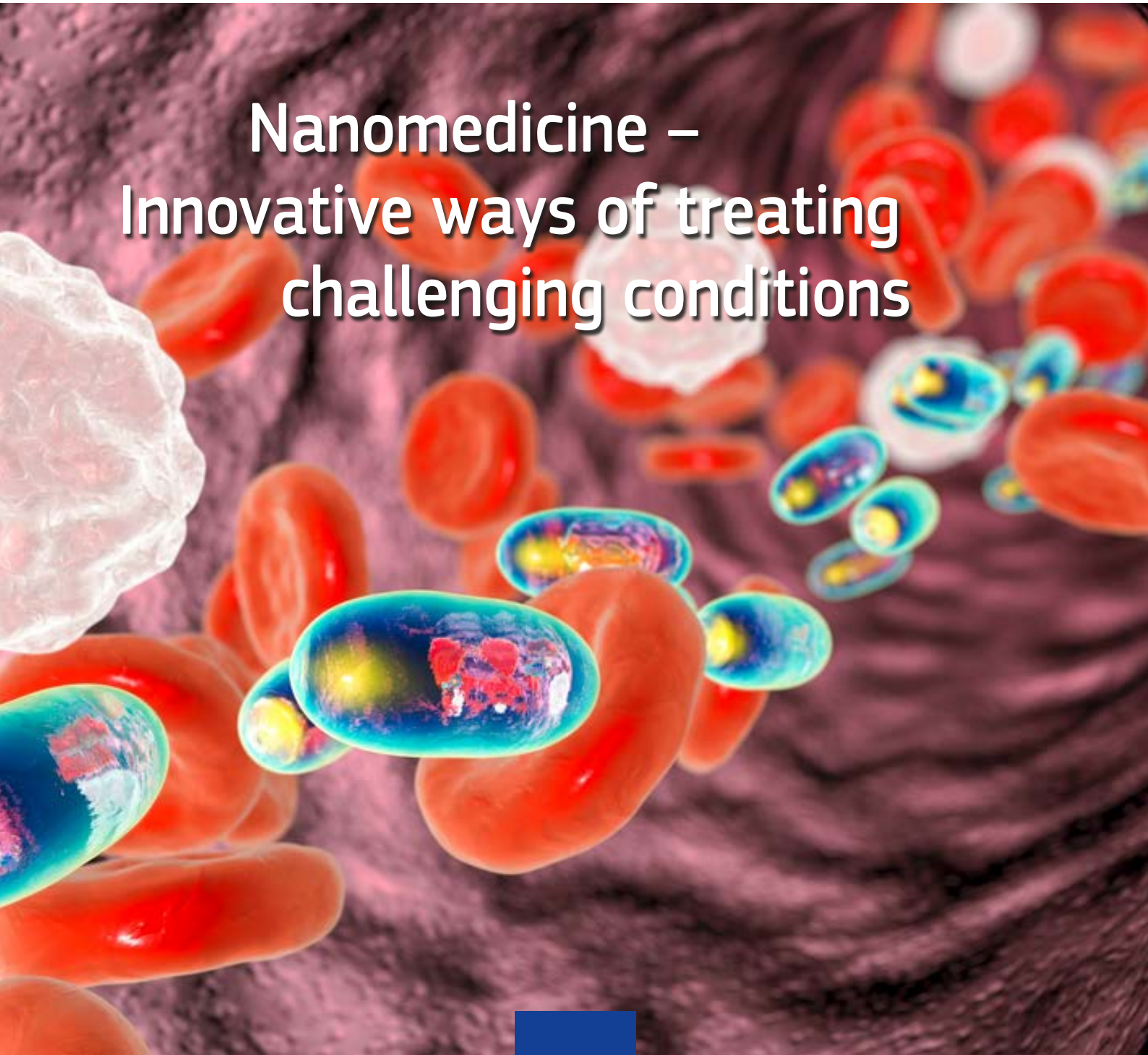
ISSN 2529-2919

European Commission

research eu

RESULTS PACK ON NANOMEDICINE



Nanomedicine – Innovative ways of treating challenging conditions

EDITORIAL

NANOMEDICINE – INNOVATIVE WAYS OF TREATING CHALLENGING CONDITIONS

Conditions often associated with later life, such as cancer, cardiovascular diseases, Alzheimer's and Parkinson's, along with diabetes, are debilitating to the sufferer and a challenge to European healthcare systems. So, the potential breakthroughs nanomedicine can offer are being carefully and enthusiastically researched by a variety of EU-funded projects, some of the most promising being highlighted in this CORDIS Results Pack.

While the proportion of working-age people in the EU is shrinking, the relative number of those retired is growing, so questions arise regarding how to pay for long-term care.

From drug delivery targeted to specific cells, to regenerative medicine for patients with organ failure or severe injury, nanomedicine opens up numerous potential pathways to improving medical diagnosis and therapy.

A strategic issue for the competitive position of the EU's healthcare industry

From 2007-2010, the Industrial Technologies programme, as part of the EU's Seventh Framework Programme for research, invested about EUR 265 million in nanomedicine-related research projects.

Horizon 2020, which has EUR 80 billion of funding available from 2014 – 2020, is building on those results with a focus on nanomedicine translation – helping innovation make it out of the lab and into healthcare provision.

The European Technology Platform for Nanomedicine, an EU initiative, is an industry-led forum that provides input on the research priorities and gives advice on innovation-related policy for the nanomedicine field.

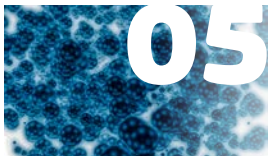
EU projects leading the field

This Results Packs showcases 10 projects whose research is opening doors to new opportunities for patients, as well as fostering the vital contacts between researchers, industry and financial intuitions to take these technologies further.

*Cancer cells are hard to treat: their microenvironment is complex and the blood vessels treating them are abnormal. EU funding through the **NeoNaNo** project helped to develop tumour-targeted combination therapies. The first in-human, clinical trial to look at their area of research is being conducted at the Centre for Clinical and Translational Research in Aachen, Germany.*

*Antibiotic resistance, potentially one of the biggest medical crises we currently face, could be around the corner. The **FORMAMP** project has harnessed nanotechnology to develop new delivery systems and antimicrobial peptides (AMPs), to put forward new tools in the fight against infectious diseases.*

Contents



Imaging technique offers cancer patient screening for nanomedicine therapies

Despite holding out promise for cancer treatments, nanomedicine lacks a means to predict patient response to tumour-targeting therapy. The CONQUEST project has devised imaging which differentiates between those likely to benefit and those unlikely to do so.

A significant barrier for the nanopharmaceutical industry tackling cancer is that while some patients treated with nanomedicine (NM) show major improvements in survival rates and quality of life, many others do not respond well.

This can result in delays to those patients receiving appropriate treatment. An effective mitigation would be the ability to stratify likely responders from non-responders, prior to their selection for studies or treatment.

The EU-funded CONQUEST (Companion Nanodiagnostics for Quantifying EPR and Stratifying Patients to Targeted Nanotherapies) project addresses this clinical gap through the development of nanomedicine imaging which uses (radio-) tracer labels to pre-select patients for inclusion in clinical trials. Additionally, market research amongst opinion leaders to ascertain which tracers, imaging methods, clinical protocols and reimbursement models were preferred has enabled the team to propose therapeutic solutions.

"Nanomedicine is still in its infancy, but with increasing discussion and success stories, I am convinced this will become a crucial therapeutic tool for cancer patients"

Individual EPR profiles for each tumour

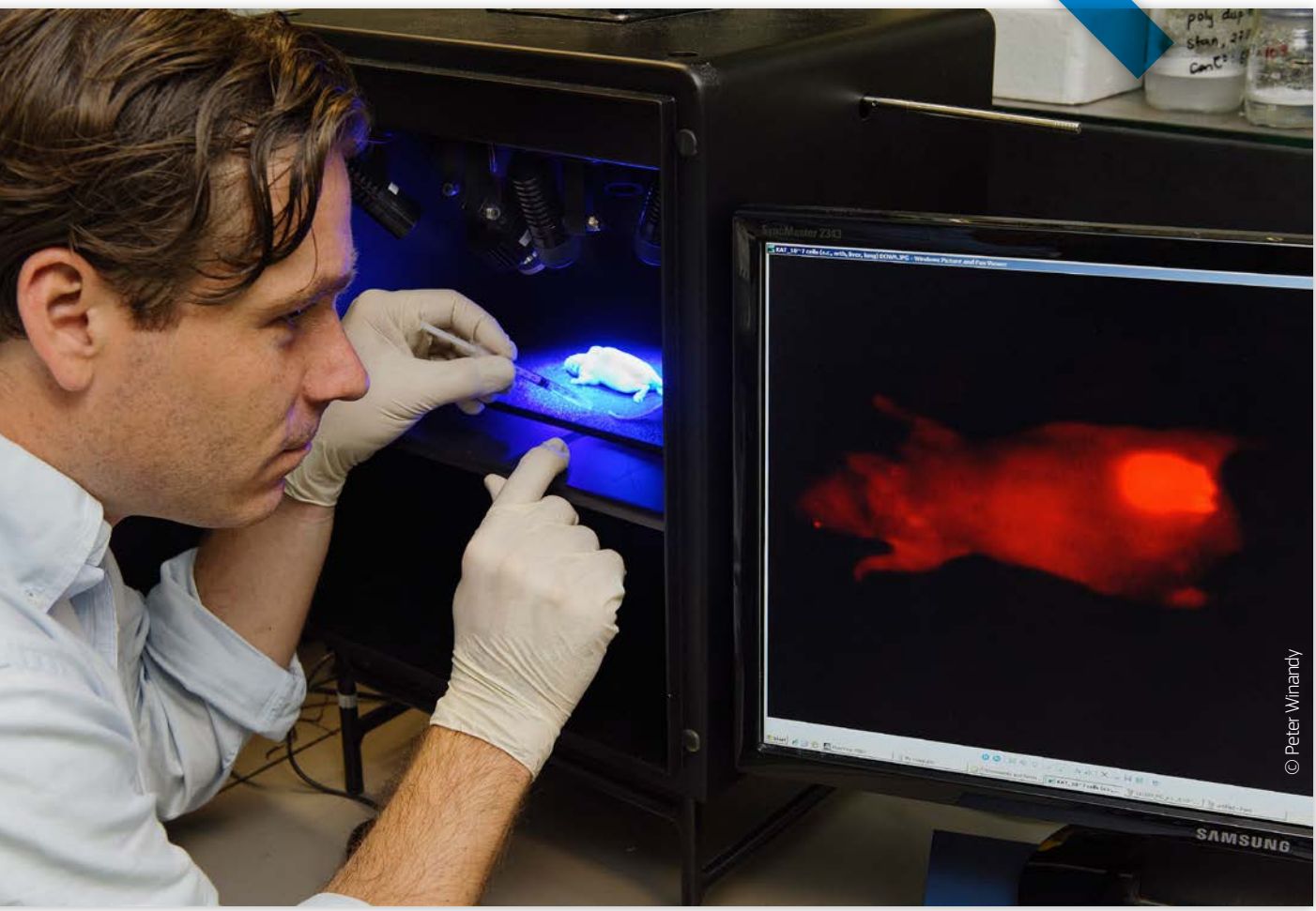
Nanomedicines depend on the so-called Enhanced Permeability and Retention (EPR) effect to work, whereby molecules are thought to accumulate more readily in tumours than in normal tissue. However, the EPR effect is variable across patients and can even present differently within lesions in the same patient.

Unlike virtually all new anticancer drugs which have protocols to stratify potential responders from non-responders, nanomedicine tumour-targeting technology does not have the same safeguard.

CONQUEST was able to conduct non-invasive imaging of tracer-labelled nanocarriers that accumulate in tumours through the EPR effect. Patients evidencing a low degree of EPR, and so unlikely to respond, can then be excluded from treatment and referred for established or experimental interventions. Likewise, patients with a high level of EPR can expect relatively impactful treatment.

"Pre-selecting patients in nanomedicine trials ensures that promising formulations transit more efficiently from phase one to two and three, and that only patients most likely to respond will be treated with the drug delivery system in question," explains Professor Twan Lammers the project's principal investigator.

The team applied both nanodiagnostic and nanotheranostic approaches. In the former case, drugs are precluded from the nanocarrier screening step. Whereas with the theranostic approach, both the drug and the imaging agent are present in the same formulation, providing valuable *in situ* information on target site accumulation of the nano-drug under investigation.



© Peter Winandy

"Options are currently being explored, by us and others; not only in terms of making and upscaling the therapeutic application of nanoproducts, but also for defining the optimal biomarkers and measures to be used for patient pre-selection," Prof. Lammers summarises. "These include things like the percentage of injected dose, distribution of dose within the tumour and the kinetics of probe accumulation."

A key therapeutic tool for the future

To exploit the technology's commercial potential for the pharmaceutical and diagnostics industry, the CONQUEST team is working to customise the technique for integration with PET-MRI imaging. However, as well as being the most promising, it is also the most difficult, costly and least available imaging technology and so they are also weighing up the pros and cons of using PET-CT or SPECT-CT.

"In future, theranostic techniques are likely to be used to exploit nanomedicine more efficiently in the clinic, for immunotherapy purposes," Prof. Lammers enthuses before adding, "We are currently exploring several options in this direction. Nanomedicine is still in its infancy, but with increasing discussion and success stories, I am convinced this will become a crucial therapeutic tool for cancer patients."

Project	**CONQUEST – Companion Nanodiagnostics for Quantifying EPR and Stratifying Patients to Targeted Nanotherapies**
Coordinated by	University of Aachen in Germany
Funded under	H2020-ERC

Nanoparticle drug delivery to tackle antibiotic resistance

There is a general consensus that drug-resistant microorganisms have emerged as an ecological consequence of the injudicious use of antimicrobial agents. To combat resistance, a European study has developed nanoparticles for the safer delivery of drugs.

Mycobacterium tuberculosis, the causative agent of tuberculosis, is an intracellular pathogen. When targeting such pathogens, the selected drug must be able to cross the eukaryotic cell membrane to ensure therapeutic success. However, not all antibiotics achieve the therapeutic intracellular concentration, resulting in antibiotic resistance and emphasising the need for improved drug delivery systems capable of penetrating infected cells.

Nanocarriers based on biocompatible cyclic oligosaccharides known as cyclodextrins (CDs) are emerging as promising delivery vehicles for antibiotics. They exhibit excellent biocompatibility in humans, low toxicity and absence of immune stimulation even at high dosages. Importantly, they can effectively incorporate a series of active molecules that protect them from degradation, thereby increasing drug bioavailability and decreasing treatment duration.

CD nanocarriers are particularly being considered for treating tuberculosis as well as bacteria species most frequently implicated in hospital infections. With a focus on tuberculosis, the EU-funded CYCLON HIT (Nanocarriers for the Delivery of Antimicrobial Agents to Fight Resistance Mechanisms) project aimed to design, characterise and evaluate the efficacy of CD-based nanocarriers.

Nanoparticles for treating tuberculosis

Tuberculosis constitutes a major health issue with millions of infected individuals worldwide. The misuse of first-line drugs may lead to multidrug-resistant tuberculosis, which is subsequently treated with chemotherapy or second-line drugs such as ethionamide (ETH). However, patients often find it difficult to comply with treatment regimens that require high doses of ETH.

Recent studies have discovered that certain 'booster' molecules significantly increase ETH efficacy, improving the clinical treatment of drug-resistant tuberculosis. "We wanted to investigate the simultaneous delivery of ETH and booster molecules using CD nanoparticles," explains project coordinator Dr Ruxandra Gref.

"We wanted to investigate the simultaneous delivery of ETH and booster molecules using CD nanoparticles"

Researchers focused on pulmonary administration of the nanoparticles, since lungs are the primary site of Mycobacterium tuberculosis infection. This approach helped achieve higher drug concentrations at the target site and less systemic side effects.

Following treatment in an animal model for tuberculosis, researchers observed a significant decrease in lung mycobacterial load. "Considering that the current regimen for tuberculosis consists of a cocktail of drugs, the CYCLON HIT approach could be employed to encapsulate synergic drugs, considerably simplifying the treatment and increasing patient compliance," continues Dr Gref.

The next era in antibiotic design

Drug resistance not only prevails in healthcare environments due to selection pressure or drug abuse, but is also increasing amongst community-acquired pathogens due to continuous exposure to low doses of antibiotics contained in foods, for example.

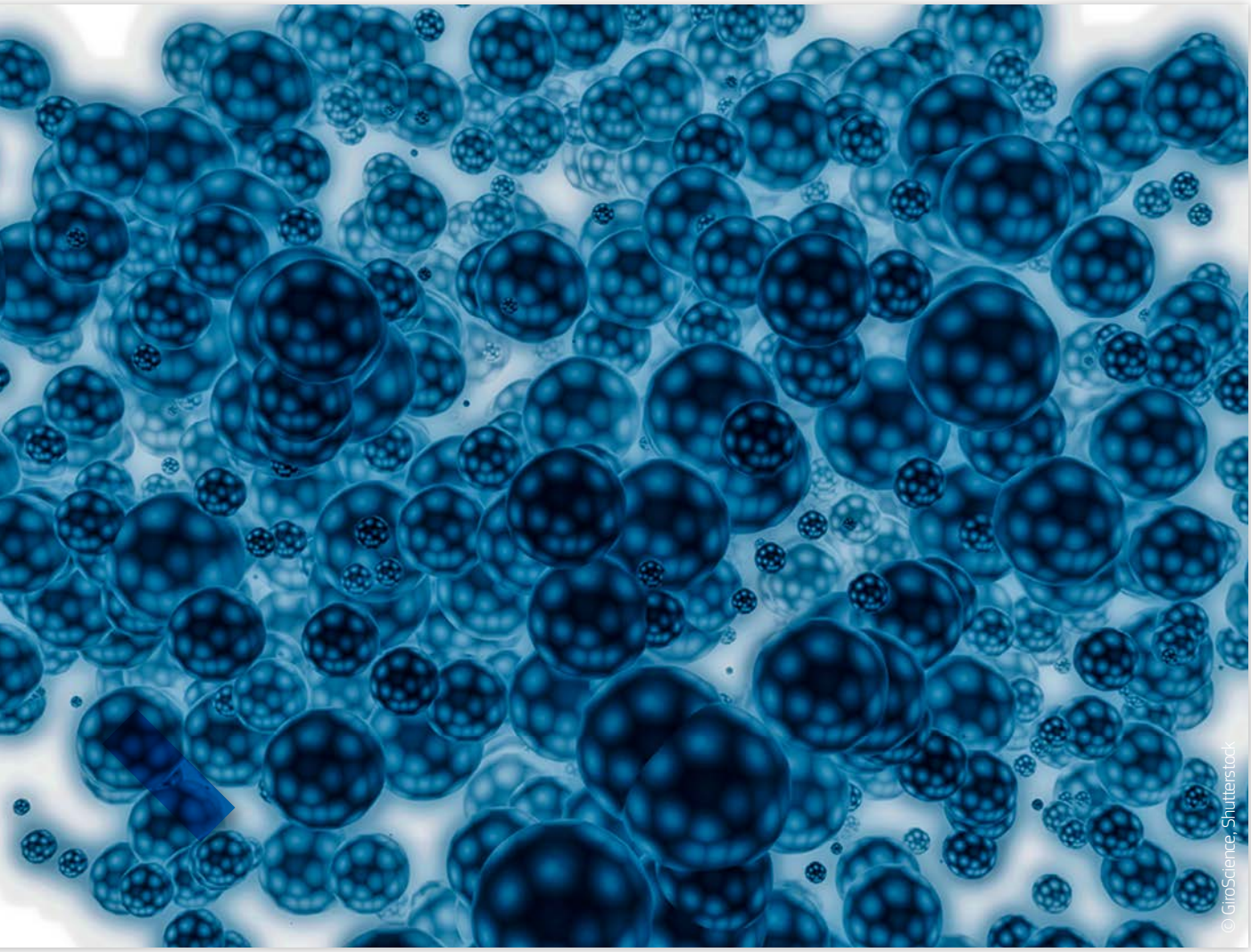
Given that bacteria respond to the selective pressure of antibiotics by continuously mutating and acquiring resistance genes, sustained research efforts are needed to deliver well-documented existing drugs in an optimised fashion.

To help achieve this goal, "The CYCLON HIT project trained the next generation of highly educated researchers to deal with the complex issues related to the increasing antibiotic resistance," outlines Dr Gref. Early Stage and Experienced Researchers undertook interdisciplinary training in chemistry, nanotechnology, microbiology and *in vivo* studies.

The consortium also organised three workshops, two summer schools and numerous outreach activities. The teams involved went beyond their initial objectives by organising a congress at the Pasteur Institute in Paris in 2015 and a 'brokerage and pitching' event in Orsay in 2018, bringing together several EU projects. Furthermore, Prof. Thorsteinn Loftsson and Dr Gref edited a special issue of a high impact journal in the field where CYCLON HIT partners made significant contributions concerning the use of CDs to fight serious diseases.

Exploiting the most recent advances in the nanomedicine field, CYCLON HIT partners envision alternative therapeutic approaches for other resistant microorganisms besides Mycobacterium tuberculosis. "Intelligent nanoparticles capable of delivering drugs to the site of infection with minimal side effects are certainly the choice of the future," claims Dr Gref.

Project	**CYCLON HIT - Nanocarriers for the delivery of antimicrobial agents to fight resistance mechanisms**
Coordinated by	National Centre for Scientific Research in France
Funded under	FP7-PEOPLE
Project website	http://itn-cyclonhit.eu/



© GiroScience, Shutterstock

Helping innovative medical research make it from lab to clinic

Multiple barriers stand between the development of nanomedicines and their passage into healthcare systems. An EU project brought together a pool of services, tools and innovation experts to help smooth the way.

Tissue engineering, organ repair, personalised medicine – the future of medicine is full of exciting new potential. But while researchers are innovating the ways we can diagnose and treat diseases such as cancer or degenerative diseases, getting these vital discoveries out of the lab and into hospitals, and the market, is beset by hurdles.

Scientists may be more than capable of cracking challenges presented by their research, but they need support to go on to crack barriers relating to regulation or financing. Taking inventions or academic research projects and translating those into therapies or medicinal products is a complex process. The SMEs and researchers developing the concepts now that will further the medicine of the future, need expert advice if they are to meet the requirements of regulatory and reimbursement agencies, the demands of large industrial companies and analyse clinical needs.

"The very complexity of the context can be off-putting. So ENATRANS succeeded in creating a network of support to SMEs by offering expert advice on issues such as intellectual property, business models and funding strategies, etc.," explains project coordinator Nicolas Gouze.

"ENATRANS succeeded in creating a network of support to SMEs by offering expert advice on issues such as intellectual property, business models and funding strategies, etc."

Guiding academia and industry on their translational journey

Partners in the ENATRANS (Enabling NAnomedicine TRANSlation) project all have a solid foothold in the European nanomedicine community and their expertise enabled the project to perform well. The nanomedicine Translation Advisory Board (TAB) was the main instrument through which academics and SMEs were given individual advice. "The board guided nanomedicine innovators, helping them translate their projects to something with market potential," says Gouze.

Having identified promising projects, the TAB helped them with issues such as accessing clinical centres for preliminary studies in patients, advice on complying with regulatory requirements or aid in finding finance to scale up processes.

The TAB has proven to be such a success that contacts have been initiated internationally to extend the concept outside Europe. Other concepts and tools have also been confirmed or validated during the project. These include the compendium for successful translation, the Nanomed Award, the Nano World Cancer Day, the Nanomed Handbook and the Nanomed Map.

Through webinars on how to get medical and pharmaceutical innovation to the market, the project put their knowledge of industrial and clinical needs at the disposal of participating SMEs and academics.

Who benefits?

A team of 12 high-level experts provided specific, individual advice to more than 80 European projects at differing stages of maturity through the TAB. Some of these projects are targeted towards global diseases such as cancer, multiple sclerosis or pulmonary infections. Business

development support provided by the TAB has contributed to nearly EUR 15 million in funding rounds creating three start-ups and two licence agreements with industry.

Partners in ENATRANS have successfully secured funding for TAB's operations for the next three years. It will be able to continue under the EU's NOBEL project to offer services to nanomedicine projects. At the same time it will support projects developing other key technologies for healthcare.

Project	**ENATRANS – Enabling nanomedicine translation**
Coordinated by	VDI/VDE Innovation+Technik GMBH in Germany
Funded under	H2020-LEIT-NANO & H2020-SME
Project website	http://www.enatrans.eu/public

Treating infectious disease with the help of antimicrobial peptides

Against the backdrop of increasing antibiotic resistance, the FORMAMP project has trialled nanotechnology-based delivery systems and antimicrobial peptides (AMPs), to deliver new tools in the fight against infectious diseases.

It has been calculated that increased microbial resistance is responsible for an estimated 25 000 deaths per year, costing EUR 1.5 billion, across the EU. In the quest for new therapies against infectious diseases, antimicrobial peptides are touted as promising candidates, as they appear to induce less resistance.

The EU-funded FORMAMP (Innovative Nanoformulation of Antimicrobial Peptides to Treat Bacterial Infectious Diseases) project was established to explore how nanotechnology formulations and local delivery strategies could improve the stability and efficiency of AMPs when applied directly to the site of skin and burn wounds, as well as to lung infections.

The project also developed a completely new type of nanomaterial, as well as generating a new approach for the treatment of tuberculosis with promising strategies for biofilm degradation.

"The combination of new treatments, such as ours, and increased public awareness of antimicrobial resistance, diagnostic tools and preventive actions against the spread of multi-resistant strains, should bring us closer to a future where resistance can be reduced"

Developing smart formulation and delivery strategies

Antimicrobial peptides are a group of molecules functioning as part of the innate immune system in most organisms. As they are fast acting against invading microorganisms and operate under non-specific mechanisms, bacteria struggle to develop resistance towards them. Despite holding out great promise for a new generation of therapeutic treatments, few AMPs have reached clinical trials, due to their sensitivity to degradation and high manufacturing costs.

The FORMAMP team evaluated various combinations of AMPs and nanocarriers for antibacterial effect against several types of strains (including multi-resistant strains). The nanocarriers investigated included lipidic nanocapsules, lipid self-assembly systems, microgels, dendrimers and mesoporous silica nanoparticles. The team also succeeded in developing a completely new type of carrier system, with a patent pending.

Summarising the nanocarrier results, the project coordinator Dr Lovisa Ringstad says, "Lipid-based systems were most successful for topical delivery. Whereas, the mesoporous silica particles were shown to be highly successful for pulmonary delivery. The polymer-based systems were utilised both for pulmonary and topical application. For bacteria biofilm degradation, one type of lipid-based nanocarrier has been shown to act synergistically with the AMPs, which is highly exciting."

As for the drug delivery systems, a thermosensitive gel formulation was shown to be most promising for topical administration, while for pulmonary delivery, inhalable powders were developed and shown to distribute well within the lungs.

Biophysical characterisations were carried out continuously to understand the interactions between AMP and nanocarriers, as well as with the surrounding environment. Cell and tissue modelling (*in vitro* and *ex vivo*) were used to investigate the effect of the peptides. At the final stage, mouse and rat models (*in vivo*) were used to assess effects. Additionally, the toxicity of the formulations was investigated.

Adding to the treatment toolkit

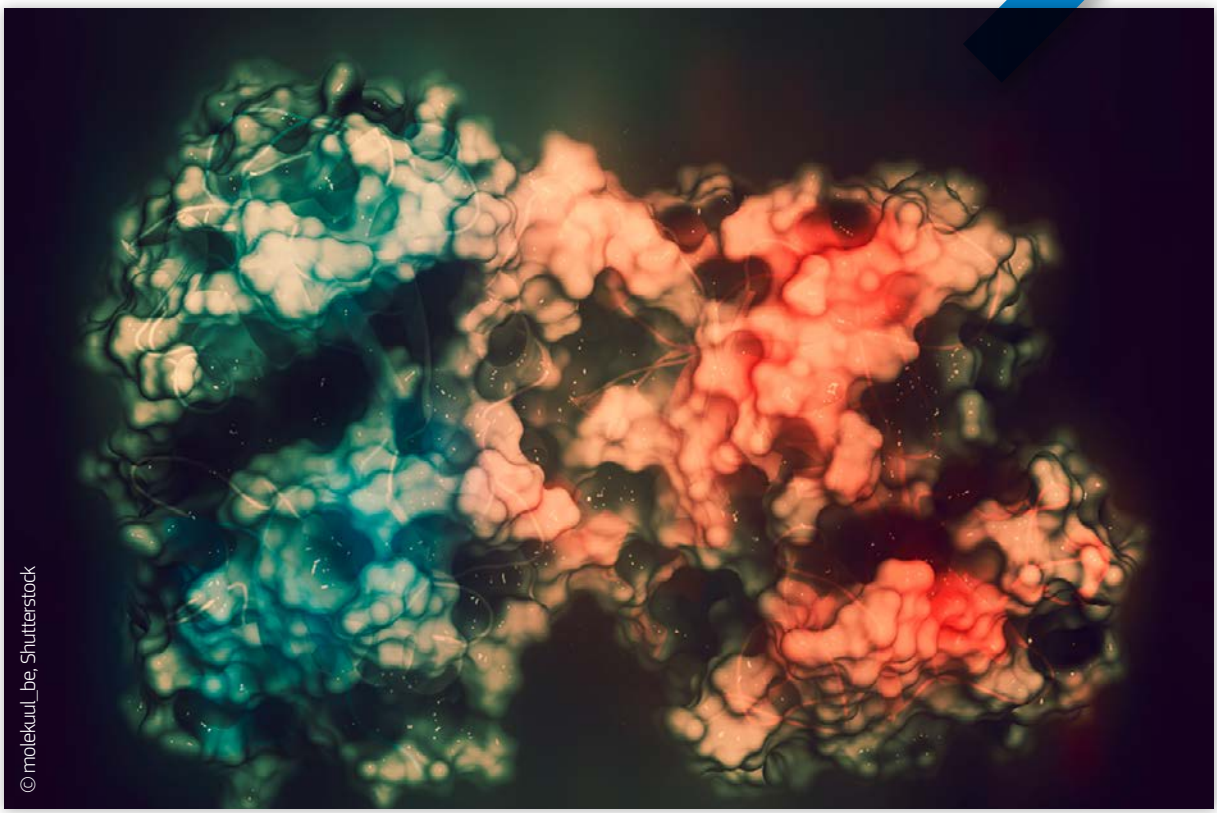
A number of tangible project results are already contributing to new treatments. For example, one of the consortium SMEs has signed a licensing agreement for further development of a peptide for the treatment of skin and soft tissue infections. Additionally, strategies to degrade biofilms, associated with several severe infections such as cystic fibrosis and burn wounds, have also been developed.

FORMAMP has also led to the development of a promising formulation for tuberculosis treatment. As Dr Ringstad elaborates, "Further development is planned for this treatment, where additional proof-of-concept in living organisms and a more detailed understanding of the precise mechanism are needed. Also, we need to fine-tune our formulation, which requires further manufacturing and quality control development."

The FORMAMP nanoformulations can also be further developed to serve as a platform for other applications delivering biologics as new therapies with fewer side effects for a range of diseases.

As Dr Ringstad says, "The combination of these new treatments, increased public awareness of antimicrobial resistance, diagnostic tools and preventive actions against the spread of multi-resistant strains, should bring us closer to a future where resistance can be reduced."

Project	**FORMAMP – Innovative Nanoformulation of Antimicrobial Peptides to Treat Bacterial Infectious Diseases**
Coordinated by	Rise Research Institutes of Sweden
Funded under	FP7-NMP
Video	http://bit.ly/2HxK7uW



© molekuul_be, Shutterstock

A new point-of-need nanodiagnostic for better healthcare

The Nano4 project takes fast, reliable and low-cost molecular point-of-care (POC) diagnostic tools down to the nano-scale, offering better healthcare outcomes for patients.

Medics working in migratory hotspots, in countries with low laboratory infrastructure or in other demanding situations often need to be able to screen large populations quickly, in a non-centralised manner. Patients who would be more comfortable not having to travel far to be screened, and those with conditions which demand swift action, such as suspected chronic myeloid leukaemia, would also benefit.

The EU-funded Nano4 (Providing the New Generation of Nano-Based Molecular Technology for the Early Detection of Bacteria, Viruses and Cancer at the Point of Care) project has developed a fast, accurate, low-cost, point-of-care diagnostic for certain viruses, pathogens and cancer types. The technology will help healthcare decision-makers to act quickly, save lives, reduce pain and avoid mistakes. It could even prevent epidemics.

"Nano4 is a disruptive technology as it may be applied to any disease or condition with a molecular (RNA/DNA) signature"

A disruptive technology for molecular diagnostics

The Nano4 platform is able to diagnose the presence of disease by using a colour revelator (colorimetric detection) to alter nanoparticle properties. After collecting a patient sample, for example sputum (saliva and mucus) or urine, nucleic acids are extracted then mixed with gold nanoprobes. The presence of signature nucleic acid sequences, as evidenced by gold nanoparticle activity in the mixture, is then picked up by the nanoprobes and so the disease-causing microbes and/or gene are identified.

The solution is initially a strong red colour. Once the revelator is added, if the solution changes to blue then it indicates the disease molecule is not present. If it remains red, it is present. "As Nano4 is very sensitive, it can provide an immediate indication of the presence or absence of disease," says project coordinator and Nano4 Global founder, Mr Filipe Assoreira.

However, this game-changing potential for molecular diagnostics also presented the team with their biggest challenge. As Professor Pedro Baptista (Nano4 Global CSO) explains, "Nano4 is a disruptive technology as it may be applied to any disease or condition with a molecular (RNA/DNA) signature. So we've had to work hard to make it clear to the market where it can be integrated in the value chain, for both the patient and the industry as a whole."

So far, Nano4 has validated the technology's diagnosis for a range of conditions including pathogens (Zika, MRSA, Salmonella) and cancer types (lung, colon, leukaemia). Testing for antibiotic resistance is likely to be the most common application, with the healthcare industry already investing in key programmes. At the same time, companion diagnostics (identifying specific biomarkers accompanying the therapy) and cancer diagnostics represent fields where Nano4 is forecast to achieve its fastest growth in the coming years.

Diagnostics for those otherwise left outside

The project has already had a number of successes. Prof. Baptista, recalling his field work with Prof. Alexandra Fernandes (Nano4 Global COO) says, "When applied to chronic myeloid leukaemia, Nano4 provided doctors with an additional tool to simplify diagnosis. During the project,

we were able to assist a clinical team to re-evaluate one patient for disease progression and therapy, which proved to be life-saving."

However, it was with tuberculosis that the team identified a unique opportunity to demonstrate Nano4's ability to scale up to meet market demand. As tuberculosis is mostly prevalent in countries with low laboratory infrastructures, it presents a good opportunity to showcase the full power of the technology in decreasing the burden, of both financial and time investment, on health systems.

Communicating the team's underlying motivation, Mr Assoreira enthuses, "Discussing Nano4 methodological applications reminds us why we started the project – to provide diagnostics to those otherwise left outside. Remembering our field trips to Africa and Brazil with the patients, clinicians and health personnel spurs us on to provide frontline workers with sustainable diagnostics to improve quality of life."

Currently Nano4 is scaling up production, getting CE certification for In Vitro Diagnostics (IVD) and undergoing a full performance validation.

Project	**Nano4 - Providing the New Generation of Nano-Based Molecular Technology for the Early Detection of Bacteria, Viruses and Cancer at the Point of Care**
Coordinated by	Nano4 Global LDA in Portugal
Funded under	H2020-LEIT-NANO, H2020-LEIT-ADVMAT, H2020-LEIT-ADVMANU & H2020-SME
Project website	http://www.nano4global.com/

© Nano4 Global

New printing techniques bring 'lab-on-paper' within reach

EU researchers have brought the prospect closer of producing cheap, disposable laboratories on a chip by printing the entire laboratory on paper. These patterned paper devices – capable of handling microfluids, filtering, biosensing and transmitting the results – could revolutionise healthcare by making advanced diagnostics available to all.

EU researchers have made progress on techniques which could soon allow us to have disposable paper-based diagnostic devices that can do everything we can currently do in a lab but much faster and at a fraction of the cost.

NANOPAD (Nano cellulose based paper diagnostic devices) has come up with ways of printing using electrical inks to turn plain paper into sophisticated electrochemical devices. The three-year project, funded as a Marie Curie fellowship, took Swedish materials scientist Max Hamedi to the United States to develop his ideas in the labs at Harvard University.

Scientists have been trying to unify biochemistry, electronics and microfluidics or the handling of liquids to build micro total analysis systems for many years. Dr. Hamedi is confident that his team's approach – using smart conductive inks to print on paper and other materials – marks a significant step forward.

The technique involves micropatterning porous electronic conductors on paper. Unlike conventional printed wires, these are porous with a high surface area, can carry liquids and electrons simultaneously and can withstand scratches or creasing of the paper.

The results of NANOPAD include integrating ion sensors into paper, developing the first printed electrical valve that can control the flow of liquids and showing it is possible to electronically control liquids by printing on textiles. "Another significant finding is that … you can coat the surfaces of cellulose so that it acts like conductive e-paper," says Dr Hamedi, now assistant professor of chemistry at KTH Royal Institute of Technology in Stockholm, Sweden. This means a piece of paper could soak up a liquid, such as blood for testing, produce a biochemical reaction, "and then you can read the signals electronically," he adds.

Diagnostics for everyone

These advances could help democratise our access to sensors and, through this, to advanced diagnostics for disease and easy monitoring of health markers.

This would revolutionise how patients in hospitals are diagnosed but it is at point of care in more difficult conditions, such as when doctors provide healthcare in rural areas or developing countries, where it could really make a difference. "Imagine I say that I have a device you can plug into your phone and it would detect which strain of malaria you are dealing with," says Dr Hamedi by way of illustration.

Integration means innovation

Monitoring the environment is a second area where Dr Hamedi believes it could have a big impact, although he readily admits

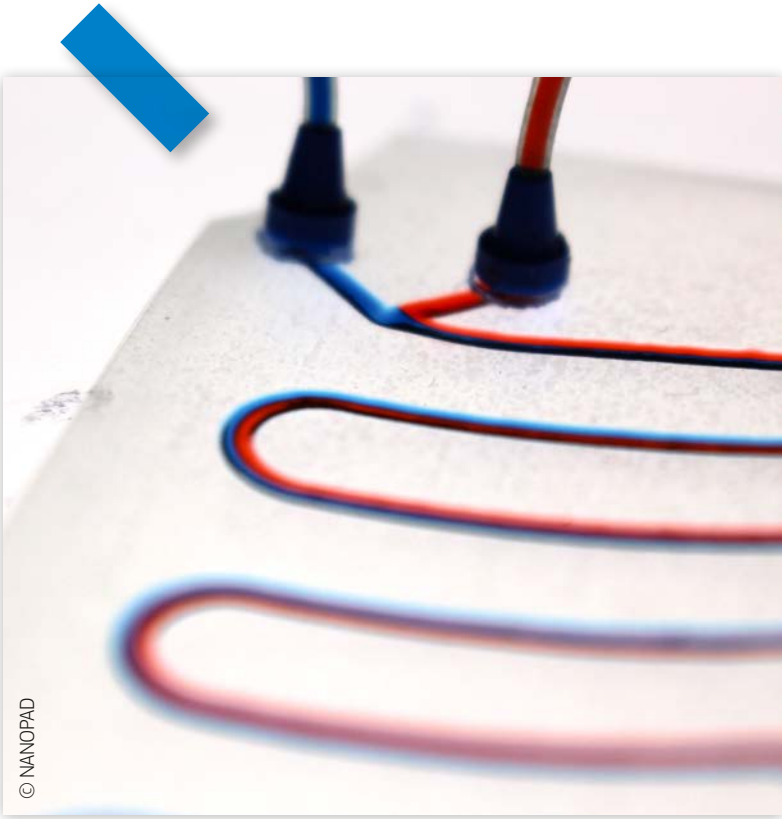
"Paper has been used for 50 years or more as a platform for making some kind of sensing device but the real innovation is to integrate how you control liquids and how you digitalise the data"

that it is hard to predict what uses such devices could be put to in future.

"Materials science is not always about coming up with new materials, it is about thinking about existing ones in new ways," he says. "Paper has been used for 50 years or more as a platform for making some kind of sensing device but the real innovation is to integrate how you control liquids and how you digitalise the data."

Seven articles have been published on the results and Harvard University has applied for five patents covering the technology. Back in Stockholm since 2016 Dr Hamedi is now running his own research group focusing on applying printing technology to DNA.

Project	NANOPAD - Nano cellulose based paper diagnostic devices
Coordinated by	Royal Institute of Technology in Sweden
Funded under	FP7-PEOPLE



© NANOPAD

New tools to boost the delivery of drugs to cancer tumours

The treatment of cancer tumours is made complex by their microenvironment and the abnormalities of the blood vessels sustaining them. The EU-funded NeoNaNo project has developed methods to improve drug delivery to tumours and improve the efficacy of anticancer therapy.

Drug delivery to tumours is difficult. The blood vessels they depend on are abnormally structured, heterogeneously distributed and relatively poorly perfused, so transporting the drugs is challenging. Once the drugs have been delivered, the dense and hostile tumour microenvironment makes it hard to target them precisely.

The NeoNaNo (Neoadjuvant Nanomedicines for vascular Normalization) project worked to establish whether, by pre-treating tumours with anti-inflammatory nanomedicines, they could improve drug delivery to the areas in which they are most needed. The project evaluated the potential of pre-treating tumours with anti-inflammatory nanomedicines to prime the tumour vasculature

"We found that we were able to lower the collagen content in tumours by using liposomal dexamethasone. This helped with the accumulation and penetration of drugs"

for more efficient drug and oxygen delivery, thereby improving the efficacy of subsequently administered chemo- and radiotherapy.

Both pharmacological (liposomal dexamethasone, anti-CCL2 agents, macrophage-modulating proteins) and physical combination treatments (sonoporation) were evaluated as means of enhancing the delivery and efficacy of both standard chemotherapeutic drugs (< 1 nm) and nanomedicine formulations (10-100 nm).

Surmounting the challenge

"The first stage of establishing a more effective treatment for cancer tumours is to get a clear idea of the structure of the tumour," explained Professor Twan Lammers, the project's principal investigator. "We then needed to accurately visualise and quantify drug delivery within that structure."

In the first half of the project, NeoNaNo optimised *in vivo* and *ex vivo* contrast-enhanced micro-computed tomography (CT) to improve quantitative 3D analyses on the vascular network in tumours.

To establish how well the delivered drug was distributed and to see if there was build-up in the tumour, the team also harnessed hybrid computed tomography, fluorescence molecular tomography (CT-FMT). This permitted them to non-invasively and quantitatively assess the biodistribution and target site accumulation of nanomedicine formulations.

Using these visualisation techniques NeoNaNo went on to assess the impact of vascular normalisation on tumour blood vessels, on drug delivery to tumours, and on the therapeutic efficacy of nanomedicine-based combination therapies.

Boosting the efficacy of drug delivery systems

"We found that we were able to lower the collagen content in tumours by using liposomal dexamethasone. This helped with the accumulation and penetration of drugs," says Prof. Lammers, based at the University of Aachen, Germany.

The project's research showed drug delivery systems (DDS) also benefited from the inhibition of CCL2-dependent macrophage infiltration, which had the added advantage of attenuating pathological angiogenesis. Finally, by using a combination of ultrasound and microbubbles, the project showed it was possible to open up blood vessels in tumours, and in the brain, enhancing DDS extravasation and penetration.

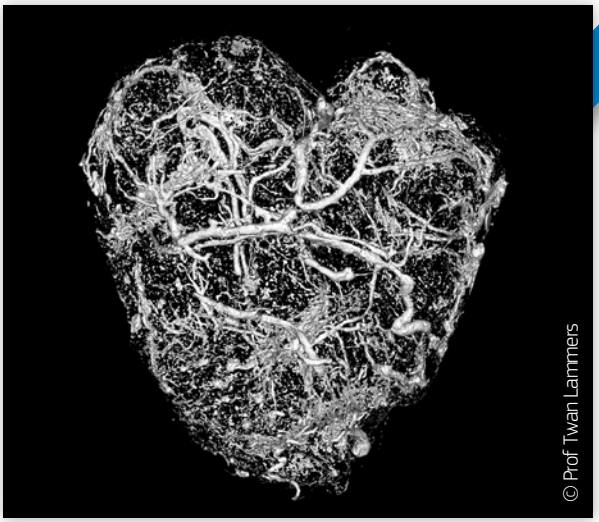
Translating findings into results

These findings add to the tools that clinicians can draw on to pre-treat tumours to make them more susceptible to the traditional approaches of chemo- and radio-therapies.

Their results showed that pharmacological and physical priming, such as broadly applicable ultrasound protocols to enhance drug delivery to, and into, tumours, can improve tumour-targeted drug delivery. Priming may also help to improve the efficacy of systemic (nano-) chemotherapeutic interventions.

Liposomal dexamethasone is now being evaluated in a first, in-man clinical trial at the Center for Clinical and Translational Research in Aachen for the treatment of multiple myeloma.

"Our EU funding through NeoNaNo has fostered the development of tumour-targeted combination therapies," says Lammers. "Through NeoNaNo, relevant steps were taken to further the pharmaceutical and clinical development of a novel liposomal corticosteroid formulation, which may have multiple advantages when applied in combination with other drugs in patients suffering from multiple myeloma."



Project	**NeoNaNo - Neoadjuvant Nanomedicines for vascular Normalization**
Coordinated by	University of Aachen in Germany
Funded under	FP7-IDEAS-ERC

New insight into why most nanoparticles don't make it through biological barriers

The biological barriers our bodies have developed evolve to keep us safe from infection and parasites. But they also filter out many of the nanoparticle drugs that hold such promise for treatment. Working out why is central to the development of next-generation drugs.

Crossing some biological barriers is fundamental to all advanced or targeted therapeutics. Different kinds of barriers present different levels of difficulties, for example the most challenging is the blood-brain-barrier, which has been a block to really effective therapeutics for the brain. Other barriers, such as the gut and lungs, are similarly difficult, but not quite as challenging. Many studies, both in academia and industry, have taken a trial and error approach to establishing why some nanoparticles just can't pass.

The EU-supported PathChooser (Innovative, mechanistic-based strategies for delivery of therapeutic macromolecules across cellular and biological barriers) project took a different approach. "Our intention was to try to understand what the processes are that prevent barrier transport, and what mechanisms could permit such transport to take place," explains project coordinator Professor Kenneth Dawson, Director of the Centre for BioNano Interactions at University College Dublin.

He explains that endocytotic, transcytotic and other cellular processes enable barrier crossing, or, in some cases, prevent it. "It has been known for many years that small numbers of particles could cross *in vivo*, for example the blood-brain-barrier and other barriers, and our intention was to enable the better design of nanoparticles as drug carriers to increase the likelihood that they can cross safely."

Taking the trial and error out of the process – reverse engineering

In order to further the design of better drug carriers, the project wanted to establish what it is about these cellular processes, and their interaction with nanoparticles, that promotes or blocks the crossing of these barriers.

To approach the problem from a different angle, the project worked backwards. They produced large groups of nanoparticles that could be tracked very easily as they cross a barrier. The team then tried to re-grow the cells composing the barrier and checked which of these nanoparticles could cross a given barrier.

"We hope our better understanding of the link between the nanoparticle design and its outcome will considerably reduce the inefficiency in drug design loops"

"We took many established barriers from the research community and developed some of our own. Using these models, we studied the mechanisms of how particles cross and what prevents some of them from crossing into the models," says Prof. Dawson.

The team then found they had increasingly fewer candidates that had some ability to cross a barrier. PathChooser studied those in more detail to see the key aspects of the nanoparticles that engage the pathways they were using to cross.

A clearer understanding of the mechanics behind barrier penetration

The project established that the molecules on the surface of the nanoparticles can prevent and inhibit their crossing. "We can actually see those particles being endocytosed, taken in, and then, trafficked away to be degraded because they have

been recognised as 'foreign'. And that has given us a much clearer focus on the need to design the nanoparticle surface very carefully."

The PathChooser project has provided valuable insight into how the mechanics of barrier crossing are impacted by the surface organisation of biomolecules. "We were able to map out the common approaches to surface organisation that were leading to the failure in barrier crossing," he says.

At the start of the project, researchers were not clear as to why some nanoparticles were not able to cross barriers. Thanks to the team's work they now have relatively simple ways of rejecting huge numbers of candidates, which they know cannot work because of their surface design criteria.

"We are no longer as discouraged as people generally are in this field because we now begin to feel there are more systematic ways to approach the problem," says Prof. Dawson.

Helping to develop more effective medication

In the long term, PathChooser should have an impact on developing more effective and 'easy-to-live-with' medicines for conditions such as diabetics and some of the most intractable diseases, such as glioblastomas, which are considered quite untreatable because of such poor access to the brain.

"We hope our better understanding of the link between the nanoparticle design and its outcome will considerably reduce the inefficiency in drug design loops." The impact on research and development costs, if nanomolecular drug design can be made more efficient, could open doors to the creation of a new suite of medications.

"The key overall result of our project is a much deeper understanding of what it is that bars the crossing and what the key access pathways to that crossing are," says Prof. Dawson.

Project	**PathChooser - Innovative, mechanistic-based strategies for delivery of therapeutic macromolecules across cellular and biological barriers**
Coordinated by	University College Dublin, National University of Ireland
Funded under	FP7-PEOPLE
Project website	https://www.pathchooser.eu/project/project-summary/

New solutions for diagnosing and treating antibiotic-resistant bacteria

Infections caused by antibiotic-resistant bacteria are a widespread health problem. To help, the EU-funded PNEUMONP project has developed new ways to both diagnose and treat infections caused by antibiotic-resistant bacteria.

A patient suffering from a respiratory tract infection caused by a bacterium goes to the doctor for help. To treat him, the doctor tries several different types of antibiotics, none of which is successful. This is because the bacteria causing the patient's infection are resistant to the most common types of antibiotics available.

This by no means is an isolated case. Every year, millions of people suffer from infections caused by antibiotic-resistant bacteria. In fact, the problem is now so widespread that the World Health Organisation (WHO) regards it as one of the major current global health crises.

In response to this crisis, the European Union has funded many research projects aimed at solving the problem, including PNEUMONP (Nanotherapeutics to Treat Antibiotic Resistant Gram-Negative Pneumonia Infections). "The objective of the PNEUMONP project was to develop a novel solution for diagnosing and treating infections caused by antibiotic-resistant bacteria," says Project Coordinator Iraida Loinaz.

"We are still far from having a new medicinal product in clinical trials, but we have generated knowledge on the systems and we really think nanotechnology will help in the design of a new generation of drugs"

A better diagnostic kit

One solution was the development of a diagnostic kit to identify the bacteria causing the infection within a single sample. Using Polymerase chain reaction (PCR) technology, the PNEUMONP multiplex kit screens for more than 30 of the main antibiotic-resistant genes. Running on standard lab equipment, the kit has the added benefit of being relatively cheap to use.

Thanks to this kit, doctors can now easily get a diagnosis at a very early stage of the disease, immediately treat with the proper antibiotics and likely save a life. "Using the kit, a doctor can quickly identify whether a patient is infected with an antibiotic-resistant bacterium and prescribe the proper treatment – all within as little as just three hours, whereas conventional methods take up to 48 hours," explains Loinaz.

The diagnostic kit will receive clinical approval in 2018 and will then be released onto the market.

Coupling antibiotics with nanocarriers

But what about those bacteria that are extremely resistant to antibiotics, where no antibiotic is available to treat the resulting infections? Here, PNEUMONP researchers created new antibiotics.

For instance, the project explored the antibacterial properties of the M33 peptide, developing a specific form of the molecule that has proved effective against such infections as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Researchers also studied the AA139 molecule, another promising antimicrobial peptide.

To ensure the efficient and safe delivery of these new antibiotics, researchers also looked at coupling the M33 and AA139 molecules with nanocarriers. Nanocarriers are nanomaterials used as a mode of transporting another substance.

As a proof of concept, the new antibiotics in nanocarrier form were delivered in aerosol form and tested against an antibiotic-resistant bacterium responsible for serious respiratory tract infections. "Attaching antibiotics to nanocarriers allows for the delivery of various combinations of antibiotics without an increase in risk to the patient," says Loinaz. "Early tests of several combinations demonstrate that this approach does improve the performance of antibiotics, and there were no signs of resistance to the new antibiotics."

The project has also been developing a manufacturing process that will allow for scalable production of the nanosystems – crucial if these new antibiotics are to break into the highly regulated pharmaceutical sector.

Overall, the PNEUMONP project successfully demonstrated the positive effect of coupling antibiotics with nanocarriers for treating multi-resistant bacteria-based infections. "We are still far from having a new medicinal product in clinical trials, but we have generated knowledge on the systems and we really think nanotechnology will help in the design of a new generation of drugs," adds Loinaz.

Project	**PNEUMONP - Nanotherapeutics to Treat Antibiotic Resistant Gram-Negative Pneumonia Infections**
Coordinated by	CIDETEC Foundation in Spain
Funded under	FP7-NMP
Project website	http://www.pneumonp.eu

Oral peptide-based nanomedicine to treat a range of diseases

The potential of peptide drugs to treat devastating systemic diseases would be vastly increased if administration was oral. The TRANS-INT project has taken us closer to this more cost-efficient and patient-friendly point through the use of nanotechnology.

The pharmaceutical industry is increasingly reliant on the potency of biological molecules, including peptides, to treat complex diseases. However, a major limitation of these drugs is their dependence on injection. Oral drug administration on the other hand is the most effective delivery mechanism, with the highest rate of patient compliance: but barriers presented by the gastrointestinal ecosystem have proven difficult to surmount.

The EU-funded TRANS-INT (New Oral Nanomedicines: Transporting Therapeutic Macromolecules across the Intestinal Barrier) project set out to overcome these barriers by better understanding the underlying mechanisms involved. The team identified key properties of nanomaterials-intestinal interaction, including instability in enzyme-containing intestinal fluids, mucointeraction and trans-epithelial transport.

The resulting knowledge has generated prototype nanocarriers for oral drug delivery that overcome biological barriers, as well as formulations of peptide and protein drugs to treat diseases with high socioeconomic impact.

"Chronic systemic diseases that could benefit from the technologies include diabetes, obesity and chronic pain, all three of which TRANS-INT studied. Additionally, in the near future the treatment of local diseases, such as intestinal bowel diseases, could benefit from targeted nanotechnology-based treatment"

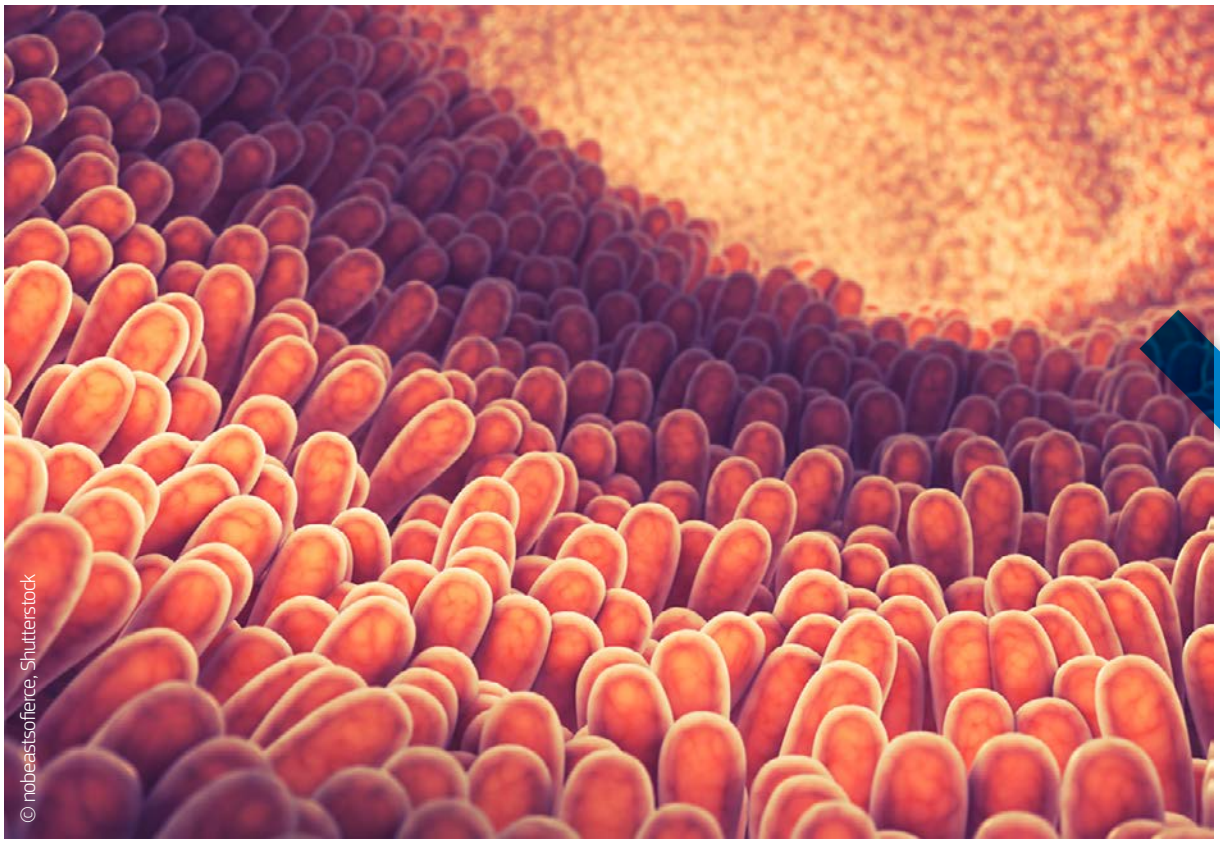
The nanocarrier prototypes

The gastrointestinal (GI) tract, as a whole, represents a very challenging barrier for the oral administration of peptides. Wrapping a peptide drug in a pH-sensitive formulation can ensure it passes through the stomach.

However, once the peptide molecules reach the small intestine they are very rapidly degraded into amino acids, leading to their inactivation. Even if peptide molecules could be protected from degradation, they would not be able to cross the intestinal mucosa due to their large size and hydrosolubility.

"Previously, efforts to use nanotechnology to enhance oral peptide delivery have been based on a trial and error approach," explains project coordinator Professor Maria José Alonso. "This is because of limited information about the interaction of nanomaterials with the gastro-intestinal environment and insufficient research on peptide nano-encapsulation and controlled release. Our goal was to use this information to design oral peptide delivery nanocarriers, for local action either in the gut or through the blood-stream."

To overcome these biological barriers TRANS-INT developed tailored nanocarriers by engineering the structural organisation of their selected components. For example, some of the customised



nanocarriers contained penetration enhancers, such as cationic polymers and oligomers, surfactants and lipids. Others were protected with hydrophilic polymer coatings (e.g. polyethylene glycol) which avoided degradation of the peptide cargo by preventing interaction with enzymatic proteins, as well as facilitating muco-permeation.

Adjusting their size and composition modulated the interaction of the nanocarriers with the epithelial tissue. The team observed that the majority of the nanocarriers investigated have a low toxicity to cells, (cytotoxicity) and selected nanocarriers exhibited very low toxicity to the immune systems of mice.

Taking one set of experiments, TRANS-INT found that selected prototypes loaded with insulin exhibited good pharmacological responses in normal and diabetic rats. However, in most cases the response was variable and highly dependent on the experimental conditions (e.g. different animal models and protocols of administration). One formulation did give homogeneous and reproducible responses and is being further investigated in pigs.

These results point to the project's central challenge of, "Conferring the nanocarriers with the desired properties that would enable them to overcome biological barriers and, at the same time, maintain a significant drug load with the necessary controlled release," as Prof. Alonso summarises.

Getting to clinical development

The knowledge generated from the project is expected to help pharmaceutical researchers design more effective formulations for the peptide drugs in their pipelines.

As Prof. Alonso elaborates, "Chronic systemic diseases that could benefit from the technologies include diabetes, obesity and chronic pain, all three of which TRANS-INT studied. Additionally, in the near future the treatment of local diseases, such as intestinal bowel diseases, could benefit from targeted nanotechnology-based treatment."

Taking project results to the next step, prototypes are currently undergoing preclinical development by TRANS-INT partners, in the hope that one can be selected for the clinical development of one or more oral peptide-based nanomedicines.

Project	**TRANS-INT - New Oral Nanomedicines: Transporting Therapeutic Macromolecules across the Intestinal Barrier**
Coordinated by	University of Santiago de Compostela in Spain
Funded under	FP7-NMP
Project website	http://www.trans-int.eu/
Video	http://bit.ly/2Iy79Dc

research*eu
RESULTS PACK

Published
on behalf of the European Commission by CORDIS at the
Publications Office of the European Union
2, rue Mercier
2985 Luxembourg
LUXEMBOURG

cordis@publications.europa.eu

Editorial coordination
Zsofia TOTH

Disclaimer
Online project information and links published in the current issue of the research*eu Results Pack are correct when the publication goes to press. The Publications Office cannot be held responsible for information which is out of date or websites that are no longer live. Neither the Publications Office nor any person acting on its behalf is responsible for the use that may be made of the information contained in this publication or for any errors that may remain in the texts, despite the care taken in preparing them.

The technologies presented in this monograph may be covered by intellectual property rights.

PRINT	ISBN 978-92-78-41773-4	ISSN 2529-3265	doi:10.2830/71463	ZZ-AD-18-002-EN-C
PDF	ISBN 978-92-78-41770-3	ISSN 2529-2919	doi:10.2830/459404	ZZ-AD-18-002-EN-N

Luxembourg: Publications Office of the European Union, 2018

ZZ-AD-18-002-EN-N

research*eu Results Pack

CORDIS brings you the latest results from EU-funded research and innovation projects, including scientific breakthroughs and exciting new products and technologies. Explore our Results Packs that focus on specific themes to make it easier for you to use the concrete outputs of projects active in your domain. Find them all and much more on cordis.europa.eu

WE SPEAK YOUR LANGUAGE

ALL ARTICLES IN THIS MAGAZINE ARE ALSO AVAILABLE ON THE CORDIS WEBSITE IN

FRANÇAIS · POLSKI · DEUTSCH · ITALIANO · ESPAÑOL

DON'T MISS AN ISSUE

- FREE DOWNLOADS
- SUBSCRIPTIONS
- ORDER PAPER COPIES
- EMAIL NOTIFICATIONS

Go to cordis.europa.eu/research-eu
to get the research*eu Results Magazines the way you want to!

Publications Office
- **eur-lex.europa.eu** · EU law
- **publications.europa.eu/bookshop** · EU publications
- **ted.europa.eu** · Public procurement
- **cordis.europa.eu** · EU research results

Follow us on social media too!

facebook.com/EULawandPublications

twitter.com/CORDIS_EU

EN

ISBN 978-92-78-41770-3