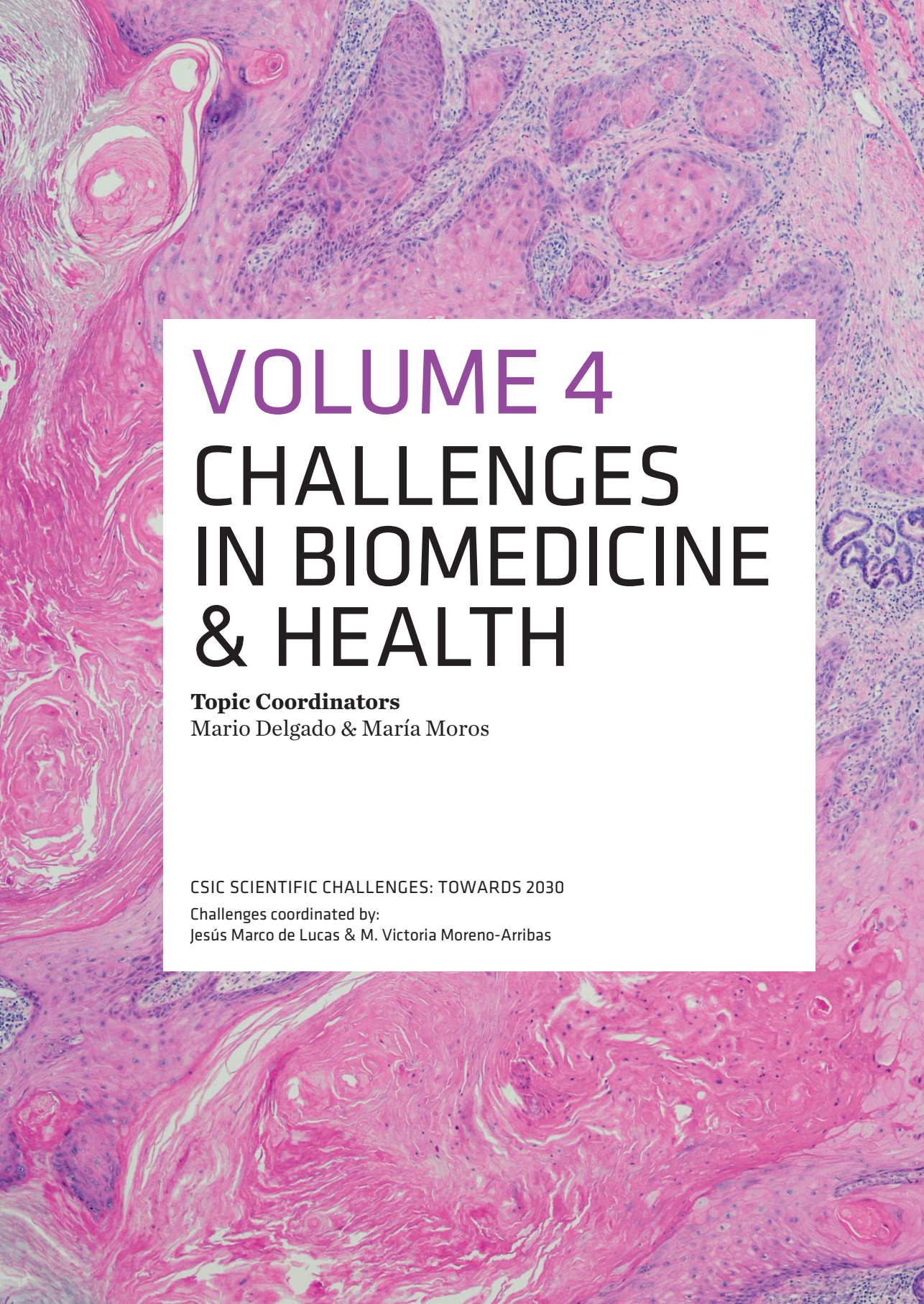

VOLUME 4
CHALLENGES IN BIOMEDICINE & HEALTH

Topic Coordinators
Mario Delgado & María Moros

CSIC SCIENTIFIC CHALLENGES: TOWARDS 2030
Challenges coordinated by:
Jesús Marco de Lucas & M. Victoria Moreno-Arribas

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CSIC SCIENTIFIC CHALLENGES: TOWARDS 2030

What are the major scientific challenges of the first half of the 21st century? Can we establish the priorities for the future? How should the scientific community tackle them?

This book presents the reflections of the Spanish National Research Council (CSIC) on 14 strategic themes established on the basis of their scientific impact and social importance.

Fundamental questions are addressed, including the origin of life, the exploration of the universe, artificial intelligence, the development of clean, safe and efficient energy or the understanding of brain function. The document identifies complex challenges in areas such as health and social sciences and the selected strategic themes cover both basic issues and potential applications of knowledge. Nearly 1,100 researchers from more than 100 CSIC centres and other institutions (public research organisations, universities, etc.) have participated in this analysis. All agree on the need for a multidisciplinary approach and the promotion of collaborative research to enable the implementation of ambitious projects focused on specific topics.

These 14 "White Papers", designed to serve as a frame of reference for the development of the institution's scientific strategy, will provide an insight into the research currently being accomplished at the CSIC, and at the same time, build a global vision of what will be the key scientific challenges over the next decade.

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1 *New Foundations for a Sustainable Global Society*
2 *Origins, (Co)Evolution, Diversity and Synthesis of Life*
3 *Genome & Epigenetics*
4 *Challenges in Biomedicine and Health*
5 *Brain, Mind & Behaviour*
6 *Sustainable Primary Production*
7 *Global Change Impacts*
8 *Clean, Safe and Efficient Energy*
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10 *Digital and Complex Information*
11 *Artificial Intelligence, Robotics and Data Science*
12 *Our Future? Space, Colonization and Exploration*
13 *Ocean Science Challenges for 2030*
14 *Dynamic Earth: Probing the Past, Preparing for the Future*

CSIC scientific challenges: towards 2030
Challenges coordinated by:
Jesus Marco de Lucas & M. Victoria Moreno-Arribas

Volume 4
Challenges in Biomedicine & Health

Topic coordinators
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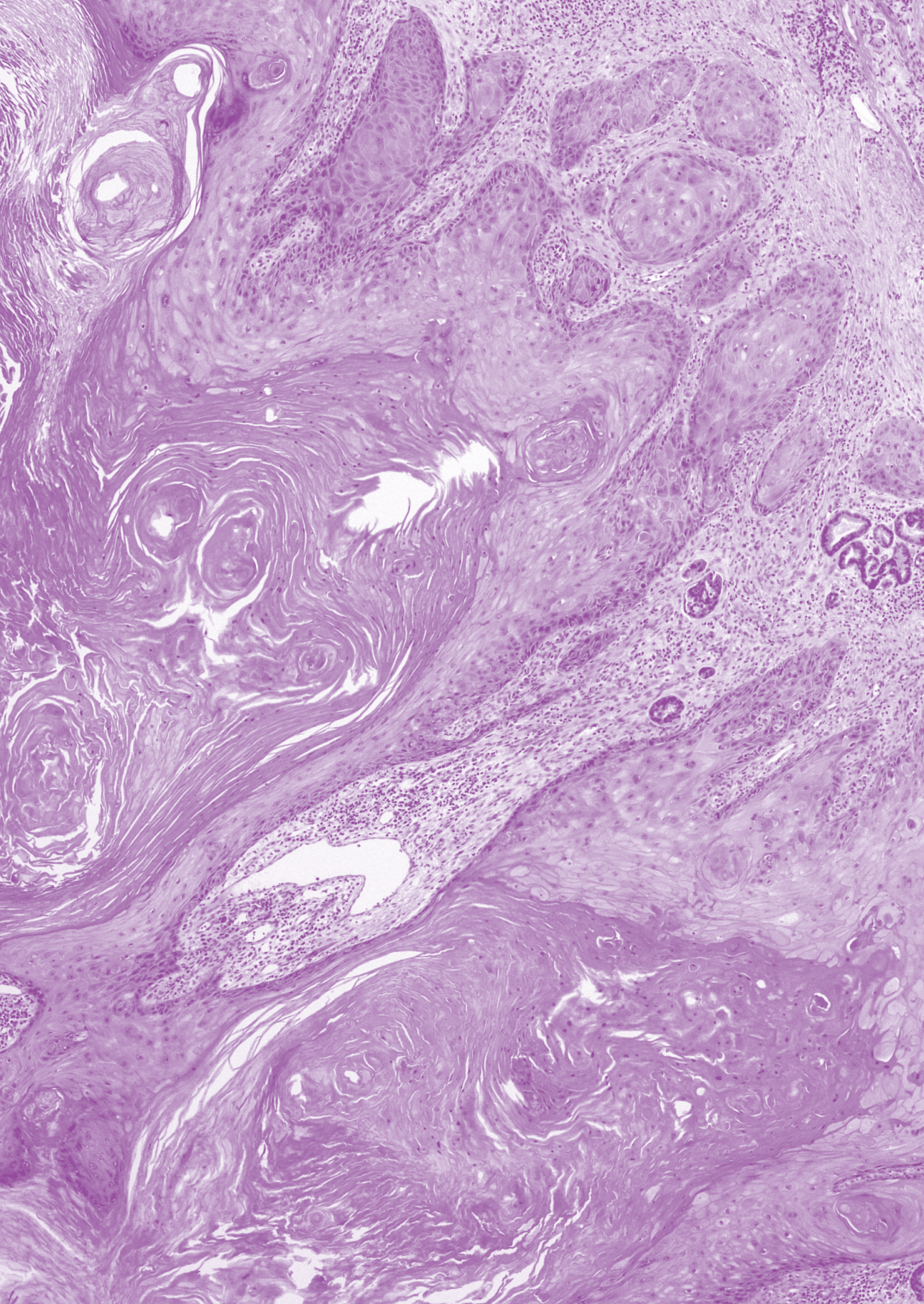

CONTENIDO

ABSTRACT

A lesson that we have learned from the pandemia caused by coronavirus is that solutions in health require coordinated actions. Beside this and other (re)emerging infectious diseases, Spain and Europe are suffering a plethora of disorders that are currently acquiring epidemic dimensions, including cancer, rare diseases, pain and food allergies, among others. New tools for prevention, diagnosis and treatment need to be urgently designed and implemented using new holistic and multidisciplinary approaches involving researchers, clinicians, industry and all stakeholders in the health system. The CSIC is excellently positioned to lead and coordinate these challenges in Biomedicine and Health.

KEYWORDS

biomedicine | therapies | diagnostic tools | cancer | chronic diseases

CHALLENGES IN BIOMEDICINE AND HEALTH

Topic Coordinators
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EXECUTIVE SUMMARY

Despite significant improvements in research, detection and treatments in recent decades, a considerable number of complex diseases have acquired epidemic dimensions and nowadays represent major health, economic and social burdens for our societies. Cancer, emerging and re-emerging infectious diseases, food allergies and rare diseases affect millions of Europeans, and all of them share the urgent need for new strategies that must be implemented at three different levels: basic research level, translational/clinical level and public/social level.

1. Basic scientific level. Because we will only cure what we fully understand, it is mandatory a better comprehension of molecular and cellular mechanisms of these disease from a patient-oriented perspective: study of genetic and epigenetic mechanisms involved in tumor initiation tumor evolution (metastasis cascade), mechanisms that pathogens use to infect and how the host responds to infection, molecular mechanisms of antimicrobial resistance and its evolution, genetic and physiological defects in rare diseases, mechanisms involved in immune tolerance against food components and novel pain targets/pathways with disease-modifying potential between others.

2. Translational/clinical level. It is obvious that we need a comprehensive view of the disease at the patient-level to translate research to the patient bedside, in order to implement new tools for early diagnosis and effective/safe

treatmentsof the disease and to ensure long-term well-being of patient. Development of vaccines against viral/bacterial threats, identification of biomarkers for drug resistance, cancer, rare diseases and chronic pain, new strategies to enhance host immune responses against pathogens, faster methods of detection of food allergens, repurposing drugs for untreated diseases are some of the challenges that we must face in a short-term frame. The support of stable and accessible technical platforms (omics, big data, data mining, machine learning,...) will catalyze the development of these challenges together with a real and immediate translation to the clinical practice of innovative ways of diagnosis by medical imaging and biosensors as well as of advanced gene and cell therapies, tissue engineering and nanoparticles.

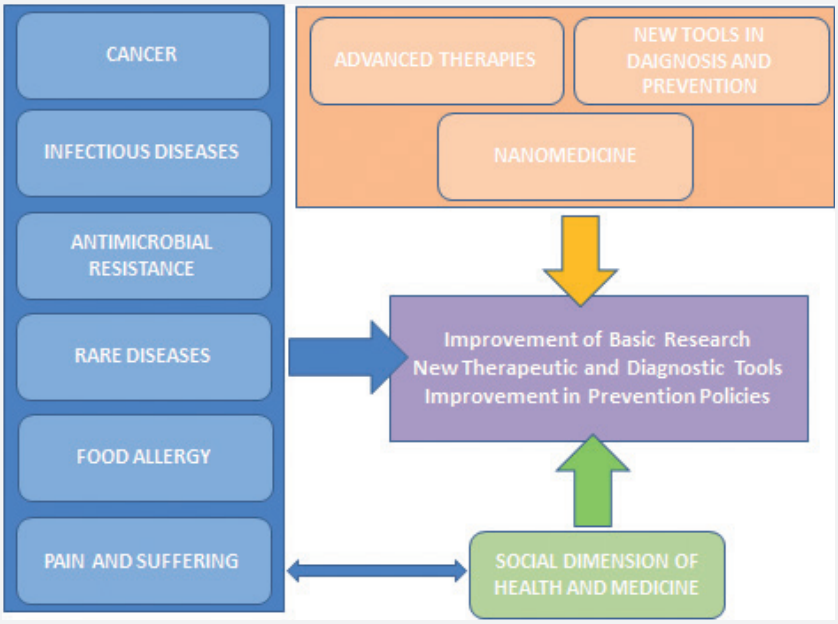
3. Social level. Because all these diseases represent health global problems, every strategy directed to solve them must include the analysis of social dimension as a challenge itself. Understanding concepts such as socio-economic determinants of health (poverty/income inequality, education, gender), complexity of healthcare policies and health systems, migratory currents, alimentary habits, globalization and urban overcrowding, visibility of research institutions by society, researcher-clinician-patient relations is critical for example to improve surveillance systems and prevent epidemic emergent and re-emergent infectious diseases, to manage chronic pain, cancer and rare diseases as a public health problem or to adequately progress in advances therapies.

Toward this goal, we need innovative types of synergistic cooperation by researchers, clinicians, industry and other actors of health system such as health and regulatory agencies, research organizations and patient advocates. Only through this inter- and multidisciplinary approach we will succeed in making real impact in the lives of millions of Europeans. Given its strong research base, the CSIC is well positioned to address these health system needs and capitalize its human and technical resources to become a major player in Biomedicine from a research, a health-oriented, and a policy-making perspective, and to participate in international initiatives for improving prevention, diagnosis and treatment of diseases of global impact.

INTRODUCTION

The present strategic topic aims at giving an overview of the current status and challenges of some important biomedical applications. Biomedicine can be defined as the application of basic sciences to solve problems in clinical

FIGURE 1–Challenges that currently represent a major health, social and economic burden for our society that are considered in this strategic topic



medicine, merging the aspects of medicine with other disciplines such as biology, biochemistry or biophysics. Widely considered as an umbrella term of modern medical research, it includes a vast range of scientific and technological approaches that try to find new treatments and diagnostic tools to improve health, cure illnesses and increase our quality of life. Consequently, biomedicine has a deep impact in our lives.

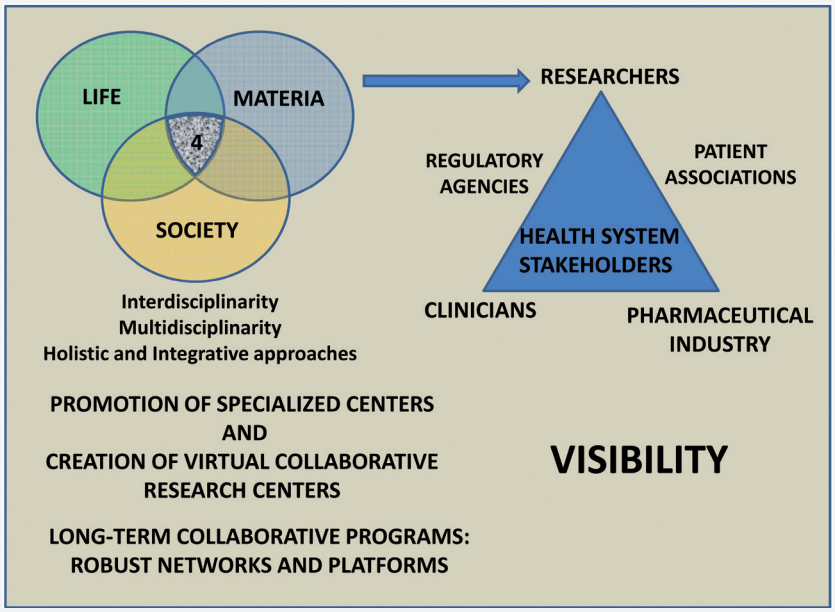
The biomedical field has witnessed outstanding advances in the last decades as a consequence of the progresses in different fields such as cell biology, biochemistry, biomedical engineering, genetics or microbiology among others. In many cases, interdisciplinary approaches are needed to struggle with the diseases. For instance, some time ago death rate caused from cancer started to increase. In that moment cancer research received considerable funding, but neither the causes of death neither the treatments were evident. Only when this research was combined with other disciplines such as

molecular biology studies, major breakthroughs were accomplished. Something similar happened with AIDS in the 1980s.

Despite major advances in health care over the past several decades, there are many complex problems and challenges that still need to be faced. Some of the challenges that currently represent a major health, social and economic burden for our society are considered in this strategic topic (Figure 1):

i. Cancer is the first and second cause of death among men and women in Spain respectively, and it is forecasted that the cases will double by 2040. Many patient and scientific associations have set the goal of improving the survival rates of cancer patients from the current 55% to 70% by the year 2030 (Challenge 1).
ii. Emerging and re-emerging infectious diseases such as influenza, HIV/AIDS, malaria, SARS-CoV-2, etc. account for a quarter to a third of estimated deaths worldwide, about 15 million of annual deaths. Resistance to antibiotics, farming activities, environmental and societal changes have favoured the rapid spread of these infections, representing a serious concern in Europe. Of note, infections caused by multi-drug resistant pathogens are considered one of the top three threats of global health (Challenges 2 y 3).
iii. Rare diseases affect to near 3 million patients in Spain. Because pharmaceutical companies generally underserve them, there is an increasing societal pressure to develop novel diagnosis and therapy methods (Challenge 4).
iv. Chronic pain has acquired epidemic dimensions from the mid-20th century, being the most common complaint for which individuals seek medical treatment. In many cases current treatments have serious adverse effects or lead to drug abuse, which converts chronic pain in a major medical and societal challenge (Challenge 5).
v. The prevalence of food allergies in the general population has been roughly estimated to be around 2-4% in adults and 6-8% in children. As the only effective strategy is to avoid the food that causes the allergy, food allergies have a great impact in the quality of life. Food producers must ensure that all the allergens are identified in the labelling. However, traces of allergens due to cross-contaminations are difficult to detect and can pose a health problem. More sensitive and fast detection methods for allergen control that guarantee the security to the consumers are needed (Challenge 6).

FIGURE 2–Strategy and interactions that must be applied and implemented in order to reach the main challenges in biomedicine and health



Currently it is clear that to tackle the majority of these scientific challenges a multifaceted strategy will be needed. Understanding the molecular processes that underlie the diseases, the physiopathology, risk factors and associated societal burden will ease the progress of therapies and diagnosis to cope with them. For instance, machine learning, artificial intelligence and -omics will help to make more accurate decisions regarding the diagnosis, therapies and prognosis in the future. Many of these diseases could greatly benefit from the use of advanced therapies to treat them (Challenge 7). Gene therapy is a powerful tool that offers the potential to cure patients with serious or fatal conditions by engineering or editing the genome. Cell therapy hold great promise on regenerative medicine and biomaterials can revolutionize the way to approach injured tissues. In addition, the development of new biomarkers for early diagnosis of disease and new methods for diagnosis is of paramount importance (Challenge 8). For instance point of care biosensors and wearable devices to monitor health and disease in a less invasive manner will have a profound global socioeconomic impact. In this sense, Nanomedicine, is a truly interdisciplinary field that holds a tremendous

potential for the development of new therapies and diagnosis tools because of the unique properties of nanomaterials when compared with bulk materials (Challenge 9).

Although the topics of the aforementioned Challenges vary widely, we can expect that multidisciplinary teams will be needed to face them, as well as the cooperation between different actors such as researchers, clinicians and stakeholders. Further, health equity must be considered when developing health policy and programmes, as health is considered as a universal Human Right that must be analysed by integrating social, physical and psychological aspects (Challenge 10).

CHALLENGE 1

ABSTRACT

Cancer is one of the key strategic missions identified by the HorizonEU research framework for the 2021-2027 period. Here, we describe the current positioning, weaknessess and potential of the CSIC in this research field. We also pinpoint the major goals to be achieved by CSIC scientists in this area at the basic, translational, clinical, and public levels. Those challenges include the understanding of tumor initiation, evolution, and metastasis, the elucidation of the crosstalk of tumors with the surrounding environment and the rest of the organism as well as the identification, validation and clinical implementation of better diagnostic and pharmacological tools for cancer patients. Given our current strengths and potential, we also propose to set up a virtual CSIC-Cancer section to foster synergistic interactions of CSIC researchers among themselves and with external stakeholders. The ultimate goal is to place the CSIC at the forefront of cancer research both at the national and international level.

KEYWORDS

cancer | tumor evolution | metastatic cascade | systemic responses to the tumor | novel therapeutic aproaches

CHALLENGE 1

CANCER

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1. INTRODUCTION AND GENERAL DESCRIPTION

1.1. Cancer: a health, social, and research challenge

Cancer is a general term for more than 200 diseases that originate from almost any cell in our body. They have in common both a genetic origin (spontaneous, inherited, intrinsic or pathogen-caused) and the presence of general alterations in cell behavior such as increased proliferation, reduced apoptosis, and changes in cell motility and migration. They also exhibit systemic alterations in the organisms due to the acquisition of metastatic properties and the rewiring of physiological interactions with healthy tissues. Although significant improvements in detection, treatment and overall survival have been achieved in the last decades, these diseases still represent a major health, social and economic burden for our societies. Thus, even now, cancer causes the death of close to 135,000 and 1,900,000 Spanish and Europeans per year, respectively. In Spain for example, cancer is currently the first (298 deaths per 100,000 population) and second (187 deaths per 100,000 population) cause of death among men and women, respectively.

In addition to its intrinsic pathobiological and clinical practice-associated problems, cancer creates more health problems to patients as well. For example, the recent Covid-19 crisis has revealed that specific cancer patients have been more prone to develop live-threatening conditions than other segments of the population. Indeed, lung and hematological cancer patients have faced death rates higher than 30% in Spain as a consequence of SARS-CoV-2 infections, 5 times higher than the average. Another problem is that surviving cancer does not mean being as healthy as before developing the disease. This is not a minor issue from a populational point of view, since it is calculated that more than 12 million people are cancer survivors only in Europe (half a million of which being pediatric cancer survivors). Now, it is becoming clear that many of those survivors experience late side effects of treatment, which may become only apparent after very long post-treatment years. Arguably, we need a comprehensive cataloguing of all these long-term comorbidities while developing new prevention and treatment measures specifically aimed at these long-term cancer survivors.

Unfortunately, these health problems will worsen in the years to come due to increasing poor social habits (diet, obesity, smoking, drinking, UV exposure) and the general aging that most industrialized societies face nowadays. In fact, it is forecasted that the number of cancer cases in both Spain and Europe will double by 2040 if no strong actions are undertaken soon.

This situation indicates that is of paramount importance to implement multifaceted research avenues aimed at understanding the biology and molecular taxonomy of each cancer type as well as at developing new diagnostic and therapeutic tools. We also need to better characterize the pathobiological processes that still hamper the survival of patients to both conventional and new therapeutics such as drug resistance, immune evasion, and tumor recurrence. In addition, we should not forget that this type of research is critical on many tumor types that, due to low epidemiological incidence, are neglected both by basic researchers and the biopharmaceutical industry. Last but not least, new efforts are needed to understand and prevent the medical problems that long-term cancer survivors face.

This health challenge has been acknowledged by the European Union, leading to the consideration of cancer as one of the leading Missions of the next 2021-2027 HorizonEU research and innovation framework program (for more information, see the web link: https://ec.europa.eu/info/horizon-europe-next-research-and-innovation-framework-programme_en). In

addition, many patient and scientific associations such as the Spanish Association against Cancer (AECC), Cris-contra-el-Cáncer, Spanish Association for Cancer Research (ASEICA), the Spanish Society of Medical Oncology (SEOM) and their European counterparts (European Association for Cancer Research [EACR], European Society of Medical Oncology [ESMO]) have set the goal of improving the survival rates of cancer patients from the current 55% to 70% by the year 2030.

Toward this goal, it is generally assumed that we need innovative types of cooperation by researchers, clinicians and other stakeholders (national health system agencies, research organizations, technical platforms, industry, and patient advocates). Only through this multidimensional approach we will succeed in making real impact on the lives of thousands of Spaniards and Europeans.

Given its strong research base, the CSIC is well positioned to address this health system needs and, in addition, to capitalize its human and technical resources to participate in these international initiatives (namely, the HorizonEU framework program, its Mission on Cancer, and other potential funding sources such as the Innovative Medicines Initiative [IMI], and the Marie Curie-Skłodowska Program). However, it also faces significant challenges due to some intrinsic weaknesses that particularly impinge on the cancer research field. In this document, we will describe in detail the strengths, weaknesses, and strategies (scientific and organizational) that have to be taken into consideration in order to make CSIC and its cancer groups key players in this new highly competitive scenario.

1.2. Cancer research at the CSIC

Although the CSIC is not generally perceived as a cancer research institution by most stakeholders, it should be underscored that cancer research does constitute a relevant and productive area in our institution. In particular, there is one institute, IBMCC-Centro de Investigación del Cáncer de Salamanca, whose main research focus is cancer both at the basic and clinical level. As such, it has assumed in a miniaturized version the philosophy of the American comprehensive cancer centers, a type of umbrella organizational structure that combines basic, translational and clinical researchers as well as a smooth integration with the University and cancer departments of hospitals. This structure, that has been unique to the American research system, is likely to be implemented in the short term in Europe according to current

discussions on the design of the next Mission on Cancer of the HorizonEU research and innovation framework. Although not exclusively focused on cancer research, the IIBB of Barcelona and the IBIS of Sevilla have also strong links with the Clínic and Virgen del Rocío hospitals, respectively. Relevant research groups are also present in other CSIC institutes, including CABIMER, CBM-SO, CIB-MS, IBBTEC, IIB-AS, IN, and IRB-CSIC (in alphabetical order). Despite this, it is clear that cancer research at the CSIC is highly dispersed and the number of cancer-specific centers is quite limited. Because of this, the creation of a virtual CSIC-Cancer institute is of capital importance. This virtual CSIC-Cancer institute should facilitate the integration of all the dispersed CSIC cancer research groups and related subjects (pharmacology, nanotechnology, photonics, etc.) in a common network. In addition to prompt cooperation, this strategy will favor a better exposure and visibility of this community at both the academic and public level in Spain.

Despite this dispersion, the productivity of the CSIC in this area is very relevant. Thus, according to the Web of Science (WOS) database, the CSIC currently ranks in the third position among all the Spanish research institutions according to the number of publications in the cancer field. In fact, it probably ranks second given that the institution that is identified in the second position is the Centro de Investigación Biomédica en Red (CIBER) that contains a significant number of CSIC cancer groups (Fig. 1). Importantly, CSIC is the first Spanish institution from both the public and private sector in numbers of cancer-related patents filed in our country during the period 2006-2017 according to the "Primer Informe sobre la Investigación e Innovación en Cáncer" published by the ASEICA, AECC and Fundación La Caixa (for more information, see the webpage https://www.aseica.es/wpcontent/uploads/2019/07/informe_investigacion_cancer_aseica.pdf). In this case, it is worth underscoring that the CSIC has filed twice as many patents as the organization who is placed in the second position (Servicio Andaluz de Salud). However, it must be also pinpointed that the ASEICA-AECC-Fundación La Caixa document reveals that, as a consequence of the decreasing funding for research in Spain, the number of patents has decreased by 37% when compared to the previous decade. Nevertheless, this impact has been lower than in other institutions (e.g.,VHIO, -46%; CNIO, -52%).

These data, collectively, indicate that CSIC is in a relatively good position to address the challenges that the cancer field will face in the near future if proper strategic decisions are set at this very moment.

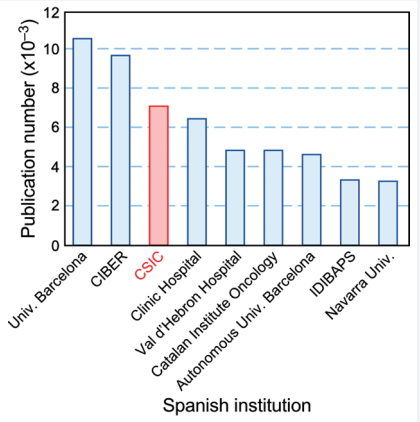

FIGURE 1.1–Ranking of the CSIC in cancer research-related publications when compared with those from top research organizations in Spain.

2. IMPACT ON BASIC SCIENCE AND POTENTIAL APPLICATIONS

In order to be a relevant player in cancer research in the forthcoming years, the CSIC has to implement a number of significant strategic decisions, both at the scientific and the institutional level. In the former case, we have identified 5 challenging points to achieve leadership in this area (see Section 3). Keywords for the future include deeper understanding of molecular mechanisms of cancer from a patient-oriented perspective, translation of research to the patient bedside, networking, multidisciplinarity, and multi-institutional cooperativity. If properly done, the implementation of these policies will allow the CSIC to become a major player in this area from a research, a health-oriented, and a policy-making perspective. We summarize below the major outcomes expected from the implementation of such measures:

2.1. Scientific level

We need to understand the intrinsic and extrinsic pathobiological programs in cancer cells from the earliest developmental timepoints in order to better diagnose and treat patients. Despite the rapid improvement and/or development of standard, targeted and immune-based therapies, there are still large number of cancer types and individual patients that are resistant or develop resistance to current therapies. There are also biological processes intertwined with the foregoing challenges, such as the biology of cancer initiating and metastatic cells that represent a medical challenge to ensure proper

treatment, lack of recurrence and long-term survival of patients. If these research needs are critical for most tumors, they are even more acute in the case of rare (both childhood and adult) and other poorly understood cancers. Arguably, the understanding of their etiology, promotion, progression, and spreading must result in new biomarkers and improved therapies in the near future for those tumors.

It is also important to note that many lifestyle, environmental, and physiological (including gender- and age-based) factors affect the development and treatment of many tumor types as well as the well-being of patients (e.g. cachexia). Although they are beginning to be grasped, many remain in the shadows. As a token, the recent Pan-Cancer studies have revealed new and unexpected patterns of mutational mechanism whose primary cause remains unknown (Alexandrov et al., 2020). Clearly, a better understanding of these causative and ancillary cancer agents must help make decisions on preventive medicine policies in the near future.

The research plan contemplated for the CSIC in this area is directly aimed at addressing those points and, therefore, it has the potential to make a significant impact on how we view all the pathological processes, intrinsic and extrinsic, that originate, influence and contribute to disseminate cancer cells. Special focus on these research challenges will provide a further boost of the contribution of the CSIC to the cancer field as a whole.

2.2. Translational level

It is of paramount importance to have a comprehensive view of cancer at the patient-level to effectively treat patients and to ensure their long-term well-being. Other challenges include finding the key drivers and best therapeutic targets in the large haystack of information in the cancer genome that has been gathered during this last decade. Another important point to be addressed is the fact that many of the most important drivers identified to date in many tumors are not druggable according to current knowledge. Likewise, we will need to devise better biological tools to screen for new drugs such as, for example, organoids and PDX either in standard cultures or in bioengineered chambers. Finally, there is a significant room for improvement in the delivery methods of drugs to patients. The effective implementation of these research goals, which will be unavoidable as well given the current funding trends in Europe, will require strong interactions with other CSIC research areas (nanotechnology, microfluidics, optics, chemistry) and external

stakeholders (universities, industry, health system agents). Outcomes from the proposed strategy will increase the impact of CSIC on cancer research and will facilitate the generation of extensive portfolios of marketable biomedical products. Given the trends seen in the Horizon2020 and the HorizonEU programs, the implementation of this strategy will be also essential to remain competitive in the capture of international funding.

2.3. Clinical level

Despite the enormous progress being made during these last decades, it is clear that the diagnosis and treatment of patients is still in its infancy. We will need in the short-term to devise optimal tools to allow the early detection of tumors (biological and physics-based), the diagnosis and taxonomical classification of tumors, and the delivery methods of antitumor drugs. We will also need to stratify in a more rationalized and empirical manner the cohorts of patients that are specifically sensitive to specific targeted or immune-based therapies. In this area, special potential is inferred from cutting-edge bioengineering strategies for guiding the assembly of next-generation organoids with improved reproducibility and physiological relevance. We are also only beginning to grasp the potential of the agnostic therapies. Only with those improvements the personalize medicine will become fully mature in the clinical setting. Finally, we need to understand the short- and long-term effects of therapies in cancer patients in order to facilitate their well-being both during and after the treatment. Linked to this issue is the problem of the long survivors.

In addition to the intrinsic challenges associated with this type of research, we believe that the current CSIC structure is poorly suited to facilitate a major role in this area. This could be solved by setting up a specific CSIC-Cancer network. In any case, the CSIC must make inroads in this direction given its direct relationship with the diagnosis, treatment, and long-term well-being of cancer patients.

2.4. Public level

As indicated in Section 1, cancer represents a heavy social and economic burden for Spain and Europe nowadays. Current epidemiological trends also indicate that this problem will become even more acute in the next decades due to unhealthy lifestyle and the progressing aging of our societies. This problem has been recognized both by the European Commission, cancer patient societies, and cancer research associations. Last but not least, the problematic associated with cancer falls within the United Nations (UN) Sustainable

Development Goal 3 that aims at ensuring healthy lives and the well-being of individuals at all ages and in all countries. Thus, active research in this area is essential for the competitiveness of the CSIC in the near future and, most importantly, to ensure the well-being of most of our compatriots. Given the costs associated to these diseases, contributions in this area will also allow significant savings for our national health services.

Although the impacts at this level will be mostly due to the progress made by CSIC researches in points 2.1 to 2.3, we must not forget that part of the CSIC role is to be a scientific loudspeaker to our society and, in this context, to be an active policy maker in the context of both public and private organizations. Success in this goal will benefit enormously by establish both continuous dialogs and cooperative initiatives with health system organizations (e.g., ISCIII, autonomous communities), industry (e.g., PharmaIndustria, ASEBIO), patient advocacy associations (AECC, Cris-contra-el-Cáncer), foundations (FERO, La Caixa), and scientific associations (ASEICA, SEOM, EACR).

Finally, the CSIC must implement clear dissemination policies, on its own or in collaboration with the foregoing health-related institutions, to create awareness about cancer and its prevention in our country that, ultimately, will be also critical to help defeat this disease.

3. KEY CHALLENGING POINTS

As indicated in Section 1, cancer is a multifactorial and challenging group of diseases that, despite significant progress in the last decades, still represents a major social, health, and economic burden for our societies. After decades of focusing on the established primary tumor, studies are now moving to a more integrative and holistic approach (Bernards et al., 2020). The main challenge at the basic science level is to fully characterize tumor transitions across space and time at the single-cell resolution (Rozenblatt-Rosen et al., 2020). Taking into consideration the key issues outlined in Section 2, we have identified a number of challenges that the CSIC will have to tackle in this area. These challenges include the understanding of:

i. The genetic and biological basis of tumor initiation and evolution
ii. The metastatic cascade
iii. The systemic responses
iv. New diagnostic and therapeutic tools. We describe below each of these challenges

3.1. New challenges in the understanding of tumor initiation and tumor evolution

Tumorigenesis follows a Darwinian evolutionary process characterized by continuous variation and selection. Variation is provided by mutations occurring randomly across the genome of our cells, most of them innocuous, as well as by environmental and physiological stresses that cancer cells face as the tumor mass grows and disseminates. Selection appears when one (or a group) of those mutations provides a selective advantage to the cell such as faster proliferation rates, use of metabolic resources, immune evasion, or apoptosis escape (Hanahan and Weinberg, 2011). The mutations conferring selective advantage to cancer cells are generally referred to as driver mutations, whose identification and functional characterization has been the focus of the cancer research field for the last 30 years. The analysis of close to 2,658 whole-cancer genomes from 38 tumor types has revealed that genomic events are at the root of virtually all tumors, each of them carrying a minimal average of 4.6 driver events (The ICGC/TCGA Pan-Cancer consortium, 2020). The advent of high throughput sequencing technologies in the last decade has revealed that driver mutations work in concert with genomic, epigenomic, and epitranscriptomic alterations in the same cells. The main challenge is to integrate all this molecular information into the cellular and organismal interactions that occur between the tumor and the host and determine cancer outcome and patient survival. However, the need to identify driver mutations still exist for a reduced number of tumor types, given that recent Pan-Cancer data have found tumors with no apparent drivers in them (The ICGC/TCGA Pan-Cancer consortium, 2020).

3.1.1. The precancerous lesions

A special emphasis should also be made in identifying the early precancerous lesions. Recent Pan-Cancer studies have described mutational signatures in humans, some of which still have an unknown causative agent (Alexandrov et al., 2020). This comprehensive study should help identifying the mutational processes in healthy tissues and preneoplastic disease states that predate tumorigenesis. The validation and subsequent studies in this direction will be crucial to implement early diagnosis criteria that twill lead to better prognosis for all cancer patients. In addition, they will be useful in the design of future cancer prevention policies in the case that such mutational signatures are caused by environmental or chemical factors.

It is well known that the development of cancer requires the stepwise accumulation of genetic alterations in protooncogenes and tumor suppressor

genes. Recent observations, however, indicate that this process is much more complex than previously anticipated. Thus, phylogenetic studies using Pan-Cancer-generated data suggest that such evolution can take place years if not decades. Some trends in how such mutations progressively accumulate in tumors have begun to emerge, although much work will be needed to fully understand this process (Gerstung et al., 2020). On the other hand, the recent characterization of the genomes in topologically distinct regions of healthy tissues have unveiled the presence of multiple clones of cells displaying genetic alterations of high oncogenic potential. Yet, these cell clones do not seem to be able to create a fully transformed phenotype (Ciccarelli, 2019). This suggest that: (i) There must be new tumor suppressor mechanisms that prevent those clones to grow in a fully malignant manner. (ii) We still do not fully understand the number of genetic alterations (or the combinations of them) that support a fully transformed state. (iii) There are cues from either the surrounding microenvironment or long-range endocrine programs that cooperate in the acquisition of a fully transformed condition.

On top of that, this feature complicates the identification of the drivers in the already formed tumors since many of them can be just carry overs from the pre-neoplastic condition. Consistent with this, low frequent mutant genes long considered as drivers are now deemed as simply bystanders that have been carried over from those genetically altered but non-transformed cell clones (Ciccarelli, 2019). Understanding this problem will require more sophisticated animal, organoid, and bioengineered models, organoids, bioengineered models to mimic this initial cellular and tissue milieu ex vivo. We will also need to develop more powerful single cell-sequencing and proteomics analyses.

3.1.2. Genomics and tumor evolution

In addition to classically identified mutations, cancer cells also accumulate large genomic alterations such as changes in number of chromosomes (aneuploidy), whole-genome duplication, focal amplifications and deletions, translocations, retrotransposon mobility across the cancer genome, and other more complex alterations. It is also clear now that chromatin structure affects mutational rates at a local scale (Gonzalez-Pérez et al., 2019). The orchestration of all these genomic alterations through time is critical to determine tumor evolution, malignancy, and clinical features (Gerstung et al., 2020). The recent description of mutational signatures after the most commonly used chemotherapies (Pich et al., 2019) should be of very much help in

understanding tumor evolution and inform the decision-making process on specific therapeutic approaches. Thus, translating the accumulated cancer genomic knowledge into improved cancer therapies and dynamic treatment regimens that consider the specific genomic alterations of a tumor, accounting for intratumor heterogeneity and its predicted evolutionary path is among the most important goals of cancer precision medicine.

3.1.3. Epigenomics

In addition to genetic alterations in cancer, the genome can alter its function through epigenetic modifications, which allow the modulation of gene expression in the absence of changes in the DNA sequence. The epigenetic language is made out of DNA modifications such as methylation and hydromethylation, a broad variety of post-translational histone modifications, chromatin accessibility and the 3D genome structure within the nucleus. The classical association between DNA methylation and gene expression is less clear nowadays, as these changes seem now to reflect a silent epigenetic memory of the maturation stage from which the tumor cells arose, a concept that can be used to classify tumors into sub-entities with different clinical behavior (Rodriguez-Paredes et al., 2018). In addition to DNA methylation, chromatin marks are rapidly gaining importance, as a new generation of regulatory elements have been found altered in cancer. They are large enhancer regions called super-enhancers, which are now converted in therapeutic targets (Lovén et al., 2013, Beekman et al., 2018). The translational benefits of these research efforts shall be a better sub-classification of neoplasms based on epigenetic imprints of their cellular origin, a more accurate prediction of future clinical behavior and a development of targeted epigenetic therapies.

3.1.4. Epitranscriptomics

We are now beginning to appreciate the multiple molecular processes that are under the regulation of mRNA synthesis, stability and translatability. In addition to conventional metabolic and stability mechanisms, we now know that specific RNAs can be post-translationally modified, given rise to the new field of epitranscriptomics. Interestingly, alterations in the post-transcriptional modification machinery have been associated with tumor-suppression or promotion. Most of these studies have focused on the molecular role and physiopathological functions of only one mark, 6-methyladenosine on mRNA (Liu et al., 2019). However, the epitranscriptome encompasses over 170 RNA chemical modifications and other post-transcriptional RNA processing events (Barbieri and Kouzarides, 2020). This implies that epitranscriptome-wide

association studies of hundreds of RNA marks on coding but also non-coding RNAs remain to be discovered and hundreds of possible epitranscriptomic mechanisms and their impact on transcription, translation and cancer cell biology remain unexplored. Only few small molecule inhibitors have been developed that can target specifically m6A regulators, yet none of them have reached clinical stages. Importantly, the high-profile publications have also excited venture capital investors that only in the last two years have invested over $200 million into five early-stage companies devoted to map and target the epitranscriptome in human disorders (Hodgson et al., 2018). This clearly opens the opportunity to basic and translational researchers to explore novel avenues to develop diagnostic tools based on epitranscriptomic modifications in cancer.

3.2. The metastatic cascade as a paradigm of evolving cellular organization in cancer

In order to fully understand cancer, it is also required to unveil the interactions of cancer cells among themselves and with the microenvironment (physical and cellular) that impinge on cell motility, plasticity, transitional states such as epithelial-mesenchymal and mesenchymal-epithelial transitions (EMT and MET), and many other cell behaviors (intravasation, survival in the blood and lymph stream, extravasation, adaption to peripheral niches, etc.) that cancer cells encounter all throughout the metastatic cascade. All these processes are of paramount importance to understand malignancy, metastasis, stemness, and therapeutic resistance. It is also clinically important, given that metastasis is responsible for more than 90% of cancer-associated deaths. Yet, its understanding and treatment is far from being achieved.

3.2.1. The interaction of cancer cells with the tumor microenvironment in the primary tumor and in the metastatic niche

Tumor microenvironments (TMEs) are highly complex, containing multitude of pro-active factors, bidirectional interactions, and a composition that depends of multiple parameters (target organ, type of cancer, individual factors) and that changes over time (Quail and Joyce, 2013). As such, it is clear that TME in the primary tumor can promote EMT and invasion, while at secondary sites it promotes metastatic success where some degree of epithelialization (MET) is required (Nieto et al., 2016). In addition, primary tumors can condition secondary sites from the distance generating pre-metastatic niches that affect metastatic efficacy and organotropism (Peinado et al., 2017). The complexity and divergence of the primary and secondary TMEs and their evolution

throughout cancer progression and dissemination represents a significant challenge to fully understand the process. As such, it is essential that future work characterizes the complex cellular states that co-exist within the primary and metastatic TME, describes the underlying mechanism and delineates ultimate biological relevance within the metastatic cascade. Altogether, these studies will likely unveil new tumor and TME features that identify patients with greater risk of malignant disease and inform of vulnerabilities that can further be exploited to adjust treatment strategies —even possibly in real time.

3.2.2. Disseminated cells and early diagnosis of malignancy

Understanding intravasation, survival in circulation and extravasation is a key challenge in the study of the metastatic cascade, as it determines metastatic potential. The vast majority of cancer patients bear circulating tumor cells (CTCs) at the time of diagnosis and thus, CTCs are considered as one of the most potent cancer biomarkers. The development of liquid biopsies in the clinic is revealing its potential in the early detection of malignancy, making informed decisions on personalized treatments, in following-up treatments and even in the diagnosis of residual cancer disease (Pantel and Alix-Panabières, 2018). Despite its extensive use in some specific tumor types (i.e, prostate, lung) technical challenges still remain before CTCs become standard tools for diagnosis and prognosis. Thus, optimized animal models should be generated where the mechanisms of CTCs availability, survival and evolution are fully understood and can be translated to the clinic. Developing state-of-the-art in vitro (organ-on-chip) and in vivo models with improved imaging capabilities to monitor spatio-temporally controlled cell lineage and phenotypic plasticity will have a significant impact on our understanding of metastasis. They could provide essential information on other important aspects such as organotropism, pre-metastastic niches or interaction with TME. With respect to organotropism, why tumors of different origin generate metastasis at preferential organs (i.e., liver in colorectal carcinoma, brain in breast cancer; bone in prostate cancer) is still an open question and an unmet clinical need. The influence of factors emanating from the primary tumors (tumor cells, TME, paracrine factors) and the organ-specific metastatic niches requires further investigation. Recent information from brain metastasis indicates that activation of a "specific brain metastatic niche" prevails over the origin of the primary tumor (Boire et al., 2020). In summary, understanding the underlying mechanisms for the colonization of disseminated cancer cells will allow the use in the clinic of newly identified prognostic biomarkers of metastatic potential in disseminated cells.

3.2.3. The threats of residual cancer disease: dormancy and relapse

Primary tumors and/or metastasis can be dormant for a ong time, only relapsing after several years. Mechanisms underlying dormancy and relapse are largely unknown and are of the upmost clinical relevance (Goddard et al., 2019). Deciphering the mechanisms maintaining dormancy and those leading to reactivation of the primary or metastatic niches and the influence of phenotypic plasticity are some of the most relevant conceptual challenges to be approached in the coming years. They entail important technical challenges, as the need of innovative in vitro and particularly, in vivo models. Advance into this challenge would have a tremendous clinical impact helping to predict cancer relapse, and hopefully providing therapeutic options to specifically target the dormant state.

3.2.4. Lymphatic dissemination as a route to distant metastasis

Although the presence of lymph node metastasis has been considered an important prognosis factor for many decades, we still have a limited understanding of the basic molecular and cellular mechanisms involved in it. However, recent data showing that local lymph node metastases are not only an indication of the ability of the primary tumor to disseminate cells but also an important route towards distant metastases (Brown et al., 2018; Pereira et al., 2018), have fueled the interest in studying the mechanisms by which cancer cells survive and grow in the lymph nodes. Interestingly, such a dissemination seems to require an unexpected metabolic rewiring towards fatty acid oxidation (Lee et al., 2019). This latter feature suggests a close link between cancer, metabolism, and a plethora of systemic responses at the organismal level.

3.3. The organismal level: systemic responses to the tumor

From a systemic perspective, tumors behave as organs which actively interact with the host (the patient). The last decades have shed light on the molecular and biological interactions between cancer cells with other cellular components inhabiting the tumor, the stroma. The latter includes different cells types such as cancer associated fibroblasts (CAF) and immune cells from both the lymphoid and myeloid lineages. The molecular mechanism behind these interactions are still in their infancy. More broadly, the tumor as a whole also establishes interactions with the vascular and the nervous system the nervous system that can affect the well-being of the patient. In this regard, anticancer strategies are currently focused on three main aspects of tumor-stroma interactions: the immune response to cancer, the

metabolic rewiring of cancer and its impact on this cross-talk, and the interaction of the tumor with the microbiome.

3.3.1. Metabolic rewiring in cancer

The rewiring of glucose metabolism towards aerobic glycolysis was the first indication of altered metabolism in cancer cells. Described by Warburg almost 100 years ago, an extensive study of other metabolic pathways had been somehow neglected. Fortunately, the field has exhibited a renaissance in the past decade. Of particular interest is the rewiring in fatty acid metabolism and cholesterol synthesis, both explored in terms of cancer therapies (Snaebbjomsson et al., 2020). Similarly, many other aspects of tumor metabolism remain to be elucidated, namely interaction of metabolism with cell signaling in cancer cells, metabolic interactions with the stroma, and metabolic control of the epigenome. As such, metabolic alterations can lead to abnormal accumulation of lactate, fumarate, succinate, acetyl CoA, alpha-ketoglutarate, and other molecules, that can reprogram the epigenome. Since epigenetic regulation is at the core of cell identity and cellular adaptation, a broader understanding of this phenomenon could harbor great potential for the development of prognostic/predictive biomarkers and therapeutic strategies. Metabolic reprogramming can be monitored by ascertaining metabolites, enzymes, or metabolic-related transcipts abundance in tumor biopsies or biofluids. Whereas expression-based analysis of metabolic genes is feasible for clinical application, metabolomics technologies are still not widely implemented in the clinical setting. On the one hand, the identification of relevant metabolites, together with the miniaturization of quantitative assays that are clinically friendly is a pending task. Metabolic genes that serve as relevant biomarkers should be identified and clinically validated.

Cancer pathogenesis and progression also result in systemic metabolic alterations, including wasting phenotypes and cachexia. This phenomenon has a profound impact on quality of life. Therefore, the molecular basis of the crosstalk between the tumor and the organism, and the therapeutic strategies that target those interactions would be instrumental for disease management (Petruzzelli and Wagner, 2016). Different diets have been associated with cancer risk for a long time (Steck and Murphy, 2020), but an experimental framework to mechanistically connect diet and metabolic alterations with patient outcome is still missing (Lien and Vander Heiden, 2020). A recent example is the description of how tumor-initiating cells rely on dietary lipids to promote metastasis (Pascual et al., 2017).

3.3.2. Inflammation, cancer and microbiome

Chronic inflammation has been long considered as a high-risk factor for cancer. As such, hepatitis, pancreatitis and inflammatory bowel disease (IBD) show a strong association with the development of hepatic and pancreatic carcinoma, and colorectal cancer, respectively. As mentioned above, dietary patterns together with patient susceptibility very much influence the development of cancer and there is a direct relationship between the diet and the microbiota. Recent advances point to a key role of intestinal microbiota in both inflammation and cancer in the gastrointestinal tract. A direct link was found between *Helicobacter pilorii* and stomach ulcer/gastric cancer on one hand, and more recently between *Fusobacterium nucleatum* and IBD/colorectal cancer on the other (Tilg et al., 2018). The microbiota represents an important fraction of our cellular composition. It is now known that our organism establishes symbiotic relationships with microorganisms in various organs. To which extent microbiota composition and microbe-derived metabolites influence cancerous and inflammatory processes remains an exciting field of study. High-throughput technologies enable the annotation and classification of microbiome alterations. The impact of such changes on cancer susceptibility and aggressiveness must remain an active field in CSIC through clinical and preclinical studies. Conversely, the correction of microbiome abnormalities in cancer patients should be evaluated as an intervention strategy in cancer management.

3.3.3. Tumor interactions with the vascular and the nervous systems and cancer cell mimicry

In addition to the well-known role of vascularization in tumor growth and dissemination, the uncertain outcome of treatments based on antiangiogenic drugs has led to the appearance of new requirements in basic research. As such, tumors resistant to antiangiogenic drugs cancer cells have been shown to reprogram to generate channels of cells that can provide blood to the tumor, through a phenomenon called vascular mimicry (Maroufi et al., 2020). In addition, recent studies have unveiled an unexpected phenomenon by which cancer cells can disseminate to distant organ without getting into the blood circulation, but rather undergoing a reprogramming process towards embryonic fates and undergo pericyte mimicry, attaching to the vessels and undergoing extravascular migratory metastasis. In addition to vascularization, innervation of tumors has been observed concomitant with cancer progression. But interestingly, and similarly to the situation in vascular mimicry, recent studies have shown that tumors can co-opt developmental neural programs to grow and progress (Zahalka and Frenette, 2020). Thus, altogether there is a need to

understand the cellular and molecular mechanisms that govern on one hand, the interaction of tumors with the vasculature and the nerves of the host and on the other hand, the reactivation of developmental vascular and neural programs that as it occurs with the epithelial to mesenchymal transition, help cancer cells to survive and progress to the metastatic disease. Optimized animal models, analysis at the single -cell level and new developments in 3D tissue engineering should help the progress in this area.

3.3.4. Immune system, cancer and immunotherapy

Tumor development activates both innate and adaptive immune responses, but cancer cells develop mechanisms to mimic immune tolerance and escape immune surveillance. How to revert or block the escape response has been one of the most challenging questions in cancer research. A breakthrough in the last decade is how boosting the endogenous immune system or using chimeric synthetic antigen receptors (CAR-Ts) engineered to selectively kill tumor cells time, could be a game changer in cancer therapy. Indeed, the therapeutic exploitation of this knowledge has led to clinical trials with unprecedented results, and the discoverers of cancer immunotherapy obtained the Nobel prize award in 2018 (https://www.nobelprize.org/prizes/medicine/2018/summary/). However, there are still many challenges ahead that require renewed efforts in basic research (Hegde and Chen, 2019, and see next section).

3.4. The therapeutic level: novel therapeutic approaches and the response to therapies

Due to their etiology, cancers are highly heterogeneous at multiple levels. On the one hand, patients affected by a common tumor type can display marked physiological and molecular differences despite the cell of origin or the clinical classification of the cancer involved. As a token, more than ten types of breast cancers are now considered according to such features. On the other hand, cancer cells from the same tumor can show different biological and clinical features depending on the region of the primary tumor mass or the metastatic site in which they are located in. To make things worse, the heterogeneity of tumors can evolve in time and space due to pressures imposed by both physiological constraints and drug treatments. We are also beginning to understand that standard chemotherapy treatments can drive the development of new genomic changes that can subsequently favor tumor recurrence. This inter- and intra-patient heterogeneity makes it very difficult the diagnosis, effective treatment, and long-term survival of cancer patients. To deal with this problem, we will need a deeper understanding of those processes at the

genomics, signaling, and cellular level with a perspective of space and time. In addition, we will have to solidify and further implement personalized precision medicine (PPM) strategies both at the basic and clinical level. Nowadays, however, these approaches are still in their infancy.

Recent genomics studies have revealed that most tumors develop during long periods of time, a process during which the accumulation of mutations and divergent cancer cell clones arise. Due to this, it is widely assumed that the detection of tumors at very early times would significantly simplify their handling at the clinical level. Unfortunately, there are no technologies (molecular or physics-based) as yet available to allow such early detection in patients in routine clinical practice.

The implementation of PPM-based tools in cancer should be based on the combination of new detection techniques (biomarkers, liquid biopsy, physics-based equipment of higher spatial resolution), a holistic understanding of tumor evolution pre- and post-treatment, the identification of actionable targets, and new strategies for patient stratification. Based on this, the following strategic goals are proposed (we do not include here other topics that have been discussed in other sections of this book).

3.4.1. Early detection

Improvement in liquid biopsy approaches (circulating tumor cells, soluble biomarkers, cells carrying cancer epitopes) and physics-based detection (PET, RMN, detection of diagnostic compounds and metabolites, new techniques) of tumors.

3.4.2. Implementation of large-scale efforts to understand the molecular mechanism behind tumor evolution in animal models but also its analysis in patients

This goal includes the routine implementation of NGS in patient samples, the generation of in silico repositories, and the development of Big-Data tools (supercomputers, algorithms, and artificial intelligence [AI]).

3.4.3. Development of new strategies to inhibit cancer-driving genes, especially those that are not druggable according to current chemical knowledge

This goal will benefit from the optimization of high-throughput structural (cryo-EM, RMN, crystal structures) and in silico modelling (e.g., structural

dynamics, IA) platforms. It will also require new inhibitory mechanisms based on peptide-mimetics and degrons, among many other potential avenues. Along these lines the recent development of PROTACs has open the possibility to target any protein using nonclassical drug strategies.

3.4.4. New tools for drug screening and clinical selection of individual patient-focused therapies

Those include resources such as high-throughput screening platforms, organoids and animal models (Zebrafish, PDXs, GEMMs).

3.4.5. Development of new drug delivery methods (antibody conjugates, nanoparticles)

3.4.6. Exploitation of intrinsic and extrinsic cancer cell vulnerabilities

Treatments to be developed or further improved in the near future as PPMs include:

- Hormone therapies
- Signal transduction inhibitors (e.g., KRAS, cell cycle inhibitors, synthetic lethals)
- Gene expression modulators
- DNA repair inhibitors (e.g., antiPARP-like)
- Apoptosis inducers
- Angiogenesis inhibitors
- Inhibitors of cancer stem cell properties (tumor-initiating capacities)
- Antibody drug conjugates
- Agnostic therapies
- Combinatorial therapies (concurrent or step-wise)

3.4.7. Cancer immunotherapy

In addition to the targeting of the intrinsic cancer cell vulnerabilities, special attention deserves cancer immunotherapies in its different modalities. Humoral and cell-based immunotherapies (CAR-Ts, check-point inhibitors, monoclonal antibodies to immune reaction checkpoints, cancer vaccines) offer a quite attractive new therapeutic approach to deal with many tumors either as single agents or in combination with other therapies. The effectiveness of such therapies has been already demonstrated in melanoma, lung, kidney and hematological tumors. Unfortunately, these new therapies are far from optimal: there are many patients that still do not respond or develop resistance to them

(and we do not know why), some develop autoimmune responses and many tumor types are still refractory to such therapies (Hegde and Chen, 2019). As in the case of the targeted therapies, it is likely that most immunotherapies will have to be optimized and implemented following a PPM-based philosophy.

3.4.8. Development of novel tools to follow therapeutic responses

- Noninvasive techniques in real time (e.g., liquid biopsy). Liquid biopsies can now be considered an alternative to tumor biopsies in the monitoring of biomarkers to design therapies and to follow treatment response and resistance (Kilgour et al., 2020).
- Ascertaining the interference/contribution of chronic therapies to anticancer treatments and immunotherapy. People in modern societies is chronically treated with a variety of agents, including anti-diabetics, anti-cholesterolemics, anti-hypertensive agents, antibiotics and other classes of drugs. To which extent chronic treatments can influence the activity or effectiveness of cancer therapies remains poorly understood. Basic research emanating from the CSIC should be aimed at defining the molecular links of these interventions with cell signaling and metabolic networks, immune status and microbiota composition. Moreover, the impact of these perturbations should be ascertained in clinical prospective studies aimed at quantifying potential drug interactions.
- Post-treatment tools to quantify amount and type of residual disease.

3.4.9. Resistance to therapies

Both targeted therapy as well as radio and chemotherapies have classically been challenged by the appearance of drug- refractory cancer cells resulting from the selection of rare pre- existing genetic alterations or the acquisition of de novo mutations. We are also beginning to understand how standard chemotherapy treatments can drive the development of new genomic changes that can subsequently favor tumor recurrence (Pich et al., 2019). Drugs designed to target specific signaling pathways can also promote a rewiring in the downstream targets leading to a "paradoxical activation" of the pathway and resistance. The recent finding of the high levels of intratumor heterogeneity has posed another challenge to the design of targeted therapies (Marusyk et al., 2020). Cell plasticity has also recently emerged as a crucial mechanism for therapy evasion (Boumahdi and de Sauvage, 2020), including the dynamic phenotypic changes intimately linked to the metastatic cascade (Gupta et al., 2019). Thus, the more we learn about the complexity of cancer biology, the

more we need to develop smarter therapeutic strategies, as this continues to be a big challenge in cancer research.

3.5. Technological challenges

The technological challenges in cancer research are so wide that cannot be summarized in a general document. We will only refer to a few recent technological developments that help understanding cancer biology, evolution, individual patient susceptibilities, prognosis and responses to therapy at an unprecedented pace, but that also pose challenges that need to be tackled.

3.5.1. The new level of resolution

Although integrative omics analyses of bulk tumors shall continue, those performed at the single-cell level will surely become standard in the next few years, with important implications in cancer medicine (Lim et al., 2020). Tumor cells show accelerated evolutionary mechanisms leading to (epi)genetic heterogeneity and phenotypic diversity, and single cell analyses are essential to tackle such tumor heterogeneity. Although optimized single cell technologies are being rapidly developed, further refinement and possibilities for simultaneous multi-omics is still needed. This single cell approach will not only serve to analyze the tumor cells but also the microenvironment. This shall lead to detailed description of functional diversity of tumor, accompanying cells and interactions with tissues and organs, all to be considered for therapeutic strategies. However, this technology is very costly and requires specific infrastructure. Special actions should be taken in this respect.

3.5.2. Acquisition, storage, retrieval, and smart use of big data

As a consequence of the omic-type analysis of the genome, epigenome, transcriptome, metabolome, etc. implemented at the single cell levels cell, the magnitude of data and their management for both basic research and clinical practice is unprecedented and requires the existence of specialized infrastructures at the local, national and international level and specialized personnel. Considering the enormous potential of big data analyses (Shilo et al., 2020), CSIC should consider the development of such infrastructures and also the coordination with European hubs, including the European Bioinformatics Institute (EBI) and favor links with Institutes in the area of Mathematics and Artificial Intelligence (see also section 5). The big data will also have to be integrated in the clinical setting.

3.5.3. New bioengineering strategies to allow the high-throughput analyses of organoids, PDX and other multicellular structures under improved reproducibility and physiological mimicking conditions

These tools will be essential for in vitro cancer models (either for cancer and cancer/microenvironment studies), drug discovery, and patient stratification according to empirical responsiveness to antitumor treatments ex vivo as indicated in challenge 3.4.

3.5.4. New biological methods for early tumor detection and treatment monitoring

Although promising, there is still room for improvement in liquid biopsies based on detection of circulating tumor cells, molecules, metabolites, and cancer cell antigen-carrying normal cells (e.g., macrophages, dendritic cells). These techniques have high potential for early cancer detection, diagnosis, molecular taxonomy classification of tumors from biopsies (including levels of tumor heterogeneity) as well as the therapeutic response (early and during subsequent treatment periods) of patients. Challenges in this area still remain in terms of detection of small amounts of samples, characterization of usual false negatives, the technological platforms to be used, and standardization protocols. The implementation of these techniques for validation using large scale clinical trials is still a challenge.

3.5.5. New physics-based methods for early detection of tumors

Detection of tumors and metastasis is still hampered by the need of relatively large and metabolically active masses of cancer cells in patients. New technology will have to be developed to lower such threshold of detection. Tackling this issue will require the implication of technologically focused CSIC institutes and groups as well as collaborations from other academic and industrial organizations.

4. CONCLUDING REMARKS

Cancer represents a major health, social and economic burden in our societies. Due to this, cancer is now being considered as one of the five key missions of the HorizonEU research framework program. Given its current portfolio of research groups working in this area, the CSIC is in a privileged position to delineate and implement an integrative program to address lingering basic (understanding the molecular basis of cancer) and translational (earlier detection and improved treatment) questions that affect both the

understanding and effective treatment of this disease. The proposed strategic program targets an ambitious, although realistic goal: to increase survival rates in cancer patients at least up to 70% by the end of the next decade.

In this document, we have identified a number of thematic priorities at four different levels of research to achieve this goal. In addition, we propose important organization issues, including the establishment of a virtual CSIC-Cancer Institute to promote interactions within CSIC, international competitiveness and increase visibility of the CSIC as a key player in cancer research. The successful implementation of this strategy will generate the right environment and momentum to achieve both the research and institutional goals in this critical health system-related area.

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CHALLENGE 2

ABSTRACT

The tremendous impact that the newly emerged virus SARS-CoV-2 is causing around the world is a clear evidence that coronavirus and other emerging, re-emerging and antibiotic-resistant infectious diseases represent the most importat threat to human kind. Tackling these diseases will require robust scientific cooperations to provide different perspectives for the design and development of vaccines, new antimicrobials or innovative tools for the prevention, diagnosis and control of the spread of infectious agents that can be applied both in the hospital sector and veterinarian sectors. In this chapter we present the most relevant advances of the last decades in the fight against infectious agents and the future challenges associated with the field of infectious diseases.

KEYWORDS

antibiotic-resistant infections

emerging infections | re-emerging infections

epidemics | pandemics

CHALLENGE 2

SOLUTIONS FOR INFECTIOUS DISEASES

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1. INTRODUCTION AND GENERAL DESCRIPTION

1.1. Introduction

Antibiotic-resistant infectious diseases, newly emerging infections, re-emerging infections and even deliberately disseminated infectious diseases (i.e. bioterrorism) represent the biggest health threat facing humanity. Bacterial infections, once controlled by the use of antibiotics, are now becoming resistant and spread rapidly without any control measurement (Morens et al., 2004). The magnitude and difficult management of pathogenic virus infections is explicitly and crudely exemplified currently by the SARS-CoV-2 pandemic. As consequence, infectious agents cause major health problems globally for a significant proportion of the population. They are the leading cause of death, accounting for a quarter to a third of estimated deaths worldwide (Tacconelli and Pezzani, 2019). Parasite-related infections and neglected tropical diseases (NTDs) also raise to alarming levels in the pasts decades. These types of infections, once exclusively associated with low- and middle-income countries, are now spreading freely and they are responsible for substantial mortality and morbidity. There are several reasons why infectious diseases in general represent a major threat to society:

1) Antibiotics have started to fail and antibiotic-resistant bacterial infections represent a serious threat to public health as many infections can no longer be treated and patient isolation is no longer feasible (Coates et al., 2020; Tamma et al., 2012). Resistant bacteria already cause more deaths

every year, with predictions to rise dramatically if radical actions are not taken. The antibiotic market is broken and companies continuously leave the sector.

2) A mix of complex environmental and societal changes in European societies favor the rapid spread of emerging and re-emerging infection diseases. Rapid population growth combined with uncontrolled urbanization (millions of city habitants live in overcrowded areas), increased trade and travel, abuse of antibiotics, as well as mutations in pathogens are the main drivers of disease spreading (Drexler, 2010). For instance, people, services, goods, capital and microbes are free to move across borders of the European Union (EU), which currently has 28 member states and an estimated population of 508 million. The capacity of people and goods to move across European areas is overwhelming that in case of pandemic, patient isolation becomes a serious challenge (Gibbs, 2005). Europe is a potential juncture for the emerging and re-emerging severe infectious disease threats (Gibbs, 2005). In this sense, Southern Europe is already colonized by insect vector of previously considered "tropical diseases" such as mosquito-borne viral diseases such as those caused by chikungunya, dengue or Zika viruses. Although not properly endemic to Europe (with the exception of several cases of locally acquired dengue virus infections have been reported in France and Spain. This, together with the fact that the vector mosquito population is endemis in areas of Southern Europe and that climate change predictions point at a widespread expansion of the vector, it has been estimated that the cases of mosquito and other arthropod-borne virus (Arbovirus) infections will become endemic in Europe in the next decades.

Climate change is also expected to alter animal distribution areas (Wu et al., 2016). Of particular interest is the continental and transcontinental bird migration patterns (Wu et al., 2016). These animals are not only asymptomatic carriers of potential human viral pathogens such as influenza or West Nile Viruses, but are also carriers of infected arthropods, such as ticks, that may carry viruses causing severe human disease such a Tick-Borne encephalitis or Crimea-Congo viruses.

3) Animal farming activities also constitute a potential source of infectious diseases that pose, not only an immediate economic threat to this important economic activity, but may also be the source of zoonotic diseases (Tomley and Shirley, 2009). A paradigmatic example of this is the recurrent emergence of pathogenic strains of influenza viruses of avian origin in the context of a reassortment of the viral genetic material with seasonal human or swine

influenza strains. This often results in self-limited, highly pathogenic infections that only cause local problems to people directly exposed to the infected animals. However, these viruses have the potential to transmit efficiently amongst humans, causing very serious health threats with the potential for a pandemic. This potential is currently exemplified by the zoonotic infections caused by coronaviruses that, when efficiently transmitted among humans cause very serious human health problems, like in the case of the severe acute respiratory syndrome (SARS) virus outbreak in Asia in 2003 and the current COVID19 pandemics caused by a related coronavirus (SARS-CoV-2).

Regarding bacterial infections, antibiotics are used in animal husbandry activities, previously used to stimulate growth and to prevent infections in farms and slaughterhouses, boosting antibiotic consumption and resistance among bacteria in the animal habitat (Landers et al., 2012). These animals are often kept under scavenging conditions with little attention to disease control, housing or feed supplementation, suffer a high burden of endemic disease and are likely to be in close contact with other livestock species and humans, and potentially in contact with a variety of non-domestic animals. The impact of epidemic diseases on the livelihoods of these poor farmers, particularly if there is high mortality or the imposition of animal movement restrictions or culling, is severe (http://smallstock.info).

1.2. General description

Infection diseases have become one of the greatest threats to global health and one of the biggest health concern in Europe. Consequently, drug-resistant bacteria, emerging and re-emerging infectious diseases spread rapidly in populated areas to dangerously high levels, threatening our ability to treat common infections and causing severe complications in medical procedures; with an estimated 250,000 deaths and 5.5 billion EURO cost per year in healthcare costs and productivity losses in the EU alone (Suk and Semenza, 2011). Antibiotic do not stop bacteria anymore and we are painfully witnessing that there is no efficient treatment to stop the spread of many pathogenic viruses. Without an efficient mechanism to prevent the spread of pathogens, deaths caused by infections will raise in the following years to unprecedented high levels. For instance, WHO predicts that drug-resistant bacterial infections will be the most important cause of death worldwide in 2050 (WHO, 2019).

Antibiotic-resistant bacteria. Antibiotic resistance are rising in hospital environments to dangerously high levels and spread globally, threatening our

ability to treat common infectious diseases. Antibiotic resistance is putting at risk all the advances that modern medicine has achieved in the past decades. Organ transplantations, chemotherapy and even minor surgeries that should not represent any technical now are much more dangerous procedures without effective antibiotics for the prevention and treatment of resistant infections (WHO, 2020). Among the antibiotic-resistant bacteria usually found in hospital settings are carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacteriaceae; methicillin-resistant and vancomycin-intermediate *Staphylococcus aureus*, clarithromycin-resistant *Helicobacter pylori*, fluoroquinolone-resistant *Campylobacter*, *Salmonella*, *Neisseria gonorrhoeae* and *Shigella*; vancomycin-resistant *Enterococcus faecium*, penicillin-non-susceptible *Streptococcus pneumoniae* or ampicillin-resistant *Haemophilus influenzae* (Wright, 2010; Gootz, 2010).

Emerging Infectious Diseases. These infections appeared recently within a population or a specific geographical area but are rapidly increasing in incidence or geographic range (Quaglio, 2012). Emerging infectious diseases have the potential to shape the course of human history due to the tremendous impact and incalculable misery and death that they create. The recent SARS-CoV-2 crisis is showing us how unprepared human kind is to address these infectious threats and it reminds us past pandemics of the human history, such as the Black Death in the 14th century, smallpox in the 16th century or the Spanish influenza in 1918. Although most of these pathogens are viral, bacterial infections are also included, like the with the foodborne *Escherichia coli* O104:H4 outbreak in Germany. Nonetheless, the major risk from bacteria comes from antibiotic-resistant strains, such as multiple- drug-resistant *Staphylococcus aureus* (MRSA) or *Mycobacterium tuberculosis* (David and Daum, 2010). As mentioned above, viral pathogens endemic from other areas of the planet are expected to become endemic in Southern European regions in the next decades, particularly those transmitted by vectors that are increasingly present in European ecosystems. These threats are extensive to highly pathogenic viruses circulating in wild animals such as West Nile Virus in birds or Crimean Congo hemorrhagic fever circulating in wild boars across Europe. The zoonotic potential of these animals is high and poses a serious threat that should not be undermined.

Re-emerging infections. These are infectious diseases that once become a serious health problem and, after a previous decline in incidence, are again becoming health problems for a significant proportion of the population

(Morens et al., 2004; Quaglio, 2012). Relevant examples are the new influenza viruses, new pathogenic microbes transmitted from animals or resurgent infections. For instance, public health officials have reported increased rates of tuberculosis in Europe, previously thought to be nearly eradicated from the continent. Many patients developed tuberculosis while they were abroad on exotic destinations and brought the disease to Europe. In addition, the incidence of tuberculosis is rather high in immigrant population and also in EU countries from the Baltic area. Among the re-emerging infections are those caused by yellow fever, Plague, cholera, meningococcal disease, Dengue, influenza, african trypanosomiasis, HIV/AIDS, leishmaniasis, multidrug-resistant tuberculosis, nipah virus or rabies.

Tropical disease caused by parasites, Chagas disease, leishmaniasis, malaria and helminthiasis, among others, seem to be the most relevant pathologies. Global mobility as well as global climate changes partly explain these are emerging diseases. Chagas disease (ChD) caused by the protozoan parasite *Trypanosoma cruzi* is endemic in 21 Latin-American countries, being its prevalence estimated between 10 and 15 million (Leslie, 2011). In non-endemic countries, the number of infected people is around one million and the number of infected patients in Spain is more than 50,000, being the vertical transmission the main route of infection. The current treatment focuses on the elimination of the parasite load in blood and it is effective in the acute phase, but its efficacy is questionable in the chronic phase due to severe adverse effects. Leishmaniasis comprises a series of pathologies caused by different species of *Leishmania* genus (Mansueto et al., 2014; Aoun and Bouratbine, 2014). In Spain and other Mediterranean countries, the predominant infecting species is Leishmania infantum, whose infection produces mainly visceral manifestations. Currently, there is no effective prophylactic method and existing chemotherapeutic treatments do not have the desirable efficiency. For leishmaniasis there are 300,000 estimated cases of visceral leishmaniasis (VL) and over 20,000 deaths annually, 1 million cases of cutaneous leishmaniasis (CL) were reported in the last 5 years and a total of 310 million are at risk.

Malaria is caused by a parasitic protozoa of the genus *Plasmodium* (Most, 1974). The disease afflicts between 400 and 500 million people worldwide and kills approximately 800,000, mainly infants every year. The spread of drug-resistance parasites has greatly reduced the efficacy of cheap, widely available compounds such as chloroquine. Anti-folates are also suffering from an upsurge in resistance and artemisinin resistance is now emerging.

In Spain, between 300-400 cases are reported annually and the incidence rate is approximately 1 case/100,000 people; practically all described cases are imported.

Livestock infections. Infectious diseases of livestock are a major threat to global animal husbandry (Tomley and Shirley, 2009). They impact severely agronomic health, national and international food supplies and represent a serious threat to human health. Livestock and farming animals usually maintained with little attention to disease control. They likely diseases with the risk of become endemic if they are in close contact with other livestock species, non-domestic animals or humans. Approximately 75% of new human pathogens that have been reported in the past 25 years have originated in animals and the risk of zoonoses will increase in the following years. Zoonotic infections that are transmissible between animals and humans pose significant threats to human health. The new influenza A (H1N1) is a classic example of a zoonotic viruses. The first cases of an influenza H1N1 illness in Mexico were reported in February 2009. In June, it had spread to 91 countries with 55,867 cases reported. Other livestock-associated infectious pathogens are African horse sickness virus African swine fever, *Babesia, Clostridium, Bacillus anthracis* (also for bioterrorism purposes), bluetongue virus, bovine leukosis, *Brucella, Burkholderia* mallei, swine fever, *Cochliomyia hominivorax, Echinococcus, Ehrlichia ruminantium,* Equine infectious virus, *Mycoplasma,* porcine reproductive and respiratory Syndrome virus, Rift Valley Fever virus, *Trypanosoma,* West Nile virus.

Crop infections. Microorganisms that cause plant diseases compromise greatly our ecosystem integrity and our society in general. Plant pathogens cause significant losses to agricultural yields and increasingly threaten food security, harming the job market and the one of most important pillars of Spanish economy, the agriculture. A special mention is to *Xylella fastidiosa* (Baldi and La Porta, 2017), one of the most dangerous plant bacteria worldwide. An outbreak of this bacterium is now detected in in France and Spain olive-growing areas (more than 2.5 million hectares) and throughout the Mediterranean agriculture. In addition to *Xilella*, other plant pathogens of importance are *Pseudomonas syringae, Ralstonia solanaceraum, Agrobacterium tumefaciens, Xanthomonas, Dickeya, pectobacterium atrosepticum, Erwinia amilovora*.

About 15 million (>25%) of 57 million annual deaths are estimated to be directly related to infectious diseases globally. In addition to this figure, millions of deaths occur as a consequence of post-traumatic infections (for

example, streptococcal rheumatic heart disease), or due to further complications associated with chronic infections. Indirectly, infections diseases cause a profound impact in our societies, threatening jobs, markets thus our European well-being standards.

Why are these diseases spreading. A complex combination of societal changes in European societies favor a rapid spread of antibiotic-resistant and re-emerging infection diseases. These factors can be mainly grouped in four pillars:

Social drivers. The demographic changes are mainly people migration between countries and rural to urban areas. Growing concentrations of people in large cities, mass migrations forced by social or economic pressures generate overcrowded cities that facilitate the spread of infections to the inhabitants (Drexler, 2010). Common facilities and societal services are not prepared for receiving such number of residents; substandard housing and inadequate sewage and water management systems favor the spread disease so that big cities have become great incubators of infections. Outbreaks of respiratory, gastrointestinal, meningeal, and skin infections are rather usual in large cities. According to the UN, 54% of the world's population lived in urban centres. Larger urban centres show greater potential for growth and development, thus the economic growth for countries is associated with urbanization thus, the most urbanized countries are also the best developed and the ones with better well-being status. However, the density of inhabitants and close contact between people allow a fast dissemination of re-emerging infectious diseases or multidrug resistant pathogens. The tremendous flux of citicens to overcrowding districts in which local governments do not have enough resources to provide safe housing or adequate sewage facilities, all of which increase the threats of spreading infectious diseases. Cities allow fast and efficient dissemination of infectious diseases at the same time as they are an essential piece of EU economy. In Europe, 72% of the total population lives in medium-to-large sized urbanized areas. As European urbanized areas contain most of the financial and political power, the economic growth is often associated with the good shape of urbanization centres. They hold 62% of the EU jobs and represent 71% of the whole European Gross Domestic Product (GDP).

Globalization. Expanding communication networks (airplanes, long-distance trains or better highways) brings people into contact with new environments and pathogens (Gibbs, 2005). Travel has increased, bringing together thousands of people from diverse geographic regions in confined spaces. They may

encounter microbes that they have never before been exposed to. In addition to travelers, migration and refugees from other countries are responsible for large amounts of globalization. ~3.3% of the world's population no longer live in their birth country. This population is susceptible to introducing infections into a population, but also is likely for them to contract new infections. The recent flow of immigration has setting Spain as the major recipient among OECD countries in both relative and absolute terms. The latest data provided by the National Statistical Institute point out that there are nearly five million foreigners living in Spain, representing 10% of the Spanish population. This population is, in general, young and healthy, but they are considered the main gateway of imported infectious diseases as they could present health problems closely related to their country of origin or their precarious living situation here, in Spain.

Tourism is a key driver of socio-economic progress in Europe through the generation of jobs, export income and infrastructure development for many destinations. Europe received 500 million tourists in 2017 and this number is expected to increase in the following years. Globalized international travel is huge in number of people, is rapid, and is concentrated on a very specific season and geographical area. Small towns that receive such amounts of tourists that cannot cope with this unprecedented increase in urban density; they cannot provide correct waste management, water resources, healthcare or even sewage management to the whole population. This causes a significant threat for the spread of re-emerging infectious disease. Human travelers can easily carry person-to-person transmitted infections and can move microbes globally. In an increasingly interconnected world, new risks (e.g. multi-drug resistant infections) and disease-causing microbes will move even more rapidly in the coming years. In 2017, Europe registered an all-time record upswing (+8%) in international tourist arrivals, above worldwide growth (+7%). EU received 671 million visitors; excluding internal tourism of Europeans, which has increased significantly with the Schengen treaty and the euro13. For the European economy alone, travel and tourism directly contributed 624.3 billion euros to GDP in 2016 and 2.28 million jobs through direct employment in the sector.

Public health. Antibiotic abuse and overuse is one more of the many key factor to the development of antibiotic-resistant bacterial infections and multidrug-resistant bacteria (WHO, 2020). These are relatively common human pathogens that were traditionally easy to treat using common antibiotic

treatments but have now developed resistance to multiple antibiotics and can cause many life-threatening infections. In the current antibiotic crisis, new antibiotics become ineffective soon after they are introduced into the clinic due to rapidly evolving resistance in pathogenic bacteria. As a consequence, bacteria have developed resistance to all antibiotics introduced thus far and the pharma industry no longer considers antibiotic development as wise investment. Evolution of antibiotic resistance is a major challenge of our time, as many multi drug resistant pathogens are often resistant to most, if not all, antibiotics and hospitals have no choice but to treat these infections with a combination of antibiotics in an desperate attempt to improve patient outcomes. A breakthrough in the field is needed to avoid that common infections evolving into life-threatening diseases. Antibiotic-resistant pathogens threatens our ability to treat common infections, causing severe complications in medical and surgical procedures and prolonged hospital stays. 700,000 people around the world die of drug-resistant diseases each year with severe healthcare costs. Thus far, efforts have been focused on the development of new antibiotics but bacteria have developed resistance to all antibiotics introduced.

The antibiotic market is a broken market (McKenna, 2020). The antibiotic market is not as strong as it used to be and several drawbacks led antibiotics to an important troubling trends escalation. As a consequence, major pharmaceutical companies are backing away from developing new antibiotics or made significant reductions. Several reasons explains the important decline of the antibiotic market. The antibiotic market is different from other drug markets as one new antibiotic is being approved, the public health sector solicits its preservation as long as possible to slow the development of resistance. This means that new antibiotics often face anemic initial sales. Patients increasingly face infections that do not respond to existing antibiotics because bacterial develop resistance to the drugs over time. This causes a new antibiotic to become ineffective in a period of a few years, making inevitable that a new antibiotic will be needed. Investors continue to leave the field of antibiotic resistance, jeopardizing the short-term future of biotech companies and Small and Medium Enterprises that operate in the sector. Without adequate incentives it is anticipated that in less than 2 years from now, many small biotech companies dedicated to the discovery of new antibiotics will have disappeared together with their innovative products and expert teams.

Based on the current situation, modern European societies deal with an important paradox; while technological and economic development allow the

design of large-scale urbanization plans, high mobility of the population and large-scale farming exploitation, the overall effect of this progress sometimes does not benefit the well-being of citizens but causes severe alterations of the surrounding environment. Together with antibiotics abuse these are the causes of the rapid spread of emerging, re-emerging infectious diseases and antibiotic-resistant infections.

2. IMPACT ON BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

The concept of potential applications is void without a strong network of basic research that supports the progress of the field. Basic research provides de essential raw material for translation and is the best chance to meet the whole set of public health challenges. But it is not a simple equation in which basic research in this or that research field translates irremediably to potential applications in this particular field. In fact, this is often not the case. Otherwise, it is difficult to imagine the practical applications of basic research that was performed into the DNA polymerase of *Thermus aquaticus*, a extremophilic bacterium with no medical, agricultural of biotechnological interest and yet it provided by the development of the PCR. Similarly, the study of fungal interactions led to the discovery of statins, which are now used as cholesterol-lowering drugs. Overall, potential applications of basic research are unpredictable but there is something highly predictable in basic research; only the formation of a robust and strong network of basic research can nurture the development of translational research. Translators (e.g. pharmaceutical companies, small biotech, genetic engineering companies, crop design companies) need something to translate, which is often provided by a solid and robust research network. Therefore, translators look to be surrounded by these networks and accumulated in the areas in which basic research network is solid (e.g. Switzerland, Boston area, Silicon Valley). In Spain, there are currently a large number of factors that impede the generation of a solid network of basic research, being the lack of sufficient resources to support early-stage investigation the most prominent one. With that in mind, and with the aim of constructing a solid network of basic research that would be attractive to translators, the most important priority in emerging and reemerging diseases basic research could be delineated as it follows:

It is paramount to understand how microbes cause disease (infection biology) and how hosts respond to this challenge (host-pathogen interactions). All

pathogens developed remarkable molecular strategies to infect their hosts, to colonize our bodies, to evade the immune system or to secure their transmission (life cycle) from one host to another. Basic research in infection biology should develop new techniques or use conventional ones to elucidate the functional and mechanistic basis of pathogen and host specific biology. This will surely open brand-new opportunities to develop new strategies against infectious diseases. It is possible to seed for the creation of such encouraging research environment by:

1. supporting the generation of relevant scientific knowledge and new technologies for the detection of emerging diseases, by expanding research on ecology and environmental biology, to understand the factors that influence disease emergence and transmission.
2. supporting the generation of knowledge in molecular biology and biomedicine to understand the mechanisms of infection; the proteins and other virulence factors involved and their role in the infection process as well as to understand that molecular mechanism elicited by the host cell to fight the infection.
3. supporting the generation of knowledge for the development of new vaccines, antibiotics and therapeutics that can help to control specific diseases as well as 4) reinforce research (large-scale facilities as well as laboratory routine equipment) and training (PhD and postdoc fellowships) infrastructures for studying infectious diseases.

With the creation of a solid network of basic research, the pipeline of potential applications and their implementation to improve citizens' lives is guaranteed.

3. KEY CHALLENGING POINTS

3.1. The need of efficient surveillance systems

It is clear that effective surveillance is critical for the early detection and prevention of infection threats, especially those with high epidemic potential like re-emerging or multi-drug resistant pathogens. Early detection is essential to the control these infectious diseases. Containing the spread of such diseases requires active vigilance for signs of an outbreak, the rapid recognition of their presence and symptoms, in addition to strategies and resources for an appropriate and efficient response. A major challenge to infectious disease surveillance and detection is the ability to detect resistant pathogens or reemerging

infectious diseases in relatively remote areas, such as small towns or the countryside. There is also need to develop complimentary detection systems beyond the more traditional surveillance approaches. This is because traditional population screening based on hospital laboratory reports both for the confirmation of clinical diagnoses and strain characterization are extremely tedious, time-consuming, expensive and potentially invasive of privacy, which altogether cause a significant delay in data processing, and thus an untimely and inadequate activation of the warning system.

One way to close the gap in infectious disease surveillance may lie with the implementation of new approaches to obtain near "real-time" data and automated tools that will allow rapid and efficient detection of infection disease threats, even in rural regions where there is no infrastructure to perform such analyses. To achieve this, new approaches in regional syndromic surveillance using societal data obtained from non-traditional sources, bioinformatics, and rapid diagnostic methods (e.g Next-Generation sequencing, NGS) have already made important contributions to infectious disease control and are thus excellent candidates to combine them synergistically with traditional surveillance detection methods, although the implementation of these technologies in personalized medicine is costly.

The use of pre-diagnostic data and statistical algorithms aims to detect epidemics earlier than traditional methods. Social media communication is increasingly used to create and to post information through the internet. News media and social media create a vast platform for minority viewpoints and personal information that is not captured by other sources. People often talk about infection and epidemics on social media using key words such as "fever", "infection" or "antibiotics" before they are officially identified. Social media provides an additional informal source of data to identify health information that is not reported to medical officials, especially of a sensitive nature. However, analyses of this data require the use of well-constructed algorithms to obtain accuracy and prevent false positives. Overall, while traditional surveillance systems are often untimely due to the number of checks to ensure data accuracy, pre-diagnostic surveillance based on Big Data mining from social media is fast and timely but maybe inaccurate, so it needs further validations. On the other hand, NGS approaches are accurate and timely but costly and effort-wise prohibitive for patient diagnosis. Thus, a trade-off is possible to combine such disciplines to gain speed and data validity for rapid detection of epidemics. Consistently, an efficient method for early detection

of infection disease threat should combine both traditional and pre-diagnostic methodologies, together with anonymized cutting-edge rapid diagnostic methods at the community level.

3.2. Exploring the mechanisms of antibiotic resistance to find new antibiotic strategies

The antibiotic market suffers from a troubling trends escalate. On one side, patients increasingly face infections that are resistant to current antibiotics. On the other companies involved in antibiotic discovery and development are backing away from developing new antibiotics. The antibiotic market will not fix by itself. It is imperative that basic research stands out in the field and supports large research initiatives to understand the mechanisms that bacteria follow to become resistant to antibiotics. This should be linked to further actions on antibiotic discovery, to find new molecules or repurpose molecules with new antibiotic properties. New antibiotics development should stands as a key priority of public research institutions. The early identification of naturally occurring resistance mechanisms and targets that can accommodate numerous structural changes should lead to the discontinuation of the development of agents that are likely to fail in the clinic as a result of resistance. Resources could instead be focused on agents that are less likely to drop out of the pipeline for microbiological reasons. Knowledge about how and when resistance occurs and potential synergies with combinations of agents will also facilitate the development of dosing regimens that can help to minimize the emergence of resistance to current and new antibiotics, enabling these drugs to be used to best effect. This is crucial in the short term as new agents are not likely to enter widespread clinical practice in the immediate future. The challenge for the field is now to make best use of the available technologies, information and expertise to ensure the impact of resistance is fully accounted for in the urgent development of next-generation antibacterial drugs.

The intrinsic resistance of a bacterial species results from the absence of a susceptible target of a specific antibiotic. In addition, bacteria can acquire or develop resistance to antibiotics by several mechanisms; Minimizing the intracellular concentrations of the antibiotic, via poor penetration into the bacterium or via activation of antibiotic efflux pumps. Modifying the antibiotic target by acquisition of genetic mutation, often associated with the mechanism of action of the compound, or post-translational modification of the target. Inactivation of the antibiotic by hydrolysis or modification of their structure. Special attention should be given to the genetic mechanisms that bacteria display to acquire

foreign DNA coding for resistance determinants via horizontal gene transfer. Acquisition of extracellular DNA material via horizontal gene transfer is one of the most important drivers of bacterial evolution and it is responsible for the development of most of the antimicrobial resistance mechanisms (Sun et al., 2019). Related to this, another efficient mechanism for acquiring antimicrobial resistance genes is represented by integrons. These are site-specific recombination systems that are able to incorporate new open reading frames within mobile gene cassettes. Integrons are an efficient bacterial mechanism to acquire new genes and ensure their expression.

To tackle the problem of antibiotic-resistant bacteria and re-emerging infectious diseases, important efforts on research and development need to be heavily increased and supported. It is absolutely essential to fully understand the mechanisms by which bacteria become resistant to antibiotics. This will be central to design novel strategies to counter the resistance threat and develop new antibiotics. Research efforts to develop antibiotics and to study mechanisms of resistance should be heavily increased, continuous and steady. Solutions require different types of research scientists, not just chemists and biologists with expertise and interest in microbiology. The field needs innovative actions that should include mathematical modelers, machine learning bioinformaticians and physicists. Similarly, it is of paramount importance to establish close and strong collaborative efforts with clinicians, epidemiologists, microbiologists and medical doctors with expertise in infectious diseases and translational medicine. The contribution of professional that work in clinical settings is key to have an end-user perspective; many times the laboratories study mechanisms of resistance of antibiotics of no relevance in clinic. So clinicians should be involved in basic research to provide an important global perspective of the problem.

It is equally important to establish new methodologies for antibiotic discovery. It comes obviously through the use of large dataset platforms. The increasing availability of high throughput datasets has transformed microbiology and have caused impact on drug discovery. The establishment of systematic platforms to screen a natural sources in large scale numbers is crucial for the discovery of new antibiotic classes. Is key to deeply screen large libraries of synthetic semi-synthetic or untapped natural products for different sources, as it this is likely the way to find a new powerful source of antibiotics. For instance, new natural product sources, such as marine plants or marine organisms or endophytes and epiphytes is expected to reveal wide class of new molecules with new

potential therapeutic applications. In addition, omics technologies have been proven a value asset as auxiliary tools for antibiotic discovery, as complimentary techniques to identify the potential mechanism of actions of the new compounds of the bacterial evolution to the presence of the compound. However, there is an important gap between high-throughput screenings and omics-centered assays in which mechanistic information is obtained. Innovative favoring high-throughput technologies that reconcile these two disciplines will be extremely welcomed in the field of antibiotic discovery.

3.3. Developing vaccines against plausible viral and bacterial threats

Bacterial vaccines

The decrease in the number of effective antibiotics is concomitant with the rising number of multi-drug-resistant pathogens. Vaccine development is one of the highest priorities for EU healthcare policies to prevent multi-drug-resistant infections and reduce antibiotic use. There is currently no effective vaccine against most of the multi-drug resistant bacteria that are catalogued as risk or high risk by the WHO. This is partly due to the complexity that entails the development of vaccines against bacterial infections, which it lies on the ability of bacteria to cause a broad range of infections using a wide and variable expression of target antigens. This means that not all the antigens are used in all type of infections or even by all the strains. There is a collection of antigens that are necessary for cause an infection and some of these antigens will play a more prominent role depending on the type of the infection and the infective bacterial strain. Based on this, it has been concluded that an effective vaccine should combine multiple antigens (i.e. a multivalent vaccine) to prevent the wide range of bacterial infections. Bacterial surfaces comprise numerous surface-exposed antigens, making them excellent candidates for the development of potent multivalent vaccines. Cell surfaces of these organisms are prominently decorated with strain-specific capsular polysaccharides or peptidoglycan, which are highly immunogenic and commonly shared among many different bacterial species.

Extracellular vesicles EVs are naturally occurring spherical nanostructures (~20–250 nm) produced constitutively by all bacteria (Kim et al., 2015). They are compact, non-living cell regions that contain specific proteins, lipids, and glycans that originate primarily from bacteria. They are attractive vaccine platform, because they are nonreplicating immunogenic entities that mimic bacteria and have natural adjuvant properties to stimulate the innate and

adaptive immune symtem. EVs from Neisseria meningitidis have been successfully developed into commercial vaccine for use in humans and is one of the few cases in which a vaccine is used to prevent a bacterial infection.

Viral vaccines

Research on vaccine development takes particular importance in virus research as viral vaccines are the most effective approach to controlling the viral diseases of mankind and animals. In the past, viral vaccine development depended upon an empirical strategy, which usually delineated tiresome and inefficient lines of research. Nowadays, new approaches in genetic engineering allow a more efficient design and test of new vaccine candidates or to circumvent existing issues in the vaccine development. Such approaches need to be consolidated and reinforced for better design of vaccines. For instance, an interesting alternative to inactivate viruses is the preparation of a recombinant viral protein for use as an immunogen. However, recombinant proteins must retain their natural conformation so they induce protective antibodies. This requires the implementation of large protein production platforms that allow to assay the conformation of any given protein under many different expression conditions. In addition to this, there has been a burst of research dedicated to develop innovative modes of antigen presentation, termed as vectors or "platforms." These new approaches include recombinant viruses, replicons, and purified DNA and are the front line in viral vaccine research. CSIC counts on the laboratory of Luis Enjuanes (CNB-CSIC) which is expert in the development of such approaches and have successfully implemented this techniques to develop a vaccine against SARS-CoV-2. Basically, vector-based strategies for immunization relies on cloning the coding sequence for a inmunogenic protein into a nonpathogenic virus that expresses the protein of interest and use it for vaccination therapy.

A special mention to future prospects in vaccine development is the recent achievements of RNA-based vaccines and holds great promise to prevent and treat a wide range of diseases (Schlake et al., 2012). RNA-based vaccines, no antigen is introduced, only the RNA containing the genetic information to produce the antigen. Upon vaccine delivery into the body, the RNA sequence is translated in the host cells to produce the encoded antigen/s, which induce the adaptive immune response to produce antibodies against the pathogen. The field of RNA vaccines is still nascent. However, their production is flexible and fast; mounting literature indicates that these vaccines could be effective against a wide range of infectious diseases and cancers. Multiple

companies are currently developing RNA vaccines, and several RNA-based vaccine candidates are developed to fight SARS-CoV-2.

3.4. Developing specific and broad-spectrum antivirals against immediate and unexpected viral threats

In contrast to antibiotics, antiviral drugs are often highly specific and designed to treat infections by a very particular virus or group of viruses. This is mainly due to the enormous diversity in the replication strategies of viruses. Moreover, viruses, particularly viruses with an RNA-based genome, often behave as mutant clouds capable of rapidly evolving (quasispecies) to circumvent the bottlenecks imposed by the host's immune response but also in response to an antiviral treatment. Thus, highly potent and specific antiviral drug combinations are often required to control viral infections. This is exemplified by the enormous antiviral armamentarium required to control HIV progression into AIDS or to eliminate chronic hepatitis C virus infection at the individual patient level.

Thus, a continued effort in the development of specific and broad-spectrum antiviral molecules is required to be prepared to respond to the emergence of viral infectious diseases. This entails a deep knowledge of basic aspects of viral replication but also of its necessary interaction with the host cell, since antiviral therapies may also be targeted towards essential cellular factors required for efficient viral spread. Even though at the general population level, antiviral therapies are secondary measures to the development of an effective vaccine, they have been proven instrumental for the control of worldwide spread human diseases such as those caused by hepatitis C and human immunodeficiency viruses.

In contrast to these highly specific therapies, broad-spectrum antivirals, such as viral mutagens ribavirin or favipiravir have been proposed for the management of emerging infectious diseases such as imported Ebola cases during the 2014 african outbreak. Currently, the only antiviral approved for clinical testing to fight the COVID 19 pandemic is remdesivir, a non-specific viral polymerase inhibitor, proven to be ineffective in the clinical setting for several viral infections for which promising in vitro data had been obtained. This antiviral is administered intravenously, making it unavailable for the general population. This crudely illustrates how poorly prepared we are to respond to emerging viral threats and advocates for a strong investment in the development of orally available, broad-spectrum antiviral drug combination treatments to face unexpected viral threats.

3.5. Prevention and control of parasitic infection diseases

Surveillance of infectious diseases imported by travelers and immigrants, many from tropical countries, is one of the main tools in the prevention and control of emerging infectious diseases in the 21st century. In addition, it is necessary to carry out the prevention and control on Neglected Tropical Diseases in endemic countries.

New entities with entirely novel mechanisms of action are urgently required to be able to address new challenges in the field of treatment of tropical diseases that are mainly arising as a consequence of the emergence of resistance or the lack of effective therapies against pathogens that are life-threatening. This need for new medicines is not only an issue in endemic countries but also areas of the developed world are more and more at risk. These are in line with the WHO 2030 Agenda for sustainable development goals; in particular, points 3.3 (WHO, 2019b).

Progress to control and elimination of tropical infectious diseases depends on the development of efficient tools, including new diagnostic tools, biomarkers, new drugs, vaccines and vector control strategies. Thus, development of biomarkers, new prophylaxis and immunotherapeutic approaches for infectious tropical diseases will help to building up the overall capacity for preparedness research against severe infectious diseases caused by emerging parasites causing significant damage to health and socio-economics in the affected areas.

Thus, the prevention and control of these sickness will require:

a. to identify and characterize parasite molecules useful as markers of pathology and therapeutic efficacy,
b. to establish specific patterns of the host immune and metabolic responses associated to pathological degree, prognosis and control of these diseases,
c. identification of virulence factors and molecular probes useful for typing the infecting strains isolated from patients and reservoirs,
d. characterization of the immunological mechanisms underlying protective response to pathogens causing tropical diseases and development of new tools for the assessment of vaccines and immunotherapies and e) identification of novel compounds with therapeutic potential and the investigation of aspects related to therapeutic failure.

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CHALLENGE 3

ABSTRACT

In the last decades, the increase of antimicrobial resistance (AMR) and the extensive use of antimicrobials have led to a global public health crisis. This might be one of the world's biggest problems, jeopardizing the treatment of infections worldwide. Alarming levels of drug resistance have been reported worldwide, with the result that common infectious diseases are becoming untreatable. AMR costs lives and economical resources and threatens to undermine the effectiveness of health delivery programs. It has recently been considered as a threat to global stability and national security. Overall, all Health Institutions and International Organizations consider that Basic Research, Development and Innovation represent critical points to increase the fight against drug resistance. AMR is a multifactorial problem that must be addressed from different disciplines. Promoting synergies and opportunities for collaboration among CSIC researchers in a One Health context is essential to implement a research program to achieve a significant impact against drug resistance in infectious diseases.

KEYWORDS

infectious diseases

antimicrobial resistance (AMR)

"One Health" | mechanisms of AMR

host-pathogen interactions

horizontal gene transfer

AMR diagnostic methods

therapeutic strategies

CHALLENGE 3

DRUG RESISTANCE IN INFECTIOUS DISEASES

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1. INTRODUCTION AND GENERAL DESCRIPTION

Antimicrobials are among the most successful drugs developed throughout history. The use of antimicrobial chemotherapy allowed to treat devastating and very common infectious diseases, such as tuberculosis, children's and water-associated infections, which caused millions of deaths worldwide. The generalized use of these drugs has dramatically modified Medicine allowing the development of new medical practices. Invasive surgeries, intubation, catheterization, immunosuppression associated with anticancer chemotherapy or organ transplantation will be unfeasible without the aid of methods for preventing and treating infections. An idyllic world in which infectious diseases were no longer ranked in the top causes of death did not last. The emergence and spread of antimicrobial resistance among pathogens currently risk the use of several methods for treating other diseases, and globally menace both patient safety and economy (European Centre for Disease Prevention and Control, 2016; World Health Organization, 2016).

Antimicrobial resistance (AMR) is a process by which microorganisms (bacteria, fungi, and parasites) can escape the action of antimicrobials (antibacterial, antifungal, and antiparasitic drugs). AMR has existed long before the anthropogenic use of antimicrobials. Nevertheless, the massive introduction of antibiotics in the last century became a worldwide experiment whose outcome was the fast selection of highly resistant microorganisms, one of the few

evolution processes amenable to experimentation and that can be followed in real (human-scale) time.

The World Health Organization (WHO) recognizes infections caused by multi-drug resistant (MDR) pathogens as one of the top three threats of global health (Rice, 2008; Bassetti et al., 2011), being responsible of at least 700,000 deaths per year globally, including 230,000 deaths from MDR tuberculosis (O'Neill, 2014; World Health Organization, 2018b), a figure that could increase to 10 million deaths per year globally by 2050, if not action is taken (The World Bank, 2017). Around 2.4 million people could die in high-income countries between 2015-2050 without a sustained effort to contain AMR (Organization for Economic Cooperation and Development, 2018). More than 214,000 newborns and babies die each year from drug-resistant bacterial infections, as 40% of infections in newborns and babies resist standard treatments (World Health Organization, 2016; Zaidi et al., 2005). In May 2015, the 68th World Health Assembly adopted the Global Action Plan on Antimicrobial Resistance (World Health Organization, 2015), with recognition among member states of the threat posed to human health by AMR and proposed specific actions to tackle the problem.

AMR has rapidly developed and disseminated in various pathogenic microorganisms (European Centre for Disease Prevention and Control, 2014; World Health Organization, 2015). Alarming levels of resistance have been reported in countries regardless of their income levels in the past decades, shortening the shelf life of new antimicrobial drugs. The perpetual increase in resistance mechanisms (Brown and Wright, 2016), the seeming sparseness of valid antimicrobial targets (Silver, 2016), and the notion that antimicrobial discovery offers a poor return-on-investment (Piddock, 2012) underline a widespread concern about the reliability of antimicrobial chemotherapy in the future (Shore and Coukell, 2016). Therefore, it is imperative to contain the spread of these highly resistant microorganisms, particularly since the antibiotic pipeline is weak, more innovative antibiotic candidates are only in the early-stage testing, and research and development for antibiotics are primarily driven by small- or medium-sized enterprises with large pharmaceutical companies continuing to exit the field (World Health Organization, 2020).

In the last years, the study of AMR has broadened its former scope, exclusively focused on human infections, to encompass a variety of scenarios. Misuse and overuse of existing antimicrobials in humans, but also in animals and plants, and their dumping or leaking to the general environment have favored

the development and spread of AMR (Wernli et al., 2017; World Health Organization, 2018a). More recently, there is a growing awareness of the participation of other natural ecosystems in the origin, evolution, and spread of AMR, although we cannot yet predict which environmental resistance genes would be functional in pathogenic bacteria and cause clinical diseases (Martínez, 2008; Martínez et al., 2007, 2015). Antibiotic-resistant organisms can move from animals to humans and vice versa (Aarestrup et al., 1998). Most human infections have a zoonotic origin, and 75% of new and emerging pathogens originated from animals, including those dubbed as foodborne (Cutler, 2015), responsible for 420,000 deaths per year (World Health Organization, 2015).

Nowadays, the use of antimicrobials as growth promoters has been banned in the EU (Aarestrup et al., 2001), but not in other places around the world (World Health Organization, 2015). The main (and nearly unique) method so far implemented for reducing the impact of antibiotic resistance has been the quantitative reduction of antibiotics in use. However, this practice alone is not sufficient to reduce the current AMR burden (Collignon et al., 2018). Although the reduction in antibiotic use can slow-down the emergence and eventual AMR dissemination, the fact is that even when an antibiotic has been fully banned in a given region, resistance may decline, but it does not disappear (Sundqvist et al., 2010).

Consequently, AMR is a clear example of the "One Health" approach: a concept that intertwines human health with those from animal, plant and environmental health. AMR is not confined to the species where it emerged first. A resistance that develops in one microorganism or location can also spread rapidly and unpredictably, through the exchange of genetic material between microorganisms by horizontal gene transfer mechanisms, such as transformation, transduction or conjugation in bacteria, or through extracellular vesicles in the case of protozoan parasites. Saturation of the environment with selective agents might cause directional selection for higher rates of mutation, recombination, and horizontal gene transfer, producing unpredictable consequences for humans and the biosphere (Gillings and Stokes, 2012; Blázquez et al., 2018). Cumulative transfer of resistance genes between different microbial species has generated multi-resistant microorganisms, the so-called "supermicrobes", resilient to most of the current treatments, including the last-resort drugs (World Health Organization, 2017).

Our current knowledge of the complexity of factors underlying the current levels of AMR is far from complete. Some of the factors are pathogen

interactions with drugs and hosts, the intrinsic and acquired genetic variability of the pathogen, cross-resistance to unrelated drugs, the emergence of successful resistant clones and transmission rates of resistant pathogens among humans, animals, and the environment. Other sociological and healthcare components of this complex equation are rates of vaccination, health care systems, and population density and mobility.

Consequently, more global actions are needed to reduce the emergence and dissemination of AMR (World Health Organization, 2015). We must acknowledge the absence of an easy solution to AMR. Bacteria, fungi, and parasites will always evolve to thrive through new drug challenges. We can smooth the problem, or prevent its worsening, but the experience dictated the inability to find a drug/condition to avoid pathogens to evolve and reach a definitive solution. The analysis and control of AMR thus require eco-evolutionary approaches involving multi-hierarchical systems linking ecological, biological, and genetic entities, to slow down AMR emergence and dissemination, even so, an unpredictable factor in future interventions will be always present.

WHO is committed to developing a global consensus approach to AMR monitoring, with predefined measures of impact and outcome consistent with the Global Action Plan. WHO and the Interagency Coordination Group on Antimicrobial Resistance have launched a call to society: "No time to wait, unless the world acts urgently, antimicrobial resistance will have a disastrous impact within a generation". Only multidisciplinary efforts could give a proper answer to address this challenge (Food and Agriculture Organization, 2018; World Health Organization, 2018a). In the last 6 years more work has been done on AMR than in the preceding 30 years, regarding surveillance, awareness building, reducing antibiotic consumption, and to boost the development of new antibiotics (Laxminarayan et al., 2020).

The emergence of coronavirus pandemic caused by the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-COV-2) has become the defining challenge of our time, affecting not only our lives, the global economy but also rising many public health issues. One of the most worrying wider health impacts could be the rise and long-term propagation of drug-resistant infections due to a widely (and often suboptimal and inappropriate) use of antibiotics in hospital settings, especially in the acute care setting, to prevent and treat dangerous secondary bacterial infections in seriously ill COVID-19 patients (Langford et al., 2020; International Severe Acute Respiratory and Emerging Infection Consortium, 2020). Also, the spread of COVID-19 has heightened

societal awareness of personal hygiene and environmental contamination due to the use of biocides and disinfectants, mainly in Hospitals, that could have an impact in the selection and spread of antimicrobial-resistant pathogens. There is a risk that the pandemic will lead to potential break-downs in well-established antimicrobial stewardship programmes and severe disruptions to immunization services, with major long-term consequences, including secondary outbreaks of vaccine-preventable diseases (Rawson et al., 2020).

In Spain, the Ministerio de Sanidad, Consumo y Bienestar Social established the Plan Nacional frente a la Resistencia a los Antibióticos, known by its acronym PRAN, which includes a total of 8 ministries, 70 scientific societies, organizations, associations and universities, and more than 300 experts in the area. The main objective of PRAN is to promote a rational control in the use of drugs in human health and optimization of their use in hospitals and health care centers. CSIC leads the ranking of public Research Institutions in Spain and holds the seventh ranking world-wide (Ranking Scimago 2019). With 120 Research Institutes, CSIC has a large potential in basic research, development, and innovation, all critical points to increase knowledge in the fight against AMR in microorganisms.

2. IMPACT ON BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

WHO has emphasized the urgency to strengthen knowledge on AMR through surveillance and research, to reduce the incidence of infections, to optimize the use of antimicrobial agents and to increase investment in new medicines, diagnostic tools, vaccines and other interventions (World Health Organization, 2015). Resistance to antimicrobials is a multifactorial phenomenon and thus requires a diverse, open-minded approach. This offers a unique opportunity to foster scientific research in basic questions that will ultimately translate into potential applications to ameliorate the AMR problem. Research in this field will be useful for (i) the Spanish National Health System, as it will improve the health of patients with infectious diseases; (ii) WHO, due to our leadership in studies of some neglected infectious diseases; this research will increase the number and quality of treatments for such infections, as such, for their assimilation and implementation by health systems with limited resources; (iii) pharmaceutical companies for the selection of new drugs/therapeutic strategies that will improve the chemotherapy of infectious diseases.

AMR itself can be viewed as a global-scale experiment where basic laws of microbial evolution can be tested. The study of AMR is closely associated with the mechanisms of horizontal gene transfer and how gene interchange modulates the evolution of the individual organisms and the population as a whole. Besides, the need for tracking antibiotic resistance is basic to understand the population biology of microorganisms, as well as the hierarchical organization of the elements driving their evolution. Studies on this field are also excellent models for systems biology researches dealing with second-order consequences of mutations, with the role of noise in the emergence of novel phenotypes and with the interplay between acquired genes, providing novel functions, and the core genome of the microbial cell. Studies dealing with AMR are not restricted to biomedical sciences. Novel systems of delivery and the development of prostheses or devices with antimicrobial properties require the use of techniques from the area of materials. Similarly, new approaches for the fast identification of resistant organisms require the collaboration of multi-disciplinary teams including engineers, mathematicians, microbiologists, and clinicians. A final aspect frequently despised is the socioeconomic impact of antibiotic resistance (Collignon and Beggs, 2019; Hendriksen et al., 2019). In particular, quantitative risk analyses are scarce and urgently needed.

Four main venues of basic and applied research on AMR were envisaged, which deal with:

2.1. Mechanisms of AMR acquisition

Microorganisms have genetic plasticity to respond to a wide array of environmental threats, including drug pressure. Resistance to anti-infective molecules is developed and disseminated via the basic genetic mechanisms of mutation (including hypermutation), recombination (which creates and combines resistance determinants) and horizontal transfer (which shares complex mechanisms of resistance among members of the same or different, even evolutionary distant species). Available sequence data from different species strongly suggest that the majority of antibiotic resistances have most likely been acquired through the lateral transfer of resistance genes from ecologically and taxonomically distant microbial species (Binnewies et al., 2006). Transformation (Dubnau and Blokesch, 2019), transduction (Torres-Barceló, 2018), and conjugation (de la Cruz et al., 2010; Grohmann et al., 2003) are the classical mechanisms of horizontal gene transfer in bacteria. Recently, exosomes and other extracellular microvesicles shed from almost every type of cells or organisms have been proved to perform relevant functions in

intercellular communication and regulation including host-pathogen interactions and, probably, in the acquisition of drug resistance (Wuyts et al., 2018; Schorey and Harding, 2016). It is thus expected the lower the impact of all these basic mechanisms, the lower the development and dissemination of resistance. Gaining insights into these mechanisms and how they are affected by anti-infective challenges is fundamental to understand the basic process of resistance acquisition and the development of novel targets for both new therapies and diagnostic tests.

Knowledge of the molecular bases of antimicrobial action, including the mechanisms of killing and the basic genetic mechanisms of evolution that allow microorganisms to evolve drug resistance, is essential for developing improved therapeutics. AMR is achieved mainly by an impaired binding of the drug to its target, reduction/abolishment of drug import or prodrug modification, inactivation of the drug, or increasing drug efflux (Munita and Arias, 2016). Besides, the study of microbial physiology is key to understand the antimicrobial action. The interplay between bacterial cell-wall integrity and the summoning forth of resistance mechanisms to deactivate cell-wall-targeting antibiotics involves exquisite orchestration among cell-wall synthesis and remodeling, and the detection of and response to the antibiotics through modulation of gene regulation by specific effectors (Dik et al., 2018). Different pathways that maintain the integrity and stability of the bacterial genome, including SOS response, mismatch repair, and reactive oxygen species detoxification, can play key roles in the development and spread of resistance (Couce and Blázquez, 2009). On the other hand, AMR genes evolve, modifying the range of antimicrobials on which they can act. Such is the case of beta-lactamases, which broadened their action spectrum from penicillins to cephalosporines through a few point mutations (Philippon et al., 1989).

Besides, microorganisms develop other drug resistance strategies including the appearance of persister/dormant or quiescent cells, as non-replicating (or replicating very slowly) cells with metabolic alterations such as diminished DNA synthesis and/or down-regulation of protein translation (Lennon and Jones, 2011). After the withdrawal of antimicrobials, surviving cells can resume growth and act as reservoirs of AMR genes, promoting their spread (Wuyts et al., 2018). Additionally, bacteria and fungi form sessile multicellular communities called biofilms that provide a structural scaffold and defensive barrier for the cells against environmental challenges and stresses, including antimicrobials (Rabin et al., 2015). Biofilms are the cause of nosocomial, recurrent and chronic

infections associated with catheters, implants, or prostheses, as can often be at the origins of food-associated outbreaks (Jamal et al., 2018). Within the population that forms part of a biofilm, phenotypic differentiation can take place, as well as social interactions, such as metabolic cooperativity or intercellular communication via quorum sensing mechanisms, mediated by chemical signals secreted by the cells. Furthermore, biofilms constitute a favorable environment for the acquisition of new traits through horizontal gene transfer (Jamal et al., 2018; Wuyts et al., 2018). In biofilms, antibiotic treatment may kill metabolically active cells and leave persisters intact, so that these can re-start colonization and biofilm development when the treatment is removed (Wuyts et al., 2018). These persister-like cells exhibit drug tolerance and are responsible for frequent drug treatment failure (Lewis, 2007; Fisher et al., 2017; Barrett et al., 2019). The research will provide new tools for understanding the molecular bases of persister phenotypes in microbial populations and the environmental cues favoring or limiting biofilm development and thus new therapeutic strategies to face AMR in persisters and biofilms.

The studies directly addressing AMR can impact the field in two different aspects: firstly, providing information on the mechanisms of antibiotic resistance that will be later tracked to map the epidemiology of such resistance and implement actions to reduce its spread. Secondly, to identify the elements involved in the selection and dissemination of resistance; these include the biological elements implied in resistance or its development and spread (genes, plasmids, clones, gene-exchange communities) and also the ecological ones (human-to-human contact, animal production, water treatment...). Knowing the drivers of resistance is a pre-requisite for tackling this problem (Bush et al., 2011). Besides, studies on microbial physiology are fundamental to develop novel targets that should include, besides classical lethal targets, virulence determinants, basic genetic mechanisms involved in bacterial evolution, elements involved in bacterial attachment and biofilm formation, the resistance determinants themselves, and the elements involved in their dissemination (Kudo et al., 2019). Similarly, it has been proposed that knowing the AMR effect in microbial physiology may help in developing drugs specifically targeting resistant microorganisms (Vestergaard et al., 2017).

2.2. Host-pathogen interactions

AMR mechanisms represent a complex interrelationship between microorganisms and host cells, and both contribute to the resistance/therapeutic failure, which represents a growing burden in the management of infectious

diseases. Perhaps the most critical case is sepsis, in which there is a complex and dysregulated host response (Grondman et al., 2020). Sepsis-specific treatment options are still lacking. Several factors related to the host, drug, pathogen, and environment are engaged in the treatment outcome, including (i) host factors, such as immune status (contribution of host-immunity to treatment), nutritional conditions, adherence to treatment and co-infections by different microorganisms in simultaneous or sequential infections; (ii) drug factors, such as quality, pharmacokinetics, route of administration, time of initiating treatment and pharmacokinetics-pharmacodynamics limitations; (iii) pathogen factors, such as acquired or intrinsic resistance, organism localization and accessibility of drugs, inherent virulence; and (iv) environmental factors, such as environmental contamination with the drug or global warming that contribute to the expansion of diseases to new geographical areas.

Many microorganisms are obligate intracellular organisms that reside inside the host cells, even in the presence of innate and adaptive immune responses. In some cases, persistent intracellular infections are asymptomatic, although the infection can pose a risk to the host, especially if the disease is reactivated from an innocuous state of dormancy, as happens in tuberculosis (Peddireddy et al., 2017). Thus, there is always intimate crosstalk between the host and the pathogen, and pathogens have evolved numerous anti-immune strategies for continuous lifelong survival to escape host immune elimination by overcoming both innate and adaptive immunity (Tang, 2015). This balance of host immune response and pathogen counter-defense contributes to the complexity of persistent infections. Additionally, antimicrobial drugs must cross the plasma membrane of host cells to be accessible for intracellular microorganisms and produce functional effects, supporting the relevant role of host cells in the antimicrobial effect of drugs. Therefore, antimicrobial therapeutic effects would be influenced not only by the ability of different drugs to cross the plasma membrane of infected host cells but the distinct immune capacity of the cells. Consequently, one of the mechanisms that could be used for intracellular microorganisms to avoid drug effectiveness, survive inside host cells and develop therapeutic failure could be that intracellular microorganisms modulate gene expression in infected host cells (Nelson and Nelson, 2018). These modifications could be at (i) plasma membrane composition (with changes in plasma membrane fluidity and functionality of drug transporters), and (ii) immunomodulation of host cells by counteracting macrophage activation leading to microorganisms survival inside host cells.

Conversely, intracellular microorganisms present altered physiological states that modify their susceptibility to antibiotics. Notably, it has been described that *Listeria monocytogenes*, considered to be intrinsically resistant to phosphomycin, becomes susceptible to this antibiotic when growing intracellularly (Scortti et al., 2006, 2018).

The involvement of host cells in the antimicrobial effect of drugs opens an innovative approach in the treatment of infectious diseases. This can be done by modulation of host cell factors essential for pathogen replication or persistence, increasing protective immunity leading to reduced inflammation or host reactions at the site of pathology. Therefore, the concept of host-directed therapy for infectious diseases may provide a new way to potentiate novel drug combinations aiming to reduce toxicity and decrease the possibility of drug resistance (Wuyts et al., 2018). Knowledge of the innate signaling pathways activated by bacteria, fungi, and parasite components is key to produce vaccines and therapeutics (Bucşan and Williamson, 2020). Host-directed therapies to boost the microbicidal capacities of immune cells may enhance the clearance of drug-resistant pathogens. Strategic targeting of the host together with appropriate antimicrobial treatment of the pathogen(s) by rational combination therapy may reverse drug resistance and thereby increase the efficacy of drug therapies. Among promising host targets, various biological processes that modify host cell function, modulate the inflammatory response or affect pathogen replication and virulence are included. Facing these challenges, successful identification and development of host-directed therapeutics will conduct to a promising adjunctive therapy to treat infections caused by MDR pathogens. Besides, understanding quorum sensing in bacterial communities, as well as between hosts and their microbiota, offers opportunities to develop quorum sensing modulators that inhibit the development of virulence and biofilm formation by pathogens. Since these modulators would not harm the beneficial microbiota, the evolutive pressure towards resistance would be greatly reduced. Moreover, modulators that boost beneficial host-microbial synergies and host-defense alliances would reduce the need for antimicrobials and represent a promising future strategy (Galloway et al., 2011; Kalia, 2015; Ansari and Ahmad, 2018; Sibanda et al., 2018).

2.3. Antimicrobial-resistant pathogen diagnosis and track

The inheritance of the AMR genes as functional units in mobile platforms from an accessible genetic pool allows them to adapt very quickly to the antibiotic selective pressure. This means that the barriers to confine AMR should

not be limited to avoid the expansion of resistant clones, but rather include a thorough examination of the dissemination routes from natural reservoirs to human pathogens and the acquisition mechanisms involved. In recent decades, the epidemiology of pathogen outbreaks changed from acute and local to diffuse and widespread. This challenge has led to the development of rapid and more sensitive molecular methods for AMR diagnosis, of which the whole-genome sequencing is the flagship, at least for epidemiological studies. This is the time to foster the research on methodologies that allow handling and extracting valuable information from a large amount of DNA sequencing data that is exponentially accumulated. The exploitation of DNA sequencing data will be key for the fast identification of pathogens, AMR genes, mechanisms of resistance, sources of origin and transmission routes, allowing the real-time genomic epidemiology and surveillance. Applying next-generation sequencing, machine learning, data mining, and predictive microbiology, the identification of high-risk pathogens, prediction of resistance phenotype directly from genotype, and depicting resistance exchange networks will speed up and allow the comprehension of the evolutionary dynamics of antimicrobial resistance worldwide (Boolchandani et al., 2019). Besides DNA-based analysis, point of care fast methods for identifying resistant organisms in less-than-hours time are still needed, and some of them are under development. Procedures based on microfluidics (Mizoguchi et al., 2020) or the use of nanomechanical cantilevers (Stupar et al., 2017) may help in this purpose. Nevertheless, all these techniques, from genome sequencing to fast phenotypic tests require the isolation of the organism first. The final goal will be to establish diagnostic methods even of identifying the organism causing infection and its resistance phenotype in a few hours, and this task cannot be achieved if the 24 h cultivation required for isolation is needed. Hence, methods that do not require isolation of the infective agent are still required, particularly in the case of infections in locations with a complex microbiome as the gut.

Besides, computer simulations of populations, either resistant/susceptible microorganisms or susceptible-infected-recovered humans, could be used as a valuable methodology to model how infectious diseases and drug resistance spread in such populations (Atkins et al., 2018; Leclerc et al., 2019), and as a suitable frame to simulate the multi-hierarchical processes involved in AMR (Campos et al., 2019). Such models could help to understand how fitness landscapes affect the probability of appearance of mutants/resistance, and the time frame until mutants/resistances appear, to characterize different growth models and the interactions between strains, and how they affect the

emergence of single/multi-resistance, to predict the selective strengths of some drugs, the effects of different rates of patient flow from hospital to the community and vice versa, and the evolution of resistance phenotypes. These predictions could directly affect the planning of drug treatment schedules and select for optimized vaccination strategies against infectious diseases.

2.4. Rethinking therapeutic strategies

Results on the above points should lead to practical strategies to combat AMR-encoding pathogens. The appearance of resistances is favored when only one antimicrobial is used. A combination of drugs targeting different pathways is currently the most studied and successful approach for creating multi-drug cocktails to fight against drug resistance. It is worth mentioning that the acquisition of resistance to a drug may concur with increased susceptibility to another, what has been called as collateral susceptibility. The identification of collateral susceptibility networks may allow the implementation of novel, evidence-based therapeutic protocols based on drug combinations or replacements (Lázár et al., 2018; Pál et al., 2015). However, since resistance is eventually generated with time, the ongoing development of novel antimicrobials is necessary. Several approaches can be put into practice. The first of these would involve overcoming the old beliefs that we exhausted the sources of new families of antibiotics and making a return to the starting point by promoting a revival of natural products. This can be undertaken from different aspects, one of which would consist in reexamining those antibiotics discarded in the golden age, in which the demands were very high and the approach different. The second aspect would be based on the observation that we have not exhausted the arsenal of compounds of microbial origin with antibiotic activity. What we have exhausted is the chemical space of microbes that can be grown in a laboratory. However, this type of bacteria constitutes only 1% of the total. 99% of the microbes would still be available, which might be able to generate the desired antibiotics if we managed to grow them. Alternatively, culture-independent approaches, such as the metagenomics, can be used to mine for new antimicrobial biosynthetic pathways (Gomes et al., 2013; Adu-Oppong et al., 2017) that could be implemented by synthetic biology techniques. The third aspect would be based on drug-delivery systems, such as nanoparticles and adjuvants, among others. Nanoparticles can be used for the therapeutic management of infections in different ways (Pelgrift and Friedman, 2013; Baptista et al., 2018). They can be coupled with existing antimicrobial agents for enhancement of their physiochemical behavior against drug-resistant pathogens, and they are released under appropriate stimuli.

Adjuvants are compounds that have little or no activity on their own but that enhance the activity of antimicrobials. They have the advantage of suppressing the emergence of resistance and rescuing the activity of certain medications, thus offering an orthogonal strategy that is complementary to that aimed at discovering new antibiotics (Wright, 2016).

The new killing strategies aim to minimize the selection of resistance. These include host defense peptides, which raise special interest for their clinical potential, both in humans and animals (Mookherjee et al., 2020; Parai et al., 2019; van Dijk et al., 2018). Due to their promiscuous mode of action and lack of specificity, the chances for the selection of resistance are low. This fact, together with their broad activity spectrum and synergy with other antimicrobials, as well as their immunomodulation activity (at least in some cases), have drawn the attention of the pharmaceutical industry. Once these peptide-codifying sequences are discovered, synthetic analogs (peptidomimetics), which are more stable in vivo and allow the introduction of new functionalities, can be produced (Perez, 2018). Another strategy is the development of species-selective drugs, such as antibodies, which attack the pathogens without harming the beneficial microbiota (Fan and Li, 2017; McConnell, 2019). This approach includes comparative in silico screening of multiple genome sequences that allows the identification of conserved antigens specifically present in pathogens but absent in the other microbiota.

Very close to this strategy is reverse vaccinology, a high-performance genome scan of a microorganism, to identify new antigens and epitopes, which could speed the development of vaccines against specific pathogens (Bidmos et al., 2018). Additionally, the photothermal therapy, based on the use of nanomaterials that convert the energy of near-infrared light into heat, is a technology able to kill agglutinated bacteria, prevent and eliminate biofilm and exotoxin production, and inhibit bacterial quorum-sensing systems (Li et al., 2019).

The CRISPR technologies can be also used to block the AMR development and increase the resistance of animals and plants to infections, thus reducing the use of drugs and phytosanitary compounds (Shabbir et al., 2019). Phage therapy historically preceded antibiotic treatment against bacterial infections. The use of bacteriophages that kill bacteria, especially from drug-tolerant biofilms, has come up as a viable alternative to circumvent the antimicrobial resistance crisis (Moelling et al., 2018; Saha and Mukherjee, 2019). Another alternative is the use of natural or engineered endolysins (phage-encoded enzymes that break down bacterial peptidoglycan at the terminal stage

of the phage reproduction cycle) to control antibiotic-resistant pathogenic bacteria. Their cell wall binding domains target the enzymes to their substrate, and their corresponding catalytic domains can cleave bonds in the peptidoglycan network (Hermoso et al., 2007).

One strategy applied to control infections in the hospital environment is to prevent bacterial growth in textiles and biomedical material that may be in contact with human tissues. An ideal antiseptic agent should have a broad spectrum of antimicrobial activity and, at the same time, low toxicity against eukaryotic cells and, besides, not generate resistance mechanisms, neither it themselves, neither cross-resistance to antimicrobials. Currently used antiseptic agents, such as quaternary ammonium compounds, present some drawbacks, and thus alternatives that would select low levels of resistance and cross-resistance and produce low cytotoxicity against human cells are needed (Jennings et al., 2015).

Nevertheless, microorganisms eventually find mechanisms to cope with new antimicrobials. Intervention strategies that do not exert a positive selective pressure of resistant variants and allow restoring susceptibility to host immune defenses are thus regarded as more efficient in the long run. The ecology and evolution of AMR players would be relevant to the eco-evo strategies focused on interfering with the mechanisms associated with the infective capacity to cancel the causative elements of the disease or those involved in AMR emergence and spreading without killing the pathogen (Smith and Romesberg, 2007; Baquero et al., 2011, 2014; Culyba et al., 2015; Cabezón et al., 2017; Blázquez et al., 2018). In this way, a softer evolutionary pressure would be exerted, drug-resistant strain selection would be limited and, contrary to traditional strategies, the undesirable effects on the host-microbiome would be minimal. Numerous anti-virulence strategies are being investigated, including inhibition of adhesion (Shoaf-Sweeney and Hutkins, 2009; Rodrigues, 2011), invasion and formation of biofilms by bacteria (Clemens et al., 2015), interference with microbial signaling and gene regulation systems (Saeki et al., 2020), targeting DNA repair, hypermutation, and damage tolerance mechanisms (Blázquez, 2003; Oliver et al., 2004; Mittal et al., 2020), inhibition of toxins (Afrasiabi et al., 2020) and specialized secretion systems (Ripoll-Rozada et al., 2016), and restraints on the dissemination of bacterial conjugation (Getino and de la Cruz, 2018).

Development of enhancers of host natural defenses, such as probiotics and proteobiotics is another line of research and intervention. Probiotics are

specifically selected to not contribute to the AMR spread. They may reduce the risk for antibiotic-associated diarrhea, as well as for certain infectious diseases, and thereby may reduce the need for antibiotics (Ouwehand et al., 2016). Through probiotic studies, novel compounds (proteobiotics) were discovered that possessed antimicrobial activity and can interrupt bacteria cell-to-cell communication (Tarsillo and Priefer, 2020). In the case of plants, defensive mechanisms can be triggered against many phytopathogens by using chemicals that do not have direct antimicrobial action, thus reducing the appearance of AMR, and the leaking of phytosanitary compounds to the environment (González-Bosch, 2018).

Besides the modulation that exerts the probiotics on the microbiomes, they could be modified by removal or addition of targeted microbial species (Feehan and Garcia-Diaz, 2020; Willis and Gabaldón, 2020). Basic research and safe procedures to develop microbiota interventions, for instance using sentinel plasmids and bacteriophages in complex communities to specifically eliminate AMR-encoding microorganisms or displace pathogens, could be explored for ad hoc domestication of microbiomes.

3. KEY CHALLENGING POINTS

Prompted by the urgency and importance of the antibiotic resistance in infectious diseases, the current and future projects need to be articulated under a robust project portfolio to feed the pipeline. Basic research intertwining with the implementation of recommendations will help to save millions of lives, maintain economic and other development gains, and secure the future in the treatments of the infectious diseases against AMR. This portfolio is built according to several key points:

3.1. Study of the mechanisms responsible for the appearance of drug-resistant microorganisms

- Identification and characterization of basic genetic mechanisms and pathways that allow microorganisms to evolve antimicrobial resistance.
- Identification and characterization of microbial genes and mutations involved in drug resistance and biofilm formation.
- To define the resistomes with relevance for human health in the microbiome of animals (humans included), wastewater, environmental bacteria, and foods.

- To analyze the dynamics of biofilm speciation and horizontal gene transfer, in combination with computer simulation and theoretical modeling of different ecosystems.
- Structural and functional characterization of the mechanisms linking bacterial cell-wall remodeling and antibiotics resistance in Gram-negative pathogens.
- Evolutionary studies of microbial resistance mechanisms and identification of potential pathogen reservoirs in natural settings.
- Characterization of the persistence/quiescence, especially in intracellular drug-resistant microorganisms.
- Analysis of exosomes and other extracellular microvesicles in drug-resistant pathogens.
- To understand the mechanism of action of current and brand-new drugs against pathogens and their development of resistance.

3.2. Search for drug-resistant markers and specific diagnostic methods

- Development of faster and cheaper methods to evaluate the drug sensitivity of pathogens.
- Development of methods that do not require the isolation of the microorganisms for the diagnosis of antibiotic resistance.
- Epidemiological survey and, eventually, diagnostics for drug resistance through Whole Genome Sequencing data, determination of the genomic location of drug-resistance genes and prediction of drug resistance using genomic techniques.
- Implementation of methods to predict drug resistance in pathogens using DNA sequence data, computer simulation, machine learning, and theoretical modeling.

3.3. Appraisal of the routes of dispersal and spreading of resistance

- To explore the mechanisms and the ecosystems underlying resistance propagation.
- Analysis of dynamics of drug resistance transmission, through the linkage of the resistance mobilome with different microorganism genomes and metagenomes.
- Studies on the dynamics of microorganism competition concerning horizontal gene transfer.

- Computer simulation and theoretical modeling to evaluate the effect of antimicrobials on the emergence and spreading of drug resistance; and risk analysis of the transfer of drug resistance among different ecosystems.
- To determine the importance of environmental factors on the proliferation of drug resistance in microorganisms.
- Risk analysis of the human and animal infectivity of species/clones of microorganisms to implement control measurements to reduce animal-human transmission of antibiotic-resistant pathogens/genes.

3.4. Modulation of infected host cells by intracellular pathogens

- Omics analyses and integration with other biological data to comprehend the modulation of the infected host cells by pathogens, and identification of the molecular markers associated with this process.
- To exploit the host-pathogen interaction as a new alternative to combat pathogens profiting from the conserved adaptation mechanism of the pathogen, evolved throughout evolution.
- Host-directed therapies and development of enhancers of the host natural defenses.

3.5. Search for alternative treatments: new drugs and therapeutic strategies

- To develop new drugs with negligible induction of resistance against novel targets, aimed at decreasing evolution of resistance, microbial adhesion, biofilm formation or dispersion, conjugative plasmids dissemination, as well as targeting persister cells, especially in intracellular pathogens. Use of phages, enzybiotics and antimicrobial peptides.
- Development of vaccines for human and animal infections, in particular those targeting resistant organisms.
- The search of new compounds with a preferential target against resistant organisms, as well as drugs that inhibit the mechanism of resistance, among them, inhibitors of efflux pumps.
- To develop quorum-sensing inhibitors to interfere with the concerted changes in microbial populations, as well as bacterial conjugation inhibitors.
- To implement adjuvant systems to maximize the efficiency of antimicrobial compounds.

- Identification of drugs able to cross the outer and inner membranes of drug-resistant microorganisms.
- To search and develop phage-derived lytic enzymes able to selectively eliminate MDR bacteria.
- Identification of new drugs for improving the immune response of patients with drug-resistant pathogens as those inducing sessile biofilms, as well as the search for anti-virulence compounds.
- Research on new mechanisms to avoid the dumping of active antibiotics into the environment, especially those with a long half-life, to prevent outbreaks of drug resistance.
- Nanotechnology applied to drug resistance to improve the delivery of drugs and to reverse drug resistance. Nanomaterials as antimicrobial agents.
- Computer simulation and theoretical modeling to evaluate the risks of induced resistance towards new alternative treatments.
- New surfaces, or surface coatings, to inhibit bacterial colonization and biofilm formation.
- To define the role of microbiomes in the development of antibiotic resistance, microbiome replacement or modification to revert drug resistance, and enhancement of host-microbiota defensive alliances.
- Microbiota interventions. Modulation of the microbiome by probiotics and removal or addition of bacterial species, plasmids and bacteriophages in complex communities, to specifically eliminate AMR-encoding bacteria or displace pathogens.
- CRISPR technologies for blocking drug-resistant microorganisms.

3.6. Social dimension and incentive structure

- Education and outreach initiatives on the correct use of drugs and disinfection procedures, as an efficient strategy to combat drug resistance in infectious organisms.
- Economic models for analyzing the impact of drug resistance in Health Care, as well as the economic cost concerning changing animal management for preventing infections.

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CHALLENGE 4

ABSTRACT

Rare diseases (RDs) have a low prevalence individually, but together affect ~6% of the population. RDs are an exceptional scientific challenge in basic biomedical research. CSIC's multidisciplinary approach is an advantage for developing frontier research and contributing to the genetic, epigenetic, molecular, cellular and physiological mechanisms of RDs, to identify therapeutic targets and develop personalized medicine.

KEYWORDS

advanced therapies | computational biology | genotype-phenotype | omics | rare diseases or disorders | translational medicine

CHALLENGE 4

RARE DISEASES

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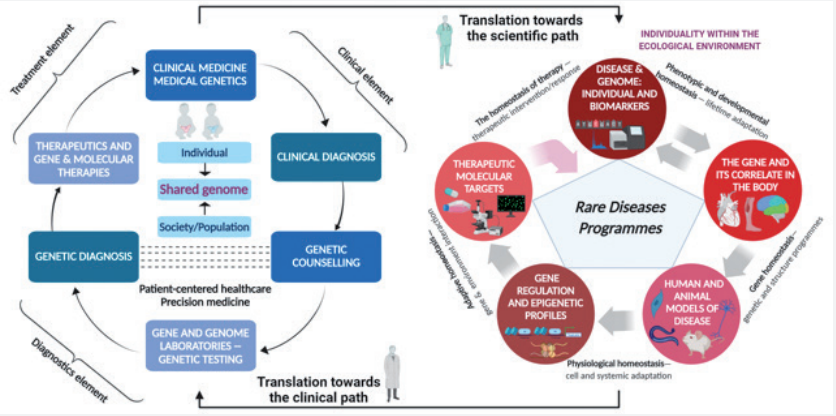
1. INTRODUCTION AND GENERAL DESCRIPTION

Physiological homeostasis depends on nature and nurture, represented by the integration of the gene pool and the interactions with the environment, both physical and cultural. The ability to adapt is maintained for most of an individual's life, although homeostasis can be compromised by disease and aging. Rare diseases (RDs) showcase a wide range of non-adaptive scenarios, and offer unique opportunities to investigate the nature of human adaptation and of its failure in pathological situations. Current scientific and technological capabilities allow the study of these diseases to advance the objectives of prevention, care and cure of RDs, and also to get insight into normal processes for which their disruption in specific RDs opens windows for in-depth study. Figure 1 represents a model with the two arms that support the translational paradigm suggested by clinical and scientific action plans. The elements of the clinical decision-making process in a patient with a genetic RD are indicated in the left panel. The right panel proposes different levels of action on altered homeostasis, showing key points for developing scientific research programs.

RDs have low individual prevalence, but together they affect 3.5-5.9% (Nguengang Wakap et al., 2020) of the population and they are estimated to

FIGURE 1. MODEL OF TRANSLATIONAL MEDICINE–The paradigm of translational research between medicine (left panel) and science (right panel). The translation paths of medical elements, focused on clinical, diagnostic and therapeutic issues, as well as the different homeostatic levels on which to influence the search for scientific solutions are represented (based on the IPER Translational Diagnostics Programme of the Sant Joan de Déu Children's Hospital and Research Institute, by F. Palau and J. Hoenicka; created with BioRender).



collectively affect ~350 million patients worldwide. In Spain, near 3 million patients suffer from a RD and there is increasing social pressure to diagnose unsolved cases and develop therapeutic options for RDs, as they are usually chronic disorders underserved by pharmaceutical companies. As recognized in the EU Council Recommendation 2009/C 151/02, RDs are a major research area that can greatly benefit from coordinated international action (www.eurordis.org). The WHO has catalogued around 7,000 RDs, which constitute a highly heterogeneous group of disorders. Many of them are caused by mutations in genes resulting in an unbalanced physiology with loss of compensatory mechanisms, leading to a complex pattern of symptoms. In most cases, current therapies aim to palliate and delay the symptoms or, if possible, to restore the altered physiology.

Modern medicine is firmly rooted in the understanding of the mechanisms of life as described by genetic, molecular, biological and behavioral sciences, from which predictions are made about the consequences of identified alterations, exemplified by genetic errors that abolish given functions (from causes to consequences). The physicians usually face the opposite view: they see manifestations leading to differential diagnosis (from consequences to causes;

abductive method) (Kliegman et al., 2017). Translational medicine fosters the fast flow between the scientific and the clinical fields, with a twofold objective: (i) to transform the clinical question into a scientific question that allows the search for scientific solutions, and (ii) to integrate and use scientific knowledge into that of differential diagnoses. RDs, including the undiagnosed or those still non-delineated (Gahl et al., 2012; Ramoni et al., 2017), offer a very wide landscape in which translational research together with precision medicine (SEBBM, 2018) can open up new paths and opportunities for understanding, as well as for diagnosis and treatment (Boycott and Ardigo, 2018), in total alignment with the IRDiRC recommendations (irdirc.org/about-us/vision-goals/). **CSIC can play an essential role in this bi-directional transmission of scientific and clinical knowledge.**

2. IMPACT ON BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

The application to RDs of CSIC's capabilities and expertise in basic sciences can advance the genetic, epigenetic and molecular understanding of RDs, identifying and validating potential disease targets and clarifying pathogenetic pathways of symptomatic patterns, while possibly also shedding light on heterogeneous phenotypes for similar genetic alterations, with the possibility of designing personalized treatments (precision medicine). Clearly, CSIC institutes can play a very important role in deciphering the biological processes associated with these diseases and in deepening the knowledge about these processes.

2.1. Diagnosis and Molecular Mechanisms

As indicated, many RDs are genetically-determined disorders. This places the need for widespread, easy and inexpensive access by CSIC groups to the most powerful genetic diagnostic technologies at the forefront. A genetic platform within CSIC, or CSIC affiliation to a shared platform with other institutions, appears vital, particularly given the current benefit of the widespread use of next-generation sequencing (NGS) for genetic diagnosis. In addition, facilities are also needed to clarify the pathogenic nature of sequence variants, in which the expression and purification of proteins, as well as the characterization of the properties of proteins and their complexes, are routine tasks, given the lack in many cases of genotype/phenotype correlations (Posey 2019; Whicher et al. 2018). Given the large number of genes (~20,000 genes; ~2% of the genome; size of haploid human genome, 3.2×10^9 base pairs; 3.2 Gbp; see ensembl.org/Homo_sapiens/Info/Annotation)

and the even larger number of encoded proteins largely due to alternative splicing, it also seems essential to set up protein engineering facilities or a network of research groups with the capacity to provide services in this field. We also need expert hubs for the characterization of gene functions, as only about 80% of the human genes (informatics.jax.org/mgihome/homepages/stats/all_stats.shtml) have a clear function ascribed. This shall need to recruit facilities for gene modification and characterization in several model organisms, from bacteria and yeasts to worms, fish and mice. Although it is predicted that two-thirds of the encoded human proteins have a structure similar to that of proteins from other organisms (Brooker, 2012), the fact that function resides mostly in protein loops that cannot be faithfully modelled and which are the sites of significant variation, makes the existence of abundant facilities for the structural characterization of macromolecular targets and their complexes important. This should be covered by macromolecular crystallography, currently accessible via shared synchrotron sources, but also, and very importantly, by promoting cryo-microscopy of single particles, a field in which the CSIC should continue its efforts to achieve a good structural characterization of the targets.

Current genetic tests used in clinical settings include chromosome microarrays (CMA) that have led to the discovery of most of the reported genomic rearrangement (~10% of disease-causing genetic lesions). NGS technologies are extremely efficient to identify disease-causing mutations, having completely transformed genetic diagnosis. As whole exome sequencing (WES) has become cheaper, it has accelerated the identification of highly penetrant pathogenic variants in the context of rare Mendelian disorders (Brown and Meloche, 2016; Yang et al., 2014), and has translated directly into clinical practice (Yang et al., 2013). However, 50–75% of patients suffering from various Mendelian disorders do not currently receive a genetic diagnosis by exome sequencing. WES is mostly blind to several types of variants (i.e. triplet expansion, epimutations, regulatory variants or mutations in non-coding RNAs, among others). Whole genome sequencing (WGS), particularly PCR-free WGS, has certain advantages over WES, not only because it can detect pathogenic variation outside coding regions, but also because this technique allows better coverage of the coding region of the genome and GC-rich regions (Meienberg et al., 2016). The emerging long-read single-molecule technologies, also referred as third-generation sequencing, generate data from single molecules. Two methods provide considerably long read lengths. Single-molecule real-time (SMRT) sequencing provides reading lengths up to 45 kb and

the nanopore technique is in theory capable of sequencing DNA strands up to 200 kb or longer in length (Eid et al., 2009; Jain et al., 2018). The possibility of obtaining these long reads reduces the problem of sequencing and assembly through complex genomic regions containing repetitive elements. Other advantages are the possibility of achieving better phasing of polymorphisms and the ability to directly detect epigenetic modifications (Furst et al., 2020). In addition to WGS for cases in which exome-sequencing fails to identify a pathogenic variant (Fresard and Montgomery, 2018), the use of the more powerful whole-genome sequencing approaches, in combination with whole-transcriptome analyses, may help to reduce the diagnostic gap (Cirulli and Goldstein, 2010; Deelen et al., 2019). Analyses combining the use of biological networks (Barabasi et al., 2011), involving either information from protein-protein interaction screens (Menche et al., 2015) or the integration of multiple -omic datasets (Mohammadi et al., 2019), have succeeded in elucidating the functional impact of certain alterations for particular rare disorders. Current techniques of genome and epigenome analysis, as well as the associated bioinformatics developments to extract information, are detailed in Volume 3 *Genome & Epigenetics* of this White Book.

Unlike other well-established public databases (Maher, 2012), those on RDs research remain highly fragmented, and lack a structured and systematic connection between clinical and genetic information. Initiatives such as Orphadata powered by Orphanet (http://www.orphadata.org/cgi-bin/index.php) and RD-Connect (https://rd-connect.eu) (Rath et al., 2012; Thompson et al., 2014) have greatly facilitated the organization of patient-related studies. However, with the ever-increasing number of large datasets, institutional efforts are required at the National and International levels in collaboration with funding bodies to ensure that these essential resources are updated, shared and maintained. CSIC could play an important role as the largest research institution nationwide.

Clinical sequencing of the genome accelerates the identification of variants, but the underlying mechanisms to connect genomic changes and clinical impact are not yet known. Experimental models (animal, cellular and organoid) are irreplaceable tools in biomedical research, especially in RDs. The generation and extensive phenotypic characterization of genetically modified mouse models is an efficient way to increase our knowledge on RDs, to find key molecular targets and to evaluate new therapeutic interventions. Ongoing international mutant mouse programs, such as IMPC (www.mousephenotype.org/), have provided key

information on human diseases over the past 20 years. To date, the IMPC has fully tested more than 6,000 protein-coding genes, but there are still over 11,000 mouse orthologues to be analyzed. IMPC resources have helped identify new candidate genes and develop mouse models for RDs, but there is not a specific program for these diseases. Additionally, iPSC-based RD models and also three-dimensional culture and tissue organoids provide a promising approach to the study of RDs pathophysiology. One of the main challenges is to generate genetically modified models for specific genes causing RDs. Since 2013, the CRISPR-Cas9 genome-editing technology has notably accelerated the generation process (Cohen 2016). Therefore, it would be feasible to have a catalog of animal and cellular models to study in depth specific aspects of the pathophysiology of RDs, as well as to carry out preclinical trials of potential therapies.

Finally, computational biology is a new ally for understanding RDs. The application of deep learning approaches can be used to improve low diagnosis rates and the classification of different diseases into a limited subset of observed phenotypes (Brasil et al., 2019). The ability to integrate data from different sources has the potential to identify novel prognostic biomarkers, thus illuminating the path to drug discovery and connecting similar diseases with potential ongoing clinical trials. Importantly, since the judgement of this data requires a significant amount of analysis and expertise, these goals must be accompanied by the generation of user-friendly software and databases to facilitate their appropriate translation into the clinic (Balloux et al., 2018). Recent advances in artificial intelligence are also facilitating the repurposing of mainstream drugs for use in orphan diseases (Xia, 2017), and are also paving the way for the rational design of novel therapeutic drugs such as Milasen (Kim et al., 2019), a recent breath-taking example of an ultimate personalized treatment. Whilst these precedents represent just a glimpse of the power of bioinformatics, the field still faces some serious challenges before it can address the functional analysis of RDs in the post-genomic era.

2.2. Development of New Therapies

Advances in NGS technologies have revolutionized our ability to discover the genetic causes of RDs, but the biggest challenge is the lack of effective treatments for most of them. Although there have been substantial efforts to promote the development of therapies for RDs in the last decades, at present, only 8% of RDs have a FDA-designated drug. Therefore, there is an urgent need to broaden the scope of applicability of therapies for RDs (Cardon and Harris, 2016).

Pharmaceutical companies in general are not interested in RDs research, since they are less likely to recover the investment required for drug development, given the small numbers of patients. However, it should be noted that the EMA authorizes an average of 9 medications for drug repurposing (DR) per year, which represents 25% of the total number of drugs approved, and therefore RDs are an opportunity for the pharmaceutical sector. There was a clear change in trend as a result of the publication of the European Union regulation in year 2000 for orphan drugs. The main efforts for RDs therapeutic discovery are focused on creating a treatment for single diseases rather than grouping patients according to a molecular etiology that would make much more sense (Ekins, 2017). Some molecular mechanisms are shared across multiple diseases and therefore, designing and implementing an efficient pipeline that handles multiple RDs in parallel to deliver new bioactive small molecules will reduce cost and will be more attractive to pharmaceutical companies (Brooks et al., 2014). Industry has traditionally focused on small-molecules chemical drugs, but advances in molecular biology and understanding of the molecular genome have expanded the drug discovery toolbox to include protein-based therapies, antisense oligonucleotide treatment such as small interfering RNAs (siRNA) therapies, or gene and cell therapies (Tambuyzer et al., 2020; Wu et al., 2019). These therapeutic modalities differ in their ability to target molecular mechanisms of disease and/or in their efficiency to reach certain cellular compartments. While protein-based therapies, antisense oligonucleotides and siRNA therapies have an specific target, gene and cell-based therapies have expanded the druggable aspects including targets and mechanisms that are difficult to address using small molecules (Tambuyzer et al., 2020). Those include genetic editing and targeting non-coding regions or 4D structural alterations (Wu et al., 2019). Gene and cell therapy are extensively discussed in Challenge 5, "Advanced Therapies and Bioengineering".

Here we present each modality with its strengths and limitations and the research and clinical success of each therapeutic modality in order to identify the plan and resources needed to achieve the therapeutic goal.

2.2.1. Small Molecule Drugs

Small molecule (SM) drugs are the best-established agents to fight diseases, and continue to be attractive drugs because of their diverse ways of administration, controlled dosing, stability, scale of synthesis and comparatively low cost. Although concerns have been raised (Scannell et al., 2012) as the rate at which SM drugs reach the clinic is decreasing, new screening technologies

and improvements in synthetic chemistry, computational analysis and structural biology are accelerating the discovery and design of novel bioactive chemical entities. There is also huge potential to expand the knowledge of previously understudied genes as drug targets, as less than 700 of the estimated 3,000 disease-associated proteins encoded in the human genome are targeted by currently approved drugs (Rodgers et al., 2018). The identification of SM candidates generally depends on the screening of cell lines with libraries that typically vary in size from thousands to millions of ligands. This approach has been boosted by the introduction of more efficient screening technologies and developments with chemical libraries to increase hit rates (Macarron et al., 2011), optimizing the effects on disease models, as well as their absorption, distribution, metabolism, excretion and toxicological characteristics, before selecting a lead-compound for clinical testing (Plenge, 2016). In parallel, drug design based on protein structure will represent a paradigm shift in the identification of SMs for RDs treatment. Therefore, active campaign of three-dimensional characterization of RDs targets seems to be an indispensable way to accelerate the process of finding novel SM leads (White et al., 2019).

The success of SMs in treating RDs has been driven by targeted screens and improved disease modelling. Several SMs are currently in clinical trials or even already available to treat RDs (Gamez et al., 2018). SMs remain at the forefront of drug discovery projects because they can be produced at reasonable costs and their manufacturing is scalable. For RDs, if the causative molecular target is in a class with established tractability for SM drugs, such as G-protein-coupled receptors or kinases, the vast scientific, clinical and regulatory experience with this platform can also be an advantage compared to emerging platforms. Furthermore, the potential for phenotypic screening to identify molecules that have the desired therapeutic effect by novel, unknown mechanisms could also be an advantage for RDs whose molecular cause is unclear or multifactorial. One of the major challenges is finding the right molecule that displays excellent pharmacological effect and pharmacokinetics but with few off-target effects, which sometimes requires extensive optimization of a lead-compound. To that end, computational analysis of experimental data using systems biology can help to reduce the number of hits to be tested and to predict off targets. The other main hurdle is the development of screens which are relevant to the disease state in vivo. Furthermore, as screening for disease phenotypes improves, many of the drugs already shown to be safe and well tolerated in one condition may be repurposed to treat a (different) RD

where there might be a common pathway for intervention. To sum up, SMs remain as a powerful source of potential new molecules for RDs therapy, complementing other new and more advanced therapeutic approaches.

2.2.2. Drug Repurposing

Drug repurposing (DRP) (or repositioning) consists of identifying new uses for approved drugs that are outside the scope of the original medical indication. It is an alternative option in drug development and represents a viable and risk-managed strategy for developing orphan drugs. DRP offers several advantages: fewer risks, lower costs and shorter timelines. Repurposed medicines have the added value of immediate use in clinical trials, as their safety is confirmed from their first indication (Masoudi-Sobhanzadeh et al., 2020).

Currently, about 20% of the orphan drugs are repurposed (pharmaceutical-technology.com/comment/). Choosing the right candidate drugs for a given disease requires in-depth basic research into the pathophysiology of the disease, the knowledge derived from this will help to tackle pathways or molecules to target. In the case of poorly characterized RDs, computational techniques for predictive repurposing offer a quick way to identify testable hypotheses that may be translated into the clinic (Talevi and Bellera, 2020). These include signature matching of transcriptomic or proteomic data, molecular similarity approximations, structure-based virtual screens, and systematic analysis of electronic health records and clinical trials. With the advancement of big-data analysis, modeling software, and high-throughput screening techniques, DRP will bring a steady rise in the number of orphan drugs (Pushpakom et al., 2019). Part of the increasing success of repurposing is that it can take advantage of the growing wealth of basic scientific research, and extract the information to match disease targets or biochemical pathways with specific drugs (Azvolinsky, 2016). Moreover, the advances in basic science in RDs may provide models, even for the most prevalent conditions in medicine.

2.2.3. RNA-based medicine for RDs

RNA therapeutics has gained new momentum, as it has effectively increased the "druggable" space, traditionally focused on small-molecules or protein-based therapies. It is easy to design, cost-effective and has already proven to represent a viable path for personalised treatment in RDs (Kim et al., 2019; Yin and Rogge, 2019). The main RNA-based therapies involve the use of antisense oligonucleotides (AONs) (Levin, 2019), which are considered SMs, thus not facing the

challenges of gene therapy or genome editing which need viral vector-mediated delivery. There are already eight AONs approved for clinical use, although current challenges for broad translation in the clinic persist, including poor pharmacological properties and difficulties in delivery to specific target organs and tissues. AONs are chemically synthesized, 15-30 nucleotides in length, and capable of specifically binding to only one target RNA following Watson-Crick pairing rules. After binding, and depending on the chemistry and positional requirements, AONs modulate RNA function by steric blocking mechanisms (blocking translation or modulating splicing) or by promoting RNA cleavage and degradation (RNAse H1 or Ago2 recruitment) (Bennett et al., 2019).

Potential applications of AON based therapy, with examples relevant to RDs, include: reducing toxic RNA levels (as in Huntington's disease and other neurodegenerative disorders (Bennett et al., 2019)) or in the production of a toxic protein (TTR in transthyretin amyloidosis); recovery of a partially functional or full-length protein by modulating splicing (Duchenne muscular dystrophy, spinal muscular atrophy, retinal diseases, inherited metabolic diseases and other RDs (Desviat et al., 2019); or forcing the selection of a non-malignant transcript by modulation of alternative splicing in cancer.

2.2.4. Immunotherapy applied to RDs

Immunotherapy is the modulation of the immune system through its activation, suppression or replacement, for therapeutic purposes. In a broad sense, it includes the alteration, modulation or adaptation of the function of certain types and subtypes of immune cells with a therapeutic or prophylactic goal, as well as the use of bioproducts of the immune system or modifications of those (cytokines and antibodies, for example) for the treatment of diseases. The first immunotherapy developed in medicine was vaccination, as early as in the 18th century. However, it was not until recently that the advances in biomedicine have allowed immunotherapy to fully develop. At this time, we have available a wide selection of immunotherapeutic products and approaches to fight a variety of diseases, both from the immune system and against different types of cancer (www.cancer.net).

Immunotherapy has already revolutionized the medical practice and has opened new opportunities for patients with RDs. Among the long list of RDs, numerous types of cancers as well as autoimmune and inflammatory diseases could be found. Some of these diseases are already treated with immunotherapy. However, most of them, including most rare cancers, are still lacking a treatment (rarediseases.org/for-patients-and-families/information-resources/

rare-disease-information/). Genome-wide analyses and the identification of new biomarkers specific to different types of tumors have resulted in tumor stratification, recognizing an increasing number of cancer subtypes from what it was initially considered a single entity. Current developments are directing us towards treating cancers based on the genetic mutations driving the disease and the biomarkers that they express, rather than the usual criteria of the cell type from which they originate. This new trend towards stratified medicine, as a prelude to personalized medicine, represents a new hope for patients with RDs, especially those with oncological and immune diseases.

One of the aspects that hamper the research in RDs is the limited number of patients suffering from each of these entities. As a consequence, the interest of pharmaceutical companies to invest in R&D is reduced by the limited profit expectations. Therefore, it is the role of institutions such as the CSIC to fill the gap, to promote the study of these diseases and to develop researchers-driven new therapies.

3. KEY CHALLENGING POINTS

There is an urgent need not only to provide general support capable of identifying novel therapies, but to do it as efficiently as possible and to translate the science to the patients "from bench to the bedside". To that end we have identified the following key challenging points:

3.1. To unravel the genetic causes and disease mechanisms underlying Rare Diseases

i) Integration of multi-omic datasets

Whilst the study of single layers of regulation can provide useful insights into the origin of a given disease (Posey, 2019), studies based on systems biology approaches, which analyse the problem from multiple perspectives, are more powerful in discerning potential downstream consequences (Zhang and Itan, 2019). Therefore, the development of novel algorithms capable of integrating multiple –omic layers, and the concomitant generation of CSIC scientific-technical platforms, will facilitate the discovery of functional and actionable pathways that could, in the near future, be subjected to target therapy.

ii) Application of artificial intelligence in Rare Diseases

The capacity to integrate data from different sources has the potential of identifying novel prognostic markers, thus illuminating the path to drug

discovery and connecting similar diseases with potential ongoing clinical trials. Importantly, as the judgement of this data requires a significant amount of analysis and expertise, these goals must be accompanied by the generation of easy to use software and databases to facilitate its appropriate translation into clinics (Balloux et al., 2018).

iii) Development of appropriated cellular and animal models for pathophysiology studies and therapy development

Experimental models are irreplaceable tools in biomedical research, especially in RDs. Generation and characterization of genetically modified animals, most frequently mouse models, cells-derived iPSC (hepatocytes, neurons, cardiomyocyte like-cells) or in vitro 3D functional tissue models, such as organoids, are an efficient way to increase our knowledge on RDs, find key molecular targets and evaluate new therapeutic interventions. There are different challenges:

a. To generate genetically-modified animal models and derive cell lines for specific RDs' causative genes;
b. To develop a standardized battery of tests for functional phenotyping of laboratory animals and cellular models; and
c. To create a network to test interventions in experimental models of RDs and other experimental models.

The solution is to create a limited number of facilities (or at least use the existing facilities), train staff, and optimize pipelines to test discrete interventions for specific Rare Diseases in a series of replicable, simultaneous assays at different centers of the network platform (Murillo-Cuesta et. al, 2020).

3.2. Bringing the scientist closer to the clinical action framework and vice-versa

In the case of Rare Diseases, in most cases the flow of research goes from the disease to the molecular mechanism, although the study of the phenotype of genetically modified animal models frequently leads to relevant findings for the study of Rare Diseases.

To join the efforts of researchers and clinicians, with the main target of improving the situation of RD patients, the key points will be:

i. to generate an added and complementary value of the translational research;

ii. to bring scientific timing closer to clinical timing;
iii. to establish a dialogue as a bridge between scientists and physicians to jointly understand clinical and scientific decision-making processes;
iv. to adequately transform clinical questions into scientific questions and vice versa; and
v. to facilitate communication between hospitals and hospital-based research institutes and CSIC biomedical centers.

Furthermore, it is essential to bring together the well-recognized new scientific and technological disciplines (experimental biology, biochemistry, molecular cell biology, genetics, genomics/omics, precision medicine, artificial intelligence, big data) with the new clinical structures such as biobanks and diagnostic image repositories, and patient participation and empowerment.

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CHALLENGE 5

ABSTRACT

Food allergy is an important health problem with an increasing prevalence that negatively affects the quality of life not only of allergic patients but also of their families. Although some of the molecular mechanisms that drive this disease have been already described, most of its causative details remain unknown. In this regard, a multidisciplinary approach of this problem may help the finding of new solutions. Thus, the development of omic techniques could allow identifying the key players of this process and, similarly, biosensors may lead to the detection of trace levels of allergens in different food matrices and hypoallergenic foods, allowing safer products and new immunotherapeutic approaches that could induce oral tolerance to food in allergic patients. In this challenge, we will highlight the most relevant strategies for the prevention, diagnosis, and treatment of food allergies.

KEYWORDS

antigen | allergen | allergic sensitization | food allergy | IgE | immunotherapy | oral tolerance

CHALLENGE 5

FOOD ALLERGY

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1. INTRODUCTION AND GENERAL DESCRIPTION

Food allergy, defined as a hypersensitive immune reaction to food proteins, is considered to be the fourth most important public health problem according to the World Health Organization (WHO). Actually, recent studies have confirmed a significant increase in the incidence of food allergies in industrialized countries over the past two decades (Loh et al., 2018; Ben-Shoshan et al., 2012), that already in 2013 was estimated to affect 6-8% of young children and about 2-4% of adults (Fox et al., 2013). This situation has raised alarm among clinical, scientific and government institutions, not only because of the health problems associated to food allergies but also for the cost associated to the prevention and treatment strategies (Kim and Burks, 2020). Clinical manifestations of food allergies range from itchy skin to an outbreak of hives, gastrointestinal dysfunction, and other anaphylactic-related symptoms (hypotension, weak pulse, and trouble breathing). To date, the most effective action to combat food allergies is the avoidance of the food(s) that trigger such an immune abnormal reaction. However, this strategy is ineffective, risky, and generates a negative impact on the quality of life (QoL) of patients and their families where the need of a balanced-nutrition requires constant attention and daily work, as well as the immediate response through the self-administration of epinephrine due to accidental exposures. Besides, several studies have shown that food allergy can persist for years in the absence of any known

exposures, and strict avoidance does not appear to facilitate food allergy resolution.

Food allergies are the result of an altered response of the oral tolerance to dietary proteins and the immune mechanisms that lead to this altered response is not yet clear. During allergic sensitization, the normal oral tolerance response is altered and the development of regulatory T cells (Treg), which orchestrate the generation and maintenance of tolerance to food antigens, is compromised and replaced by the generation of an effector immune response deviated towards an allergenic T cell response leaded by Th2 cells, which are characterized by IL-4 secretion. This cytokine is required for B cell class switching, synthesis of antigen-specific IgE, expansion of allergic effector cells, and development of clinical symptoms (Tordesillas et al., 2017a). Regarding the potential origins of tolerance rupture, innate triggers and external damages have been pointed out as responsible of the alarmins secretion (IL-33, IL-25, and thymic stromal lymphopoietin, TSLP), by intestinal epithelial cells (IECs), which play a key role in the induction of allergic responses at mucosal level (Roan et al., 2019). Alarmin secretion by IEC has been shown essential for the sensitization to dietary proteins, whereas the single production of any of these cytokines can maintain an established food allergy (Khodoun et al., 2018). In addition, IECs have been reported as a major source of eotaxin in the gut that regulates the recruitment of eosinophils, cells that have been positevely related to the severity of intestinal manifestations against food allergens (Kim et al., 2018).

Since most of the main immune mechanims underlying the origin of food allergy in humans are still unclear, mouse models have been essential for the understanding of this disease because they are able to mimic food allergic sensitization by using exogenous adjuvants. In fact, oral administration of cholera toxin (CT) as an adjuvant induces OX40L expression on CD103+ dendritic cells (DCs) and their migration to the mesenteric lymph nodes which results in Th2 cell skewing and food allergy development (Chu et al., 2013; Blazquez and Berin, 2008). Induction of Th2 responses to food antigens in presence of CT is associated with the suppression of antigen-specific Tregs at gastrointestinal level (Tordesillas et al., 2017b). In addition, the use of other adjuvant as staphylococcal enterotoxin B (SEB) promotes maturation of intestinal DCs and enhances the expression of TIM-4, which is required to induce Th2 polarization (Yang et al., 2007). Taken together, the use of adjuvants in mouse models of food allergy has revealed the key role played by CD103+ DCs and how variations in their tolerogenic phenotype drive a suppression of antigen-specific

Tregs and prime naïve CD4+ T cells towards Th2 responses to food antigens. Moreover, production of IL-4 by these primed Th2 cells induces B cell class-switching and synthesis of antigen-specific IgE, which is central to the maintenance of food allergies and the development of clinical manifestations (Dolence et al., 2018). Most of the allergen-specific IgE secreted by plasma cells binds to high-affinity receptor FceRI expressed on the surface of basophils and mast cells. After re-exposure to the allergen, it will be recognized by the IgE bound to these cells and the cross-linking of the IgE molecules will induce the release of preformed and newly synthesized mediators that are responsible for the symptoms that characterize the effector phase of food allergy including swelling, hives, itching, eczema, wheezing, abdominal pain, diarrhea, nausea, vomiting, dizziness, lightheadedness, fainting or anaphylaxis.

In the recent years, increasing evidence suggests that sensitization to food allergens can be developed through non-oral routes, in particular the skin. There are several lines of evidence that support that early cutaneous exposure to food proteins through a disrupted skin barrier promotes allergic sensitization prior to the first ingestion of food, as opposed to the tolerogenic nature of oral exposure. This led to the formulation of the dual exposure hypothesis, which suggests that exposure to food allergens through altered skin promotes sensitization, while early exposure to food allergens through oral route promotes tolerance (Lack, 2012). In fact, there is a strong association between atopic dermatitis or eczematous skin and sensitization to food allergens (Tsakok et al., 2016).

Although topical exposure has been proposed as a main route of sensitization to food allergens, experimental models have demonstrated that skin is not inherently sensitizing, as topical application of food allergens such as milk in the absence of external adjuvants leads to tolerance (Dunkin et al., 2011). In addition to allergen exposure, epicutaneous sensitization to food allergens may require the effect of additional factors, including skin barrier damage (Oyoshi et al., 2010) and presence of exogenous adjuvants such as toxins produced by microbes colonizing eczematous skin (Tordesillas et al., 2017c). Some allergens such as peanut present intrinsic adjuvant activity being able to activate dendritic cells and to sensitize epicutaneously without the use of external adjuvants (Shreffler et al., 2006; Shimura et al., 2016). Taken together, these evidences support the hypothesis that, under conditions of skin barrier dysfunction or inflammation, sensitization to food allergens can be elicited through the skin.

Along with the dual theory, the hygiene hypothesis also gains interest. Urbanization and industrialization increase early contact with antimicrobials and cleaning products, which positively correlate with the humanized (not-soiled) microbiota found inside homes (Dominguez-Bello et al., 2019). Early age microbial exposure has implications with food allergy as demonstrated with germ-free mice, which are highly susceptible to anaphylactic responses to food (Stefka et al., 2014). The exposure of newborn infants to maternal vaginal tract, breast milk, and the outer bacteria results in stimulation of microbial pattern recognition receptors and the development of tolerogenic mucosal immune networks that protect against allergic hypersensitivity reactions. Increased rates in caesarean section deliveries, perinatal antibiotic use and urbanization have been reducing the neonate microbial exposure to the maternal microbiota and the soil and farming animals and pets microbiotas. All these have been associated in human health with the increased transition from infectious to non-communicable diseases, including food allergy. Furthermore, diet is one of the most potent modulators of microbiota, inducing changes in microbiome composition and bacteria-derived metabolites. In fact, dietary fiber and short-chain fatty acids (SCFA) promote regulatory immune responses (Benedé et al., 2019) and protein breakdown by intestinal microbiota gives rise to amino acid derived metabolites with immunomodulatory properties (Lozano-Ojalvo et al., 2019).

However, the study of immune basis that drive food allergies, that could help in their diagnosis and identification of therapeutic targets, is still a work in progress. In this context, the quick development of effective and less invasive methods of diagnosis is essential for the prevention of the allergic course, accidental exposure, cross reactivity and, finally, for improving the quality of life of allergic patients.

On the other hand, the identification of specific allergens in foodstuffs at the molecular level is essential to avoid accidental exposures. For this reason, molecular allergology also includes the determination of the precise reactivity of patients to allergen sources in the form of well-characterized panels of allergens, either purified from their natural sources or as recombinantly expressed molecules. Such approach corresponds to the so-called "component resolved diagnosis" (CRD), and often offers enhanced analytical sensitivity and specificity, particularly in the case of underrepresented molecules in the extracts, cross-reactive processes and polysensitization (Matricardi et al., 2019).

Fourteen foods have been declared by the European Food Safety Authority (EFSA) as allergens: fish, crustaceans, molluscs, egg, milk, nuts, peanut, soybean, celery, mustard, sesame, sulphur dioxide/sulphites, lupin, and wheat. To guarantee consumer safety, a number of regulations in terms of Food Allergy have been implemented (Regulation (EU) No 1169/2011). In the EU, these regulations require food producers to label the fourteen food allergens when they have been intentionally introduced. However, some products on the market could contain traces of allergens due to cross-contaminations during the food manufacturing processes. The EFSA recognized the existing high risk throughout the world and identified the need for developing new methods of food safety and control. As a consequence of these regulations and recommendations, accurate, sensitive, and fast detection methods for food allergen control that guarantee the security to the consumers are highly recommended, as well as effective and less invasive methods of diagnosis are essential to improve the QoL of these patients and their environment.

Nonetheless, the development of efficient and safety allergen-specific immunotherapy (AIT) is the major challenge in the field of food allergies. Although the first immunotherapy records date from 1911, there is still no effective vaccine or a general (universal) efficient treatment for food allergy. In fact, there are still many concerns of safety, long-term treatments, adverse reactions and cost for a wide application of AIT. Moreover, the induction of tolerance or permanent sustained unresponsiveness after desensitization is not evident in many cases and a deeper analysis of the underlying mechanisms and more clinical trials are required to assess the viability of the immunotherapy treatments.

The discovery of IgE during the 60s and the improvement of better information related to the mechanism and the molecular bases of the immune response has led to more efficient and consistent treatments. Besides all this progress, immunotherapy is still, in most of the cases, an experimental approach to food allergy. In addition, the lack of well-stablished standard protocols for AIT (protein source, dosage, route of administration, use of adjuvants, etc.) has impeded the normal application of AIT as a therapy for food allergies. Based on experimental data and on the ratio adverse reactions/successful cases, the mode of administration has changed during all these years.

There are four main administration routes: oral, subcutaneous, sublingual and epicutaneous. Subcutaneous immunotherapy has been the most popular one during long time; although it has been discarded as a therapy nowadays.

Then, oral immunotherapy (OIT) was extensively used due to the advantages of the easy administration and also, taking into account the amount that can be swallowed easily to achieve successful desensitization. Although apparently OIT should appear as a very promising therapy, unfortunately, the risk factors and safety concerns with documented severe reaction events in several clinical assays led to consider other routes of administration. In 1986, sublingual immunotherapy (SLIT) was introduced as an alternative approach. Several successful studies are reported using SLIT but still there are many problems of standardization for practical and routine application. Due to the safety profile in comparison with OIT, but with the limitation of the allergen threshold that can be used, a new intervention has been considered by combining a first SLIT followed by an OIT, decreasing the risk of OIT and speeding the treatment to reach the maintenance dose. More recently, epicutaneous immunotherapy (EPIT) has been introduced as an alternative route for immunotherapy administration. This route of administration has been successfully used in other applications (for instance, controlled nicotine administration to help persons quit smoking) but in the case of food allergies, few documented studies with success ratio are not sufficient to consider this route as the most convenient one.

The efficacy of the AIT could be evaluated based on adequate biomarkers that should indicate the progression of the disease. The identification of these biomarkers is a big challenge and are necessary to develop new and efficient AIT strategies than can be considered as promising therapies.

Progress in the understanding of the stimulating and anti-inflammatory properties of food peptides on cells of the innate and adaptive immune system has made apparent that peptides represent an attractive alternative to whole allergens to enhance the safety and efficacy of immunotherapy treatments. Food peptides influence intestinal homeostasis by maintaining and reinforcing barrier function or affecting intestinal cell-signaling to nearby immune cells and mucus secretion. In addition, they can stimulate cells of the innate and adaptive immune system while suppressing inflammatory responses (Lozano-Ojalvo et al., 2016a, b). The conclusions drawn from curative and preventive trials in mouse models are promising, although there is a need for more pre-clinical studies to further explore the immunomodulating strategy and its mechanisms to maximize effective clinical translation.

Within the 14 allergens to be declared, most Food Allergies are caused by the Big Eight (milk, egg, fish, crustacean shellfish, tree nuts, peanuts, wheat and

soybean), where most of them are essentials in the formulation of a large number of elaborated foods, not specially for nutrient considerations but for functional or rheological reasons such as texture, appearance, taste or colour. In this sense, the presentation to society of hypoallergenic ingredients/foods for the prevention of food allergies and suitable for specific sectors of the population is necessary. Moreover, the uptake and impact of the new guidelines recommending early allergenic food introduction are still being determined, but this may prompt some food companies specifically manufacturing a whole new category of baby food add-ins, for exposing an infant to specific food allergens in the hope that this will induce oral tolerance (Perkin et al., 2016).

I2. MPACT ON BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

In food allergy, within the scientific panorama, six highly relevant topics have been identified, which are set out below.

2.1. Mechanisms underlying sensitization and the allergic responses

Mechanisms involved in food allergy development

The mechanisms underlying food allergy development and maintenance remain unclear in humans. In this regard, the major role played by the allergen-specific Th2 cells in initiating and orchestrating the allergic response has recently been described (Wambre et al., 2017). They defined a subset of human allergen-specific memory Th2 cells characterized by a terminally differentiated phenotype (CD27-CD45RB-) and the co-expression of CRTH2, CD49d, and CD161. These cells exhibit numerous functional attributes distinct from conventional Th2 cells observed in animal models, including the combined secretion of multiples Th2-related cytokines (IL4, IL5, IL9, and IL13). In addition, this pathogenic T cell subset has showed a stable allergic disease-related phenotype. These results indicated that the elimination of these cells is an indicative of clinical responses induced by immunotherapy.

These findings have opened a window to new opportunities for the whole description of the mechanisms involved in the development of food allergies, improved diagnostic methodologies, recognition of successful treatments, and identification of new targets for the development of specific therapies.

However, the functional properties of the allergen-specific Th2 cells need to be deeply studied as well as the effect exerted by these cells on B cell subsets and the generation of allergen-specific IgE responses.

The specific role of Tregs in humans

Animal models have shown that the administration of food antigens by the oral route is effective in the induction of oral tolerance. Feeding mice with oral antigens induces a population of peripheral allergen-induced CD25+-Foxp3+ Tregs and mediates regulatory responses in a mechanism dependent on TGF-b secretion (Curotto de Lafaille et al., 2008). Other Treg subsets that have been associated with the induction of oral tolerance are Th3 and Tregs type 1 (Tr1). Th3 cells are identified by surface expression of latency-associated peptide and they also have suppressive functions mediated by TGF-b secretion (Carrier et al., 2014). Tr1 cells are characterized by expression of the lymphocyte activation gen 3 (LAG-3) and CD49b in the face of absent Foxp3 and CD25 expression (Gagliani et al., 2013). Their role in the oral tolerance induction has been related to the suppression of immune responses via IL-10 production (Zhou et al., 2010).

However, the antigen-specificity of human Tregs in the context of food allergies is still largely unknown, preventing analysis of fundamental aspects of Treg biology, such as antigen-specific tolerance, antigen-induced functional differentiation, and spatial distribution or memory. Although Tregs specificity seems to direct tolerance versus allergy against aero-antigens, allergen-specific Tregs against food antigens have never been shown altered in allergic patients compared to healthy donors (Bacher et al., 2016). These facts limit our understanding of the role of Tregs for food allergy in humans and the development of Treg-based treatment strategies.

Early introduction of food antigens

Several studies support that the early oral consumption of allergenic foods, such as peanuts, fish or wheat is related with a reduced incidence of food allergy (Du Toit et al., 2008; Kull et al., 2006; Poole et al., 2006). Additional trials have been performed to study the effect of early introduction of other foods such as egg, with different results (Tan et al., 2017). In addition, a meta-analysis of randomized controlled trials concluded that early egg or peanut introduction to the diet was associated with lower risk of developing allergies to these foods with moderate certainty (Ierodiakonou et al., 2016).

These studies show that the early introduction of food allergens in the diet may significantly reduce the incidence of food allergies. However, it remains unclear whether the early introduction of modified allergens such as hydrolyzed hypoallergenic food may also induce a similar oral tolerance with some benefits, abrogating the potential sensitization to food antigens of children with high risk for development of food allergies and avoiding the progression of the atopic march.

Immunomodulating effect of matrix compounds

Several food components have been shown to have a regulatory effect on the immune system. For example, lipids of the egg yolk provide Th2-adjuvant stimuli to the immune response that may increase the susceptibility to develop egg allergy (Pablos-Tanarro et al., 2018). Co-administration of egg yolk may promote sensitization to egg white through activation of innate immune cells, such as IECs, and DCs, that are central to the progress of allergies (Pérez-Rodríguez et al., 2020). In addition, peanut lipids can be presented by CD1 molecules expressed on the surface of antigen-presenting cells to iNKTcells, acting as adjuvants for sensitization through the skin (Tordesillas et al., 2017a). iNKT cells from cow's milk allergic children produce higher levels of IL-4 and IL-13 than those from non-allergic children in response to stimulation with the lipids present in milk, thus suggesting their contribution to food allergy (Jyonouchi et al., 2011).

Since the conventional exposure to the food allergens is rarely to their isolated and purified form in humans, the modulatory effects exerted by other compounds that join antigens in the food matrix need to be studied. The better understanding of the cellular and humoral immune modulation caused by components such as lipids may help to explain the mechanisms involved in the sensitization to allergens and whether an adjuvant effect is necessary in the development of food allergy. In addition, this knowledge could be used in the design of strategies for the safe introduction of foods in the diet that lead to prevent allergic sensitization.

2.2. Microbial, dietary and environmental factors involved in the development and maintenance of food allergy

Both cultured and (mainly) non-cultured dependent methods have suggested that individuals with food allergies have distinct gut microbiome compositions compared to those tolerant to food proteins, although other than protective early exposure, causality in terms of specific microbiota dysbiosis are not yet conclusive.

Among mechanisms of protection against food allergy by the intestinal microbiota, butyrate-producing bacteria (class Clostridia) have been described to protect against food allergy by eliciting protective mucosal Treg cell responses and enhancing intestinal barrier integrity. On the other hand, some Proteobacteria genera such as *Citrobacter*, that have been related to food allergic mice, employ a mechanism of epithelial attachment and effacement that triggers reactive inflammation and gut permeability damage, which are thought to underlie the pathogenesis of food allergy.

The role of microbial SCFA, mainly propionate and butyrate, has been extensively researched while the potential for modulation of immune pathways of the vast majority of other microbiome-derived metabolites is poorly understood. Moreover, the urban and industrial lifestyle has undergone dietary habits with processed foods poor in plant-based carbohydrates (e.g. dietary fibre) accessible for the gut microbiota, which are the fuel for SCFA production. These dietary changes have resulted in the loss or reduction of phylogenetic microbial groups and functions (Sonnenburg and Sonnenburg, 2019).

Experimental approaches using murine models of allergy to select specific microbial species that would be identified as biomarkers for allergy prevention and to promote tolerance have identified a butyrate-producing clostridial species, *Anaerostipes caccae*, as candidate for protection against allergic responses to dietary antigens (Feehley et al., 2019). Another Clostridia species, *Subdoligranulum variabile*, underrepresented in infants with food allergy (irrespective of the food allergens) can suppress antigen-specific allergic responses and attributes of anaphylaxis (Abdel-Gadir et al., 2019). On the other hand, the supplementation of an isolated *Citrobacter koresi* strain aggravates allergic symptoms by inducing allergenic Th2 responses.

These studies carried out with murine models of food allergy raise an important translational question of whether the gut microbiota can be harnessed in treating and preventing human food allergy.

2.3. Molecular identification and characterization of allergenic components

Detailed molecular characterization of allergens may also result in increased basic knowledge, on the basis of allergens playing physiological roles of paramount importance in biological systems. As an example, seed storage proteins of the 11S-, 7S- and 2S- types are important biomolecules for seed physiology enabling seed to germinate autonomously and allowing plant reproduction,

as well as major food allergens. Their biosynthesis, storage and mobilization in plant endosperm and embryo tissues are important topics for plant physiology and agricultural yield (Jimenez-Lopez et al., 2016). At the nutritional side, they represent the major protein components of seeds, and have a high relevance in human and animal nutrition, even regulating inflammation and insulin levels in humans (Lima-Cabello et al., 2017; 2018a; 2018b; 2020). The structural knowledge of allergenic molecules also helps to improve our understanding of the mechanisms that trigger allergy and the modulation of the immunological response.

From a clinical point of view, accurate molecular characterization of allergens improves the identification of allergenic sources (as well as the ability to label food), the standardization of the extracts used for diagnosis and the efficacy of immunotherapy, maximizing the personalization of treatments like immunotherapy by matching the allergenic compositions of the extracts to those of the natural source of food.

Methods for molecular characterization of allergens and their reactivity are diverse, including:

- ELISA (enzyme-linked immunosorbent assay) is commonly used for allergen analysis, although this method relies on the specificity of antibodies to the allergenic proteins, which may lead to false-positive results because of cross-reactivity. Alternatively, Western blotting (WB) following 1D or 2D electrophoresis may provide further information regarding, for example, molecular weight, isoelectric point or the presence of different allergen isoforms. In the case of allergenic enzymes, enzymatic activity and the presence of isoenzymes can be assayed using biochemical methods like spectrophotometry or *in gel* activity assays. WB is also becoming a quantitative method after the recent appearance of easy and simple alternatives like equimolecular protein conjugation to fluorophores. Both ELISA and WB can be multiplexed to detect and characterize multiple allergens simultaneously (Zienkiewick et al., 2015). Other electrophoresis methods (*e.g.* capillary electrophoresis) are prone to their implementation for the standardization of allergen content in extracts for diagnosis and therapy (Zienkiewick et al., 2014). Recently, broad panels of biotechnologically engineered allergens have been made available to perform singleplex and/or multiplex assays based in microarrays and macroarrays platforms. Even disease-oriented,

customized multiple tests to assess IgE reactivity have been designed (see review of Matricardi et al., 2019), by using sometimes minute amounts of patient's serum. Their performance has to be compared with that of whole allergenic extracts.

- Identification and bioinformatic analysis of allergenic sequences, either by standard PCR amplification followed by cloning/sequencing or after data-mining using genomic and transcriptomic datasets. Bioinformatic analyses of these sequences may include the prediction of relevant physical and chemical features (molecular weight, isoelectric point, etc.), 2D and 3D protein structure, the presence of predicted epitopes recognized by T- and B- cells, and the prediction of potential post-translational modifications (Zafra et al., 2016; Jimenez-Lopez et al., 2013). The molecular variability of an allergen can be also assessed within a given food or among related products, in order to evaluate potential cross-reactivities.

2.4. Food allergen detection methods in complex food matrices

The most common and most promising methods, with their applications are:

i) Immunoassays and PCR-based techniques

Immunoassays and PCR-based techniques have been for a long time the elective methods for food allergens detection. Several polyclonal and monoclonal antibodies have been developed enabling a quick and relative sensitive detection and quantitation of specific food allergens. However, recent studies proved that different immunoassays suffer of a scarce reproducibility, besides of cross-reactivity problems with matrix components, altered immunoaffinity of epitopes from processed foods and the lack of multiplexing capability (Schubert-Ullrich et al., 2009). On the other hand, PCR-based detection and quantitation of the allergens have been developed (Monaci and Visconti, 2010). However, this method only detects the DNA molecule and not the allergenic proteins, resulting in inaccurate results, especially in the case of low amount of available DNA and in the case of food processing treatment that alter the DNA molecules. Therefore, the development of alternative and direct fast methods that presents high reproducibility, sensitivity and specificity are necessary.

ii) Proteomics and Mass Spectrometry methods

Given the limitations of the methods described above, proteomics and mass spectrometry (MS) methods can provide good alternative tools for a confident detection and quantitation of food allergens in complex food matrices.

Bottom-up targeted proteomics scanning modes have been applied to detect and quantify in the foodstuffs the presence of several food allergens (Carrera et al., 2018). Thus, using a LC-SRM/MRM assay on QqQ or Q-Trap instruments the reliable specific detection and quantitation of the presence of gluten, egg, milk, soy, hazelnut, walnut, almond or peanut in complex food products was effectively performed. Moreover, strategies for the multi-allergen detection were performed for up to seven different allergens (milk, egg, soy, peanut, hazelnut, walnut and almond) by LC-SRM/MRM. An innovative workflow published by the IIM-CSIC group permits the rapid detection of the major fish allergen (β-PRVBs) in any food product. The strategy is based on the use of a fast purification step of the β-PRVBs by treatment with heat, the acceleration of in-solution protein digestion by high-intensity focused ultrasound (HIFU), and the monitoring of several β-PRVBs peptide biomarkers by LC-MS/MS SMIM. The procedure allows the rapid detection of β-PRVBs in any foodstuff, including precooked and processed products, in less than 2 h. To our knowledge, this is the quickest methodology to achieve the detection of this allergen in foodstuffs. This workflow was also applied for the rapid detection of the allergenic protein Ani s 9, characteristic of the *Anisakis* species. The use of data-independent acquisition (DIA) mode is currently emerging due to recent innovations in MS instruments. Thus, combining DIA with high resolution mass spectrometry (HRMS), researchers detected traces of egg-white powders and caseinate in white wines. In addition, DIA coupled with ion mobility mass spectrometry (DIA-IM-MS) was used to investigate the allergen composition of raw peanuts and roasted peanut flour ingredients used in challenge meals.

Top-down proteomics can support the development of methods for the detection and quantification of food allergens at the intact protein level. The absolute quantitation of the whey protein β-lactoglobulin using LC-MS in various milk products was performed using appropriate internal standards. In addition, the detection of cow's milk protein in mixed-fruit juice samples was achieved. An easy and robust method for fish allergen detection has been developed utilizing the high speed, high resolution and fragmentation capabilities of the Orbitrap mass spectrometer implemented with an UVPD source. Using β-PRVBs as a signature for the allergen detection the method showed several benefits such as, minimal sample preparation, high sensitivity, throughput and practically a complete protein sequence coverage. Therefore, the use of reliable and sensitive MS-based proteomics approaches, for both discovery and monitoring of food allergens, will enhance the safety to the

consumers. The application of absolute quantitation by AQUA-LC-MRM, the use of CE coupled to a top-down proteomics approach to detect intact protein allergens in HRMS instruments, and the employ of new complementary top-down MS/MS fragmentation modes like HCD, ETDhcD and UVPD, for the characterization and *de novo* sequencing of whole allergens, are new directions that will provide new valuable insights. We also considered that incorporating these results into microfluidic CE systems coupled to MS and to portable biosensor devices based on lab-on-a-chip will be very advantageous.

iii) Biosensor devices

Biosensor devices have acquired a great significance during the last years in a wide variety of industrial sectors (i.e. food industry, agriculture, clinical diagnostics), because they are highly sensitive, selective, and accurate detection methods. A biosensor is an analytical device that incorporates two basic elements: a bio-recognition element (i.e. antibody, enzyme, aptamer) used for detection of a specific analyte and a transducer, capable of interpreting this recognition and give a qualitative/quantitative signal. There are three different types of biosensors according to the bio-recognition element (Alves et al., 2016) listed below.

Immunosensor. The great majority of biosensors for food allergens analysis developed are immunosensors. Target molecules (allergenic proteins or antibodies) are immobilized on the surface of such devices and the binding activity between one or more molecules can be measured by different types of transducers. The principles of the layout assays are usually similar to classic immunoassays. Thus, using different types of biosensors the reliable specific detection of the presence of fish, shrimp, egg, milk, hazelnut, peanut and soy in complex food products can be effectively performed (i.e. Ridascreen®). A direct immunosensor that allowed the detection of several allergens, namely ovomucoid and ovotransferrin (hen's egg white), β-lactoglobulin (cow's milk), tropomyosin (crab meat), and proteins from hazelnut, peanut, and sesame was recently described using a biosensor chip and affinity purified polyclonal antibodies (PAbs) raised against those proteins. The interactions of food allergens with respective antibodies were detected by an optical transducer (SPR). Nanotechnology or nanobiosensing uses innovative nanosensors (i.e. nanoprims) and specific signal-transforming systems (i.e. heatsens, SPCE) to obtain fast detection methods for different allergens (i.e. casein, peanut, gliadin, etc).

Genosensor. It is an analytical device where the biological recognition element is a single strand oligonucleotide sequence. These sequences referred as capture probe are capable to recognize selectively a complementary sequence (RNA or DNA), named target, by a hybridization reaction. Recently, an innovative silicon-based optical thin-film genosensor chip able to identify eight food allergens (DNA) simultaneously was developed. In brief, aldehyde-labeled probes from soybean (lectin), peanut (Ara h 3), wheat (gliadin), cashew (Ana o 3), beef/chicken (mitochondrial DNA), and fish/shrimp (16S rRNA) were arrayed and covalently linked to a hydrazine-derivatized biosensor chip surface. When biotinylated amplification products were hybridized with the probes, the interference pattern of light on the biosensor surface changed, producing a color modification from gold to blue/purple. However, only few multitarget genosensor have been reported for food allergens analysis.

Aptasensor. Aptamers are single-stranded DNA or RNA oligonucleotides with a specific sequence that holds a high affinity toward a particular target molecule. Aptamers can be selected using SELEX (Systematic Evolution of Ligands by EXponential enrichment), an in vitro procedure where target-binding oligonucleotides are selected from a random pool of sequences through iterative cycles of affinity separation and amplification. Preliminary approaches for food allergens detection applying versatile RNA and DNA aptamers technology to an optic transducer have been described for the development of a highly specific and high-affinity optical biosensor assay based on a selected aptamer for detecting lysozyme and Ara h 1 protein in food matrix samples (candy bars).

The advantage of using biosensors instead of other methods consists especially in the possibility to miniaturize the device and perform a real-time in-situ analysis. In addition, traditional lab-based detection methods require trained personnel, scientific knowledge, and often expensive equipment. By linking rapid lab-based biosensors with a smartphone readout system, they become more user accessible. These devices will make possible for the food industry companies and food control authorities to perform the food routine allergy control test in their own facilities without the need for expensive instrumentations and/or qualified staff.

2.5. Novel safe and efficient therapies for Food Allergy treatment

The development of analytical tools to detect and characterize new allergens in natural food matrices and in tiny amounts will be of remarkable relevance.

This should produce a real impact in safety of packing and processed foods (product labels). The identification and selection of specific epitopes is crucial for achieving efficient AIT.

The number of allergens that can be extracted from natural sources in many cases are not enough to cover the needs. The development of molecular biology tools and bioengineering methods to produce allergens, modified allergens or epitopes in large scale with high purity will provide the stuff required for preparing the required doses for a massive AIT vaccination.

Understanding how the immune response is produced and the factors that can influence or interfere in these responses is of crucial relevance. This knowledge can be used for the development of new complements capable to induce specific immune reactions to revert a Th2 allergic reaction to tolerogenic responses. To unravel the mechanisms and the signaling processes involved in this defense should help to the development of adjuvants that can be used in AIT, reducing the risk of severe reactions that can happen during the treatment.

Historically, AIT uses the whole allergens to induce desensitization of patients but not always this treatment proceeds successfully. In fact, the clinical assays reported in the literature described several cases that require the interruption of the treatment due to adverse reactions without arriving to the desensitization process and impeding to reach the adequate amount of allergen for a successful immunotherapy. For this reason, it is necessary to find alternative approaches to overcome this problem. One approach is the use of small peptide epitopes that need to be carefully selected. In fact, more than one epitope can be built in a single peptidic structure increasing the effectivity of the treatment. Therefore, the identification of T- and B-epitopes in an allergen is a relevant task to be addressed in allergies. Another approach that can be considered is the use of a modified engineered allergen presenting attenuated effect on allergic patients. The used of these modified allergens should allow achieving the threshold for desensitization faster and safer with clear advantages. This is not an obvious task and a lot of work needs to be developed in this topic.

AIT using exclusively allergens is a common strategy that has demonstrated some success in several clinical studies. Although desensitization has been achieved in many cases, the final aim of AIT is to induce tolerance and this cannot be achieved in some cases only with the administration of increasing

amounts of the allergen. The development of AIT combined with adequate adjuvants that revert a Th2 allergic polarization to a Th1 non-allergic one is a big challenge that has to be solved to achieve safe and efficient therapies against food allergy. The use of biologics has attracted the interest in the development of new strategies in AIT. Based on the safety concerns of AIT, other complementary strategies, in particular, non-allergen-specific treatments have been evaluated. The most promising approach is the use of biologics as combined together with OIT or as monotherapy. The development of specific monoclonal antibodies against IgE (for those IgE-mediated allergies) or some of the well-known pro-inflammatory cytokines have been evaluated. Some of these antibodies are under different phases in clinical studies with promising results but still more data and tests are need to be confident with the development of therapies based on these biologics.

Among all of them, Omalizumab, a selective antibody binding IgE is the most promising one. Its application to multi-food allergy is the main goal. At the moment, its use is only approved for severe asthma and chronic idiopathic urticaria. In peanut and milk allergy the effect of Omilizumab resides on the increment of the protein threshold tolerated during immunotherapy, accelerating the desensitization process in OIT. However, the adverse reaction was not completely eliminated in several cases and this depends on the patients.

Dapilumab is capable to block the IL-4 receptor, inhibiting the interaction of IL-4 and IL-13 that induces a Th2 and macrophage pro-inflammatory polarization. This biologic is approved by the FDA and the EMA for the treatment of moderate-severe allergic diseases.

Mepolizumab and Reslizumab bind IL-5, a cytokine produced by Th2 lymphocytes, have been approved by the FDA for some specific phenotype asthma treatment, although preliminary clinical results are not very promising. Benralizumab binds IL-5 receptor and it is also approved by the FDA for similar cases than the previous ones. It remains to be explored their use in food allergy immunotherapy as adjuvants.

Development of anti-alarmins (IL-25, IL-33 and TSLP) is a new targeting. Etokimab, a monoclonal antibody against IL-33 that induces the reduction of IgE production, is currently in phase II study in peanut allergy. Tezepelumab binds TSLP and very preliminary results indicate its beneficial effect in asthma. Apparently, it is necessary the use of all 3 anti-alarmins antibodies to suppress food allergy, although additional studies on safety and efficacy are required.

2.6. Production of hypoallergenic foods/ingredients focused on specific sectors of the society

According to the pathophysiology of food allergy, approaches for the production of hypoallergenic foods/ingredients could be made through two approaches: (a) foods where immune reactions cannot be triggered and, hence, cannot give rise to the food allergy (ExAllergyFoods); b) foods, food ingredients or other compounds (may or not may be involved in the allergic reaction) that combat in any way the immunomodulatory, oxidative and inflammatory activities that accompany the triggering of food allergies (Allergy Food Fighters).

ExAllergy Foods

Hypoallergenic foods would be defined as foods that do not have or have lost their allergenic reactivity, or they present an immune reaction below the threshold that can be tolerated without producing any adverse reaction, due to an elimination or reduction of the responsible epitopes. Replacement of traditional crops by new emerging crops, Design of new genetically modified strains/lines/species (GMs) and Application of conventional and novel technological processes would be three interesting research areas to be deepened and exploited. For these aims, identification and characterization of the food epitopes and a greater knowledge of their reactions with our immune systems at cellular and molecular level will be essential to define the key points for the development of specific techniques for their mitigation or reduction.

As commented previously, many raw materials are chosen by the food industry for the rheological properties rather than nutritional considerations. New emerging plant crops such as pseudocereals are attracting the attention of researchers and industries for their excellent production, biological activity and nutritional value in the elaboration of gluten-free food products for celiac population (Martínez-Villaluenga et al., 2020). Although nutritional and biological value have been extensively studied and documented, future directions in cultivation and commercial exploitation, technological behaviour together with consumer acceptance of the elaborated foods with these crops are necessary for proposing replacement strategies. Genetic transformation techniques offer considerable advantages in plant and animal breeding from which it is possible to design living organisms without the responsible proteins or epitopes for allergic foods. This approach becomes more feasible at plant level, where transgenic low-gliadin wheat, as an alternative to the gluten free diet, has shown no subchronic adverse effects in Sprague Dawley rats and good

breadmaking properties as well as excellent sensory assets (Ozuna and Barro, 2017). At the animal level, researchers have genetically modified (GM) and/or cloned mammals (including farm animals, pets, and laboratory animals), birds, fish and insects. Many GM animals (mainly mice) are used in laboratories for medical research. There are also concerns about introducing meat, milk and fish from transgenic or cloned animals into the human diet, but they are still at the research stage and have yet to be successful on the commercial market. Actually, the AquAdvantage salmon, was approved for human consumption in 2015 in USA and, since GM crops were first commercialized over 20 years ago, there is no evidence that novel protein(s) introduced in any approved GM crop have caused food allergy (Ladics, 2019). However, genetically modified animals are banned from the EU food chain. More scientific, safety and taste questions will have to be overcome before their introduction in the human diet.

Another possible approach, within this first focus, is the application of conventional or new technological processes for the production of hypoallergenic foods. Processing of foods to increase preservation, palatability, digestibility and/or assimilation is nothing new. As revised by Vanga et al. (2017), the processing of foods involves a wide array of physical, chemical, and biochemical changes which induces alteration of various components including protein and thus the allergenicity of the specific protein epitope. Food processing methods can be divided in conventional or new food processing methods and such processing methods can be subdivided in thermal or non-thermal processes. Within conventional ones, moist or dry heat as well as fermentation or proteolysis are the most studied processes, with a high scaling-up of processes to industrial size due to its easy handling and control of operational parameters. New food processing methods are linked to concerns about the environment and the emission of Green House Gases (GHG's) looking for alternative methods for the development of energy efficient and green technologies in food processing industries. Its main handicap lies in its main virtue, current and innovative, and, therefore, its availability on a large scale, as well as the reduction of experiments carried out in food allergy issues. Among them, high hydrostatic pressure seems to have played a leading role in this area probably because of the availability on the market of high scale equipment.

Allergy Food Fighters

If in the former case it was crucial to know the sequence, structure and function of the epitopes found in foods, in this type of approach it becomes

essential to know in depth what cellular metabolic pathways and components influence the triggering and maintaining of allergic reactions with the idea of establishing which compounds might be incorporated into foods so that they could combat such abnormal reaction of our immune system.

Many therapies are currently under investigation for the treatment of food allergy, being the most commonly studied those based on allergen specific immunotherapy, being the main routes of administration: oral (OIT), sublingual (SLIT), or epicutaneous route (EPIT) (Yang and Kulis, 2019; Burks et al., 2018). From these three approaches, oral immunotherapy (OIT) appears to be a more promising option. It could be adapted to foods supplemented with those bioactive compounds that combat allergic reactions, always under strict control and medical supervision, and considering that following therapy through foods could be more psychologically beneficial for the quality of life for patients than a clinical therapy. In this sense, peptides (Lozano-Ojalvo et al., 2019, 2017), polyphenols (Barbosa et al., 2018), recombinant allergens (Zuidmeer-Jongean et al., 2015), DNA vaccines (Scheiblhofer et al., 2018), probiotics (Santos et al., 2020), and prebiotics (Wopereis et al., 2018), are being considered good candidates in the fight against food allergies by being involved in the modulation of the immune response, including decreasing of allergen-specific IgE levels, basophil activation, and IL-4, IL-5 and IL-13 cytokine levels and increasing of regulatory T cells, expression of Foxp3 and TGF-β, and IL-12 and IFN-γ cytokines, among others. In fact, plants provide a promising platform for the production of recombinant proteins opening a real possibility of using this technique as vehicle to treat or prevent high-impact diseases included food allergies (Rosales-Mendoza and Nieto Gómez, 2018). In the case of biopharmaceuticals stored in seeds are highly stable without degradation, even if stored at ambient temperatures for several years (Takaiwa et al., 2015). Likewise, production of new hypoallergenic foods including some of these compounds in the final formulation could perhaps be a faster industrial and commercial option of being introduced onto the specialized food market.

In any case, further studies, particularly in human subjects, will be necessary to confirm safety and efficacy of these compounds and ensure the viability of their incorporation in foods as an efficient and safe hypoallergenic alternative in the daily diet of allergic population.

3. KEY CHALLENGING POINTS

- Full understanding of the immune mechanisms involved in the sensitization to food allergens and the development of the disease in humans.
- Unravel the role of allergen-specific Tregs in the prevention and resolution of food allergy as well as in the generation of oral tolerance in humans.
- Clarify the effect of the early introduction of intact and modified allergenic proteins on the prevention of food allergy and the establishment of an oral tolerance in high risk children.
- Identification of the cellular and humoral immune responses exerted by the food compounds that join allergens during the sensitization phase and their potential effects on the prevention or induction of food allergies.
- Identification of consortia of microorganisms that could have positive or negative influence on tolerogenic mechanisms (therefore contributing the gut microbiota to the complex etiology and course of food allergy) as no specific bacterial taxa could be consistently associated with food allergy onset yet.
- Identification of skin commensal microbiota that are critical for creating and maintaining a non-inflammatory, tolerogenic environment remains to be addressed.
- Since tolerance to dietary antigens begins with their absorption in the small intestine, microbiota in the ileal epithelium and its role to the maintenance of tolerance to dietary antigens has not been well understood and should be studied in more detail.
- Although commercial probiotics belonging to genera *Lactobacillus* and *Bifidobacterium* (included in the European Qualified Presumption of Safety list) have proven useful in modulating the immune system, approaches to test live intestinal commensal Clostridia strains (no QPS) to prevent or treat food allergy in humans still have a long way to develop.
- Translation to food allergy treatment of studies focused in microbial particles/structures mediating antiTh2 inflammation activity requires further investigations. Likewise, studies using helminth and microbial structures that mediate human tolerance to food antigens should be explored.

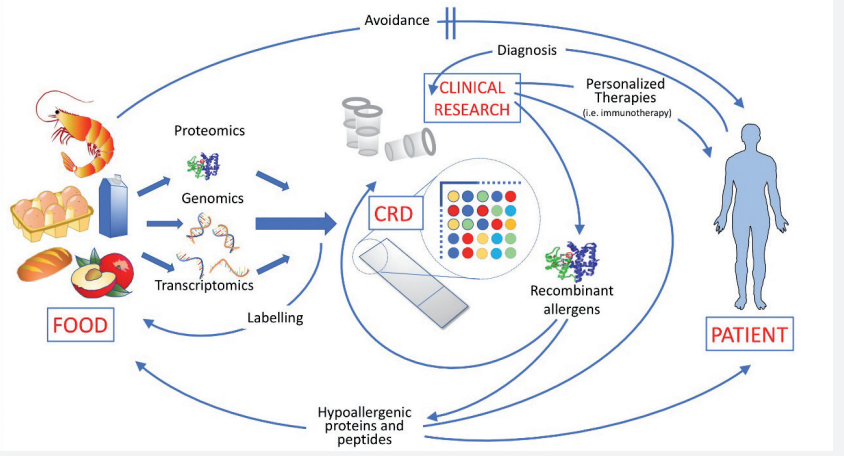
- The knowledge that food allergy is associated to urbanization and industrialization requires that scientific advances and prevention strategies have to be communicated to less developed areas of the world. Food allergy would heighten the burden of health-care in developing countries.
- Molecular characterization of the allergens present in foodstuffs is still quite limited to a scarce number of allergens. Numerous novel foods are fully unexplored.
- There is a need for the design of sensitive, reliable and inexpensive methods to detect allergens in food samples, particularly those undeclared allergens in order to reinforce allergen labeling.
- High-throughput diagnosis tools for allergy (i.e. multiplex tests for IgE reactivity and allergen micro/macroarrays) need to be tested with new allergens. For this purpose, easy recombinant expression, folding and generation of PTM tools should be used.
- Innovative immunotherapy methods should be designed, based in epitope-specific antibody binding profiles and other approaches inspired by molecular (precision) allergology.
- Standardization and certification of the allergenic composition of diagnostics tests and extracts used for AIT is still a challenging topic particularly for manufacturers.
- The use of recombinant allergens for in vivo diagnosis and therapy is limited by legal constrains in Europe, with the exception of several clinical assays. They are powerful tools with extreme plasticity and numerous advantages. Molecular variability of the allergens from natural sources might be underrepresented in the recombinant forms used, unless detailed studies of variability and immunological reactivity are performed.
- Risk-based approaches to manage allergens in foods are to be developed by the food industry and regulatory authorities to support food-allergic consumers to avoid ingestion of their problem food, especially in relation to the traces of unintended allergens.
- More studies are needed for the search of best combination of biologics with AIT to reduce the risk of adverse reactions and to improve the scope of the immunotherapy to multi-allergens.
- Adequate animal models are required to test the experimental immunotherapies against different allergens. Although some of these models are well stablished and are available as those for peach, olive tree pollen, etc., others are still demanded (gluten, fish and selfish, peanuts

and other nuts, etc). The availability of these animal models will propel the development of new therapies.

- A personalized medicine should be considered due to the different response of each individual to the AIT. Up to date, it is highly demanded to find an adequate combination of all the factors required for an efficient AIT that should be tuned specifically for each person.
- Well-defined biomarkers and a deep knowledge of the factors that influence in the different outcome of the therapy (genetics, exposure to external contaminants, expression of receptors and chemical transmitters, etc.) are needed. The challenge is to find a universal therapy to convert immunotherapy in a standard treatment for food allergy.
- There are currently no hypoallergenic foods on the market, with the exception of products for infant nutrition and pets (almost exclusively for dogs and cats) where there does seem to be a firmly established and growing market. There are two main key challenging points, which could help to the rapid expansion of these kinds of hypoallergenic foods: (a) to establish viable bioprocesses, transferable to industrial scale, and (b) to design and carry out in vivo clinical data from these bioactive and hypoallergenic compounds or foods.
- It should be established a production as well as a manufacturing process and storage with quality and consistency at industrial level, without this implying an excessive increase in cost of raw materials and marketing and sales of the final hypoallergenic products.
- Human studies and meta-analysis of scientific evidence related to long term effects, interactions with drugs in human body, and other ingredients in the hypoallergenic food preparation as well as mechanisms of action and dose response of these special foods will be crucial to address this challenge with certain guarantees of success.

The challenges and trends in food allergy research to be faced through a multi-disciplinary approach are summarized in Figure 1.

FIGURE 1–Some **FOOD** contains dietary antigens which may elicit an allergenic response in a susceptible **PATIENT**, through a sensitization process not yet fully understood. Although the best possible treatment consists in the **Avoidance** of the particular food ingestion, this is not always possible. Successful avoidance requires both a good **Diagnosis** of the patient's reactivity, and a full characterization of the allergenic profile of the most common foods, which is fulfilled nowadays through the combined use of classical and most recent "-omic" approaches (**Proteomics, Genomics, Transcriptomics**, etc). Such analyses contribute to improved and accurate **Labeling** of food products (largely claimed by food authorities), which is an obvious way to increase avoidance control by patients. **CLINICAL RESEARCH** is responsible for diagnosis and also for the improvement of treatments when avoidance is not sufficiently implemented or even risky for patients because of anaphylaxis threat. Clinical research is fed by basic knowledge (i.e. -omics) and therefore, diagnosis is highly based in a deep molecular background, with the development of "component resolved diagnosis" (**CDR**) technologies as an increasing trend. CDR helps to precisely identify patient's reactivity to particular allergens, and therefore also helps to design **Personalized Therapies** (i.e. immunotherapy) for these patients. Clinical research also makes a broad use of molecular tools like **Recombinant allergens**, which are profusely used for CDR. Molecular definition of allergens and their epitope regions is used by Clinical research in order to engineer Hypoallergenic forms of **proteins and peptides**, which represent a current alternative used to modulate and re-shape the allergenic response of patients.



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CHALLENGE 6

ABSTRACT

Pain and suffering have acquired epidemic dimensions since the mid-20th century and, nowadays represent a major social, medical and economic burden. Pain is difficult to manage because of its complex nature, unclear aetiology, cultural components and poor response to therapy in many individuals. Therefore, progress in the field necessarily will require the coordinated efforts of multi- and interdisciplinary teams. The identification of CSIC researchers involved in this area and the exploration of synergistic collaborations could be the seed that catalyze the advanced development of all aspects related to pain and suffering in Spain.

KEYWORDS

pain | suffering | signaling pathways,therapeutic agents | biological targets validation | diagnosis

CHALLENGE 6

PAIN AND SUFFERING

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1. INTRODUCTION AND GENERAL DESCRIPTION

1.1. Acute Pain and Chronic Pain

There is no single medical condition that we may call pain. What we describe by the use of that word involves a great variety of experiences. The image of pain associated to an essential mechanism for our survival has been challenged very often. From the mid-20th century onwards, pain medicine began to distinguish between useful pain and useless suffering, between laboratory pain and clinical anguish, between peripheral and central pain, between pain in the limbs and pain of the internal organs. Pain became the object of three related medical discourses: the symptomatic relief of acute pain, the treatment of severe pain in the terminally ill, and the management of chronic pain in cases of migraine, rheumatoid arthritis, trigeminal neuralgia, neuropathic pain and other syndromes of an unspecific nature.

In 1982, Patrick D. Wall and Ronald Melzack published a text that used as its starting point the distinction between acute pain—one of the visible signs of illness since antiquity, and chronic pain, described as an illness or, more precisely, as a set of symptoms, a disorder that causes a great deal of suffering for the patient, without any clinical justification. Although that distinction was already present in Romantic physiology, it only emerged explicitly in the second half of the twentieth century. The International Association for the Study of Pain (IASP) was so much dependent on that difference between the transitory and the chronic pain that, when pain achieved full visibility in the field

of clinical research, it came as a polymorphic set of conditions. Some of these specific pains, like causalgia, phantom limbs, or trigeminal neuralgia, were already known by medicine, although not always under these names. Many others, however, appeared along with the new subdivisions and led to the multiplication of theoretical frameworks and explanatory hypotheses.

According to the IASP, chronic pain is defined as "pain without apparent biological value that has persisted beyond the normal tissue healing time (usually taken to be 3 months)" (Harstall and Ospina, 2003). Chronic pain is often caused by nerve lesion or dysfunction in the nervous system. It can appear as a result of trauma, surgery, systemic disease (diabetes, cancer), infection (herpes zoster), tissue aging or as a side effect of drug treatment, as in chemotherapy, for instance. The prevalence of these conditions continues to increase, as they are more common in the elderly population. Altogether, this indicates that the prevalence of chronic pain is expected to grow in the future.

1.2. Pain Epidemics

Chronic pain has acquired epidemic dimensions from the mid-20th century. The National Academy of Medicine of the United States noted in a 2011 report that 100 million Americans live with some form of lasting pain. In Europe, a survey study lead by Harald Breivik found that 19 % of the adult population suffers from disabling pain. Today, all experts agree that pain is the most common complaint for which individuals seek medical treatment. In an early European survey published in 2006, it was estimated that around 12-30 % of adults in Europe suffer from chronic pain (e.g. migraine, diabetic neuropathy, sciatica, osteoarthritis, cancer-related pain, etc.) (Breivik et al., 2006; Breivik et al., 2013). It was highest amongst the age group of 41 to 60 and among woman. Moreover, severe pain has a major negative impact on many quality-of-life measures, leading to frequent loss of work and emotional problems, including depression, anxiety and suicide.

Clearly, persistent pain is an unmet medical need, with a large percentage of patients expressing dissatisfaction regarding their treatment (Turk et al., 2011). In many cases, current treatments produce limited relief and have serious side effects. For example, anticonvulsants (e.g. gabapentin and pregabalin) and tricyclic antidepressants are currently the choice medication for peripheral neuropathic pain. However, they are only effective in about one third of patients and physicians are unable to predict who will respond to treatment (Finnerup et al., 2015). The opioid Tramadol is a second-line choice, while stronger

synthetic opioids like fentanyl, hundred times more potent than morphine, are considered as the third line choice due to serious adverse effects. They may also lead to substance abuse. The long-term use of opioids is associated with the risk of development of tolerance and addiction, and are currently at the centre of a substance abuse epidemic in the USA (Phillips KJ, 2017).

Chronic pain is widely recognized as a major medical and societal challenge. The aging of the European population has certainly had an impact on the dimension of long-term diseases, including chronic pain. And yet, the creation of pain units and medical specialties related to pain without a clear etyology does not occur without friction or resistance. On the contrary, the position in relation to chronic pain, including pain in terminally ill patients, has been, and continues to be, a problem of clinical and political nature, which demands collective reflection and academic clarification.

According to a report published in 2013 by the Societal Impact of Pain platform (https://www.sip-platform.eu), yearly costs, including loss of work and medical bills directly linked to therapies or chronic pain have been estimated around 441 billion € across the European Union (https://www.hsimagazine.com/press-release/impact-of-pain-costs-eu-up-to-441-billion), or around 3-10% of gross domestic product (Breivik et al., 2013), an enormous sum that continues to rise. These costs far exceed costs associated with other major causes of disability in developed countries such as heart disease and cancer (Breivik et al., 2013). Despite this prevalence and social cost, investment in the study of pain mechanisms is comparatively low and progress in advancing therapies limited. The same applies to the prevalence of chronic neck pain, low back pain or fibromyalgia which has increased in the last five years in Spain, while the prevalence of migraine or frequent headaches has remained stable. Back and neck pain is the fourth most common condition in the burden of disease in the European Region. [http://www.euro.who.int/en/data-and-evidence/news/news/2016/09/what-is-the-burden-of-disease-in- the-region]

1.3. Towards a bio-cultural model of pain

Charles Sherrington, a neurophysiologist of the 1900s, defined pain as "the psychical adjunct of a protective reflex" (Cervero, 2012). We touch something hot and our brain triggers a reflex action that causes the withdrawal of our hand from the object and thus protect us from injury. He coined the term "nociceptor" to describe specialized sensory nerve endings for the detection of harmful stimuli. The value of Sherrington's definition was the separation between

perception and processing. There is, on the one hand, the protective reflex component of the definition which is, certainly, in the brain, but there is also what Sherrington called the "psychical adjunct", the sensory perceptual part added to nociception. More than one hundred years after Sherrington's remarks, the former President of the International Association for the Study of Pain, Fernando Cervero wrote: "how we match pain and nociception is very much a question of personal values well beyond the realm of science, at least until we know a lot more about the working of the brain. Although nociception is easily approached with the scientific method, understanding human pain is, at present, beyond that method's capabilities" (Cervero, 2012). The tenants of this approach depend on different reasons, the most important of them being that many forms of pain are unrelated to protective reflexes.

The historical tensions between pain and nociception have been studied by different authors in recent years. At the same time, medical anthropology has begun to stress the regimen of visibility of different forms of human suffering depending on geopolitical circumstances. In *Pain. A Political History*, Johns Hopkins University Professor Keith Wailoo, for example, has explained how the question of other people's pain remains a recurring site for political battles (Wailoo, 2014). What the book suggests and what many other examples come to confirm is that pain has never been only a clinical problem. On the contrary, the understanding of pain requires the mobilization of differente approaches and communities. The understanding of pain involves a political dimension, since what counts as pain depends not just on the testimony of those who complain but also on the negotiations of our standards of trust.

Visualizing pain and accepting other's complaints requires a joint effort of agreement between not only medical doctors, but also politicians, pharmaceutical companies, and different kinds of associations. Conversely, however, the public comprehension and understanding of pain also works as a Trojan horse, in the sense that, once it enters the public arena, what counts as pain will also determine or challenge our ideas of compassion and sympathy. This means that the social and political dimension of pain cannot be avoided. It is not simply a social feature that will have to be added to some other physiological or psychological characteristics. On the contrary, the public dimension of pain implies that pain, real pain, and not feigned or exaggerated pain, for example, lies embedded within political concerns and social values. The distinction between the hopelessly ill, the chronically ill, the terminally ill or the incurably ill concerns not only the new geriatric science, but also economics and

demographics. Meanwhile, certain expressions like "quality of life," "trust," or "dignity" abound in the media and are extolled in research centers, especially in connection with the treatment of terminal cancer. In all the cases examined, pain's temporal dimension – its duration and not merely its intensity – constitutes one of the crucial aspects overarching the new forms of objectified experiences.

2. IMPACT ON BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

One of the lessons that should have already been well learned, but that the recent COVID-19 pandemic has widely revealed, is that health problems demand coordinated answers. The platforms and Research Centres on Global Health, spreading now throughout the planet, reveal the need for tendering points of confluence and cooperation between researchers and institutions. The case of pain and suffering is very telling on this regard. After all, pain is a problem of immense social and economic impact that affects large population groups. In addition, pain is crossed by age and gender conditions, as well as by many other political interests. The biocultural dimension of pain must necessarily have an impact on the way in which our challenge would shape its contribution in terms of basic science and potential applications.

We should begin by remembering that the most significant reference for the grounding of pain medicine was the publication of the American anaesthetists John J. Bonica's book *The Management of Pain* (Bonica, 1953). The expression "Pain Clinic," introduced by Bonica himself, called attention to the collective effort to transform private suffering into an issue of collective responsibility. A delimited medical practice and a new social cohesion were required to make pain, and especially chronic pain, a matter of public reflection and medical care. The majority of the new clinics, initially created to respond to the suffering associated with terminal cancer, soon began to treat other pains of a non-specific nature, like phantom limbs or facial neuralgias.

"A disease does not exist as a social phenomenon until we agree that it does," the historian of medicine Charles E. Rosenberg wrote in 1989 (Rosenberg, 1989). This Harvard Professor considered that the existence of a condition was determined by the way in which the ailment was named and isolated. From the mid-nineteenth to the mid-twentieth century, what we now call "chronic or degenerative diseases" were often associated with incurability or other life

circumstances that prevented the patient's experience from being considered a disease. In some cases, medicalization went as far as behaviours that today we would not consider illnesses, such as neurasthenia or homosexuality; in other cases, the same social compartmentalizing of supposedly pathological behaviour was regarded as a success in medicine's progress and an effort to secularize the care of incurable and terminal patients. History shows how the scientific and cultural intervention in harmful experience neither obeys nor can be explained through a teleological sequence in which medicine of pain were the logical conclusion of humanitarianism (Arney and Bergen, 1983).

Pain's cultural centrality did not only take place in the biomedical sphere. On the contrary, never before in the history of the West had physical or moral suffering been so visible in all aspects of public life, from the world of the arts, including cinema, to journalism or consumerism. In the twentieth century, suffering was always connected to a collective reflection on the uses and abuses of pain, as well as its visual, literary, or historical representations. From art criticism to anthropology, the New Humanities also turned physical and symbolic pain into an object of academic research, especially with respect to the theory of culture, but also in relation to some others philosophical aspects.

The works of Ludwig Wittgenstein, Richard Rorty, Charles S. Peirce, Daniel C. Denett, George Ryle, Hilary Putnam, P. Strawson, John Deway, Paul M. Churchland, John Searle, Saul Kripke and Willard van Orman Quine were full of references to pain in the context of their investigations into solipsism, the contents of consciousness, private languages, referential opacity and, in general, the philosophy of language and of the mind. All their research made use of physical suffering to examine the characteristic traits of mental activity, such as consciousness, intentionality, subjectivity or causation. Continental philosophy also played their part. Enveloped in ontological and epistemic disputes, some European philosophers also began to question how we could be sure that others understand our pain or, on the contrary, how we could know about the pain of others. Under the wing of critical theory and its reflection on the great genocides of the twentieth century, so-called Continental Philosophy also developed in relation to the cultural understanding of historical memory. The political philosophy derived from the old Frankfurt School understood pain to be the inevitable consequence of reason's autonomy. The anthropology of memory constitutes yet another phenomenon in the proliferation of studies on the materialization of the emotions or performance practices linked to the rituals of mourning.

The emergence of critical medical anthropology in the mid-eighties, in Anthropology Departments in prestigious universities such as UC Berkeley or Harvard, was also a significant step in the evolution of the discipline from the analysis of non-western folk medicinal beliefs and practices to the study of biomedical models of disease and healing. All these debates were connected to long standing discussions on the very significant transformations in infrastructures, specialists and resources everywhere in the world, in terms of medical pluralism (the availability of different models of disease and healing in a given social environments, and the circulation of patients among them). With this shift to the study of biomedicine, medical anthropology started to focus on the medical encounter as the crucial settings where frictions between the different explanatory models of disease between specialist and patients conditioned the efficacy of treatments and generated a myriad misunderstandings and forms of non-compliance, particularly in multi-ethnic setting. One of the first milestones was the study of the AIDS pandemic. Increasingly, these studies coalesced with Science and Technology Studies (STS) and, incorporating may of its main theoretical developments and opening up to new ethnographic research scenarios (pharmaceutical labs, biomedical facilities of all kinds). Ethnographies of medical settings affected all medical specialties, including mental health or problems related to the difficulties of communicating pain and more generally, discomfort and suffering.

The groups and researchers that contribute to this Challenge must still integrate their confluence zones and learn to establish new forms of cooperation. For now, each group has a remarkable success in its own disciplinary field of expertise and willingness to cooperate and learn from one another. The Challenge should provide a collective clarification of the problem of pain in the 21st century, contributing to its management and treatment, both in the clinical setting, in the context of health policies and with regard to its social representation and its political dimensions. To this end, and as way of clarification, we may cite some of the areas in which we expect contributions. On this regard, it has to be underlined that CSIC lacks a Medical Research Branch. There is basically no or very few CSIC scientists (medically trained or not) working in hospitals, and treating patients. This is a serious disconnect of CSIC with clinical medicine that should be amended, as this challenge comes to confirm.

2.1. Pain management and safety issues

Recommendations on treatments to manage pain have been published by various international scientific societies. Some of them are related to specific pain

conditions such as neuropathic pain, osteoarthritis, fibromyalgia, and low back pain. There is also some focus on special populations like women, the elderly, or children. The optimal management of pain is multidimensional and includes pharmacological treatments, psychological support and physical therapies. Though there is an agreement that appropriate analgesic therapy first requires adequate pain measurement, the question still remains on how different expressions of pain are going to be socially accepted and eventually treated.

For mild pain, acetaminophen (paracetamol) and non-steroidal anti-inflammatory drugs (NSAIDs) are extensively used (Goya and Martín-Fontelles, 2010). NSAIDs are classified in 2 groups, the so-called traditional NSAIDs such as ibuprofen, naproxen, aspirin, and diclofenac and the cyclooxygenase 2 inhibitors (COX2s), such as celecoxib or rofecoxiv. The main negative outcomes of these drugs are hepatotoxicity for acetaminophen, gastrointestinal and renal issues for traditional NSAIDs and cardiovascular events for COX2s. Thus, the benefits and risks for each particular condition and treatment need to be seriously taken into account.

Opioid-based medicines, mainly fentanyl and morphine, are the pharmacological treatment of choice for moderate to severe pain conditions. Opioid tolerance and withdrawal symptoms are the consequences of long-term use of opioid-based medicines. Respiratory depression is also a very serious concern. Pharmacological options are available to help in the opioid discontinuation process. Opioid crisis due to overuse does not seem a likely scenario in Spain compared to US. However, special health surveillance for this question is required.

Due to the mechanistic complexity underlying certain neuropathic pains, a polypharmacotherapy is necessary. Thus, to achieve an optimal pain relief, combination of antidepressants (serotonin/norepinephrine-based drugs), anticonvulsants (Na^+-channel blockers or Ca^{2+}-channel modulators), tramadol, and/or opioids is often used. Topical medicines such as capsaicin and local anaesthetics may be useful in specific conditions. Unfortunately, many patients suffering from neuropathic pain do not receive effective treatments. The degree of efficacy of the currently used analgesics varies with the different pain states and with each individual patient. Thus, pain management can be very challenging considering the various side-effects of analgesics and certain untreatable pain conditions. Moreover, very serious critical issues appear when pain lasts over a long period of time or is resistant to current therapies.

2.2. New pharmacological approaches

Recommendations for pain management are not always satisfactory due to the complex nature of pain and diverse origins. During these last years, there has been an increasing number of pain clinical trials in which pain management did not show the expected efficacy despite previous successful pre-clinical studies. Some of these failures have been suggested to be due to methodological weaknesses in the design of those clinical trials, and/or to species differences (i.e. results obtained in studies performed on mice or rats) that do not reflect the human condition, and/or target selections. For optimal outcomes, individualized pharmacological therapy needs to be developed. To address this issue, different approaches are currently being explored at the pre-clinical stage:

i. target-based drug discovery, including validation of new biological pain targets
ii. novel strategies to develop analgesics with safer profile, such as biased-signalling opioids
iii. improvement of human surrogate models with cellular models from stem cell-derived patient-specific sensory neurons
iv. overcoming the limitation of current pain assess with monoclonal antibodies
v. design of novel gene therapy strategies based on combining vectors, transgenes, and promoters
vi. improved analgesic delivery
vii. optogenetic approaches to modulate neural activity

Target-based drug discovery for new analgesics

The development of effective and non-addictive therapeutics for acute and chronic pain conditions requires the scientific discovery and validation of new biological targets. This is not an easy task since several new analgesics have been developed during these last years but ultimately failed during clinical trials. That was for instance the case of an angiotensin II receptor type 2 blocker and a transient receptor potential channel vanilloid 1 inhibitors whose efficacy in preclinical stages could not be confirmed in clinical trials (Fernández-Carvajal et al., 2020). Another example is the fatty acid amide hydrolase inhibitor BIA 10–2474 that led to fatal outcomes in phase II clinical trials due to suspected off-target effects. Nowadays, there is an increased knowledge regarding the different physiological mechanics involved in the different pain conditions, but further efforts are clearly needed for a globlal comprehension.

Developing analgesics with safer profile

To exploit the analgesic potential of opioids but avoiding their serious side-effects, new approaches involve for instance shifting their functional selectivity for G-protein signalling vs β-arrestin recruitment, mainly responsible for developing tolerance. Increasing benefits of NSAIDS vs their risks has been partially fulfilled with selective COX-2 inhibitors. However, there is still a risk of myocardial infarction associated with their use in patients with cardiovascular conditions, a common situation in the elderly. Natural products mainly polyphenols obtained from plant-derived sources (e.g. *Porphyromonas gingivalis* fimbriae, *Rhizoma coptidis*, *Rhododendron brachycarpum*, *Caesalpinia sappan*) have been suggested to be an interesting approach since they act on multiple signalling cascades implicated in inflammation processes.

Development of improved pain models

A number of animal models for pain, including orofacial chronic pain, traumatic nerve injury, chemotherapy-induced neuropathic pain, chronic pancreatitis models have provided great insight regarding mechanisms underlying specific painful conditions. The main challenge for an increased predictive reliability of preclinical models has been suggested to be the capacity for dissecting different components of the pain experience, or the possibility to use human neurons derived from pluripotent stem cells for disease modeling and phenotypic screening.

Monoclonal antibodies

Monoclonal antibodies are emerging as possible alternatives to small molecules in pain treatment, due to high affinity and specificity for predetermined proteins involved in pain transmission. Reduced administration frequency is one of the advantages over conventional analgesics. Monoclonal antibodies focus on the following processes involved in pain transmission: nerve growth factor, calcitonin gene-related peptide pathways, various ion channels, tumor necrosis factor-α, and epidermal growth factor receptor. Some of them are already prescribed for migraine (Erenumab y Garlcanezumab) and for rheumatoid arthritis (Sarilumab). The most recent advances attempt to avoid hydrophilicity, gastric degradation, and immune issues, such as anti-drug antibodies response. Another current limitation involves their high costs.

Gene therapy

Gene based strategies to manage persistent and intractable pain include targeting specific proteins involved in nociceptive process and delivering a

protein encoded in viral vectors. For instance, this is the case of primary sensory neurons virally transduced with a vector coding for the glutamic acid decarboxylase enzyme to release the neurotransmitter GABA, which produces an analgesic effect. Viral vectors are also used to increase the production of endorphins, anti-inflammatory cytokines, and growth factors. Another strategy that can be mentioned here is targeting miRNA pathways since they are involved in initiation and development of chronic pain. These approaches can target specific signaling pathways with long lasting effects, which is an advantage over conventional analgesics. However, despite their promise, the potential consequences of long-lasting adverse effects and the blood-brain barrier penetration need to be addressed. Another consideration that will be addressed thanks to big data analyses is identifying polygenic risk for pain on a large number of patients.

Analgesic delivery

Oral and parenteral drug deliveries are the most common routes for analgesic administration. However, other routes have shown therapeutic utility such as spinal, transdermal or transmucosal deliveries. Formulation and safety are the main issues for spinal delivery. Nanoparticles might be very useful for improved analgesic transdermal and transmucosal diffusion.

Optogenetics

Optogenetics is a transformative, rapidly expanding technology that allows the fast and precise control of neuronal firing with light, using the targeted expression of genetically encoded light-sensitive ion channels or pumps.

The development of flexible, implantable optoelectronic devices makes their clinical application possible, although the challenge of safety for exogeneous gene products remain to be addressed.

2.3. Medical Anthropology

Apart from the development described above regarding the crucial role of culture in experiencing pain and disease, medical anthropology also branched out to critically study the consequences of different forms of social suffering related to situations of pain and violence, from every day or structural to mass crime, on local populations around the world. The increasing influence of human rights discourses and practices (fight against impunity, victim centered cosmopolitan memory models, etc.), and the expansion of humanitarian action in situations of catastrophe, created new spaces of global solidarity but

also of friction between local models of dealing with pain and suffering and the mostly biomedicalised operatives that accompanied these interventions. While there are many angles to this type of research, one clear example is related to how to confront social trauma and the one-size-fits-all operatives deployment of western oriented diagnostic categories such as Post Traumatic Stress Disorder (PTSD), with its associated specialists, diagnostic forms and protocolised treatments (Fassin and Reichman, 2009). Thus, the analytical scene grows large from the initial study of one-on-one clinical settings to multinational and rather complex operatives. Obviously, social suffering entails the parallel process of social healing. Starting with the premise that that the social dignification of victims in their different modalities has a healing effect, out of recognition and legitimation, the CSIC group SUBTIERRO (https://politicasdelamemoria.org/en/) pays attention to the ways in which social trauma (in this case, long term or intergenerational trauma) is handled in different settings, from families, to the associative memorial movement, to the media, to specialized biomedical treatment (Ferrandiz 2014, 2019).

2.4. Phenomenology of pain

This is a field of inquiry of growing interest that assumes a first person perspective in order to gain a much more precise descriptive analysis of the painful experiences undergone by the living body. Far from any introspective theoretical scheme, this philosophical approach seeks to provide adequate description of general structures characterizing the body in pain. In the last two decades, some main and fruitful directions related to the phenomenology of pain have been: the relationship between pain occurrences and attentional distorsions —trying to illuminate how and why pain captures attention—; the distinct alterations of body schema as a result of affliction —particularly focusing on the bodily motor functions that are at the same time urged and disturbed when the living being is in pain—; the necessary review of the comprehensive categories for immanent clarification of pain —affective sensation (Stumpf, Husserl), self-affection (Levinas, Henry), negative agency (Scarry), blocked flight response (Grüny).

The development of the phenomenological description of physical pain makes the lived body the subject of pain, not the physiological body, nor the mind. Physical pains are, with equal originality, unshareable conscious experiences and spatial events that take place in one's own body. But being undeniable that pain is an experience that shows up the original bond between spatiality and intimacy, our current knowledge of pain is marked by the difficulties to fully

understand the general structures of embodied feelings. A series of related problems come up in order to deal with the questions of how sense and lack of sense intertwine in painful experiences: in what way the lived body itself is marked by vulnerability and to what extent is physical pain an experience of negativity. In this respect, it is relevant to discuss the different theoretical models that have faced the decisive connection between pain and negativity: Scheler, Ricoeur, Scarry, Leder, Toombs, etc.

2.5. Spreading depolarization waves (SD) on acute brain injury

The relevance of SD waves to the Pain and Suffering topic is that they are known to initiate the aura phase in migraineurs. These waves arise in response to a variety of stimuli, but they also ignite in the penumbra of brain infarcts and are considered the cause of the gradual expansion of the ischemic core into the penumbra zone, killing neurons on their passage. Thus, they have two major implications in clinics, migraine attacks (in normally perfused brains) and brain stroke (so-called peri-infarct waves). Such SD waves are considered the initial cause of migraine attack and in the late years a number of genes in families with a history of hemiplegic headache have been reported to render the brain more susceptible to them. Diminishing the number of waves or preventing them to appear is the main goal of some clinical and pharmaceutical research in this field. In certain cases it is the main focus of attention of modern "electroceutical" approaches, which use transcranial magnetic or electrical stimulation to prevent them.

2.6. Pain and suffering in extreme situations

The impact of total confinement on individuals' health during the Spanish Civil War and its aftermath, 1936-1950 Wars commonly have huge public-health consequences, both direct and indirect. Among the direct ones are death, injury, sexual assault, disability and psychological trauma. Among the indirect ones are hunger (including intentional food deprivation), mass migration, and collapsed health services (by diversion of resources to warfare expenses and/or intentional destruction), these circumstances lead to varying levels of malnutrition (up to mass starvation), diarrhoea, infectious diseases (pneumonia, measles, malaria, tuberculosis, STD, and so on). All of them cause two distinct forms of distress, pain and suffering, at a high degree.

The sub-challenge is focused on the impact at short- and long-term that individuals' seclusion in spaces of confinement (camps, prisons, insane asylums) had on their health (mostly emotional and mental) during the Spanish Civil

War and its aftermath (1936-1950). The methodological approach will be qualitative, and the main kinds of sources resorted to will be memoirs and other ego documents by individuals (including health practitioners) having experienced or witnessed the confinement experience; and expert reports by transnational relief organisations involved in their care. The research has a double purpose: it aims to deepen the knowledge of this biographical experience and to better understand its meaning for those persons (men and women) who went through it, as much as to stimulate the reflexion in the face of current refugees crises, about the lessons to be learnt from that past confinement experience.

3. KEY CHALLENGING POINTS

Visualizing pain and accepting other's complaints requires a joint effort of agreement between not only medical doctors, but also politicians, pharmaceutical companies, and different kinds of associations. Let us take, for example, the case of childbirth pain. While the early twentieth century saw the hospitalization of childbirth, the main discrepancies regarding labour analgesia were not only related to its use, but to the practicalities involved in its employment (Leavit, 1986; Moscoso, 2016). More than being a lineal and progressive story, the history of labour pain treatment involved the superposition of different strata. During the twentieth century, the connection between physiological pain and religious guilt was still well extended. For many, pain was an essential part of motherhood, which implied that seeking relief equated an explicit renounce to develop what they understood as a "noble instinct" of women. For others, labour pain analgesia was identified as a key element within the struggle for women's rights. In the USA, the National Twilight Sleep Association counted among their members with very active suffragists. And yet, then as today, many other women thought of labour pain as an extraordinary sensation that could only be labelled "pain" in case of being pathological. This point of view was in accord with the ideas expressed by Grantley Dick-Read, one of the advocates of the so-called "Natural childbirth", for whom the principal source of pain during birth was fear. His method, which included relaxation, exercise and diet, aimed at the reduction of the pain threshold through the gain of confidence. Thus, while many feminists regarded labour pain as natural and opposed to clinical interventions, many others considered labour analgesia as another right that women deserved to gain social visibility and control on their bodies.

Similar analysis may be made regarding paediatric pain, animal pain, menstrual pain, traumatic or unconscious pain, peripheral neuropathies, animal

pain in sport injuries, etc. One the most pressing issues nowadays is the case of fibromyalgia, studied at large by one the CSIC groups involved in this research line, and of which, they have also produced a documentary in cooperation with New York based film producer Horns and Tails (Consulta 32. https://www.consulta32.com)

The following Key Challenging Points are only a tiny selection in which the different groups that form this Challenge could have an effect. It should be understood, from the very beginning, that cooperation among different methodologies and research strategies does not come up naturally and that it requires, on the contrary, a permanent and systematic attempt to find a common problem, a common language and a sharable solution. In this case, as in many others, the Key Challenging Points also reflect the deep social interest that pain and suffering has acquired at the beginning of the 21st century. The role that may CSIC scientists will have to develop in the next decades will be certainly framed by social and political demands for which we all should be prepared.

1. Identification and validation of novel pain targets/pathways with disease-modifying potential. This may include the pharmacological search for modulators of underexplored protein therapeutic targets, miRNA-based agents, and the pursuit of antibodies and degradation strategies of pain-involved proteins, among others, as alternative approaches toward innovative analgesic agents.
2. Planning work for a framework of clinical biomarkers differently expresed in pain diseases, which can quantify disease progression, and treatment response for optimal patient stratification. While doctors still mainly rely on patients' subjective feeling to determine pain treatments, evolution to objective measures of pain in the form of biomarkers is needed. In addition to the identification of some objective blood biomarkers for pain, the field of neuroimaging-based biomarkers is advancing rapidly.
3. The poor outcome of many chronic pain patients with current treatments stresses the urgent need for the development of new therapies. A major bottleneck in the development of safer and more effective pain therapies is the limited knowledge regarding basic aspects of nociceptive transduction and signalling mechanisms.
4. Experts indicate that pain management needs to become more personalized, using an evidence-based approach. To reach this goal, clinicians need to learn how patient characteristics correlate with

treatment outcomes. Mechanisms responsible for chronic pain are diverse and produce different manifestations, leading to a complex constellation of positive and negative sensory symptoms and signs (von Hehn et al., 2012). In addition, there is a strong variability in the reaction of the nervous system to the etiological pathology. These variations need to be understood at the cellular and molecular level.

5. At present there are numerous analgesic therapies available in the clinic however, their chronic use result in a large number of negative side effects. Moreover, their repeated administration often result in a reduced efficacy, leading to the danger of overuse. Thus, the scientific community maintains a continuous search for effective alternative treatments based on other mechanisms of action, which present grater safety, reducing the adverse effects (Allerton, 2013).
6. Basic research to extend existing knowledge on pain mechanisms are enabling the development of new analgesic drugs and treatments which, in the last instance, will redound in benefits for the patients and in a reduction of the economic and medical cost (Pérez de Vega et al., 2019). To give an idea on the importance of this subject nowadays, publications find in Scopus, using PAIN as unique term (all fields), indicated more than 1,600,000 entries in the last 20-year, with constantly increasing contributions (>100,000 publications annually from 2013).
7. Analysing how the notions of habit of the self and memory of the body bring clarification to situations of chronicity. These break down the familiarity of "bodily being in the world", whereas it is not possible to become effectively accustomed to pain. The same applies to a renewed phenomenology of pain in non-human animals. The eidetic perspective on the immanence of suffering and the way to intersubjectivity through intercorporality promote the recognition of suffering in non-humans. But it is fundamental to avoid the complete separation, within the experience of pain, between sensibility and sense, that is, between the mere sensation and the meaning it entails, and to delve deeper into how aversive self-affection is the source of the recognized and understood negativity of pain.

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CHALLENGE 7

ABSTRACT

Advanced Therapy is considered one of the fastest growing areas of biomedical research and with the greatest potential to influence society by offering solutions for unmet medical needs, including the possibility of curing diseases with negligible or scarce therapeutic alternatives and developing personalized medicine. We describe here the current positioning, weaknessess and potential of the CSIC in Gene therapy, Cell therapies and Bioengineering and analyze the major goals to be achieved in these research fields in order to translate basic science to clinical applications.

KEYWORDS

gene therapy | stem cells | biomaterials | immunotherapy | bioengineering

CHALLENGE 7

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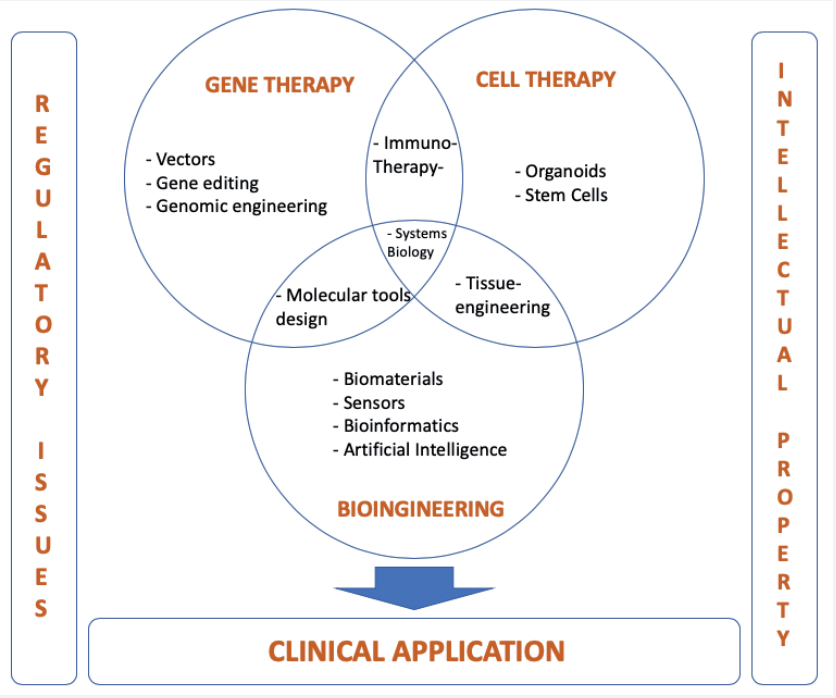
1. INTRODUCTION AND GENERAL DESCRIPTION

According to the definition provided by the European Union Framework for Advanced Therapies: "Advanced therapy refers to new medical products that use gene therapy, cell therapy, and tissue engineering. They can be used to treat diseases or injuries, such as skin in burns victims, Alzheimer's, and cancer or muscular dystrophy, and have huge potential for the future of medicine" (European Commission, 2020; Gran View Research, 2017).

In addition to these concepts, it is necessary to always keep in mind that the ultimate goal of Advanced Therapies is their clinical application. This means that the technical and scientific activities carried out in this area are subject to regulatory restrictions on safety, as well as to intellectual property precautions, which influence their development. In accordance with these ideas, we have organized the information on CSIC's Advanced Therapies sector as shown in the following diagram:

The intervention of external actors like public and private health systems, end users and other stakeholders of critical importance in the field, but alien to

FIGURE 1–The playing field of Advanced Therapies



the traditional CSIC approach to research, makes necessary to create new tools to facilitate cooperative relations with them. This aspect is especially relevant in regard to regulatory authorities, like the Spanish Agency for Medicines and Health Products (AEMPS), and with institutions dedicated to clinical research, such as the Carlos III Health Institute as well as with public and private hospitals and foundations.

The urgent need for these actions has become evident during the current COVID-19 health crisis. The gravity of the situation has forced CSIC to improvise, in a very short time, with diligence but with the inevitable mistakes, a Global Health Platform that would create some of the structures and protocols mentioned in the previous paragraph —and that were already presented some months ago at a preliminary dialogue with CSIC about a future Advance Therapies Platform—. This unplanned experiment has taught several lessons that should be held in consideration for the future implementation of the new platform.

1.1. Economic and social impact

Advanced Therapies is the fastest growing area of biomedical research in recent years. It is also the one with the greatest potential to influence society by offering solutions for unmet medical needs. These include the possibility of curing diseases with negligible or very limited therapeutic alternatives (rare diseases, cancer), the elimination of lifelong dependency of medications (hemophilia, HIV) and a clear shift towards personalized medicine from the conventional "one size fits all" approach.

During the last few years, an increasing number of CSIC research groups have started to work on different aspects related to Advanced Therapies. These have been joined by new teams working in the field since the beginning. As a result, the number of patents in the area has grown gradually, with a total of 78 since 2002. At the same time, CSIC researchers have created 4 biotechnology companies and, from 2010 to date, established 39 contracts with industry.

Projections about Advanced Therapies' future economic impact reflect these facts. Although as any prediction the figures vary, estimates for some of the sectors included in the Advanced Therapies field are impressive. The compound annual growth rate (CAGR) at which the global market for stem cell therapies is expected to expand range from approximately 9.2% to 15% (Mordor Intelligence, 2018). One market research firm predicts that the value for the market will reach $15.63 billion by 2025 (ISCT, 2018). Gene therapies are expected to grow at a CAGR of 33.3%, reaching a value of $4.402 billion by 2023 (Allied Market Research, 2017; Grand View Research, 2017); while estimates for Immuno-oncology fluctuate between a market value of more than $100 billion by 2022 (Mordor Intelligence, 2018b) and $34 billion by 2024 (Grand View Research, 2018).

According to these numbers, it is easy to see that Advanced Therapies are an emerging field with an extraordinary future, both economically and in terms of their ability to offer returns to society. These two circumstances, novelty and growth possibilities, make it a strategic sector that remains open to new participants. For historical reasons, the CSIC is active in a wide variety of fields, many of which are already mature and in which it does not seem feasible for it to play a relevant role in the short or medium term. On the other hand, Advanced Therapies is a field in which a determined and coordinated initiative between the research groups involved and the institution's management can place the CSIC at the world level that corresponds to its size and trajectory.

1.2. Alignment with European and national initiatives

We would like also to stress that all future CSIC actions in the field of Advanced Therapies should be aligned with both the European and Spanish initiatives, not only to obtain financial support, but also to follow the policies of both institutions in regard to the transfer of new therapies to the industry (i.e., IMI2, Innovative Medicine Initiatives 2), as well as their translation to the bedside.

In this context, it is important to mention the most relevant guidelines affecting the Advanced Therapies field. In October 2017, the European Commission and the European Medicines Agency (EMA) published a joint action plan on Advanced Therapies Medical Products (ATMPs) which aims to streamline procedures and better address the specific requirements of ATMP developers. The main document was published on 20/10/2017 and updated on 17/02/2020 (European Commission, 2020).

In addition, some ATMPs may contain one or more medical devices as an integral part of the medicine, which are referred to as combined ATMPs. The Agency's Committee for Advanced Therapies (CAT) plays a central role in the scientific assessment of advanced therapy medicines. It provides the expertise that is needed to evaluate advanced therapy medicines. During the assessment procedure, the CAT prepares a draft opinion on the quality, safety and efficacy of the advanced therapy medicine. It sends it to the Committee for Medicinal Products for Human Use (CHMP). Based on the CAT opinion, the CHMP adopts an opinion, recommending or not the authorization of the medicine by the European Commission. The European Commission makes its final decision on the basis of the CHMP opinion.

The CAT also:

- Gives recommendations on the classification of advanced therapy medicines;
- Evaluates applications for certification of quality and non-clinical data for SMEs, following which the Agency issues a certificate;
- Contributes towards giving scientific advice on advanced therapy medicines;
- Is involved in any procedure regarding the provision of advice for undertakings on the conduct of efficacy follow-up, pharmacovigilance and risk management systems of ATMPs;
- Advises, at the request of the CHMP, on any medicinal product which may require, for the evaluation of its quality, safety or efficacy, expertise in ATMPs;

- Assists scientifically in the elaboration of any documents related to the fulfilment of the objectives of Regulation (EC) No 1394/2007;
- Contributes towards an environment that encourages the development of advanced therapy medicines;
- Provides, at the request of the European Commission, scientific expertise and advice for any initiatives related to the development of innovative medicines and therapies.

The regulatory activity of the European authorities had its reflection on similar initiatives of the Spanish Government. The Spanish Ministry of Health approved on November 15, 2018, through the Interterritorial Health Council, a program of Advanced Therapies, which together with medicines of high economic and health impact, poses a challenge for the National Health System (SNS). The plan pursues three objectives:

a. The care provided is organized in a planned, safe and efficient manner;
b. Independent clinical research is promoted;
c. Their own and public manufacturing is promoted, guaranteeing safe, equitable and sustainable access to these high-impact medicines through the SNS.

These initiatives are discussed in a working document entitled "Plan de abordaje de las terapias avanzadas SNS 15112018" (Ministerio de Sanidad, 2020), which provides the description of the objectives and proposed activities supported by the SNS.

Once these general aspects and pivotal considerations on regulatory issues regarding Advanced Therapies have been stated, we will now focus on the specific description of the three major scientific components identified at CSIC for this challenge: Gene therapy, Cell therapy and Bioengineering.

1.3. Gene therapy

As defined by the EMA, gene therapy is a medicinal approach for the delivery of genes that lead to a therapeutic, prophylactic or diagnostic effect. It works by inserting 'recombinant' genes into the body, usually to treat a variety of diseases, including genetic disorders, cancer and long-term diseases. A recombinant gene is a stretch of DNA that is created in the laboratory, bringing together DNA from different sources. To this aim, different types of vectors can be employed, including viral-derived vectors (e.g., lentiviruses and adeno-associated viruses) and engineered non-viral methods (e.g., dendrimers, cationic liposomes, and inorganic nanoparticles).

Gene therapy represents a new frontline in science with the potential to provide a cure to many patients with serious or fatal conditions. It offers tools to address significant unmet clinical needs. Many genetic and acquired diseases that produce highly disabling or life-threatening clinical consequences, and for which no effective treatments beyond palliative care are available, could benefit from gene therapy. A gene therapy treatment, which can effectively cure the disease, could not only prevent much suffering but also lead to cost savings in the long-term from a reduction in the costs of care.

More recently, powerful molecular tools such as Zinc Finger Nucleases (ZFN), Transcription activator-like effector nucleases (TALEN) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), capable of editing the genome of livings cells, have emerged. This new type of sequence specific nucleases can be directed to modify the genome at chosen locations and, therefore, can be used to either repair mutations or create new functions both in-vivo, in gene therapy protocols, and ex-vivo, in combination with cell therapy approaches.

1.4. Cell therapy

The pioneering work on bone marrow transplantation, demonstrating that adult stem cells could restore hematopoietic function in lethally affected mammals, opened a new therapeutic research field. Currently, cell therapy aims to repair a tissue or restore a lost or impaired function. Cells used for this purpose could be either autologous or allogeneic adult stem cells or differentiated cells. In addition, tissue-like structures composed of cells ensembled into synthetic or organ-donor scaffolds can be also employed to substitute damaged tissues. Furthermore, to circumvent cellular transplantation and engraftment limitations, endogenous adult stem cells or progenitors can be activated and mobilized to proliferate and self-regenerate non-functional tissues. Finally, induced pluripotent stem cells (iPSCs) have powerfully emerged in the last decade as a versatile cell source for human cell transplantation, disease modelling and drug discovery. Overall, cell therapy-based strategies hold great promise on regenerative medicine.

In the next decade, basic research will be crucial to generate the essential preclinical knowledge required for transferring these promising advances into the clinic. Accordingly, CSIC should be a leader on promoting basic and translational research in this area by facilitating platforms at the intramural level.

For therapeutic actions at the nervous system, several CSIC research groups work on advanced cell therapies using whole bone marrow or bone marrow-derived mesenchymal stem cells (BM-MSC), neural stem cells (NSC) and carotid body populations. For the regeneration of the visual system, epithelial limb sclerocorneal cells (CLET) and BM-MSC are being evaluated. For pancreatic diseases, bone marrow and pancreatic stem cells are under study. In the lymphohematopoietic system, improvements in the current bone marrow transplant procedures are being investigated. Finally, cardiac progenitor/stem cells and MSC are being explored for acute infarct intervention.

Isolation of embryonic stem cells gave rise to a new era in the regenerative medicine field. However, technological advances have substituted them by iPSCs, which offer the advantage of being obtained directly from the patients, providing excellent disease models. Many CSIC research groups are actively working in the generation and applicability of iPSCs, targetting the treatment of varied diseases including cystic fibrosis, Alzheimer, pigmentary retinosis, macular degeneration and Friedreich ataxia. Alternatively, fetal stem cells are progenitor cells with reduced multipotency and committed to differentiate into fewer specific cell types. They can be useful for cell therapies as well as for the study of basic biology aspects, for instance, of the hematopoietic and the nervous systems. In this field, many research groups at CSIC are working on hematopoietic and vascular progenitors from the fetal liver, epithelial limbal stem cells located in the cornea and interneuron progenitors derived from the medial ganglionic eminence in the fetal brain.

At the basic research level, several CSIC research groups work to understand the intrinsic and extrinsic factors that regulate the quiescence-proliferation and the proliferation-differentiation switch of adult stem cells in the neural and reproductive systems. Moreover, other groups are trying to define the transcriptional networks responsible for generating and maintaining specific cell types, such as neurons and pancreatic cells, in an effort to better understand neurological, liver and reproductive disorders. To this end, different animal models are employed including mice, flies and worms. Interestingly, several groups are modeling human diseases (e.g., cystic fibrosis and Alzheimer) using iPSC-organoid technology. At the applied research level, two good manufacturing practices (GMP) Cell Production Units, both licensed by the AEMPS according to the EU guidelines on cell therapy production for human use, provide cells to several clinical trials on ocular, vascular and articular pathologies, among other targeted diseases.

1.5. Bioengineering

The third main theme identified in this challenge is bioengineering, for which the CSIC also has an important group of internationally renowned scientists whose work focuses on four main topics: Sensors and Micro/nanotechnologies, Biomaterials, Immunotherapy, Proton Therapy and Big Data. The specific situation of these different topics is described below.

Biomedical engineering is a relatively new, interdisciplinary, and an exciting field. Sitting at the cross-section of medicine, biological science, and engineering, biomedical engineers design the advances in equipment, devices, computer systems, and/or software used to improve human health. Bioengineering for advanced therapies can also be divided into two large groups depending on whether they have a direct or indirect interaction with the patient. Both cases have different basic sciences where the work is based on. Biomedical engineering applications directly related to patients include the development of new materials (biomaterials) and technological processes that allow a better biological-artificial interaction for the treatment and/or diagnosis of pathologies. On the other hand, there is an increased need to improve healthcare and the way clinical decisions are made for the welfare of patients. Therefore, the collection and processing of enormous amounts of data will help to make more accurate predictions in the future, specifically in diagnosis, therapies and prognosis of diseases so as to advance personalized medicine and targeted drug/gene/cell therapies. Biomedical engineering disciplines are close linked. For example, remote monitoring technologies (sensors) can capture motor features that may be clinically useful in identifying patients who may be candidates for the application of a concrete advanced therapy (such as deep brain stimulation or levodopa-carbidopa intestinal gel). This could then lead to the development of automated screening algorithms, improve referral efficiency and expand access to advanced therapies for patients with advanced Parkinson's disease.

Advances in micro- and nanotechnologies, along with progress in materials science, have provided new tools for the development of better medical solutions to improve healthcare (e.g., organ-on-a-chip devices for massive drug screening and neural interfaces for neural recording and stimulation). Non-invasive and minimally invasive analytical devices (biosensors) with the potential of measuring biomarkers and other target analytes in biological fluids such as sweat or tears are highly desired. For example, sweat monitoring devices in the form of patches and tattoos are being developed to apply screening programs in certain diseases.

The use of biomaterials as therapeutic tools to approach the regeneration of diseased/injured tissues is also receiving an expanding interest worldwide. The plethora of materials with utility in diagnosis and/or therapeutics that are under investigation by CSIC research groups is enormous, including natural polymers (e.g., chitosan, collagen, alginate, hyaluronic acid, gelatin, cellulose), synthetic polymers (e.g., PLLA, PLGA, PCL, polydiolcitrates, PUs, PMMA, PDMS, PEG), molecular materials (e.g., DNA/PEI complexes, quatsomes, protein nanoparticles, lysyl oxydases coupled with bone morphogenetic proteins), carbon materials (e.g., graphene and carbon nanotubes) and hybrid composites (e.g., calcium phosphates integrated into organic matrices, biohybrid systems combining biomolecules and inorganic solids such as silicates, layered double hydroxides and perovskites). Additionally, CSIC researchers are able to use different manufacturing processes to tune biomaterials characteristics and investigate their resulting physico-chemical and biological properties (e.g., fluorescent dyes and nanovesicles prepared by sustainable CO2 based processes, graphene oxide aerogels for diagnosis).

Polymer therapy is an important focus of some researchers at CSIC, being responsible for the design of novel vascular devices such as coronary stents with anti-thrombogenic and anti-proliferative properties (marketed, CE labelled), ophthalmic devices such as intrastromal rings and contact lens with anti-proliferative properties, tissue regeneration membranes and coatings for abdominal meshes for antibiotic release.

Importantly, many groups working on this topic are focused on nanotechnology strategies to approach less invasive and toxic therapeutics with a high efficiency and capacity to be customized to give response to this new era of personalized medicine. In this sense, we can outline prominent progress carried out by CSIC scientists in nanoparticles for cancer treatment, hyperthermia, immunosuppression therapy, bacterial infection, and MNR diagnosis of cancer and atherosclerosis, as well as carbon nanodots for advanced targeting and diagnosis. A majority of these nanoparticles are based on iron oxide magnetic nanoparticles, but other compositions including other metals such as gold and cobalt, carbon dots and liposomes are also under exploration.

Other biomaterials currently investigated at CSIC with promising features include dendrimers for diagnosis (MRI), MOFs, nanocellulose hydrogels, thin films and bioactive surfaces for therapy (e.g., cancer, Alzheimer, plant healing and regeneration therapies) and carbon nanomaterials and microparticles for theranostics. From these, those with the most promising features are assessed

in-vivo in pre-clinical studies in collaboration with hospitals, such as boron clusters-hybrid materials for multimodal cancer therapies, polymeric theranostic nanocapsules for brain repair and sepsis treatment, electroactive hybrid materials for neural therapies and quatsome vesicles as a KET for gene therapy.

Immunotherapy is a flourishing interdisciplinary medical field, based on harnessing the immune system of patients, which is giving encouraging results in the fight against cancer. CSIC researchers are developing biomaterials and protocols to improve scientific and technical limitations associated to the personalized adoptive cell therapy, including the synthesis and fabrication of hydrogels to efficiently culture immune cells, in order to translate such therapies to public health care systems.

Since the early definition of Tissue Engineering in the 1980's by Professor Langer and Professor Vacanti, progress in the creation of artificial 3D substitutes for tissues and organs for regenerative medicine purposes is vertiginous, concurring with the emergence of the fourth generation of biomaterials, the so-called smart/biomimetic materials. In this sense, progress by CSIC researchers is providing novel 3D scaffolds with regenerative potential for bone, cartilage and neural tissue repair. For these strategies, technological developments such as 3D bioprinting and organ-on-a-chip instruments, along with tools derived from other fields such as gene and cell therapies, are undoubtedly serving to create advanced therapeutics. Another attractive material in this context are hydrogels as they are structurally similar to the extracellular matrix of many tissues, can often be processed under relatively mild conditions, and delivered in a minimally invasive manner, as well as fabricated by using 3D printing technologies to more precisely reproduce native tissue architecture. Additional advantages include soft mechanical properties, hydrophilic nature, biodegradability, biocompatibility, and versatility to achieve high loads of bioactive molecules. Bacterial inclusion bodies, which are mechanically stable, biocompatible protein particulate materials with genetically controlled nanoscale properties, are being also explored for the decoration of biomaterials whose surfaces act as stimulators of the colonization and proliferation of mammalian cells.

Finally, proton therapy is a technique that uses proton beams instead of X-rays as ionizing radiation, to which healthy tissues are not immune. It has a far higher selectivity than conventional radiotherapy, what makes it ideal for the treatment of localised tumours in highly sensitive areas (e.g., brain and spinal cord). Proton therapy, with more than 150,000 patients treated worldwide and new centres under construction in Europe, is experiencing an

exponential growth. In Spain, the two first proton-therapy facilities will start treatment of patients in 2020: Quirónsalud and Clínica Universitaria de Navarra (Madrid). The application of proton therapy is not exempt of difficulties. The precision in the determination of the distal position of the distribution of dosage is crucial for complete irradiation of the tumor and dose restriction to organs at risk. In this context, it is of high relevance to investigate on: new techniques of dosimetry, proton-range verification during treatment, proton-imaging prior to treatment and proton beam production and optimization. These research fields are covered by different groups of around 10 different research institutes at the CSIC. On the basic science, it is crucial to count on accurate models of the chemical and biological effects of low-energy protons and secondary particles in living cells and tissues. Several groups of physicists, chemists and biologists at CSIC investigate along these lines, by irradiating materials and living cells with proton and electron beams either in singular facilities, such as CNA (Sevilla) and CMAM (Madrid), or at hospitals and outside research institutes of current collaborators.

2. IMPACT ON BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

2.1. Gene therapy

The era of simple treatable diseases is over. During the 20th century, an enormous number of drugs, mostly in the form of small molecules, have been developed and several diseases are now easily targeted though pharmacological approaches against single molecular targets. Nevertheless, several other major pathological states still wait to be efficiently addressed. Therefore, our Healthcare System faces a number of syndromes and complex diseases that are not easily cured. Gene therapy is a promising strategy to treat these complex diseases.

The development and improvement of already existing methods for gene delivery in specific cells and tissues represent a challenging task that requires broad research in basic scientific areas, from chemistry and biophysics to virology and molecular biology. Technological advances in bioengineering are mandatory to develop improved methods of therapeutic vectors production at industrial scale.

A special mention deserves the burgeoning and successful field of Immunotherapy in its different forms: development of immune check points inhibitors, production of monoclonal antibodies and development of engineered T cells for adoptive cell therapies. The latest mostly sat at the intersection of

gene and cell therapies, but the need to expand T cells in high quantities, with a determined phenotype and at a reasonable cost, has prompted CSIC bioengineers to develop supramolecular 3D PEG-based hydrogels to mimic the lymph nodes, organs where T cells proliferate in-vivo.

Finally, a new generation of nucleases has opened up the possibility of modifying ex vivo the genetic composition of stem cells obtained from patients and then, transplanting their derivatives back into the original donor to rescue the initial disease phenotype. This disruptive technology has made possible, for the first time to stop, and even revert, hereditary, degenerative and infectious diseases currently lacking curative treatment.

2.2. Cell therapy

Generally speaking, cell therapies are still an emerging clinical field with few products in the market. Nonetheless, their use, either alone or in combination with biomaterials, bioengineered devices and nanotechnology tools, hold promise for new and definitive treatments for many human diseases.

For instance, iPSC-based approaches can be applied in medical treatments, but also at the basic research level to develop disease models. This is especially relevant for rare diseases and central nervous system pathologies that have been historically hampered by the lack of study models and the difficulties associated to samples restricted access. On their turn, iPSC-derived cells and tissues will improve our basic knowledge of the physiopathology of many organs and respective systems. In the case of numerous hereditary diseases, iPSCs will allow the development of ex-vivo gene repair protocols for subsequent autologous transplantation, thus, avoiding immune rejection drawbacks. In this context, collaboration with gene therapy and tissue engineering experts is absolutely required. Additionally, iPSCs allow the development of large-scale drug screening procedures, application that is especially relevant for mental disorders such as schizophrenia, epilepsy, Alzheimer and autism. In combination with organoid technology, iPSCs are serving to provide a deeper understanding of the pathophysiology and therapeutic potential of patients with airways-related diseases such as cystic fibrosis.

Hematological transplant, a well-established clinical procedure that saves millions of lives every year, needs to improve its current success rate. Several CSIC research groups are working at the moment in this task, as well as on hematopoietic and vascular progenitors from the fetal and adult liver.

Regarding immune-associated diseases, Diabetes Type II continues to be a major global health problem. Several CSIC researchers are using bone marrow and pancreatic stem cells in the search for better treatments. Immunoregulatory-based interventions in which BM-MSCs are transplanted are currently on a phase III clinical trial to assess the recovery of articular cartilage in knee osteoarthritis. BM-MSC have also been clinically investigated (phase III trial) in lumbar intervertebral disk degeneration, both with the concourse of CSIC associated groups. Moreover, the use of allogeneic adipose-derived stem cells has been translated to the market in the unique stem cell-based medicament (Alofisel) authorized by EMA for commertialization and treatment of Crohn disease-associated compliation. In addition, based in their immuneregulatory actions, a phase-II clinical trial is now in course for the treatment with adipose-derived stem cells of patients with pneumonia and systemic inflammatory symdrome, which has been in part the base to the initiation of more than 50 clinical trials worldwide for treating COVID-19 and the acute respiratory distress syndrome.

Within the group of highly prevalent cardiovascular diseases, ictus and large infarcts urgently require novel therapeutic approaches. In this field, the characterization of a bona fide adult cardiac progenitor by CSIC researchers has demonstrated the feasibility and safety of this cell therapy approach.

Infertility is considered a top priority research topic in the EU demographic change challenge. In western countries, human sperm quality is declining whereas the incidence of testicular germ cell tumors is steadily increasing due, most probably, to a higher exposure to environmental pollutants. Several CSIC research groups are trying to understand the regulation of the germ cell line, as well as its niche, to identify novel therapeutic targets.

Finally, several promising strategies, some under evaluation in clinical trials, are being explored for the treatment of degenerative and mental disorders. Basic research is conducted to evaluate the functionality of neurogenic niches in healthy and pathological conditions. A clinical trial (Phase I/II) on the treatment of amyotrophic lateral sclerosis (ALS) is being conducted using BM-MSC to protect motoneurons. The therapeutic use of BM-derived populations is being also investigated in multiple sclerosis (MS). Other groups are using BM-derived cells (e.g., mesenchymal, hematopoietic, cord blood) and fetal interneuron progenitors to treat different forms of ataxias, epilepsy, ictus and stroke. On their turn, neuronal stem cells and carotid body-derived cells are being evaluated for Parkinson disease (clinical trials Phase I/II). In addition, a proof-of-concept clinical trial based on cell therapy approaches has been conducted with 37 patients

with severe corneal affectation, yielding good results to date. Stem cells, in combination with biomaterials, are being also explored for spinal cord injury. Finally, organoid technology based on iPSCs is being used to provide a deeper comprehension of the pathophysiology of Alzheimer's Disease and Cystic Fibrosis.

2.3. Bioengineering

The use of biomaterials is being currently explored by CSIC scientists for a wide range of current and future applications including:

a. 3D tissue and/or organ reconstruction for pathologies in which the native tissue/organ is functionally and/or structurally damaged and an "artificial" replacement is needed to overcome organ donor limitation.
b. Drug, gene and/or cell delivery to address diseases in which small molecules and cells of diverse nature must be transported to slow down tissue damage and/or recover functionality.
c. More innocuous and sensitive diagnostic systems to allow faster, sooner and less toxic detection of pathologies and disease progression.
d. Advanced microfluidic devices, in combination with cellular organoids, are been used to produce organ-on-a-chip platforms that enable the culture of human organ-like structures in biomimetic environments. In these devices, the use of primary cells from patients could serve to predict individual drug effects and investigate new therapies within the context of personalized medicine. In addition, organs-on-a-chip can improve the efficiency of high throughput assays, drug screenings and decrease animal testing (3R's principles).

It is important to note that all these are current and future challenges for the use of biomaterials in biomedical applications. Advances in these lines will led to a better understanding of the physiopathology of diseases and to more efficient, personalized and biocompatible therapies.

Recently, the most innovative biopharma industries have increased their interests towards nanobiomaterials, whose technological breakthrough potentiality is widely acknowledged. It is envisaged that the unique properties of these materials will make, in the near future, a strong impact in solving some of the global challenges towards which our society is heading; in particular, those relative to Health and Societal Wellbeing. As its main contribution, CSIC researchers devoted to this field will deliver highly-performing biomaterial platforms, patent protected when appropriate, which will open the possibility

to new advanced therapies to solve unmet medical needs and new contrast agents for advanced bio-imaging techniques and diagnostic platforms.

Several research groups at IFIC (Valencia), IEM (Madrid), CNA (Sevilla) and IMB-CNM (Barcelona) are developing instrumentation for proton tomography, proton-range verification using prompt gamma rays and novel micro-dosimeters. These works have a direct impact on the application of proton therapy itself. The instruments that have been developed are protected by patents and object of present and future technology transfer agreements. At the basic research side, irradiation of living cells or nanomaterials is carried out by some other groups from IFF (Madrid), ITQ (Valencia), I3M (Valencia) and CABIMER (Sevilla). Finally, novel techniques for proton acceleration using lasers are investigated at I3M (Valencia).

3. KEY CHALLENGING POINTS

3.1. Gene therapy

Gene therapy, as an emerging area of therapeutics, faces the following key challenges:

- The targeted delivery of therapeutic nucleic acids into disease sites has been the subject of intense efforts for decades, but neither viral nor non-viral vectors have yet met our expectations. Viruses, as naturally evolved infectious agents, are highly efficient vectors and a number of them have successfully progressed to clinical trials for gene therapy applications. However, although the use of site-specific promoters represents a promising strategy, the size limitation of the payload, their inherent immunogenicity and the difficulty (and extremely high cost) of large-scale production seriously hamper translation from bench to bedside. This has been spotted by several cases with important setbacks, including unforeseen serious side effects of treatment. Non-viral vectors, at their turn, provide opportunities to overcome these boundaries, but they are not fully exempt of toxicity and their delivery efficiency remains far from satisfactory.
- The production of the gene therapy itself at a sufficiently high scale is not resolved either. Even if the ideal vector for a given application is identified and the risk and selectivity issues are overcome, the production of the nuclei acid therapeutic under high level GMP compliances and at a sufficiently high scale to allow for practical application of the treatment poses technological challenges not currently solved.

- The business risks of developing gene therapies are also very high, especially for small biotechnology companies. If these therapies are to reach patients, manufacturers are likely to require these risks to be balanced by sufficient financial returns such that investment in the science underlying these technologies can be sustained. Given that most of the biotech companies (as well as most ongoing clinical trials) focus on adeno-associated viral (AAV) vectors, addressing the current challenges for the large-scale implementation of AAV production is critical to decrease that risk, which poses specific challenges:
  - The current technology makes difficult to produce AAV vectors in the quantities necessary for the treatment of a high number of patients. Moreover, current procedures are extremely expensive. For instance, the cost of producing 250 L of AAV vectors under GMP (equivalent to 1016-1017 vg) reaches 2.5 M€. It is then mandatory to develop more efficient methods for viral production.
  - Gene therapy directed to nervous system diseases is hindered by the need to generate AAV vectors capable of crossing the blood brain barrier (BBB) in humans. Although serotypes capable of doing so have been generated in mice (e.g., AAV.PHP.B, AAV.PHP.eB), there are no identified serotypes that could cross the human BBB.

The development of non-invasive methods for administering AAV vectors in the brain (e.g., through the cerebrospinal fluid) that do not require the use of large resources in hospitals (e.g., operating rooms, patient hospitalization) is also a priority in gene therapy at the nervous system.

All these challenges could be addressed by the development of a technological platform in which CSIC should be involved as a current and future strategic issue of enormous interest.

3.2. Cell therapy

Cell therapy, by definition, needs orchestrated multidisciplinary approaches to address unmet clinical needs. This integrative approach always relays on a solid understanding of the physiopathology of the target disease and the real medical need and a good control of the envisioned cell-based product. Currently, CSIC activity predominantly concentrates on the most basic aspects of cell therapy, i.e., the study of stem cells biology and the development of disease models, based both on animals and on emerging organoid and organ-on-a-chip technologies. As new areas of knowledge, these activities face outstanding challenges

that can be only addressed by strong initial investments on basic research.

In addition, when promising results emerge, their eventual commercial development and translation to the clinical arena is frequently hampered by a weak or inexistent CSIC experience. At the administrative level, CSIC Technology Transfer Office provides legal support for intellectual property management; however, a complementary department in charge of developing competitive business plans and actively searching for investors is missing. This frequently leaves CSIC research groups with the need to manage the commercialization of their research results by themselves, a task for which they are not prepared and is difficult to address without appropriate partners and advisors.

On the clinical arena, CSIC research groups are in clear disadvantage because of the lack of solid institutional long-term alliances with the healthcare sector. This is further complicated for the ban on CSIC research groups to apply for specific translational funding unless being associated with a clinical partner. So, highly motivated consortia integrating both clinical and basic research with a competitive critical mass and funding opportunities are essential to deliver novel cell therapies.

Based on this, the specific challenges of CSIC research teams working on cell therapy could be summarized as follows:

- Basic research on stem cell biology as well as on tissue development and engineering.
- Institutional support to create highly competitive multidisciplinary consortia and basic and translational research networks focused on strategic clinical needs.
- Professional pre-evaluation (due diligence) of cell therapy programs and proactive search and negotiation with appropriate biotechnology partners.
- Integration of CSIC in on-going national and international cell therapy initiatives.
- Granted and co-financed access to leading-edge technological facilities.

3.3. Bioengineering

Some of the most relevant challenges for bioengineering approaches rely on technological issues such as miniaturization and 3D bioprinting. Miniaturization is a key development needed for wearable technology, as well as for delivering drugs in-vivo, designing sensors for controlled prosthetics and deep

brain stimulation and creating microneedles for drug delivery systems, to cite a few. On its turn, 3D bioprinting is being largely boosted in the recent years by the immersion of the technological industry. This technology will become integral for the development of human artificial organs and the production of intricate circuitries in temperature-controlled clothing.

Regarding biomaterials, one of the most important weaknesses that CSIC teams face to transform knowledge into new therapies, diagnosis and better life for citizens is the limited connection with the end-user sectors, including both healthcare professionals and industry, as pointed out earlier for cell therapy approaches. Scientists at CSIC have been developing outbreaking biomaterial-based technologies that have an almost negligible translation to real therapeutics for clinical use. Based on this, a pivotal need is the creation of highly collaborative networks of scientists, clinicians and industrial companies and entrepreneurs who could work together to impulse this research to a next step of usefulness for society. This is a remarkable and common challenge for numerous disciplines within CSIC research to which CSIC has to respond by the establishment of more efficient ways of interdisciplinary work inside and outside the institution, including more effective international and multidisciplinary collaborations. By doing so, CSIC would be able to contribute to the increase of the Spanish innovation level, at present at a 3rd level (Regional Innovation Scoreboard, 2019), very low in comparison to north European countries. CSIC researchers need to be in constant contact with clinicians and biomedical companies to gain insight and refocus the materials developed to better comply the medical need and regulations and this need must be strongly supported institutionally. Besides, adequate infrastructure for the up-scaling under GMP conditions of some of the most promising biomaterials developed would clearly benefit the translation of therapeutics from the bench to the bedside.

In the radiotherapy field, new equipment enabling more selective and effective tumor treatments would be pivotal for advancing cancer therapies. Big data and smart data analyses will open new avenues to optimize computing capacities when focused on problems solving. Smart data advances would benefit a wide range of biomedical applications, from oncology and radiotherapy to neural signals recording and even sending valid information, for example, for prostheses.

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CHALLENGE 8

ABSTRACT

New Methods for diagnostic tools and prevention is a wide and active area comprising groups across physics, chemistry, engineering and biomedicine. The area encompasses novel imaging techniques (optical, ultrasound, atomic force microscopy-based, and molecular imaging) and biosensing (chemical, biological, including microfluidic-based devices and biocompatible, biodegradable sensors). Biomarkers and physical and functional properties (at the cell, tissue and organ level) are used to guide personalized treatment and formulate preventive health measures. The groups of CSIC have contributed to results ranging from basic science to clinical translation and industry transfer.

KEYWORDS

diagnosis | prevention | biosensors | bioimaging | biomarkers | artificial inteligence | personalized medicine

CHALLENGE 8

NEW METHODS FOR DIAGNOSTIC TOOLS AND PREVENTION

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1. INTRODUCTION AND GENERAL DESCRIPTION

The area is highly interdisciplinary, with a wide range in areas of research, methodologies and applications. We have widely classified the contributions according to imaging modality for diagnostics, detection/screening methods, and prevention and personalized treatments.

1.1. Medical Imaging Techniques for diagnostics

Optical and Ultrasound Imaging

There is a need for non-invasive imaging instrumentation in the clinical environment that allows in vivo geometrical and functional imaging of organs and tissue at high resolution.

Optical technologies include Optical Coherence Tomography, Wavefront sensing, Adaptive Optics or Ocular Section Microscopy. Given the transparent nature in the eye, with relatively low scattering, these quantitative 3-D imaging techniques are highly suitable for fully non-invasive applications in

ophthalmology. The Visual Optics and Biophotonics Lab (VIOBIO-LAB, IO-CSIC) is a worldwide leader in the area, supported by ERC Advanced Grants (PRESBYOPIA, SILK EYE); Coordinated H2020 Innovation Action IMCUSTOMEYE and Marie Curie ITNs, among others. Diagnostic tools in the eye have made the way to the clinic in the form of a wavefront-sensor autorefractor (Quicksee) and a Vision Simulator of presbyopic correction (SimVis), among others (Durr et al., 2014; Dorronsoro et al. 2016). Besides, the combination of imaging technologies with optical modeling and Finite Element Modelling, and Psychophysical/Perceptual quality metrics, allow new functional tests beyond those purely optical (for example corneal biomechanics), customized optical modeling to guide surgery, and the visual effects of optical manipulations (Marcos et al. 2017).

Ultrasound Imaging technologies outperform standard diagnostic technologies such as X-ray, as they do not use ionizing radiation, and can be more specific to diagnose certain pathologies with high incidence (such as breast cancer, particularly in radiologically dense breasts - 4 out of 10 women). In addition, ultrasound imagng techniques can be performed in the patient bed, resulting in a more comfortable and cost-effective exploration than magnetic resonance, computerized tomography, PET, etc. Three different groups at ITEFI-CSIC collaborate in the development of ultrasound imaging medical applications: the Group of Ultrasonic System and Technologies (ITEFI-CSIC), the Group of Ultrasound for the analysis of liquids and Bioengineering and the Group of signal processing in multichannel ultrasound systems. Their advances include automated acquisition and multimodal image (including doppler and elastography) to provide complementary information on the tissue (morphology, density, stiffness, etc), for applications in breast cancer (González-Salido et al., 2016); high resolution image acquisition at high frequency (Camacho, 2009) to study serous tissues and liquids in the body (for example to monitor meningitis in newborns (Jimenez et al. 2016; Elvira, 2019)); and the development of electronic systems for brain image in animal models.

Atomic Force Microscopy for Mechanobiology

The forces of mechanical origin at the cellular level are involved in growth regulation, cellular differentiation and tumor progression, therefore showing an important diagnostic potential. Mechanobiology addresses two main objectives: on one hand understanding the mechanism associated to the detection and response to mechanical forces by proteins, cells and tissues, and on the

other hand solving the relationship between the mechanical state of a cell and its physiological state (Dufrêne et al., 2017; Guerrero et al., 2019). The Force-Tool Group at IMM-CSIC develops new methods based on Atomic Force Microscopy to dilucidate basic relations between the mechanical properties of cells and tissues and disease (particularly cardiovascular disease) and to develop early diagnostic tools for the clinic.

The Laboratory of Protein Nanomechanics at ICN-CSIC, also uses Single-Molecule Force Spectroscopy based on Atomic Force Microscopy for early diagnosis of amyloidogenic diseases, with the aim of identifying specific conformers that trigger the amyloid cascade (Hervas et al., 2012; Oroz et al., 2012 ; Weaber, 2012) and/or quantifying the conformational polymorphism observed in the amyloidogenic proteins (Fernández-Ramírez et al., 2018) and use either (or both of them) as molecular reporters for the propensity of forming pathological amyloid in samples of blood or cerebrospinal fluid from human probands.

Molecular imaging

Several groups at CSIC address improvements in Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) from different perspectives: improvements of clinical sensitivity, modeling of positron-tissue interaction, new detectors, and new nanoparticles for MRI and PET.

In particular: I3M-CSIC investigates the increase of image quality and clinical sensitivity in PET through both photo-electric and Compton effects, and develops new detectors for molecular Imaging and new high resolution PET for cardiovascular imaging with high temporal resolutions. The group has been successful in EU project turnovers (FP6, FP7 and ERC Advanced Grants) and technology transfer (spin-out Oncovision having deployed 30 clinical PET units, and Bruker BioSpin which commercializes pre-clinical PET units); The Radiation-Mater Interaction (RMI) Research Group at IFF-CSIC that studies positron-tissue interactions in scattered media (Stevens et al., 2018; Robson et al., 2015). The Image Reconstruction, Instrumentation and Simulations in Medical applications Group (IRIS) at IFIC-CSIC, which leads the development of Compton cameras for hadron therapy treatment monitoring in Europe, having developed a prototype to operate in a clinical environment and demonstrated the possibility of imaging the distribution of photons emitted by an irradiated material similar to human tissue (Solevi et al. 2016). The experimental nuclear physics groups at IEM-CSIC and IFIC-CSIC, which jointly develop a 3D proton- CT scanner with proton-range verification capabilities for real-time

therapy monitoring, by minimizing image blurring due to multiple scattering (MSC) (Albiol et al., 2019; Ytre-Hauge et al., 2019). The Namomedmol Group (IQM-CSIC) develops nanoparticules for use in molecular imaging, in particular first combination of 68Ga and iron oxide nanoparticles for the combined use of T1-MRI and PET (used for the early in vivo detection of angiogenesis, microcalcifications, neutrophils and oxidised phospholipids) and iron oxide nanoparticles with enhanced properties as T1 (positive contrast) probes for magnetic resonance imaging in preclinical applications.

1.2. Biomarkers and Biosensors

Primary goals of the imaging techniques described above are the deployment of biomarkers for early disease diagnostics. For example, from Air Puff/Acoustic Stimulated Corneal Deformation Imaging the VioBio-Lab at IO-CISC obtains biomechanical biomarkers of corneal disease, or from Adaptive Optics/SimVis Simulation, perceptual quality markers of multifocal corrections of presbyopia, among others (Curatolo et al., 2020); from AFM, the ForceTool Group at ICMM-CSIC obtains nanomechnical biomarkers (elastic modulus or disipation coefficients) to track disease; or from Ultrasound Imaging, the Ultrasounds Groups at ITEFI obtain tissue stiffness from full angle elasticity as an indicator of tumor malignancy, or obtain a marker of the concentration of leukocytes in the cerebrospinal liquid as an indicator for meningitis.

Besides, other groups focus on the development of disease biomarkers to be applied in biosensor approaches. The ultimate goal is point-of-care applications and wearable devices to monitor health and disease. The Advanced Sensor Technology Group at ITEFI-CSIC develops chemical and biological sensors (resistive, surface acoustic waves, and magnetic) to detect markers (gaseous compounds) in the breath for early diagnosis of disease (Le Maout et al., 2018) (for example diabetes, kidney, liver or respiratory diseases), such as carbon monoxide (inflammation of the lung), dimethyl sulfide (liver disease), and nitric oxide (asthma), as well as surface acoustic wave sensors in combination with microfluidics to detect biological targets (Matatagui et al., 2014) (antibiotics, growth factors, etc.). The Biomedical Applications Group at IMB-CMM-CSIC develops biocompatible and biodegradable implants for neurological applications, and wearable devices (implants and external) for the real time and continuous monitoring, and early diagnosis for in vivo applications. Additionally, this group and the Chemical Transducers Group at IMB-CNM-CSIC develop chemical sensors integrated into microfluidic systems for applications of cellular studies, including single cell analysis and tissues, and for producing

multiplexed analytical tools for simultaneous detection of biomarkers at the point-of-care. The HPLC-CE Lab of the Instrumental Analysis in Environment, Food and Health Group at IQOG-CSIC develops high resolution separation methods that reveal alterations in glycoproteins due to changes in their glycosylation or other post-translational modifications (PTMs) which are related to certain diseases and therefore can be used as a disease biomarker (other applications include measurement of the alteration of immunoglobulins in breast milk) (Farina-Gomez et al., 2017; Puerta et al., 2011). The Nucleic Acids Chemistry Group at IQAC-CSIC develops oligonucleotides for the functionalization of biosensors, including DNA capture probes based on DNA-clamps for triplex formation, DNA-directed inmbobilization probes, and derivatives for detection of thrombin (Aviñó et al., 2016; Oroval et al., 2013). In turn, the Structure of Nanometric Systems Group (ESISNA) at ICMM-CSIC produce chemically functionalized graphene with nucleic acid aptamers. These molecules (RNA or single-stranded DNA) can bind with high affinity and specificity to a given target molecule, for example to recognize a a viral protein or tumor markers (Bueno et al., 2019).

1.3. Machine Learning and Artificial Intelligence in medical diagnostics imaging and biomarkers

Imaging and selection of biomarkers benefit from machine learning and artificial intelligence. Several groups working on different imaging modalities count on resources in their teams for automated image processing. However, with the potentially large spatially and temporal datasets, potential integration of multimodal information, and potential for automated prognosis and diagnostics, machine learning and artificial intelligence hold promise to become critical across the field. The VioBio Lab Group uses automatic image processing for segmentation of ocular structures, image distortion correction and quantification in Optical Coherence Tomography and Ocular Section Microscopy. The ForceTool Group is already implementing big data and machine learning algorithms for rapid diagnostics, and the Ultrasonic System and Technologies Group has also identified deep learning algorithms as promising line for further exploiting the outcomes of the ultrasound-based imaging technique and is also working on artificial intelligence tools to aid in the diagnosis of COVID-19 disease using lung ultrasound images.

There are two groups which focus primarily on the development of machine learning and AI for medical imaging application. While the main application for these groups is MRI, all mentioned imaging modalities can benefit from

these developments (conveniently adapted), to help radiologists and specialists in the quantification and detection of anomalies in the medical images. The Plasticity of Brain Networks at INA-CSIC combines multiple imaging modalities and texture analysis in machine learning platforms to define "disease signatures" in brain imaging, the generation of "functionalized biomarkers", and the use advanced imaging protocols and sophisticated mathematical models in diffusion imaging. The Medical Physics Group at IFIC-CSIC develops new AI algorithms for medical imaging, including the use of Annotated data, combination information from different medical devices, retrospective and device biased learning and continuous learning (De Santis et al., 2019a; De Santis et al., 2019b; De Santis et al., 2019c; Toschi et al., 2020).

1.4. Prevention and Treatment customization

The imaging and biomarker tools described above have an early diagnostic purpose but also the personalization of treatment and care. In many regards, the information gathered from those tools must serve to inspire treatments, guide surgery at the individual level, and in general, offer therapeutic solutions based on sound understanding of mechanisms underlying disease. We have grouped very diverse therapeutic solutions to a large range of medical conditions under this general section of prevention and treatment customization.

Custom implants

The technologies developed by VioBio Lab of the IO-CSIC for 3-D Quantification of ocular geometrical and biomechanical properties of a patient's eye lead to the generation of opto-mechanical customized model eyes that serve as platforms for virtual (cataract and corneal) surgery. Quantification of the cornea and crystalline lens has also stimulated bio-inspired implants for the correction of cataract and presbyopia (including the extended-depth-of-focus Isofocal IOL, licensed to/commercialized by PhysIOL, Inc and already implanted in patients) or the Accommodating IOL LightLensTM engaged by photobonding, among others (outputs of the ERC Advanced Grant Presbyopia and ERC Proof-of-Concept Grants OCT4IOL, SimVisSim and LightIOL). Also, new materials (more biocompatible and fine-tunable) will allow new corneal onlays, inlays and intraocular implants (in the new ERC Advanced Grant Silk Eye).

Organ-on-chips

The Biomedical Applications Group at IMB-CNM-CSIC Organ on a Chip combines 3D microfluidics and sensors integration to simulate organ and tissue

specific micro-environments. These systems are applied for toxicological studies and personalized medicine and represent a clear alternative to minimize animal experimentation. An example is the retinal system, which, among others, mimics the blood-brain barrier in the eye.

Personalized nutrition

The relevance of the microbiome and adequate nutrition on health cannot be underemphasized. The Microbiome Ecology, Nutrition and Health Group of IATA-CSIC works on artificial/synthetic microbiota to reduce vulnerability to disease. The understanding of the relation of structural/metabolic compounds produced by the microbiota with biological function can lead to personalized diets and therapeutic strategies of a large range of diseases. This group has coordinated one of the most competitive grants on the human microbiome in Europe (MyNewGut) and is involved in other H2020 EU initiatives.

Vaccines

Vaccines are by far the most powerful strategies for disease prevention. Various well-known groups at the CSIC, are publicly relevant these days for their work on a vaccine against COVID-19. Their work may be highlighted in other sections of the White Book. Here we report the program of Microorganisms for Health and Well-Being Group at CNB-CSIC, who investigates the of Foot-and-mouth disease virus (FMDV), as one of the microorganisms more importantly compromising animal (livestock) health and as an interesting model system for understanding the interactions of a highly variable virus and its natural hosts and the implications of these interactions on disease control. The group works on the development of new FMDV peptide marker vaccines that can induce protective humoral and cellular immune responses in pig and cattle as hosts, as animal models.

2. IMPACT ON BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

The reported research in New Methods for Prevention and Diagnostics is in many cases at the fore-front of science in Europe, and has enormous potential, not only to advance basic science but also as a generator of technologies with a clinical impact and potential for commercialization, therefore creating an impact also on economy and society.

Identified impact on basic science and technology:

1. Recognized high-risk high-impact research by the European Research Council, with ERC Advanced Grants to Susana Marcos at IO-CSIC(2, Presbyopia and SilkEye), to Ricardo García at CMM (3DNanomech) and to Jose M Benlloch (4D-PET).
2. Many of the groups have been competitive in EU grants (FP6, FP7, Horizon 2020), having been coordinators of large consortia in the area of diagnostics and prevention. For example, the I3M has coordinated several EU consortia (particularly relevant are the MAMMI and MindView) and a FET Open, the IRIS Group of IFIC is part of ENLIGHT (European Network for LIGht ion Hadron Therapy) platform, the VioBio Lab coordinates H2020 ICT Innovation Action IMCUSTOMEYE (with 10 partners) to develop imaging-based biomarkers of corneal disease. The group of IATA CSIC has coordinated MyNewGut and is involved in several other EU projects (MicrobiomeSupport, CIRCLES, miVaO) and co-chairs the EU platform Food for Life.
3. The groups have custom-developed unique competitive technologies including:
   - 3-D Fully Quantitative Anterior Segment Optical Coherence Tomography
   - Eye Wavefront sensing
   - Adaptive Optics Simulators
   - Wearable Simultaneous Vision Visual Simulator
   - Ocular Section Microscopy
   - Multi-meridian Air Puff corneal deformation OCT imaging
   - Improved Ultrasound imaging Methods
   - Full-angle spatial compound of reflectivity imaging
   - Acoustic Radiation Force Impulse (ARFI) imaging
   - Phase Coherence Ultrasound Imaging
   - Ultrasound-based screening for leukocyte screening
   - Atomic Force Microscopy based nanobiomechanics in cells and tissue
   - Single-Molecule Force Spectroscopy based on Atomic Force Microscopy to detect "missing link" conformers in the amyloidogenic cascade and quantifying the conformational polymorphism observed in the amyloidogenic proteins
   - New PET devices
   - Semiconductor positron detectors for β spectrometry
   - Positron traps and moderators to generate high energy resolution

positron beams for scattering experiments
- Models for Positron scattering and to simulate tracks and positron transport in biologically relevant media
- Compton cameras for hadron therapy treatment monitoring
- 3D proton-CT scanner with proton-range verification capabilities
- Nano-radiotracers
- Nanoparticle probes for positive contrast magnetic resonance
- Atomic Force Microscopy based nanobiomechanics in cells and tissue
- Detection methods of glycoproteins isoforms
- Chemical and biological sensors (resistive and magnetic) of breath
- Surface acoustic wave sensors (biosensors)
- Oligonucleotide-based functionalized biosensors
- High-performance devices for advanced biosensing (viral proteins and tumor markers)
- Functional two-dimensional materials (Graphene) chemically linked to RNA or single-stranded DNA oligonucleotids
- Implantable flexible and biocompatible neuroprobes
- Epidermal flexible sensor wearable devices
- Biosensors and lab-on-chip devices for measuring biomarkers in biological fluids
- Machine learning platforms for data processing and functionalized biomarkers
- Corneal and Intraocular Lens implants
- Retina on-a-chip
- Artificial and synthetic microbiotes
- Peptide marker vaccines

4. Some of the groups excel in technology transfer with patent licensing to industry and successful spin-out ventures (i.e. 2EyesVision, Plenoptika, Oncovision, Bruker BioSpin) or are in the process of launching one (i.e. PetInnovation SL). Demonstrated and potential applications include:
   - 3-D Quantitative image-based cataract surgery, being cataract the most frequently performed surgery in the world.
   - Accessible refraction with low-cost, portable wavefront-based autorefractometer (in low resource countries)
   - Selection of contact lens and intraocular lens with SimVis Technology
   - Early detection of keratoconus (a disease affecting 4% of the population)
   - Breast cancer screening through ultrasound

- Early detection of micro-calcification
- Screening of meningitis
- Early diagnostic and disease evaluation at the cellular level of cardiovascular disease and tumors through nanomechanical markers
- Prevention of Post-Traumatic Stress Disorder
- Brain tumor detection
- Heart evaluation
- Tumor limit delineation
- Early in vivo detection of angiogenesis, microcalcifications, neutrophils and oxidised phospholipids.
- Hadron therapy
- Non invasive estimation of the dose distribution in cancer therapies
- Exhaled air detection of diabetes, kidney, liver and respiratory disease.
- Fast, reusable, portable, sensitive, real time, low sample volumes biological sensing
- New disease markers and markers of alteration of immunoglobulins in breast milk
- Neural probing
- Real time and continuous monitoring, and early diagnosis of disease though wearable devices
- Improved detection of thrombin
- Activity measurement of DNA repair enzymes for chemotherapy-induced DNA damage
- Detection of anabolic androgenic steroids
- Detection of biomarkers of inflammation processes
- DNA sequence detection in genetically-modified organisms and bacteria.
- Toxicological studies of drugs for retinal disease on a retina-on-chip
- Impact on development of advanced materials for implants (for ophthalmology, orthopedics, etc), spinecord lesion repairs and foreign body reaction
- Correction of presbyopia and cataract through novel intraocular lenses
- Biocompatible corneal bandages for corneal wound healing
- Corneal onlays, inlays and implants for corneal treatment
- Personalized diet and microbiome-based strategic therapeutic interventions
- Zoonosis and prevention and animal and human virus-borne diseases

3. KEY CHALLENGING POINTS

Although each specific area underpins a series of specific challenges, we summarize here those that we consider common to multiple areas:

1. Challenges in translating results on phantoms or preclinical data to patients.
2. Establishing fruitful collaborations with clinical doctors in the hospital is challenging, particular as CSIC institutes are generally not hosted in hospital campuses.
3. Areas requiring high levels of multi-disciplinarity, which may be challenging to access funding and even to select the appropriate forum for publication. It is an area requiring continuous leaving of the comfort zone.
4. Commercial potential not fully exploited, as it requires an appropriate ecosystem: patent attorneys with specific knowledge, access and knowledge of the specific corporate environment, challenges to license and spin-out creations, and long regulatory processes in medical devices and therapeutic products, requiring costly clinical trials.
5. Added difficulties by the requirement of ethical committees and strict protocols for human studies.
6. Highly experimental area, requiring high levels of funding.
7. Not all institutes (particularly those in physics/engineering/chemistry) have access to biology labs with trained technicians.
8. Need to prove safety and specificity of the diagnostic techniques and therapies.
9. The wide spread of areas make it difficult to identify all possible groups, and sure enough several are missing.
10. Groups perceive as a thread scarce human resources and bureaucratic hurdles that place them in a lower competitive level than their counterparts in other institutions.
11. With so many projects and responsibilities on the Group Leaders' plates it is difficult to secure everyone's attention to creating collaborative programs or a comprehensive and strategic analysis of the area.

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CHALLENGE 9

ABSTRACT

Nanomedicine uses the size-dependent properties of nanomaterials and the control of interactions at the nanoscale to solve biomedical problems. A plethora of new applications has appeared in the last decade boosting the use of this new field. CSIC groups are very active in Nanomedicine and related areas, with outstanding results in the diagnosis and treatment of pathologies, from cancer and cardiovascular diseases to infections and rare diseases. This leading role in Spain and our international impact will benefit from the coordinated effort we propose here.

KEYWORDS

nanoparticles | drug delivery | molecular imaging | regenerative medicine | nanosensor

CHALLENGE 9

NANOMEDICINE

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1. INTRODUCTION AND GENERAL DESCRIPTION

Nanomedicine, in a nutshell is the application of nanotechnology to solve biomedical problems. Following the definition given by the European Science Foundation, Nanomedicine is the use of nanotechnology for diagnosis and treatment of diseases, and to increase our knowledge of the pathophysiology associated with them. The ultimate goal is to improve the quality of life. Nanomedicine is an ever-expanding discipline that is profoundly interdisciplinary, involving traditional research areas like chemistry, physics, biology and medicine.

A key aspect in Nanomedicine is the use of nanomaterials: chemical entities with at least one dimension smaller than the 100–200 nm size range. Because of their size, these nanomaterials have a unique feature: their properties are size-dependent. Contrary to standard chemical molecules, their properties don't change only due to their composition or structure but also due to nanometer size. The possibility of producing these nanomaterials at nanometric scale, with properties (magnetic, optical, electrical, mechanical) completely different from the same material at bulk scale has allowed the development of new applications in many fields, particularly in medicine. One of the strengths of Nanomedicine is the variety of nanomaterials at hand, with more being continually developed. Some widely used examples are liposomes and solid lipid

nanoparticles and nano Metal-organic Frameworks (NMOFs) for drug delivery, gold nanoparticles and quantum dots for in vitro point-of-care kit, and iron oxide nanoparticles for biomedical imaging and hyperthermia treatment. In all the cases, an important advantage is their large surface area per unit volume, and their ability to provide molecular platforms. Other attractive features are their ready shape/size control and their optical responses.

Another relevant aspect in Nanomedicine is the development of nanoscale characterization tools that provide the molecular landscape of the chemical and mechanical properties of the biological systems that are involved in a given disease. Those tools offer a single-molecule or single-cell view of the first steps, for example, of a virus-cell infection or provide our understanding of how mechanical properties at the single-cell level are transformed in the physiological response of an organ and eventually into cardiovascular or neurological diseases.

Nanomedicine is being applied to virtually all kinds of diseases, for diagnosis (in vitro and in vivo), therapy, or both simultaneously (theranostics). Most nanodrugs have been developed for theranostics of cancer, inflammation/pain, and infection, but there are also uses for cardiovascular diseases, hormonal disorders, and rare diseases, among others.

Nanomedicine (NM) presents several advantages with compared to the traditional drugs

a. Nanomedicine can meet unmet needs, eg. they allow the use of potent drugs that cannot be used by themselves due to their toxicity, poor pharmacokinetic/pharmacodynamic (PK/PD), etc.
b. Nanomedicine increases efficiency/selectivity, thus reducing dose and toxicity: NM improve transport across biological barriers, as well as targeting and controlled and site-specific release in the presence of stimuli (eg, pH, temperature, light, magnetic fields, metabolic processes). The PK/PD are also improved (from absorption to clearance). Besides, many drugs are more stable *in vivo* as Nanomedicines than as free drugs.
c. Nanomedicine facilitates useful combination therapies: Nanoparticles (NP) are used to release the appropriate drug ratio for maximum synergy in the precise location. If the drugs are administered separately, they may not reach the target cells/tissues in the optimum ratio for synergy (Barth et al., 2018).

In simple terms Nanomedicine needs two things: new nanomaterials (new properties, production routes and characterisation techniques) and new applications to solve diseases diagnosis and management. This challenge must be addressed both from basic and applied research, using the interdisciplinary approach that characterise the field of Nanomedicine. Being critic, it's possible to see that basic research in Nanomedicine is working quite well, in terms of scientific and patent production, but the transfer of these results to the clinic is being slower than predicted. Within the last 20 years of development Nanomedicine is characterised by a tremendous potential for new therapies and diagnostics tools, many of them already used at the preclinical level, but a slow transfer to the clinic. The reasons for this are manifold, on one side the lack of standardised characterisation protocols for nanomaterials and problems when scaling production, but also the increased number of variables affecting the *in vivo* behaviour of nanomaterials compared with traditional drugs. A more clinically-focused approach might be needed to crystallize the amazing basic developments in Nanomedicine towards more products approved by regulatory agencies. Examples of these developments are many at CSIC: from the "smart" delivery of drugs after an external stimulus is applied to the enhancement of biomedical imaging diagnosis or the development of in vitro diagnosis kits.

In this chapter we will analyse the current situation of Nanomedicine in Spain and, particularly, at CSIC. Focusing on the elements needed to transform knowledge at the nanoscale into advanced new products, drugs and analytical tools that would benefit the entire society.

Some definitions and acronyms are introduced below:

ADMET: absorption, distribution, metabolism, excretion and toxicity in pharmacokinetics.

Antibody-drug conjugate: are monoclonal antibodies attached to a biologically active drug by chemical linkers with labile bonds.

Biomarker: a naturally occurring biomolecule found in fluids or tissues that is a sign of normal or abnormal biological processes and can be used for disease monitoring.

BNCT: boron neutron cancer therapy.

Contrast agent: a substance used to increase the contrast of structures or fluids within the body in medical imaging, normally without any biological

specificity, and commonly used to improve the visibility of blood vessels and the gastrointestinal tract.

CRISPR-Cas: Gene editing system adapted from a naturally occurring genome editing system in bacteria that may be used to delete, add or change DNA sequences in the genome.

GLP: good laboratory practice, is a set of protocols intended to assure the quality of non-clinical laboratory products in order to help achieving permits for products regulated by government agencies.

GMP: good manufacturing practice, is a system for ensuring that products are produced and controlled according to quality standards, designed to minimize the risks in products intended for clinical use.

Imaging probes: chemical compounds providing signal in, at least, one imaging technique and in vivo selectivity towards a biomarker.

microRNA (miRNA): is a small non-coding RNA molecule that functions in RNA silencing and post-transcriptional regulation of gene expression.

Molecular imaging: the remote detection and quantification of biological processes at cellular and molecular levels.

Nanomedicine (NM): the application of nanotechnology for medical purposes, the use of nanomaterials for diagnosis, monitoring, control, prevention and treatment of diseases

Nanoparticles (NP): materials with dimensions measured in nanometers, showing size dependent properties.

Nanotechnology: the use and manipulation of matter measured in nanometers, i.e. nanomaterials.

Point-of-care testing (PoC): or bedside testing is defined as medical diagnostic device at or near the point of patient care.

Protein corona (PC): ensemble of proteins that dynamically binds to the surface of nanoparticles in a biological media.

QSAR: quantitative structure–activity relationship models are classification models used in the chemical and biological sciences to relate the value of diverse variables.

ROS: reactive oxygen species

Theranostics: the simultaneous diagnosis (-nostics), treatment (thera-) and follow-up of a disease using one single nanoparticle.

RNAi: RNA interference is a regulatory mechanism of most eukaryotic cells that uses small double-stranded RNA (dsRNA) molecules as triggers to direct homology-dependent control of gene activity.

Sensor: devices that convert chemical or physical properties into a measurable signal.

siRNA: small interfering RNA. Double-stranded RNA that interact with RNA interference silencing complex (RISC) directing the degradation of the complementary messenger RNA (mRNA).

2. IMPACT ON BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

Nanomedicine as a major research field is expanding the repertoire of applications in the basic sciences. The interplay between "traditional" sciences (physics, chemistry, biology, medicine) is continuous and mutually beneficial. Nanomedicine-based pharmaceuticals are continuously growing and so are the clinical trials where Nanomedicine is being tested. However, it is also true that the number of papers and possible applications greatly outnumber clinical applications.

The ongoing appearance of new nanosystems and medical applications is part of this feeling. Researchers and clinicians have to assume that the path for a nanosystem to become an approved Nanomedicine is more complex than the path of a traditional drug, and many nanosystems will fail during early development. At this point, a change from "looking for possible biological applications of new nanosystem" to "a rational design of Nanomedicines based in a biological problem/medical need" is needed. To get to that point, it is necessary to understand the biological barriers that Nanomedicines must cross and wisely choose the biological targeting. On the other side, the nanomaterial should be kept as simple as possible for production and biological assessment reasons. Complicated designs make production or clinical approval even harder. The enormous number of specific requirements to translate a potential Nanomedicine from academia to the industry explains the scarce number of

Nanomedicines in our lives nowadays. Nanomedicine community should be more critical: the fact that a particular material has a fancy property doesn't mean is the best option for clinical translation (Eaton, 2012).

The number of applications where Nanomedicine has a role greatly exceed the size and aim of this chapter. For this reason, we briefly summarize here those we consider have a deeper impact or promising future in basic science.

2.1. Production and characterisation of nanoparticles for medical uses

Nanoparticles (NPs) are the main players of Nanomedicine. The variety of NPs in use is overwhelming: lipid-based nanoparticles, inorganic nanoparticles, polymeric nanoparticles, etc. Most projects begin designing and synthesizing new nanoparticles for a particular biomedical function. This rational design (equivalent to QSAR for traditional drugs) still needs considerable optimisation requiring an intense interdisciplinary work. Many challenges have to be addressed before a Nanomedicine is approved for clinical use, such as cytotoxicity and clearance from the body, which strongly depend on the shape, size, surface charge, coating and chemical nature of the nanoparticle. Even small changes in these properties may significantly affect the function and ADMET of a Nanomedicine. The production and characterisation of tailored NPs (with optimised surface decoration and/or compound load), and the scale-up of their production is a research priority. In other words, the standardisation of purification and characterisation techniques is a must.

2.2. Nanoparticle-based delivery

Nanomedicines allow selective delivery, thus increasing efficiency and reducing the required dose and toxicity. Although for some diseases (eg. cancer, infectious diseases) passive accumulation could be a limited option in certain conditions (like the enhanced permeability and retention effect), the challenge for future research would be to improve active targeting systems, reducing off-target accumulation.

A better understanding of interactions with biological targets, as well as processes such as endocytosis, intracellular traffic/processing, *in vivo* degradation, cytotoxicity, immunogenicity, and clearance will be key to design improved delivery systems.

The controlled and site-specific drug release in the presence of stimuli (e.g. pH, temperature, light, magnetic fields and metabolic processes) will be

another active research area. Particular attention should be paid to controlled release in combination therapies, to provide the appropriate drug ratio for maximum synergy (which is quite difficult to achieve with conventional therapies).

2.3. Gene-based nanotechnologies and CRISPR

Gene-based technologies are emerging, such as gene silencing for eukaryotic cells, where gene expression is inhibited by molecules such as RNAi, siRNA, microRNA and antisense oligonucleotides. The development of Nanomedicines for the targeted delivery of these molecules, either alone or in synergistic combination with drugs (for example to block drug-resistance mechanisms), will elicit much attention, and multidisciplinary groups should be encouraged.

Besides, the CRISPR system (clustered regularly interspaced short palindromic repeat) from prokaryotic cells can be adapted to regulate gene expression. The use of the CRISPR-Cas complexes to silence the expression of certain genes, or even repair these genes, will be an extremely important research area. However, issues as the safe delivery to the target cells will require considerable effort, and nanotechnology offers the possibility of better selectivity and cell penetration.

2.4. Nanomedicine for diagnosis

Nanoparticle-based diagnosis is one of the two more important areas of Nanomedicine, together with therapy. The use of NP in diagnosis can be either *in vitro* or *in vivo*. The development of diagnostic kits for the rapid detection of a particular biomarker is widely pursued, from pregnancy tests to the detection of covid-19 antibodies. Nanoparticle-based POC (point-of-care) is one of the most successful fields where Nanomedicine is being applied.

The in vivo diagnosis is based on the use of different imaging techniques. NPs are being used for all the different techniques available: magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT) and optical imaging. In addition, some imaging techniques have been specifically developed based on the specific physicochemical properties of nanoparticles, like Magnetic Particle Imaging (MPI) due to the superparamagnetism of iron oxide NPs. In all these cases the rationale is the same: benefiting from the tailored biodistribution and multifunctionalisation of the NPs while providing useful signals in vivo. This approach is being used for most

diseases, from cancer to cardiovascular, infectious diseases and rare diseases, to name but a few. Most promising approaches are based on the use of nanoparticles providing clinically useful signals: like positive contrast in MRI or the use of nuclear imaging probes in combination with nanoparticles, an approach combining excellent sensitivity with all the good features of many nanomaterials in imaging.

There are many challenges ahead of the in vivo diagnosis: from reducing off-target accumulation to toxicity concerns. As for other applications of NPs, one key aspect is to keep things simple; adding many components to a NP-based imaging probe only complicates regulatory approval and translation to the clinic, the lower the number of components you add to your diagnosis NP the better.

2.5. Nanoanalytical tools for medicine and clinical research

Although Nanomedicines have been proposed for numerous biomedical applications, little is known about their long term fate, biotransformation and long-term toxicity in vivo. It is important to better characterize the interaction macrophage-nanoparticle. More basic knowledge of the cellular mechanisms and the routes that regulate Nanomedicine biodistribution and degradation in vivo are needed to facilitate the approval of Nanomedicines for clinical use. Those aspects could be addressed by the development and improvement of the nanoscale characterization tools based on advanced force and optical microscopes and well as in nanomechanical platforms.

Those tools are also having an impact in mechanobiology. This activity demands the development of high-spatial resolution, high-speed and sensitive microscopes based on atomic force microscope technology to describe in situ the changes of the mechanical properties of single cells as a function of the chemical and physical environment. In addition to the aforementioned applications, mechanical markers (elastic and loss moduli) are being developed to characterize different diseases from cancer to obesity to cardiovascular diseases. The final goal of this activity is to develop of a high-throughput mechanical microscope for applications in clinical research.

2.6. Infectious diseases

Infectious diseases caused by (multi)drug-resistant bacteria and fungi or by new viruses are a growing problem as we have recently experienced. Nanomedicine is dealing with this group of diseases in several different approaches (Lam et al., 2016; Zaidi et al., 2017):

1. Pathogen-specific nanoparticles which do not harm the beneficial microbiota. Two important goals would be: a) specific targeting in systemic infections, and b) detection and eradication of intracellular pathogens.
2. Anti-biofilm NPs, such as using selective delivery of quorum-sensing modulators, reducing the generation of resistance.
3. Anti-viral NPs, from trapping the extracellular viral particles or hindering their attachment to the cells, to inhibiting expression of the viral DNA/RNA
4. Nanovaccines. Although they generate a potent immune response, many variables should be better understood, such as the influence of administration route, the adjuvant/antigen, and the platform. In this field, Reverse Vaccinology can accelerate the development of potent and safer vaccines, and will require multidisciplinar teams.

2.7. Cancer

A vast part of Nanomedicine research is centred in cancer. Nanoparticles are being applied at different stages in disease development: from early diagnosis, to the identification of metastasis and treatment by many different approaches. In this line, we can distinguish three main research lines, many times complementary:

Nanoplatform-based diagnosis of cancer: In this line, a very active area would be the development of tumour-specific probes for the early detection of cancer by imaging, such as MRI, CT, PET/SPECT, photoacoustic imaging and others.

Nanoparticles as vehicles of cancer drugs or as anticancer drugs: Moreover, since nanoparticles can be used not only for diagnosis but also for treatment of cancer (theranostics), efforts should be focused on selective NP drug carriers, and the control of factors such as optimum drug loading (covalent or non-covalent), and controlled release under appropriate stimuli. To achieve good results, the design of the NPs (size, composition, magnetic anisotropy, etc) and the extrinsic factors (magnetic field properties, etc) should be rationalised. This promising area is open to further optimisation.

Nanomedicine for immunomodulation and immunotherapy: Cancer immunotherapy recruits the patient's immune system to prevent tumour progression and eradicate cancer cells. Hot areas are active immunisation, immunotherapy or immunomodulation. Although these techniques often present

systemic toxicity problems, they could be overcome using low systemic amounts of target-selective Nanomedicines, which can be guided to the tumour and concentrated therein using either functionalisation and/or an external magnetic field. Adoptive cell therapy (ACT) improves the patient immune cells either by selecting and expanding *ex vivo* or by genetic manipulation of effector cells to make them recognise a tumour, and is one of the most promising immunotherapy strategies.

2.8. Cardiovascular diseases

Cardiovascular diseases are the main cause of death worldwide. The use of Nanomedicine for these diseases is somewhat newer than for other diseases like cancer. However, this situation is rapidly changing with numerous examples at preclinical and clinical levels where the use of nanoparticles is being demonstrated. Nanoplatform-based therapy is quite challenging due to the systemic nature of atherosclerosis, however also here there are numerous reports on the positive use of Nanomedicines of very different nature.

More successful is the use of nanoparticles in the diagnosis stage. The combination of imaging methods and nanoparticle-based probes is being used for the early diagnosis of cardiovascular diseases. This is particularly true for the silent disease behind most of the cardiovascular problems, atherosclerosis. In this case the early, pre-symptomatic stage, imaging-based diagnosis and characterisation of the disease is the key factor. As for most applications, the critical aspect is the development of diagnostic nanoparticles that can be translated into the clinic. On this sense, not only the imaging probe must demonstrate its usefulness but the design must always keep in mind what is needed, keeping the nanoparticle as simple as possible to increase the chances of a successful approval by regulatory agencies.

3. KEY CHALLENGING POINTS

Nanomaterials have a tremendous potential for medical applications. Most of the efforts are concentrated in the field of drug delivery as well as diagnostic purposes as we have already mentioned.

3.1. Drug delivery

The search for new drugs to treat diseases considered "undruggable" with small molecules has led to the use of complex molecules, such as, antibodies and other biomolecules. In this context, Nanomedicines can play a key role in

the near future therapies. Among the existent nanodrugs, nanoparticles present advantages in terms of protecting the active principle, which is strongly required specifically when using antibodies or gene products, targeting the cells of interest.

Basic nanomaterials used in biomedicine can be classified in:

1. metallic and non-metallic nanoparticles such as gold, silver, nanodiamonds, silica, magnetite (Fe2O3), quantum dots;
2. biocompatible nanopolymers: poly(lactic-co-glycolic acid (PLGA); Poly-L- lactic (PLL), polyethylenglycol (PEG), polyethylenimine (PEI), cyclodextrines, chitosan;
3. nanovesicles (lipid nanoparticles, liposomes, niosomes);
4. bio-inspired nanomateriales obtained from the assembly of biomolecules such as proteins or nucleic acids; and
5. hybrid nanomaterials formed by two or more inorganic/organic nanocomponents which, in addition to combining the properties of its constituent materials, have other properties arising from the synergy between their components.

These materials are functionalised with drugs and molecules for enhancing cellular uptake and delivery to the target tissue. Understanding the behaviour of these nanomaterials in vivo is of paramount importance for the development of efficient Nanomedicines. A key factor in their preparation is the control of the functionalisation and size as well as the characterisation of the number of functional units as drug pharmacokinetics and biodistribution may depend on the functionalisation method.

An important issue is also the interaction of the nanodrugs with the blood components as nanomaterials can be altered by binding of the blood proteins. Protein corona (PC) influences fate, uptake and stability and it may also affect the biodistribution and excretion pathways of nanomaterials, giving rise to toxicity issues due to unwanted and unexpected increased accumulation in specific organs. Although PC may have positive outcomes such avoiding renal clearance and increase cellular uptake, the adsorbed proteins not only may hinder targeting ligands conjugated on the NP surface but also could lead to aggregation and removal of the nanodrug by the reticuloendothelial system (RES). In general, PC formation is reduced by covering the nanodrug with a neutral polymeric material such as PEG. However, as it has been shown that to completely avoid PC formation is a difficult task, strategies

focused on controlling protein adsorption on the NP surface are also being investigated.

This complex scenario explains why, despite the great potential of Nanomedicines in therapy, clinical translation of NPs still remains difficult. Indeed, few examples of Nanomedicines are commercially available such as Abraxane (paclitaxel bound to albumin), Doxil (doxorubicine encapsulated in liposomes), Onivyde (irinotecan encapsulated in liposomes) and several ADCs (Brentuximab-vedotin, trastumubmab-Kadcyla). As both active and passive targeting has shortcomings, extensive research still needs to be undertaken to develop targeted nanocarriers with a significant increase of the overall efficiency of the therapy in the region of interest.

In this sense, the development of personalised Nanomedicine approaches capable of being controlled in a spatio-temporal manner by external stimuli to exert their therapeutic effect (e.g. by light, magnetic field, ultrasounds, etc.) are very promising and thus being actively investigated. In addition, due to the recently acquired knowledge of the critical role of tumour stroma in tumour initiation, progression, and metastasis, novel treatment strategies should combine antitumoral with antistromal agents.

At the same time, this should be accompanied by:

i) development of better cancer biomarkers to be used for tumour-specific targeting, and ii) the development and use of models that offer better predictive values for what is observed in the clinics together with standardisation of techniques to acknowledge NPs biodistribution, metabolism, excretion as well as acute and chronic toxicity.

3.2. Nanoparticles as therapeutic agents

In addition to act as drug carriers, nanomaterials can be used as therapeutic agents by themselves. For instance, to kill cancer cells by thermal heating or mechanical vibration using magnetic fields, IR or neutron irradiation near the skin. Moreover, the development of nanoparticles for efficient photodynamic therapy (PDT) is a very active field. PDT is a clinical treatment based on the activation of light-absorbing molecules, or photosensitizers (PSs). Upon light irradiation at a specific wavelength, PSs generate reactive oxygen species (ROS), which are toxic to the targeted disease cells. Boron Neutron Cancer Therapy (BNCT) is a versatile, non-invasive and promising chemoradiotherapeutic technique that targets and destroys malignant tumor cells, while

restricting damage to healthy cells. This dual technique is based on the high nuclear cross-section of the 10B nuclei for neutron capture resulting in cellular damage. Therefore, potentially effective boron carriers are required for this therapy to successful.

One of the most exciting ideas is the design of nanorobots where nanodevices can perform several coordinated actions directed to treat a disease (Li et al., 2018). For example a DNA structure has been designed to transport thrombin inside a cylinder and this structure is opened by the action of nucleoline that is present only in cancer cells, liberating the thrombin at the tumour site.

3.3. Infectious diseases

Thanks to the developments in antibiotics, vaccines and antivirals, infectious diseases seemed a problem of the past in developed countries. However, there are at least two aspects that remind us that this is a continuous fight whose importance is growing, particularly in two cases:

First, regarding (multi)drug resistant bacteria. Infections caused by MDR bacteria is a major health problem that could cause millions of deaths in the next decades. Bacteria like Pseudomonas aeruginosa, Acinetobacter baumannii and Haemophilus influenzae are three major Gram-negative causative agents of infections, included in the WHO global priority list, and for which the traditional direct-acting antibiotic approach is limited by resistance to antibiotics. There is widespread consensus on the need of new drugs which elicit negligible antimicrobial resistance, driving the large-scale research effort to develop innovative antimicrobials. In this field, Nanomedicine should have a leading role: from the delivery of new antibiotics to the use of NPs that, by themselves, have a bactericidal effect. Examples include traditional NPs like Ag particles to new designs based in Cu or Fe. Other approaches that target the biofilm or the quorum sensing are also being investigated with Nanomedicine tools. Many challenges are still present to develop clinically useful NPs against this type of infections but this is certainly one of the key challenges in Nanomedicine.

The second important aspect, sadly very present for all of us, is the use of nanoparticles as antiviral medicines. In this sense the use of nanoparticles as antiviral agents has been demonstrated for many diseases like Hepatitis B, Foot and mouth disease or SARS. But perhaps the most important role of Nanomedicine can be in vaccine development. For example, in the case of SARS-CoV-2 one of the most promising vaccines, at the time of writing this chapter,

is centered in the use of nanostructured lipid carriers loaded with a viral mRNA fragment (RNA vaccines) (Alameh et al., 2020).

3.4. In vivo imaging

In medical practice there is an increasing trend to reduce the number of biopsies by employing non-invasive imaging techniques. In this sense, another important area of development of nanomaterials is the design of probes for molecular imaging with good biodistribution and an improved signal for very early detection of diseases by a variety of in vivo imaging methods such as magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), ultrasounds, optical imaging, etc. However, imaging probes for only one imaging modality cannot provide enough and exact information in the human body concerning anatomical, physiological, or molecular information. The role of NPs in molecular imaging is two-fold: first it is possible to tune the biodistribution and pharmacokinetics in a way impossible to achieve with traditional (molecule-based) probes. Secondly, nanoparticles facilitate the incorporation of two or more contrast agents in a single NP, allowing to achieve wide dynamic ranges for penetration depth and size resolution (being able to achieve single cell or even subcellular resolution). The development of multicomponent NPs that could enable to obtain simultaneously deep-body imaging (e.g. by MRI, CT) together with the high resolution required to guide real-time surgery (e.g. fluorescent optical imaging, SER, etc.). Besides, the addition of a drug cargo to the imaging properties allows the detection and treatment of a disease in the same nanodevice. This concept is known as theranostics. The development of all-in-one nanodevices integrating therapeutic, imaging, specific targeting and controlled drug release/therapeutic activity is very promising. However, still several challenges must be addressed to ensure their clinical transference (Padmanabhan et al., 2016). These include:

- the development of well-defined and reproducible synthetic protocols able to be scaled-up,
- the search for optimal dosage regimes (dose level and frequency) to ensure optimal imaging and therapeutic outcomes,
- the reduction of non-premature release of any of its multi-components, and
- the study of their metabolism, excretion, safety issues and so on.

3.5. Nanobiosensors

As the life expectancy is increasing, there is also an increase need for the development of simple analytical devices that can measure the progress of a treatment or a disease condition. Current gold standard detection assays in clinical diagnostics are limited to laboratories and implied to carry out long procedures, the need of large quantities of samples and skilled personnel to perform both the assay and the analysis. This current scenario prevents simultaneous on-site measurement of different analytes from a single sample at a doctor's office, a hospital or even resource-limited settings. In this sense, biosensors integrating nanomaterials can be small laboratories in where several operations such as separation and addition of dyes could be more easily integrated and miniaturised. Indeed, the so-called labs-on-chip are miniaturised devices that can analyse several parameters in a simple read-out without the requirement of complex instrumentation. This is also important to control populations in remote areas as well as to detect rapidly potential pathogens. In this area, important issues are the development of technologies for the deposition of several sensing molecules on surfaces for the multidetection of several relevant in vivo metabolites. Besides, due to the critical demand of portable point-of-care simple and affordable detection systems for massive screenings and/or their use in resource-poor environments, new technologies are being developed and even older technologies are being refreshed by the use of nanomaterials (e.g. nanobioconjugated paper/plastic settings, lateral flow, microfluidic or inkjet-based biosensors, etc.).

3.6. Blood purification

This is another area of important development. Dialysis has been used for long time in the medical field for the non-selective removal of small molecules from blood based on their size-related diffusion or ultrafiltration across semi-permeable membranes. However, magnetic microparticles and nanoparticles such as Dynabeads can also be used to eliminate toxic compounds or even cells from the blood. This approach offers new therapeutic possibilities such as the direct treatment of systemic infections (e.g. sepsis), and the specific targeting of larger compounds that are not dialyzable (e.g. cytokines, endotoxins).

3.7. Tissue engineering and regenerative medicine

The fabrication of artificial tissues by growing cells in scaffolds functionalised with nanomaterials is a boosting field. Indeed, nanomaterials could provide high control over the properties of scaffolds such as the tuning of their mechanical strength or the controlled release of bioactive agents. This allows

obtaining tissues for bone regeneration, healing devices as well as obtaining artificial tissues that can be used for simulating in vivo conditions for drug testing. Indeed, a very active area of research involved the development of solutions for tissue engineering incorporating even multiple nanomaterials to have in the future a higher control over in vivo integration/maturation, monitoring and long-term safety of engineered tissues.

Regenerative medicine in the nervous system through the use of growth factors or applied electric fields is heavily based on the controlled release and modulation of dendrite growth with optimal electrode materials. Nanostructuring bioactive and electroactive components has yielded to impressive advances in biocompatible electrodes that can be use by direct contact control o by induction methods.

CHALLENGE 9 REFERENCES

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CHALLENGE 10

ABSTRACT

The analysis of socio-cultural, historical, political and economic dimensions of health is fundamental to understand the factors shaping individual and social well-being. This multidisciplinary focus addresses a wide range of health-related challenges. From the relations of human societies with the natural environment and biodiversity, to the plural medical cultures and the variety of resources available to the population to maintain or recover its health. From the social determinants of health and how to overcome health inequalities and its impact on vulnerable groups, to the organization of public health systems and the design of policies to manage national and global health crisis. It is out of doubt the need for research associated to the analysis of (re)emerging infectious diseases and microbial resistance, and the challenges they pose to public health and health systems. The researchers in humanities and social sciences at CSIC are well positioned to lead and coordinate these research challenges, as described in this chapter.

KEYWORDS

ethnobotany | ethnopharmacology | health inequalities | health policies | health systems | history of medicine | medical cultures | microbial resistance | (re)emerging infectious diseases | translational research

CHALLENGE 10

SOCIO-CULTURAL, HISTORICAL, POLITICAL AND ECONOMIC DIMENSIONS OF HEALTH AND MEDICINE

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1. INTRODUCTION AND GENERAL DESCRIPTION

Health is one of the most important areas of human development and, along with quality of life, occupies a prominent place in the academic world, being studied from various theoretical perspectives and from different scientific disciplines. These studies try to explain, from the micro to the macro, what it means and what elements are involved in the health of individuals in particular and of society in general. However, given the diversity of perspectives, there is no consensus on the definition of health.

At the beginning, the study of health focused on biomedical research into disease. This approach has shown its limits in understanding health in its most complete dimension, as defined by the World Health Organization as early as 1946: "health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (preamble to the WHO Constitution, 1946). This "positive" orientation of health is later taken up as a universal human right (Declaration of Human Rights, art. 25), being considered today not only a right, but also a value in itself, an aspiration and a social demand (Palomino et al., 2014).

This implies a change of paradigm, since it implies that the analysis of the health of individuals and society requires a joint examination of the physical, psychological and social elements involved. It must be an integrative approach; it is not enough to have one based on complementarity —that is, one that studies separately the physical aspects (biomedical paradigm), the psychic aspects (mental health) and the social aspects (social determinants model) as if they were three independent spheres of the human being. Body, mind and environment are part of a symbiosis that is difficult to compartmentalize. In this sense, health is understood as a (non-linear) process in which biological events, lived experiences (personal history and lifestyles), the physical environment (environment), social environment (social structure, health care system, social services, work, housing, etc.) and cultural environment (norms and values) of people, as well as their emotions and perceptions, are involved. In the words of Canguilhem, every human being is inseparably a biological, social, emotional and sensational being, and a being of knowledge, so that health is expressed in these four dimensions together (Contandriopoulos, 2006).

We have to take into account that the health of a society is not the mere sum of the health of each of its individuals (Benach and Muntaner, 2005). The study of the health of a population involves, in the same way and inexorably, the study of the social factors associated with it. This broad conception of the study of health contemplates multiple social dimensions that make it possible to investigate the origin of numerous illnesses, as well as to offer suggestions for the design of initiatives aimed at prevention and for their treatment.

From this perspective, this challenge has a multidisciplinary orientation, starting from the humanities and the social sciences. The objective is the study and understanding of cultural, historical, economic and social factors that affect outstanding aspects of public health. From these areas of knowledge and research, relevant contributions can be made to health challenges such as: (i) the social determinants of health (the relationship between population health and income level, educational level, nutrition, living and working conditions, unemployment, housing, supplies, transport, etc.); (ii) the organization of public health and health systems (the different health policies, the sustainability of public health systems, and the viability of certain models of care such as universal care or the commitment to a hospital-centric model); or (iii) challenges in the field of global health (the fight against (re)emerging infectious

diseases and microbial resistance, which can generate pandemics and social alarm), cross-border, intergovernmental and intersectoral management of health crises.

2. IMPACT ON BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

From the perspective of the humanities and the social sciences, and taking into account the competencies of the CSIC groups involved in this challenge, the main topics raised by the participants were articulated around the following axes:

i. ethnobotany and ethnopharmacology, past and present; natural and cultural heritage, tangible and intangible;
ii. medical cultures, past and present;
iii. inequalities in health, environment, health policies and public management of health crises;
iv. evaluation of the scientific and social impact of scientific collaboration networks in biomedicine;
v. (re)emerging infectious diseases and microbial resistance.

2.1. Ethnobotany and ethnopharmacology, past and present; natural and cultural heritage, tangible and intangible

Ethnobotany (Harshberger, 1896), a multidisciplinary subject at the interface of the natural and social sciences (Barrau, 1971), studies the relations between human societies and plant biodiversity, from which a body of traditional knowledge originates that is interesting in itself and as a basis for the development of products beneficial to humanity (Garnatje et al., 2017a,b; Vallès, 2019 and references contained in this review). The same approaches to other living organisms (ethnomycology —often dealt jointly with ethnobotany— ethnozoology and others such as ethnoecology) complete the panorama of ethnobiology, whose thematic breadth encompasses both nature and culture, i.e., tangible and intangible heritage.

The definition of health established by the WHO in 1956 ("A state of physical, mental and social well-being, and not merely the absence of disease") and that which, with a similar intention, was proposed at the 1976 Congrés de Metges i Biòlegs de Llengua Catalana ("A way of living that is autonomous, supportive and joyful") are not at all alien to the interaction between people and plants

(and fungi). The first is the management of the natural environment by human groups and the popular knowledge that is generated, called ethnobiodiversity by some authors and which constitutes the fourth pillar (together with genes/genomes, taxa, and ecosystems/biomes) of biological diversity. Going one step further, the Ulrich effect, according to which the contemplation of vegetation (and water) causes a decrease in stress and the induction of a state of lucid relaxation, manifested by the greater amplitude of the alpha waves of the electroencephalogram (Ulrich, 1986), is also linked to the relationship between people and the plant world.

Ethnobotany (hereinafter considered to include ethnomycology) has basically two aspects linked to health that make it a necessary discipline to address future challenges in biomedicine and health in general. On the one hand, there are medical or pharmaceutical aspects, which are closely related to ethnopharmacology (Etkin, 2001). Medical or pharmaceutical ethnobotany affects the field of medical culture or the anthropology of medicine and is of interest both for knowledge of the uses and customs of a society in terms of prevention, treatment of diseases and even their conceptualisation, and for the development of new drugs (Prance et al., 1994; Brower, 2008; Tringali, 2012; Skirycz et al., 2016). An example of the latter is the development of artemisinin, a powerful antimalarial, from a plant used in Chinese pharmaceutical ethnobotany, which won the Nobel Prize in Physiology or Medicine in 2015 (Tu, 2016). Furthermore, the history of science and historical or diachronic ethnobotany allows not only to establish the traceability of traditional uses of plants in medicine, but also a more adequate understanding of the mechanisms of construction of knowledge about plants in various cultures in time and space (Fresquet and Aguirre, 2005; Gras et al., 2017). All this gives clues to establish intercultural patterns of knowledge exchange between different medical cultures (past and present, Leonti and Casu, 2013), whose application can lead to the discovery of drugs to which we alluded, in perfect harmony with the idea of the socio-cultural, historical and economic dimension of the field of work that concerns us.

On the other hand, directly related to health, there is also everything that has to do with food, where again the contributions resulting from the dialogue between the history of science and ethnobotany can be decisive in providing a historical, economic and socio-cultural dimension to many issues related to the challenge posed by the population of our planet today and even more so in the future. Within this area of food-related studies, the issue of food

security (both in its concept of providing food for all human beings and in the fact that this food does not entail health risks) deserves a specific chapter; the scientific resources to face this double challenge have an effective ally in the knowledge accumulated by generations and generations of people about the plants used in food (wild, large crops, minority crops, local breeds) (Rigat et al., 2016).

Both ethnobotany and history of science study the past and present of human-plant relationships in order to project them into the future of humanity, among other things. In this sense, some currently burning issues in medicine, such as emerging (infectious) diseases or pain and suffering, can certainly benefit from the knowledge of intertwined nature and culture, which ethnobotany and the history of science help to inventory, analyse and interpret (Brancher, 2015; Touwaide, 2020).

2.2. Medical cultures, past and present

Medical pluralism is a concept of absolute validity in contemporary societies, as it has been in any society and historical period, despite the different relations of hegemony between medical systems and cultures that coexist within the same social organization (Perdiguero, 2006; Jütte, 2013). In order to adequately address the way in which health is understood and signified, it is necessary to take into account the existing medical pluralism, trying to overcome the limitations offered by a vision in which only academic medicine is taken into account and considered (Brockliss and Jones, 1997).

Medical pluralism means the variety of resources available to the population to maintain or recover their health, which are not limited to the care possibilities offered by the institutionalized medical system based on academic medicine (López Terrada, 2009). There is a wide range of preventive and therapeutic practices coming from both the so-called traditional medicines and the so-called alternative and/or complementary medicines, not to mention the practices carried out without leaving the domestic environment (Gentilcore, 1998; De Blecourt and Usborne, 1999). Obviously these "non-regulated practices" are a huge drawer, as they include very varied ways of understanding health and illness, but also very different means of healing, from those based on empirical treatments to recourse to means and practices of belief or religion, more or less sanctioned by custom, and including the survival of past medical systems such as homeopathy, animal magnetism or phrenology. All this without forgetting the ubiquity of popular healing practices -where

academic medicine, the empirical use of plants and religious rituals are mixed-among people belonging to different social groups throughout Europe (López Terrada, 2009).

Furthermore, as has been widely demonstrated, the presence in a particular society of different resources and types of practices, i.e. the possibility of a patient to choose between different alternatives for treating his or her illness, was and is due, not necessarily to a lack of doctors and other practitioners of academic medicine at any given time, which is how the more traditional historiography has justified the presence of other forms of health seeking, but to complex cultural reasons, in which the patient is the central agent (Schmitz, 2018).

Therefore, patients should be included as active elements of medical pluralism in order to understand fundamental aspects such as demand, decisions, social relations and healing practices (Zarzoso, 2001). Only in this way can the coexistence of medical systems be adequately analysed and explained, avoiding falling into clichés such as attributing the presence of these resources to "popular" ignorance, "superstition" or the poverty of the rural world or colonial society, both in past societies and in our contemporary societies (Morales, 2017).

The historical dimension of the encounters between different medical cultures is the contribution to a future development of research, in which we would converge with other researchers in the area in order to design strategies to face the challenges in the field of health. Basically, and from social and cultural approaches to the past, typical of the social and cultural history of medicine, in which we have been trained and which we practice, the field of interest of the history of health and disease has been extended beyond the hegemonic medical culture to also include non-regulated empirical practices, magical-creative resources or health strategies resulting from the cultural encounter or hybridization between medical cultures in contact (Slater, 2014).

We are therefore faced with a complex problem. In a large part of the world, it is necessary to resort to traditional medicine to ensure the care of the entire population. In rich countries, alternative and/or complementary medicines are a growing option, despite the accessibility and diagnostic and therapeutic capacity of scientific and experimental medicine. In order to try to understand this phenomenon, it is necessary to consider the contributions made by the socio-health sciences in the last fifty years, but also the perspectives provided by the history of medicine and science.

2.3. Inequalities in health, environment and health policy

What are social inequalities in health?

Health is not a linear process from total well-being to illness, nor can it be measured as if it were temperature, with some system determining which person has a higher level of health than another. Neither can we pretend that the whole population has the same level of health, if this were possible to test, it would not even be desirable. Thus, any difference in health should not be understood as something that must necessarily be avoided or diminished. For example, the higher prevalence of coronary heart disease among the older population compared to the younger population cannot be considered an unfair difference, since it is the cause of a natural ageing process. Thus, some differences in health between men and women would also fall into the category of non-unfair biological variation, such as health problems specific to different reproductive systems. However, many of the differences that occur between different populations or social groups (including differences by gender) cannot be considered biological, but must be sought in other inequalities considered unfair and avoidable (Whitehead, 1992). Injustice here introduces a moral and ethical dimension that indicates that such differences are unnecessary, avoidable and unjustified. Thus, the study of social inequalities in health involves going to the causes of inequality.

WHO defines social inequalities or inequities in health as health disparities within a country and between countries that are considered inappropriate, unjust, avoidable and unnecessary (not inevitable or irremediable) and that systematically burden populations made vulnerable by underlying social structures and political, economic and legal institutions.

In the study of health inequalities, it is known that different types of inequality (of income, quality of life, status, social capital, etc.) often accumulate in social groups with well-defined characteristics. People with greater economic, social and political disadvantages have worse levels of health and get sicker and die more, have a greater number of chronic and disabling diseases, than those in more favoured positions. Hence, the social, political and economic structure must be considered as part of the structural determinants of the health of the population, since it has an unequal impact on the health of the population.

For all these reasons, the objective of an equitable health policy is not to eliminate differences in the health of the population, but to eliminate or reduce those factors that are avoidable and unfair and affect the health of citizens. These are related to health services (care, prevention, living habits, access to

health services), but also to basic public services (housing, work, social services, education, reconciliation, etc.), to the environment in which one lives (pollution, water quality, energy, etc.), to personal and community economics, etc. All these elements act in an interactive way, as determinants or explanatory factors of the state of health, as shown in the Lalon Report by Palomino et al.(2014). This is why health must be understood within a social and collective context, in which as important as medical progress (understood as the fight against disease) is social, political and ethical progress towards reducing social inequalities. In this sense, theories and models have been developed that widely explain the effect of social determinants on health (Dahlgren and Whitehead, 1992; Daponte, 2009).

As was the case when explaining the symbiosis between the physical, the psychological and the social to describe the concept of health, it is necessary to take this trilogy into account in the study of health inequalities, because they are not three independent axes that influence one another, but are in constant interrelationship. Social inequalities are incorporated into physical and psychological inequalities from the womb. It has been shown that abuse before birth (poor intrauterine care, consumption of toxic substances, poor diet, etc.), poverty and marginality can modify the brain of the fetus and condition its future physical and psychological health (Sherindan et al., 2012).

In conclusion, if as stated in the Charter of Human Rights of the United Nations we consider health as a right, social inequalities in health are a limitation to the achievement of this right and should therefore be sought to be eliminated.

Determinants and impact of national and global health policies. Managing health crises.

Along with socio-economic determinants, national and international health policies are also a powerful determinant of population health that can be studied from three perspectives.

Firstly, the content of national health policies, the way and even the place where health is provided, its financing, its coverage in terms of the population covered by the system or the scope and quality of its professionals, services and benefits are factors that have proven to have an explanatory potential in the population's health status. Despite the interest of the UN (Resolution of 12 December 2012), not all countries can guarantee universal health coverage (UHCS): 1) today half of the world's population lacks comprehensive access

to basic health services (promotion, prevention, treatment, rehabilitation and palliative services); 2) 100 million people are pushed into extreme poverty after having to pay for health care out of their own pockets; 3) 10% of the world's population spends at least 10% of the family budget on health care. We find cases like this even in developed countries like the United States where health expenses explain more than half of family bankruptcies or in Spain, which stands out for the existence of unmet medical needs specifically in the area of oral health, which is key to the health of the mouth and many other associated diseases; 4) thanks to comparative studies we know that allocating too many resources to certain services —such as hospitals— may mean neglecting other areas —such as community health—; 5) the reforms carried out during the Great Recession have allowed us to estimate the impact on health of measures such as the withdrawal of the CSU or the reinforcement of co-payments for medicines (Moreno Fuentes, 2016).

Secondly, health policies can also be studied as a dependent variable. Different factors such as the ageing of the population, government ideology, economic development, the pressure capacity of some actors, membership of supranational institutions, the type of political system, as well as factors intrinsic to the type of health policies existing to date in a country —e.g. the rate of incorporation of technology or existing population coverage—, substantially influence the health model of the states and, more importantly, their capacity to reform health.

In more developed countries, and generally in democracies, there are complex health systems that try to respond to the needs of their populations. In contexts of fiscal austerity and in view of the constant increase in health expenditure, health authorities have raised the need to introduce reforms that, at the very least, limit the increase in expenditure. Some of these reforms may be more or less acceptable (and therefore easy to implement) and more or less compatible with maintaining the health of the population. These include: the generation of information systems with the participation of different levels of government and health professionals, the evaluation of therapies and drugs to promote evidence-based medicine, the evaluation of the performance of health practices by agencies, professionals and institutions, the development of clinical practice guidelines or the dissemination of reference models and good practices, among many others (Moreno Fuentes 2015, 2018).

Thirdly, and not only due to the crisis of the COVID-19, there is increasing interest in the implications of local and global health policies in what has been

called Global Health, both in situations of normality and in those other circumstances, increasingly frequent, health emergencies such as pandemics, which represent "a serious threat to the basic structures or fundamental values and norms of a social system, which under time pressure and in uncertain circumstances requires critical decisions" (Rosenthal et al., 1989:10).

In addition to global health policies, it is specifically important that states and civil society (including scientists) are prepared for crisis management, a task that places us all in a difficult position where strategic actions with profound implications must be taken. These strategic tasks imply:

1. understanding what has happened (causes of a crisis), what is happening (the immediate threat and the time frame for mitigating it) and what may happen next (likely course of events, consequences of possible decisions) (Weick, 1995);
2. taking decisions and implementing them in coordination with other levels of government and with the private and third sectors, and between different sectors of public policy (social, health, security or economic, among others);
3. socially construct the "definition" of a crisis, which, regardless of other implications, and in terms of management, can have important consequences in terms of ensuring that decisions are acceptable, as well as shortening or lengthening the duration of the crisis by reducing or extending the objectives or the results sought.

2.4. Assessment of the scientific and social impact of scientific collaboration networks in biomedicine

Scientific performance evaluation systems put increasing weight on demonstrating both the scientific and social impact associated with publicly funded projects (Bornmann, 2013; REF, 2011). In the biomedical field, the growing demand for demonstration of social impact is reflected in the expectation of achieving research results that are useful for medical professionals and patients. The perception that the advances achieved in basic research in biomedicine have not contributed as systematically as expected to the generation of new treatments or improvements in patient care has led to the design of numerous public initiatives to address this issue (Ioannidis, 2004).

These initiatives aimed at facilitating the "translation" of scientific discoveries into beneficial applications and practices are often geared towards the formation of networks involving actors from multiple disciplines and

institutions. The idea behind these initiatives is that the formation of networks composed of heterogeneous actors favours the existence of multidirectional information and knowledge flows, as well as new learning processes. For example, knowledge flows ranging from basic scientific advances to the development of new medical treatments and clinical practice, and from clinical practice to basic science through the generation of evidence-based research questions (Barberá et al., 2014; Molas-Gallart et al., 2016; Llopis & D'Este, 2016).

Despite the importance of these collaborative networks, the interaction between a wide variety of actors from different professional communities and institutional settings poses significant challenges from an organizational perspective. These challenges provide an opportunity to examine how interprofessional links are created and effectively articulated, involving actors with different knowledge bases. For example, advances related to the introduction of digital technologies in areas such as genomics and radiomics are associated with collaborative networks that incorporate new actors, such as researchers in computer science and artificial intelligence. Moreover, these new technologies are modifying the role of actors such as geneticists and radiologists, who become the interface for the translation of computational knowledge into clinical applications, with profound consequences on the organization of research activities and clinical practice (Hilgartner, 2017; Meseguer et al., 2019).

In addition to cognitive heterogeneity, these collaborative networks lead to increased institutional diversity (Greenwood et al., 2011), since they involve actors who are subject to different social norms and organizational cultures. For example, research in areas such as rare diseases and chronic diseases (such as diabetes or cardiovascular conditions) crucially incorporates the input of patient associations in the framework of collaborative networks between basic and clinical researchers (Barberá et al., 2014). The different institutional logics governing the behaviours of these actors have demonstrated their importance as a source of opportunities, but also of barriers and resistance (Llopis and D'Este, 2016).

In this context, the analysis and evaluation of scientific collaboration networks in the biomedical field aims to understand how the various actors involved overcome the organizational challenges associated with the cognitive and institutional complexity of these networks, or why they fail in their ability to mobilize the human and physical resources necessary to achieve the

proposed objectives. The results of these analyses should lead to relevant recommendations for medical professionals and policy makers in support of collaborative research in health.

2.5. The global challenge of (re)emerging infectious diseases and the new social epidemiology

In the late 1970s and early 2000s there was a jubilant health optimism in the international community. By 1977, smallpox had been officially declared extinct worldwide. In 1978, WHO proclaimed in its Declaration of Alma Ata "Health for All by the Year 2000" that, thanks to the development of primary health care on a global scale, by that year all humanity would be immunized against most infectious diseases and basic health care would be guaranteed for all men, women and children, regardless of social class, race, religion or place of birth. That same year, 1978, the identification and isolation of the first retrovirus pathogenic to humans - known as HTLV-1 - by the research team led by Robert Gallo at the National Institutes of Health (NIH) in Bethesda, seemed to augur well for research into cancer, so-called "slow virus infections" and a number of enigmatic conditions, including multiple sclerosis and the set of conditions included in that "catch-all" known as "systemic autoimmune diseases". For a moment, humanity seemed to have within its grasp not only the utopia of a definitive victory over infectious diseases, but also the key to resolving many of the chronic non-infectious conditions with the greatest social and health impact.

Under these circumstances, the new infectious diseases detected since the 1960s (for example, Machupo and Marburg hemorrhagic fevers, or Lassa fever), were considered mere "anomalies" insignificant for a medicine that seemed to be managing to free humanity from one of its heaviest burdens. In the course of the following decades, these health "anomalies" have grown unstoppably in number and relevance (think, for example, of the impact of epidemic outbreaks of new conditions such as legionellosis, Ebola hemorrhagic fever and the various respiratory syndromes caused by coronaviruses: SARS, MERS, COVID-19). And, in fact, since the 1980s there has been an upsurge of infectious diseases all over the planet (including the developed West), at the expense not only of the emergence of diseases apparently unknown to date - the so-called "emerging infectious diseases" - among which HIV-AIDS should be highlighted, but also of the return of old, known and supposedly controlled diseases - called "re-emerging infectious diseases" - such as malaria, tuberculosis, syphilis and other sexually transmitted diseases (STDs).

This change in global health trends has fuelled in recent decades a growing response to the biomedical model from very different sides, raising many questions about the appropriateness of its discourse and practices in the face of infectious diseases in a planet increasingly subject to their designs. In fact, the current health situation of the planet raises quite obvious questions, although not sufficiently addressed, which are essential keys to understanding and successfully facing the new health challenges of the 21st century:

1. the never-ending and irreversible nature of the health achievements of the 20th century;
2. the questioning of the validity of linear and progressive models to explain the evolution of the epidemiological profiles of human populations, which can both progress and return over time;
3. the growing interdependence between the levels of health of the different regions of the planet, inherent in globalisation and, ultimately, the impossibility of solving in isolation the health problems of certain populations or social groups while ignoring others, much less in the case of the majority of humanity; and
4. the close connection within human societies between levels of health and those of socio-economic well-being (Arrizabalaga, 2000, 2016, 2018b).

In the new circumstances, social epidemiology is experiencing a renewed boom. This is reflected in a proliferation of studies that focus on health inequalities and include social factors to explain health and disease phenomena in societies and human groups (Wemrell et al., 2016, pp. 157-158). But also in the appreciable efforts to integrate social parameters and theories in different lines of development of epidemiology, such as ecosocial theory (Krieger, 2012), Latin American social medicine (Tajer, 2003), theorization of psychosocial determinants of health and disease (Wilkinson, Pickett, 2009), and analytical frameworks based on complex systems science (Jayasinghe, 2011).

In the last two decades, new conceptual tools have also been developed and applied to this field in order to address the challenge of aligning empirical results of epidemiological research with social theory and environmental sustainability (Wemrell, 2016: 160-163). Three of these conceptual tools are particularly relevant: (1) the determinants of health and disease at the macroscopic level, which include concepts such as "social gradient" and "lifestyle" in contrast to "way of life" (Perdiguero, et al., 2001); (2) the categories of human and health differences such as race/ethnicity, sex/gender and

socio-economic/class position (Ng, Muntaner, 2014); and (3) the concept of embodiment to understand the distribution of disease patterns as biological expressions of social relations within a framework of renegotiation of the boundaries between "the social" and "the biological" (Krieger, 2005).

The emphasis of all these innovative currents on the diachrony of epidemiological ideas and practices reminds us that epidemiology has always been essentially a historical science except after World War II, when risk factor-based epidemiology (RFE) became hegemonic. Not by chance, RFE have been one of the privileged fields for the cultivation of modern game theory whose Cold War origins lie, as in the case of rational choice theory, in Pentagon-funded research to guide military decisions and win the ideological battles of our time (Amadae, 2003, quoted by Jacoby, 2010: 211). Today there is a growing perception that the RFE is a model that has been overcome by its inability to provide adequate responses to the new challenges of global health. These new challenges have led to a renewed emphasis on the "eco" and "social", associated with the growing concern about the climate crisis and the loss of biodiversity as a result of intensive exploitation of land and water natural resources and the disrespectful use of the territory, among others (Arrizabalaga, 2018a).

3. KEY CHALLENGING POINTS

Based on the discussion developed in the previous section, we highlight the main challenges that we consider fundamental to address in the coming years within the framework of the challenge of health and medicine:

The social determinants of health: The relationship between the health of the population and its level of income, level of education, food, living and working conditions, unemployment, housing, supplies, transport, etc., in addition to access to health care systems, will continue to be a fundamental issue that can be summarised in the need to continue to take the relationship between health and inequalities very seriously.

Organisation of public health/health systems: The study of health systems and models of care is needed in the coming decades. Policies to reduce public spending on health and policies to privatise put at risk the management of public health, which is universal and equitable. Reflections and proposals are needed to ensure the sustainability of public health systems and the viability of certain models of care such as primary health care.

Global health: Some of the great challenges in the field of what we understand as global health, would be

- The fight against emerging infectious diseases that can become pandemic and generate great social alarm because of causing high numbers of sick and dead: Ebola and other pathogens, coronavirus - to give a recent example - a possible new flu pandemic, etc.
- Microbial resistance to antibiotics that make it difficult to treat certain bacterial diseases (tuberculosis, gonorrhoea, salmonellosis, etc.).
- Health problems resulting from population movements for various reasons (migrants, displaced persons and refugees due to war and other types of catastrophes, travellers, etc.).
- Impact of climate change on health: as the WHO states, "the climate crisis is a health crisis", which increases respiratory diseases due to environmental pollution, malnutrition and famine due to major droughts, or the spread of infectious diseases (e.g. malaria, dengue and zika) beyond their usual habitats.

Ethnobotany, human societies and biodiversity: Ethnobotany, along with ethnomycology and other related ethnobiological disciplines, uses the approaches of the natural and social sciences in the study of the relationships between human societies and biodiversity. Ethnobotany and the history of science study the past and present of human-plant relationships to project them into the future. Three aspects linked to health make these disciplines necessary to face future challenges in biomedicine and public health in general:

- Medical issues: pharmaceutical ethnobotany, closely linked to ethnopharmacology and relevant to the field of medical cultures and anthropology, is basic both for the knowledge of pluralism in the uses, traditions and customs of a society in terms of prevention, treatment and palliation -including conception- of diseases, and for the development of new drugs.
- Historical ethnobotany, looking towards the future: in connection with the preceding point, historical and current ethnobotanical knowledge allows the establishment of patterns of (inter)cultural exchange of medical cultures and the traceability of plant uses, both of which are useful in the development of drugs and in the delineation of the socio-cultural, historical and economic dimension of health in the society of the future.

- Food security, food sovereignty and health: ethnobotany, historical and current, is fundamental to providing humanity with the necessary food without risks to health. The scientific resources to face this complex challenge have an ideal ally in the traceable knowledge accumulated over generations of human groups on food plants (wild crops, minority or forgotten crops, local varieties or breeds).

New therapies: new therapies are currently being developed based on greater scientific knowledge and technological advances, such as stem cell treatments or genetic therapies. These new therapies raise a number of questions in terms of their social and ethical dimensions:

- The role of ethics in the development of these new therapies: will they be of equal access?, will they stigmatize those people who genetically present poorer standards of health?, will they introduce new inequalities in health or help to reduce them? These are questions that must be asked from the critical and social vision of health.
- Transhumanism, eugenics, genetics: the growing technification of medicine and the possibilities of intervention on bodies, varying them to heal or improve them, opens up a field of ethical reflection of great interest.
- Dazzle by technology: we are witnessing a culture of bio-technological development which, backed by the media, is creating a public opinion that is eager for improvements in the diagnosis and treatment of any disease, returning to the biomedical paradigm that has already been shown to be necessary but not sufficient to improve the health of the global population, and in contrast to the importance of the social determinants of health.
- Pseudosciences and the anti-vaccine movement: the growing use by part of the population of pseudoscientific resources to preserve health or treat diseases, as well as the anti-vaccine movement, which denies the validity of vaccination with arguments that are not based on evidence and threatens to reverse the achievements made in the control of numerous infectious diseases, are fundamental challenges for the coming decades. It is essential to understand the social dimension of these movements, which have a much greater presence in the media and in public opinion than their share of the population.

Gender approach to health: among the various social inequalities in health, gender inequalities affect more than 50% of the world's population in terms of the diagnosis, treatment and study of diseases that are more prevalent

among women, or have a differentiated impact on men; and to which other discriminations and inequalities (economic, labor, social, etc.) are added, which have a specific impact on women.

Non-communicable diseases, lifestyles and health education: chronic diseases (diabetes, cancer, heart disease, etc.) are the cause of increasing morbidity and mortality in younger patients. The relationship between the incidence of these diseases, and health education and lifestyles, are issues that should be investigated from a critical and social vision of health.

The social dimension of pain: pain is one of the most universal elements in human life, but it has been little studied from a social point of view, with the biological dimension and its relationship with the disease taking precedence. As in the case of health, pain cannot be understood in a global way, without taking into account its social dimension, the social, economic and cultural structure, in which "sufferers" live and which determines the way in which they experience this physical and emotional sensation.

Doctor-patient relationships: this is an essential issue in a context of increasing technification of medicine and deteriorating conditions of consultations. The need to improve communication between doctor and patient, bearing in mind their concept of health and illness, and to generate a climate of trust is a fundamental challenge for the coming decades.

Impact of translational research: networks of scientific collaboration in the biomedical field seek to integrate communities of actors from multiple institutional settings and scientific disciplines in order to contribute to the prevention, diagnosis and treatment of diseases. These research networks pose challenges such as:

- the difficulty of integrating heterogeneous and potentially conflicting incentive systems and forms of knowledge
- evaluation of the scientific and innovative performance of these networks: in particular, evaluation associated with inter-disciplinary research processes aimed at generating scientific and applied knowledge.

Palliative and end-of-life care: the study of the end of life is fundamental in an increasingly ageing world in which people's will must be respected and individual choice on how to make the transition towards the end of life must be politically recognised as a right. This is an issue of great ethical and moral content, as well as of great social and cultural significance.

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A lesson that we have learned from the pandemia caused by coronavirus is that solutions in health require coordinated actions. Beside this and other emerging and re-emerging infectious diseases, millions of Europeans are suffering a plethora of disorders that are currently acquiring epidemic dimensions, including cancer, rare diseases, pain and food allergies, among others. New tools for prevention, diagnosis and treatment need to be urgently designed and implemented using new holistic and multidisciplinary approaches at three different levels (basic research, translational/clinical and public/social levels) and involving researchers, clinicians, industry and all stakeholders in the health system. The CSIC is excellently positioned to lead and coordinate these challenges in Biomedicine and Health.

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