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Immunometabolism pathways as the basis for innovative anti-viral strategies (INITIATE): A Marie Sklodowska-Curie innovative training network

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ABSTRACT

The past century has witnessed major advances in the control of many infectious diseases, yet outbreaks and epidemics caused by (re-) emerging RNA viruses continue to pose a global threat to human health. As illustrated by the global COVID19 pandemic, high healthcare costs, economic disruption and loss of productivity reinforce the unmet medical need to develop new antiviral strategies to combat not only the current pandemic but also future viral outbreaks.

Pivotal for effective anti-viral defense is the innate immune system, a first line host response that senses and responds to virus infection. While molecular details of the innate immune response are well characterized, this research field is now being revolutionized with the recognition that cell metabolism has a major impact on the antiviral and inflammatory responses to virus infections. A detailed understanding of the role of metabolic regulation with respect to antiviral and inflammatory responses, together with knowledge of the strategies used by viruses to exploit immunometabolic pathways, will ultimately change our understanding and treatment of pathogenic viral diseases.

INITIATE is a Marie Sklodowska-Curie Actions Innovative Training Network (MSCA-ITN), with the goal to train 15 early stage PhD researchers (ESRs) to become experts in antiviral immunometabolism (https://initiate-itn.eu/). To this end, INITIATE brings together a highly complementary international team of academic and corporate leaders from 7 European countries, with outstanding track records in the historically distinct research fields of virology, immunology and metabolism. The ESRs of INITIATE are trained in these interdisciplinary research fields through individual investigator-driven research projects, specialized scientific training events, workshops on academia-industry interactions, outreach & communication. INITIATE will deliver a new generation of creative and entrepreneurial researchers who will be able to face the inevitable future challenges in combating viral diseases.

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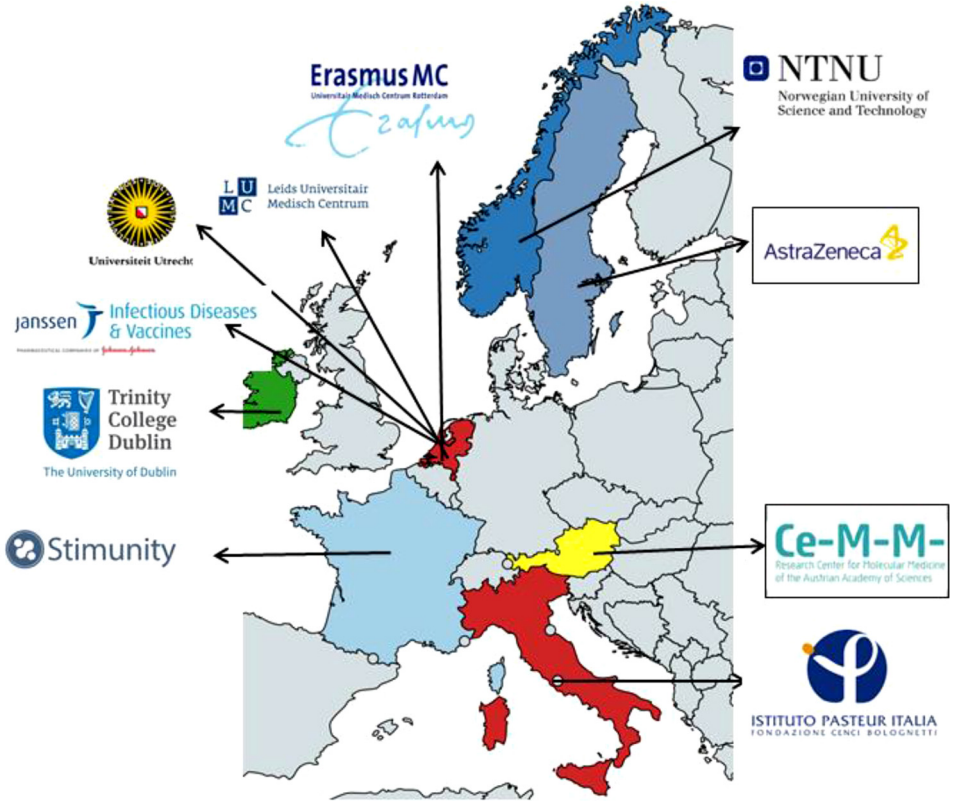

Fig. 1. Countries involved and the INITIATE partners.
INITIATE consists of 10 laboratories in seven countries (colored): Erasmus MC, Leiden University Medical Center (LUMC), University Utrecht (UU), Norwegian University of Science and Technology (NTNU), AstraZeneca Gothenburg (AZ), Research Center for Molecular Medicine (CeMM), Istituto Pasteur Italia (IP), Stimunity (STIM), Trinity College Dublin (TCD), Janssen Infectious Diseases &Vaccines. More information on partners can be found at https://initiate-itn.eu/.

1. Background to the INITIATE program

The past century has witnessed major advances in the control of many infectious diseases, as well as the mechanistic basis of the immune responses that limit virus replication and pathogenesis. Nevertheless, viral outbreaks and epidemics caused by (re-) emerging RNA viruses continue to pose an imminent global threat to human and animal health. During the early years of the 21st century, prominent examples of such viral outbreaks included the H1N1 influenza pandemic of 2009, SARS- and MERS-coronavirus outbreaks in 2003 and 2012 respectively, ebola virus in 2014, 2018 and 2019, zika virus in 2016 and annual outbreaks of dengue viruses in tropical regions - each of which resulted in a dramatic strain on health care infrastructure and major societal disruptions. But since the start of 2020, the global community has been engulfed by the current pandemic of coronavirus induced disease (COVID-19) caused by the novel SARS-CoV-2. At the time of writing, the pandemic rages on, and in the absence of effective therapeutics or a vaccine, as well as a strong international political strategy, the only defence available to humankind is the use of masks, social distancing and personal hygiene. Despite an unparalleled effort by the global biomedical community, knowledge of the mechanisms of pathogenesis remains limited (Vabret, 2020; Blanco-Melo et al., 2020).

Such outbreaks are expected to continue because the environmental, demographic and behavioural drivers of infectious disease emergence are likely to remain intact for the foreseeable future. As illustrated by the current COVID-19 pandemic, high healthcare costs, economic disruption and loss of productivity are additional pandemic consequences that reinforce the urgent medical need to develop new antiviral strategies and therapeutics to combat, not only the current pandemic, but also future viral outbreaks (Hiscott et al., 2020).

The innate immune system is pivotal in host defense against virus infection and this field is now being revolutionized by the recognition that cell metabolism is also critical to host defense against several diseases (Ryan et al., 2019; Zaslona and O'Neill, 2020). Immunometabolism is defined as "the changes in intracellular metabolic pathways in immune cells that alter their function" or "the interface of immune and metabolic responses in disease" (O'Neill et al., 2016). It is now clear that immunometabolism pathways also have a major impact on the host antiviral and inflammatory response to virus infections.

2. Scientific objectives of INITIATE

To drive the emerging field of antiviral immunometabolism, a new generation of scientists is needed with expertise in the interrelationships between viral pathogenesis, host metabolism and immune defences. **INITIATE** MSCA-ITN addresses this unmet need by bringing together multidisciplinary European academics and private sector partners with a passion to understand interrelationships between antiviral innate immunity and host metabolism. INITIATE includes researchers from 7 academic institutions and 3 industrial partners from 7 European countries (Fig. 1); 15 PhD students – Early Stage Researchers (ESRs) have been recruited to INITIATE and each ESR is pursuing an individual research project at one of the partner institutions (Table 1). The research components of INITIATE are complemented with specialized scientific training events, workshops on academia-industry interactions, outreach, communication and public engagement activities. These components of the network are organized by outstanding partners - **Agilent, Biocrates, Elsevier and Sovalacc** – who will provide essential training to the ESRs. The INITIATE training program will deliver a new generation of creative biomedical entrepreneurial researchers at the forefront of antiviral immunometabolism. To meet the ESRs and the research projects, visit: https://initiate-itn.eu/early-stage-researchers/.

3. Innate immunity and immunometabolism

Antiviral immunity is initiated with the sensing and recognition by a set of pattern-recognition receptors (PRRs) of pathogen-associated molecular patterns (PAMPs). Best-studied are the Toll-like (TLRs), RIG-I-like (RLRs), and NOD-like (NLRs) receptors, cGAS-STING and the

Table 1
Early Stage Researchers and their INITIATE Projects.

Early Stage Researcher	Principle Investigators	Project Title
1.Coralie Guy France	Andrew Bowie Trinity College Dublin Dublin Ireland	To determine the role of mitochondria in human inflammasome activation by RNA viruses
2. **Maria Soultsioti** Greece	Eric Snijder Leiden University Medical Center Leiden The Netherlands	To define innate immune- and lipid metabolism changes associated with viral replication organelles
3. **Alix Spahn** Germany	Marit Anthonsen Norwegian University of Science & Technology Trondheim Norway	To unravel changes in metabolic pathways associated with type III IFN-mediated signalling by pneumoviruses in mucosal cells
4. **Chiara Aloise** Italy	Frank van Kuppeveld Utrecht University Utrecht The Netherlands	To determine the role of stress granules as platform for antiviral signaling
5. **Balasubramanian Susma** India	Ron Fouchier Erasmus Medical Center Rotterdam The Netherlands	To unravel the impact of influenza virus and pneumovirus proteins on immunometabolism pathways
6. **Hauke Weiss** Germany	Luke O'Neill Trinity College Dublin Dublin Ireland	To unravel the effect of pneumovirus and influenza virus components on innate immune cell metabolism
7. **Pau Ribo Molina** Spain	Bernadette van den Hoogen Erasmus Medical Center Rotterdam The Netherlands	To determine the role of pneumovirus infection in regulation of MAVS signalling and its effect on immune and metabolic pathways
8. **Xavier Martinez Vendrell** Spain	Marjolein Kikkert Leiden University Medical Center Leiden The Netherlands	To determine the role of picorna- and coronavirus proteases in manipulating cellular protein metabolism and innate immunity
9. **Magdalini Alexandridi** Greece	John Hiscott Istituto Pasteur Italia Rome Italy	To determine the effect of dengue and SARS-CoV2 infections on Nrf2 activation of the oxidative stress response
10. **Juliya Mazej** Slovenia	Angela Santoni Istituto Pasteur Italia Rome Italy	To unravel how innate lymphoid cells modulate inflammation by targeting STAT4
11. **Lorenz Wirth** Germany	Katerina Pardalli AstraZeneca Stockholm Sweden	To determine the effects that IFNs play in the plasticity of innate lymphoid (ILC2) cells
12. **Zsofia Keszei** Hungary	Andreas Bergthaler Center for Molecular Medicine Vienna Austria	To investigate the role of cytokine-induced metabolic rewiring of the liver in chronic viral infections
13. **Mihai Sularea** Italy	Cliona O'Farrelly Trinity College Dublin Dublin Ireland	To determine the interaction between viral detection and innate immune responses in inducible pluripotent stem cell (iPSC)-derived hepatocytes from hepatitis C virus resistant individuals
14. **Adrianna Loverre** Italy	Nicolas Manel Stimunity Paris France	To determine how *in vivo* STING stimulation activates adaptive immune responses
15. **Sonia Marquez Martinez** Spain	Roland Zahn Janssen Vaccines & Prevention Leiden The Netherlands	To unravel the innate and metabolic signature of mucosal vaccination by adenoviral vectors

PYHIN proteins. Activation of PRRs triggers downstream signaling through a myriad of adapters (MyD88, STING and MAVS), protein kinases (Jaks, TBK1, IKKs) and transcriptional regulators (STAT, IRF and NF-κB) to trigger the induction of type-I and III interferons (IFN), and hundreds of IFN stimulated genes (ISGs) that culminate in the generation of the so-called antiviral state; inflammatory signaling converges on the assembly and activation of the inflammasome (NLRP3, Asc, Pyhin). However, what has emerged more recently, is that cells activated by PAMPs also undergo profound metabolic changes. These changes are required for biosynthesis and energy production, but also drive key changes in immune signaling processes (Zevini et al., 2017; Bowie, 2018).

The innate immune response also relies on a wide range of innate lymphocytes (ILCs), which are now considered as critical players of the immune response. Natural killer (NK) cells are the founding member of this family and have been studied for decades and their role in providing early protection against viral infections. The ILC family comprises other four prototypical subsets including ILC1, ILC2, ILC3, and lymphoid tissue-inducer (LTi) cells. Independence from antigen recognition, tissue residency, and poised nature of key genomic loci (*e.g.* those encoding cytokines and other effector molecules) are the main features that make ILCs unique in regulating the early events of viral infection (Vivier et al., 2018).

Increased glycolysis is the hallmark metabolic switch in most immune cells undergoing rapid activation in response to detection of viruses and stimulation of PRRs, cytokine receptors or antigen receptors (Ye and Medzhitov, 2019; Ayres, 2020). In addition, viruses are likewise dependent on host metabolism for energy production and macromolecular synthesis, in order to complete efficient replication. While many mammalian viruses have evolved ingenious strategies to reprogram immune pathways to secure their maintenance in the infected host (Kikkert, 2020; Garcia-Sastre, 2017), few studies have investigated the crosstalk between virus infection, innate antiviral activity and metabolic pathways (Jordan and Randall, 2016). In addition, by-products from the Krebs cycle such as succinate and itaconate act to stimulate or repress the antiviral and inflammatory response to infection (Mills et al., 2018).

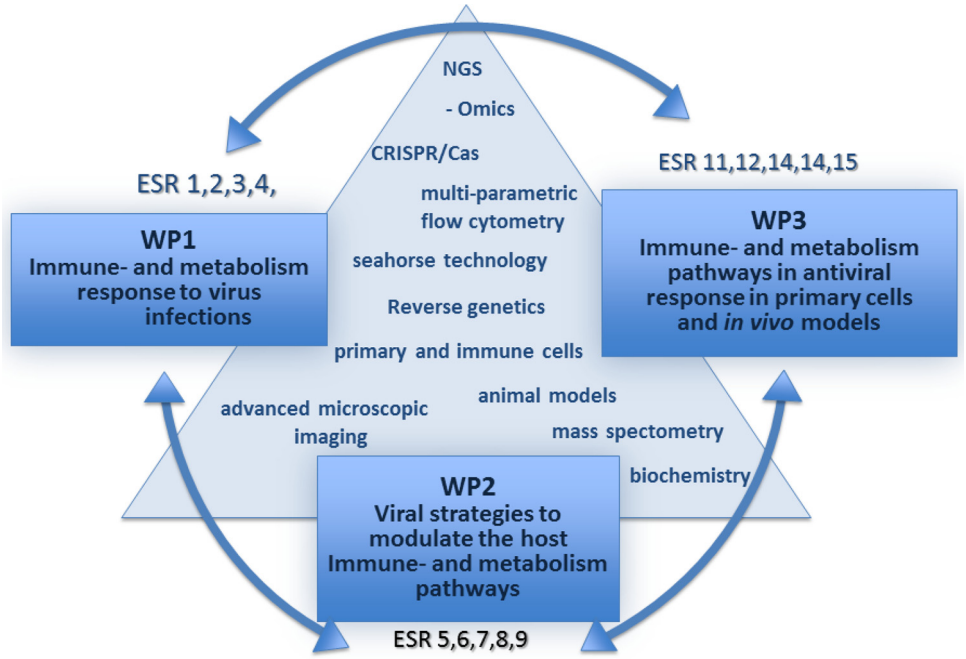

Fig. 2. INITIATE Research WPs.
The interdisciplinary approach and synergy between individual research projects. ESRs are collaborating between and within WPs, all using techniques from the three research disciplines.

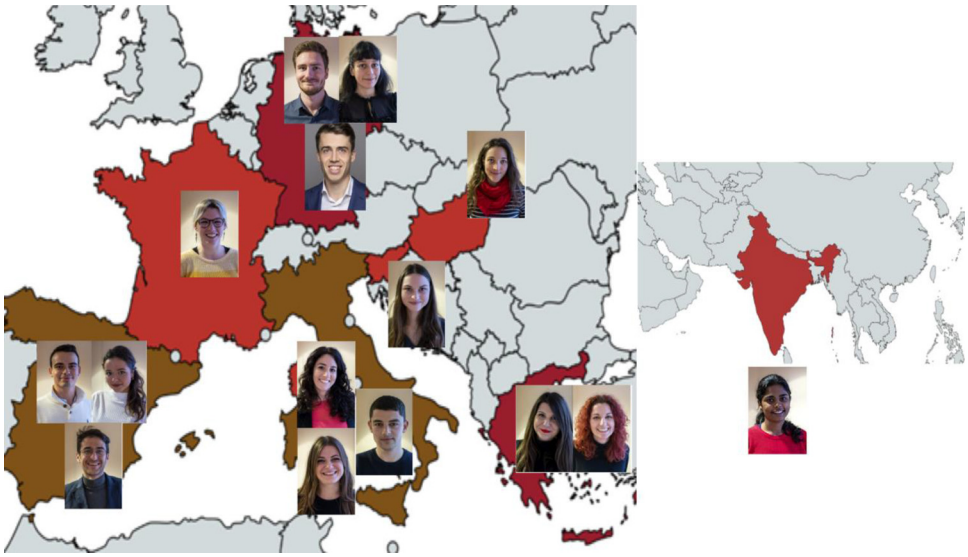

Fig. 3. Origin of INITIATE's ESR Spain: Pau Ribó Molina (left), Sonia Marquez Martinez (right), Xavier Martinez Vendrell (bottom); **Italy:** Chiara Aloise (top), Mihai Sularea (middle), Adrianna Loverre (bottom); **Greece:** Magdalini Alexandridi (left), Maria Soultsioti (right); **India:** Balasubramanian Susma; **Slovenia:** Juliya Mazej; **Hungary:** Zsofia Keszei; **Germany:** Hauke Weiss (left), Alix Spahn (right); Lorenz Wirth (bottom); **France:** Coralie Guy.

4. The INITIATE research program

The research program is divided into 3 work packages (Fig. 2):

A **Identification of the host innate immune and metabolic responses to virus infection, and their interactions.** The objective of WP1 is to obtain in-depth knowledge of the roles of different organelles and signaling complexes (such as mitochondria, signalosomes, stress granules, replication organelles) in modulation of immune- and metabolic pathways in response to RNA virus infections, including influenza virus, pneumoviruses and coronaviruses.

B **Identification of viral strategies that modulate the host immune- and metabolic pathways.** The objective of WP2 is to obtain in-depth knowledge of the interaction of viruses, viral products and viral mechanisms that modulate the immune-and metabolism pathways and the interaction between these pathways. Viral interaction with these pathways will elucidate new functions of viral proteins and modulatory interactions.

C **Determination of the interplay of immune and metabolic pathways in antiviral responses in human derived primary cells and*in vivo*models.** In WP3, innate lymphoid cells, natural killer cells, hepatocytes, dendritic cells and *in vivo* animal models relevant for RNA virus infection will be used to investigate the regulatory interface between immune and metabolic pathways on antiviral immunity.

5. A historic kick-off

INITIATE funding began in May 2019, and in the following months (June-October) the principle investigators recruited an international group of highly motivated ESRs who began working on their respective PhD projects between October 2019 and March 2020 (Fig. 3). The kick-

off meeting took place in Rotterdam, January 20–24, 2020 with introductory greetings, research presentations by the investigators, instruction from industrial partners and a visit to the facilities of Janssen Biologics in Leiden. The investigators and students also had the opportunity to meet the distinguished members of the External Advisory Board – **Dr. Adolfo Garcia-Sastre**, Professor of Microbiology and Director of the Emerging Pathogens Program, Mt. Sinai School of Medicine in New York and **Dr. Albert Osterhaus**, former Chairman of the Dept. of Virosciences at Erasmus Medical Center, current Head of the One Health Platform and Professor of Virology at Hannover University.

News of a novel viral outbreak originating in Wuhan China was already circulating during the kick-off and with new sequencing information available on January 11, it became clear that the infectious agent was a novel coronavirus. Like its SARS predecessor from 2003, SARS-CoV-2 also uses the human angiotensin converting enzyme 2 (hACE2) as receptor to gain entry into host cells. Coronavirus virologists within the network (Kikkert, Snijder, van Kuppeveld) were already planning the next steps of their research program. Within days (January 23, 2020), China announced a strict lockdown of Hubei province and later, all regions of the country. Within weeks, the epicenter of the epidemic moved to northern Italy, and then all of Europe, with devastating consequences. The imposition of lockdown measures in European countries saved countless thousands of lives, but severely halted the economy, business and travel. Likewise, the ESRs and their research projects - suddenly brought to a halt by a moment in history, except for the ones working on SARS coronaviruses. The experience of young virologists locked down in foreign countries during a viral pandemic is recounted on the INITIATE website (https://initiate-itn.eu/blog/).

As the lockdowns ease and Europe returns to a 'new normal', the focus of INITIATE on RNA viruses and immunometabolic pathways has altered its directions. With the support of the European Commission, and together with the coronavirus expertise in the network, INITIATE now includes eight research projects with a focus on SARS-CoV-2. INITIATE thus provides an ideal training opportunity for the ESRs to bring their technical and theoretical knowledge to the cutting edge of current RNA virus-host immune interactions.

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