IMMUNOWATCH

EDITION N°11 - JULY 2025

INFECTIOUS DISEASES

mabdesign
THE BIOTHERAPY EXPERT

INTRODUCTION

MabDesign's ImmunoWatch is a specialized intelligence newsletter focused on the evolving field of biologics. It has been designed to deliver to actors and stakeholders in the field, timely and high-value insights curated through the combined expertise of MabDesign and its network of contributors in scientific research, business intelligence, market analysis, and intellectual property.

Each edition is dedicated to a specific therapeutic area or trending class of biologics, providing a comprehensive overview of current trends and strategic developments. The content typically includes market research, financial and economic data, expert perspectives from academic and industrial teams, and an in-depth intellectual property analysis. The editorial direction is ensured by a chief-editor board of at least two field experts, with theme selection and content development overseen by MabDesign's permanent editorial team.

Finally, we would like to acknowledge the continued support and strategic oversight provided by MabDesign's Comité d'Orientation Stratégique et Scientifique de Filière (COSSF) for their significant contribution to the quality and relevance of each edition of ImmunoWatch.

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Rana LEBDY
Project Manager

Fabrice PORCHERAY
Head of Department

Strengthening Preparedness: Advancing Medical Countermeasures for Emerging Infectious Diseases

The COVID-19 pandemic marked a turning point in global public health, revealing both the strengths and weaknesses of healthcare systems as well as lack of availability of medical countermeasures, even in high-income countries. Despite years of warnings, many nations were unprepared for a crisis of this magnitude. In its aftermath, the urgency to invest in pandemic preparedness has never been greater. Growing threats such as climate change, zoonotic spillovers, urbanization, and global mobility mean that local outbreaks can now escalate into international crises within days.

Yet the pandemic also had a silver lining: it sparked unprecedented scientific collaboration even though a need for better coordination and alignment of activities remains. Rapid data sharing, open-source research, and public-private partnerships significantly accelerated the development of vaccines and diagnostics. This collaborative spirit should serve as a model for tackling future global health challenges. Today, pandemic preparedness spans several key areas, including research and innovation, vaccine development, surveillance, and emergency coordination.

At the global level, the WHO Pandemic Accord aims to enhance international cooperation and ensure fair access to medical countermeasures. The WHO Hub for Pandemic and Epidemic Intelligence supports real-time threat detection. The CEPI 100 Days Mission seeks to dramatically shorten vaccine development timelines, while the Pandemic Fund, led by the World Bank and WHO, provides financial support for health preparedness in low-income countries.

In Europe, the creation of HERA will ensure access to vaccines, diagnostics, and treatments, and the EU FAB network will secure manufacturing capacity during health emergencies. Initiatives such as Be Ready and VACCELERATE focus on coordinated research and clinical trial readiness. Meanwhile, the ECDC and the EU Health Union Strategy play key roles in strengthening regional surveillance and emergency response capabilities.
As part of France's approach to pandemic preparedness, the ANRS Emerging Infectious Diseases Agency (ANRS MIE) plays a central role. Originally established in 1988 to coordinate HIV/AIDS research, ANRS merged in 2021 with the Inserm REACTing task force to form ANRS MIE, broadening its scope to cover a wider range of infectious diseases. Today, the agency coordinates research on HIV/AIDS, hepatitis, STIs, tuberculosis, and emerging infectious diseases. It actively promotes national, European and international collaborations to strengthen research ecosystems and bolster global readiness.

Among its key initiatives are the European Be Ready partnership, the CORC consortium on filoviruses in collaboration with the WHO, and the European STI initiative with the International AIDS Society, which coordinates vaccine research for STIs, including HIV and mpox.

The agency has also launched 4 major platforms:

- EMERGEN is a national consortium coordinated in collaboration with Santé publique France and ANSES, focused on developing a genomic surveillance and research system for infections caused by emerging pathogens.

- OPEN-ReMIE is a network designed to coordinate and prepare interventional clinical research, both academic and industry-sponsored, on emerging and re-emerging infections. It operates in alignment with international research networks and is led by Inserm/ANRS MIE, with co-leadership from Hospices Civils de Lyon (HCL).

- The I-REIVAC Emergence platform will allow France to conduct vaccine trials, whether academic or industry-led, in anticipation of or in response to crises involving emerging infectious diseases. It is coordinated by Inserm/ANRS MIE and co-led by AP-HP.

To further support innovation, ANRS MIE has created an Innovation Department that helps researchers find the right funding opportunities and partnerships to advance the development of drugs, vaccines, and diagnostics. Through its coordination of diverse national and international initiatives across basic science, clinical research, and public health, ANRS MIE stands at the forefront of efforts to prepare for future pandemics and to combat emerging and re-emerging infectious diseases.

EDITORIAL

INSTITUT Pasteur

Sotiris MISSAILIDIS
Head of Vaccine Innovation

The Institut Pasteur has been an internationally recognized player in the fight against infectious diseases since it was founded in 1887. The Institut Pasteur works for sustainable and equitable global health. This collaborative approach aims to reduce inequalities in access to vaccines, therapeutics and diagnostics and prevent epidemics. Our policy is to make our vaccines available where there are most needed, working with national and international industrial partners. Contracts for the use of Pasteur technologies include obligations for manufacturers to:
- Ensure that the products are accessible to as many people as possible,
- Ensure distribution of the technologies in all countries,
- Promote marketing at affordable prices, considering countries' economic capacity.

The COVID-19 pandemic has illustrated the ability of the Institute to engage into vaccine development. Institut Pasteur is so far the only academic institute in France to have brought a COVID-19 vaccine candidate into clinical development and was the third academic institute worldwide (after the Jenner Institute, UK, and the University of Queensland, Australia) to start clinical trials. However, the COVID-19 vaccine and immunotherapy projects have also emphasized that there is need for a more robust nucleation of the somewhat dispersed assets of the Institute in this field.

To address these issues, the Institut Pasteur has harnessed its collective expertise to launch the Center for Vaccinology and Immunotherapy (CVI). The CVI, launched in 2024, aims to spearhead the development of next-generation vaccines and immunotherapies through collaborations with hospitals, academic and industrial partners, aligning with France Vaccins 2030 initiative and leading the IP's participation in the European Vaccine Hub (EVH). The CVI prioritizes vaccine development on emerging or re-emerging infections and addresses the growing threat of antibiotic-resistant microbes. In coordination with government initiatives, the CVI contributes to pandemic preparedness by accelerating research and development procedures for health emergencies. It leverages the strong expertise on campus in immunology, structural biology and microbiology with the capacity of the Institute for vaccine development and tech transfer to further promote a continuum between discovery, clinical research and tech transfer for product commercialization.
A major asset of the Institut Pasteur is its membership of the Pasteur Network. Several projects are underway to improve our collaborations together, to better explore our complementary expertise in developing and bringing new vaccine products in the market and guarantee regional production and equitable access. For instance, in October 2024, key members of the Pasteur Network, including Fundação Oswaldo Cruz (Fiocruz), the Institut Pasteur de Dakar, the Institut Pasteur Korea, the Institut Pasteur, and the Institut Pasteur de Tunis, have signed a strategic Memorandum of Understanding (MoU) to strengthen collaboration in mRNA vaccine research and development, part of one of the strategic pillars of the Pasteur Network.

We worked hard in 2024 to see two vaccine candidates enter clinical development in 2025, one against Lassa fever and the other against Shigella. For the vaccine candidate against Lassa fever, an hemorrhagic fever responsible for several thousand deaths worldwide each year, a phase 1 clinical trial is scheduled.

EDITORIAL

This will be the first candidate from the MOPEVAC platform, an original viral vector vaccine platform developed by Sylvain Baize's team at IP, expected to reinforce the Institute's pandemic preparedness initiatives and deliver new vaccine candidates. For the vaccine candidate against Shigelle, a highly contagious diarrheal disease in Africa, we have secured external funding to launch a phase 1/2a clinical trial at the end of 2025/early 2026 and with GMP material ready for the trial. Other vaccine candidates, against Malaria, both falciparum and vivax, flavivirus and bacterial infections, currently in development using mRNA technology, have shown promising pre-clinical results and we expect to develop them further with different partners.
And of course, we have a rich portfolio of projects at an earlier stage, with very promising results, and we're working on accelerating their development to guarantee the future impact of the Institut on Public Health.

EDITORIAL

Paul BADUEL
Head of Bacterial Target Realization

sanofi

The pharmaceutical industry has a long-standing history in the anti-infective field, encompassing both biologics and small-molecule drugs. Small to medium-sized molecules, many derived from fermentation or semi-synthetic processes, revolutionized the treatment of bacterial infections through antibiotics. More recently, antiviral compounds have driven major advancements in the treatment of diseases such as HIV/AIDS and Hepatitis C.
Biologics also play a crucial role in combating infectious diseases, particularly through vaccines and therapeutic sera targeting bacterial and viral pathogens. While vaccines continue to evolve and are now widely accessible, one lesser-known area is the use of sera and polyclonal antibodies, primarily for post-exposure treatment of infectious diseases.

To focus this editorial on biologics, several key products and approaches deserve mention: vaccines, monoclonal antibodies, and innovative strategies such as phage therapy against antimicrobial resistance (AMR), as well as the role and applications of the microbiota, including microbiota transplantation (FMT).
Vaccine research and development has always been a dynamic field, with a significant surge in interest since the COVID-19 pandemic. Current efforts are increasingly targeting unmet medical needs, focusing on novel Diseases and Targets or those that have proven challenging for traditional vaccine approaches.

The evolution of vaccine research has progressed from classical methods based on whole pathogens to reverse vaccinology, which identifies relevant antigens through genomic analysis. This has further advanced into next-generation technologies, including structural vaccinology, synthetic biology, and RNA-based platforms, emphasizing the design and optimization of antigens.

These innovations have led to the implementation of platform-based approaches in both vaccine Development and Industrial Manufacturing, with a strong emphasis on recombinant proteins (produced in yeast, CHO cells, E. coli, or baculovirus systems) and mRNA technologies.

The field of monoclonal antibodies (mAbs), traditionally focused on oncology and immune-related diseases, is now expanding into the anti-infective domain. Like the historical use of sera, mAbs can be employed for passive immunization or post-exposure treatment. In recent years, monoclonal antibodies have been developed and launched against pathogens such as SARS-CoV-2, respiratory syncytial virus (RSV), HIV, and C. difficile.
Biologics continue to evolve in the anti-infective space, combining well-established products—proven to be safe and effective in preventing or treating many infectious diseases—with innovative new therapies. These next-generation biologics are based on rational design approaches, utilizing precisely targeted antigens and antibodies. Whenever possible, they are developed using advanced manufacturing platforms that enhance scalability, improve developability, and accelerate the overall development process.

Paul Baduel has over 35 years of experience in the pharmaceutical and vaccine industries. He began his career in research focused on therapeutic recombinant proteins before transitioning to the research and development of antibiotics. For the past 25 years, he has held various leadership roles in Chemistry, Manufacturing, and Controls (CMC) activities, working with recombinant proteins, monoclonal antibodies, and vaccines. Since 2023, Paul has been leading the Bacterial Target Realization team within Vaccine Research.

Promega

HiBiT-PsVLP Bioassay: A Safer, Faster Way to Profile Neutralizing Antibodies

The assay supports the collaboration between Charles River and CEPI's Centralised Laboratory Network to harmonize and accelerate immune response evaluation worldwide.

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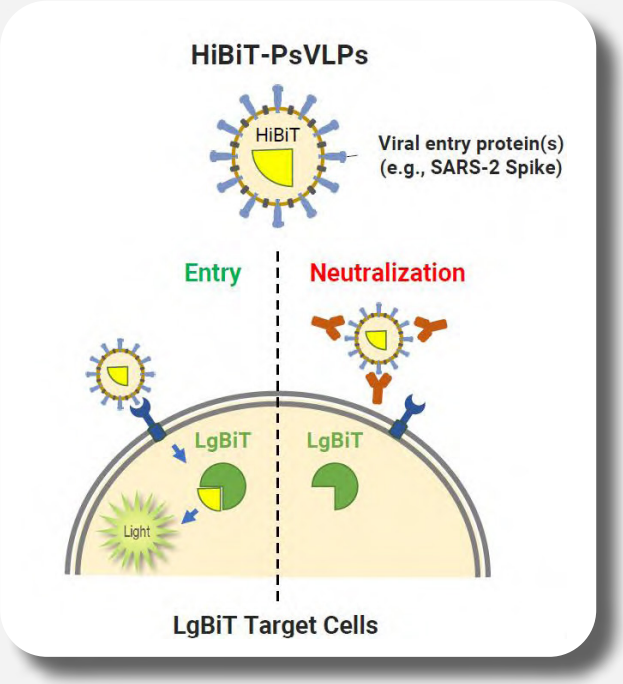
The Promega HiBiT-PsVLP Bioassay is a safe, scalable solution to assess neutralizing antibody responses against emerging viral threats — including Ebola Sudan, Marburg, and other infectious viruses — using bioluminescently tagged pseudotyped virus-like particles (PsVLPs) that mimic viral entry without containing infectious genetic material.

The assay reveals whether antibodies effectively block viral entry by binding to surface entry proteins.



Advantages of this assay include:

- **Safe:** No virus generation; suited for BSL-1/2 environments
- **Simple:** Ready-to-use kit format; just add, mix and read
- **Fast:** Get data in hours instead of days
- **Flexible:** Adaptable for multiple viruses

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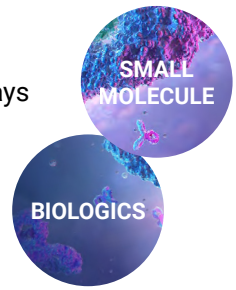

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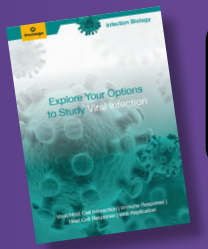

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Global market & pipeline analysis and latest trends in infectious diseases

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OVERVIEW OF THERAPEUTIC STRATEGIES AGAINST INFECTIOUS DISEASES:

By MabDesign

Infectious diseases have shaped the history of medicine and continue to represent a major public health issue worldwide. Long dominated by the major bacterial and viral pandemics, modern infectious diseases research is faced with two pressures: on the one hand, the persistence or resurgence of known pathogens (tuberculosis, HIV, malaria), and on the other, the emergence of new infectious agents, as illustrated by the Ebola and Zika epidemics and, more recently, the COVID-19 pandemic.

The diversity of pathogens - bacteria, viruses, fungi, parasites - implies a wide variety of therapeutic strategies. Previously ruled by therapeutic chemical antimicrobials such as antibiotics and antivirals and classic prophylactic vaccines, the fight against infections must now take on new dimensions. The rise of antibiotic resistance, the variability of immune responses and the limitations of certain conventional approaches have paved the way for a broadening of the therapeutic and prophylactic arsenal, incorporating new biomedicines other than conventional vaccines, including innovative therapies (gene, cell, therapeutic RNA, microbiota, etc.).

In this rapidly changing context, this article provides an overview of the major therapeutic classes used in infectious diseases, highlighting the dynamics of innovation, products under development and market prospects. From the chemical approach to the most advanced biotechnological platforms, the aim is to explore how research and industry are reinventing anti-infective treatments to meet current and future challenges.

I. Conventional therapeutic approaches: chemical antimicrobials

Chemical antimicrobials have historically been the first line of defence against infectious diseases. Since the discovery of penicillin in the 1920s, antibiotics have transformed the management of many bacterial infections, drastically reducing the mortality associated with previously incurable conditions. The major therapeutic classes today include β-lactams, macrolides, fluoroquinolones, aminoglycosides and glycopeptides, each targeting specific microbial mechanisms (wall synthesis, DNA replication, protein synthesis, etc.).

Antivirals have emerged more recently, with notable successes in the treatment of diseases such as HIV - lenacapavir (Sunlenca) - or hepatitis C - Glecaprevir / Pibrentasvir (Mavyret). Reverse transcriptase inhibitors, protease inhibitors and nucleoside analogues have made it possible to control certain chronic infections and significantly reduce the viral load, or even cure certain patients, as in the case of hepatitis C.

Despite these major advances, the effectiveness of chemical antimicrobials is now being seriously called into question by antibiotic resistance. The massive - and sometimes inappropriate - use of antibiotics in human and veterinary health and in the agri-food industry has led to selection pressure that encourages the emergence of resistant strains. According to the latest estimates, antibiotic-resistant infections are responsible for more than one million deaths every year*, a figure likely to rise to 10 million by 2050 if no major action is taken.

Faced with this global threat, efforts to develop new antibiotics are struggling to keep pace. The pipeline remains limited, mainly because of an unattractive business model: high development costs, low return on investment, use restricted to targeted situations. To overcome these obstacles, international initiatives such as CARB-X, GARDP and the AMR Action Fund have been set up to support innovative projects in this strategic field.

international initiatives such as CARB-X, GARDP and the AMR Action Fund have been set up to support innovative projects in this strategic field.

While chemical antimicrobials remain a central pillar in the treatment of infectious diseases, their future efficacy will depend on a strategy combining the development of new molecules, rational management of their use and the emergence of complementary therapeutic solutions.

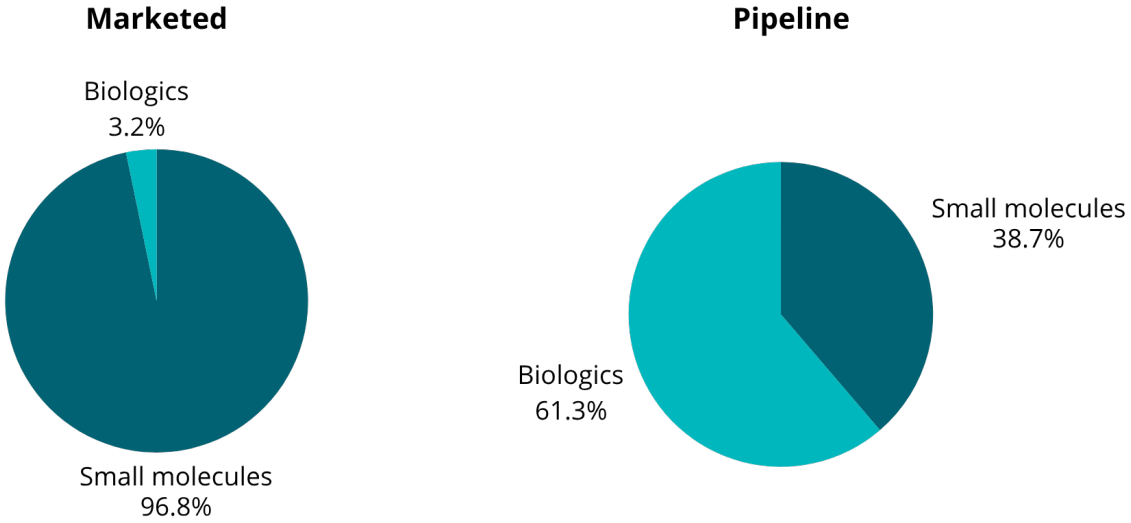

Figure 1 : *Distribution of small molecules and biologics on the market and in the pipeline for infectious diseases.*

II. Biologics in infectious diseases: vaccines, antibodies, proteins

While chemical antimicrobials have long been the mainstay of anti-infectious treatments, recent decades have seen a rise in the use of biologics to prevent and treat infections. This trend accelerated with the outbreak of the COVID-19 pandemic, revealing the potential of innovative approaches such as messenger RNA vaccines and monoclonal antibodies to provide a rapid response to an emerging infectious threat.

Prophylactic and therapeutic vaccines

Vaccines are the ultimate prophylactic weapon against infectious diseases. From traditional platforms (live attenuated, inactivated and subunit vaccines) to new-generation platforms (viral vectors, messenger RNA and DNA), the technological landscape has diversified considerably.

The COVID-19 pandemic marked a turning point, with the very first RNA vaccines (Pfizer-BioNTech, Moderna) authorised for use in humans in record time, illustrating the speed and flexibility of this platform in responding to a global health emergency.

The global vaccine market has grown spectacularly, reaching more than USD 50 billion in 2023, according to our database estimates, with projections that it will remain at a high level beyond the pandemic thanks to the extension of vaccination campaigns (influenza, RSV, dengue, malaria). Therapeutic vaccines - particularly in oncology and infectious diseases (e.g. HIV, hepatitis B) - are also the focus of an active development pipeline.

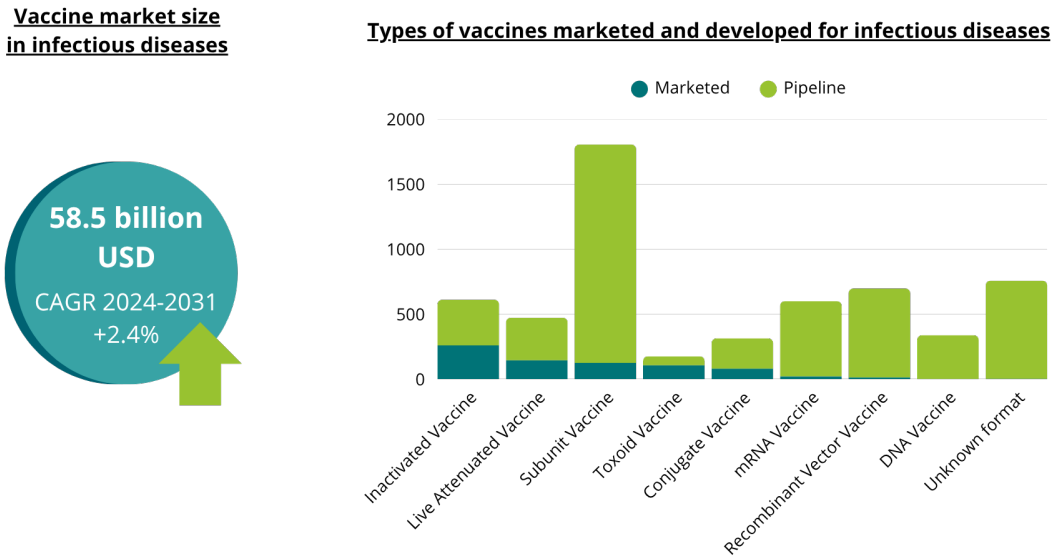

Figure 2: *Key figures for vaccines*

Anti-infectious monoclonal antibodies

Monoclonal antibodies (mAbs), long reserved for oncology or autoimmune diseases, are now finding increasing application in infectious diseases. Some products have already been authorised:

- Palivizumab (Synagis®) against the respiratory syncytial virus (RSV),
- Anti-Ebola antibodies (e.g. Inmazeb®),
- Evusheld® (tixagevimab + cilgavimab) for the prevention of COVID-19 in immunocompromised patients.

mAbs have major advantages: high specificity, ability to neutralise pathogens, and prophylactic or therapeutic potential. However, their high cost, parenteral administration and limited half-life remain obstacles to widespread adoption.

Innovation is continuing around new formats: bispecific antibodies, conjugated antibodies, or antibodies associated with half-life extension technologies. In addition, platforms for the rapid production of antibodies based on pathogen sequencing (e.g. rapid identification using artificial intelligence) offer the prospect of accelerated responses in the event of a future pandemic.

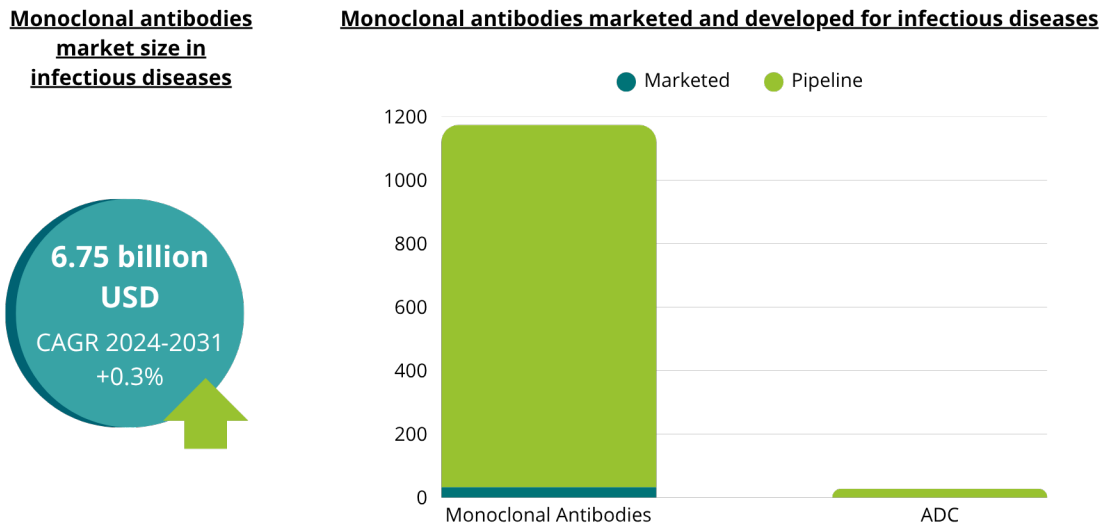

Figure 3: *Key figures for monoclonal antibodies*

Other recombinant proteins or enzymes

In addition to vaccines and antibodies, other biomedicines biologics are being developed or used to combat infections:

• Recombinant proteins or enzymes that neutralise bacterial toxins,

• Biological immunomodulators that stimulate the innate response (e.g. TLR receptor agonists),

• Use of recombinant cytokines in opportunistic or chronic infections (e.g. interferons).

These products are still limited infew in numbers, but their development could increase as our detailed understanding of host-pathogen interactions progresses.with a better understanding of host-pathogen interactions.

In short, biomedicines biologics are playing an increasingly important role in the anti-infectious arsenal. Their technological diversity, targeted efficacy and potential for rapid adaptation make them major tools for dealing with emerging infections and situations where there is a high unmet medical need. Their integration into combined therapeutic strategies (biotherapy + antimicrobial, vaccine + antibody) also paves the way for a more personalised, proactive approach to modern infectious diseases.

II. Innovative therapies: genetic, cellular and emerging technologies

Faced with the limitations of conventional approaches to certain complex or resistant infections, innovative therapies are attracting growing interest in infectiology. While these modalities were initially focused on oncology or rare diseases, they are now being explored for their potential to modulate the immune response, correct genetic susceptibilities, or even eradicate chronic pathogens.

Gene therapies

Gene therapy aims to introduce, correct or suppress a gene in a patient's cells to treat a disease. In infectious diseases, this approach is still in its nascent stageinfancy, but several avenues are currently being explored:

• **Correction of hereditary mutations** responsible for increased susceptibility to infections.

(e.g. innate immunity deficiencies such as mutations in the IFNAR gene).

• **Direct targeting of persistent viruses**, in particular using CRISPR/Cas9 technologies to excise integrated viral genomes such as in HIV or certain herpes viruses.

(e.g. EBT-101, Excision BioTherapeutics in phase II.)

• **Ex vivo gene therapies** to reprogram immune cells to make them more resistant or more effective against pathogens.

(e.g. INOVIO Pharmaceuticals products in phase II)

Although these approaches are mainly at the pre-clinical or phase I stage, they represent a promising development, particularly for chronic infections that cannot be cured or for immunodeficient populations.

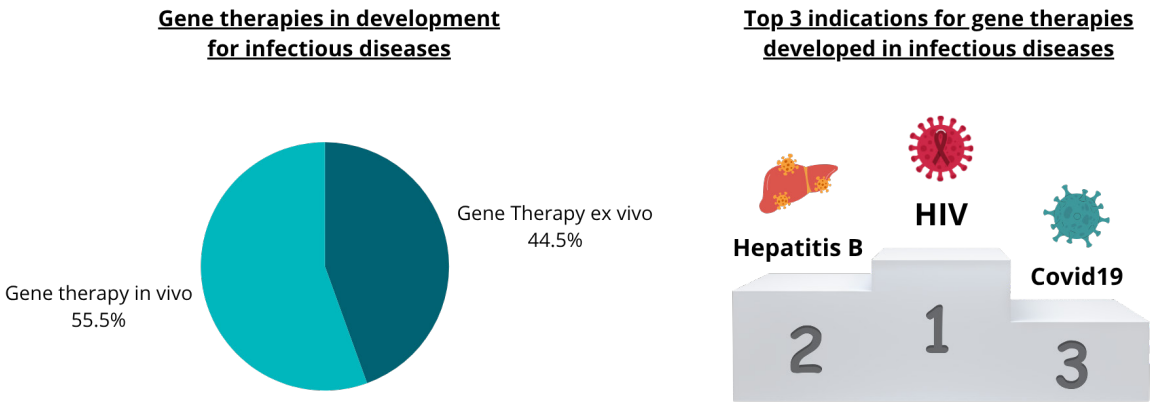

Figure 4: *Key figures for gene therapy*

Anti-infectious cellular therapies

The use of cell-based therapies (genetically modified or otherwisenot) to treat an infection represents a new therapeutic frontier. The first clinical applications mainly concern :

• **Virus-specific T lymphocytes** (CMV, EBV, Adenovirus), used in adoptive therapy for transplanted or immunocompromised patients. These products are derived from a donor or from the patient himself, activated ex vivo and then reinjected.

• More experimental approaches aimed at using or modifying **regulatory cells (Treg)** or **macrophages** to limit the immunopathological damage caused by chronic inflammatory infections.

• (Ex: Cellenkos' CK0802 in phase I for severe COVID-19)

These therapies are part of the wider trend towards personalised medicine, in which the aim is to restore or adjust an individual's immune responses to a given infection.

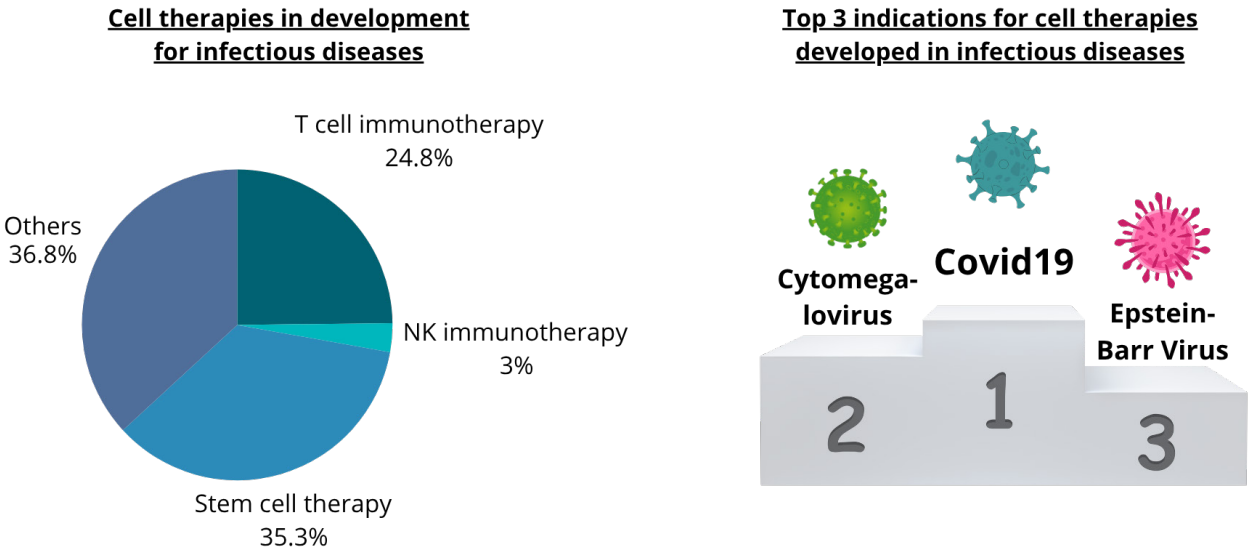

Figure 5*: Key figures for cell therapy*

RNA therapeutics, phages, microbiota

The therapeutic arsenal is also being enriched by so-called **unconventional approaches**, often resulting from advances in biotechnology and understanding of the microbiome.

• **RNA therapeutics**: in addition to mRNA vaccines, approaches using **siRNA or antisense RNA** directly target virus replication (e.g. RSV, influenza, hepatitis B). Platforms are now available for rapid, adaptable design in response to new pathogens. (e.g. Vir Biotechnology's elebsiran in phase III against hepatitis D.)

• **Phage therapy**: with the rise in antibiotic resistance, there is renewed interest in the **re-use of bacteriophages** - viruses that specifically target bacteria. Although complex to regulate, it has shown encouraging results in resistant infections. (e.g. APPA-02 from Armata Pharmaceuticals in phase II against Pseudomonas infections such as (e.g. Pseudomonas, Staphylococcus).

• **Therapies targeting the microbiota: faecal transplants** and microbiota modulators (probiotics, prebiotics, bacterial consortia) are showing benefits in infections such as Clostridium difficile, and are being studied for other infectious or inflammatory indications. (Ex: BGY-1601 from Nexbiome Therapeutics in phase II against vaginitis.).

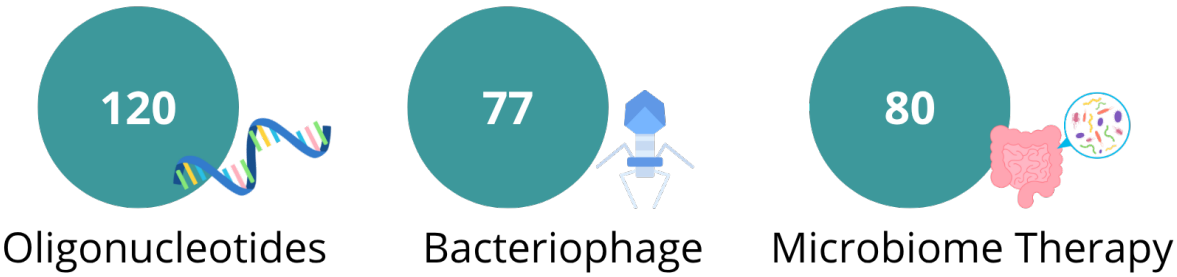

Figure 6: *Key figures for non-conventional technologies*

These innovative therapies, many of which are still in the early evaluation phase, herald a new era in the treatment of infections. They open the way to individualised, mechanistic and sometimes curative strategies, in a field historically dominated by standardised treatments. There are still many challenges ahead - cost, industrialisation, regulation - but their disruptive potential could profoundly transform infectiology in the decade to come.

Outlook and conclusion

Infectious diseases remain a major challenge for global health, at the intersection of health, economic and geopolitical issues. While chemical antimicrobials are still an essential pillar of the therapeutic response, their limitations - particularly in the face of antibiotic resistance - underline the urgent need for innovation. The rise of biologicsmedicines, new-generation vaccines and monoclonal antibodies, as well as the exploration of gene, cell and RNA therapies, are evidence of a profound transformation in the infectious landscape.

This transition is being accompanied by a reconfiguration of the market: the development pipeline is becoming increasingly diversified, with a growing emphasis on biologics, disruptive technologies and public-private partnerships. The COVID-19 pandemic acted as a catalyst, accelerating the development of flexible platforms, shortening regulatory lead times, and revealing the importance of responsive, localised industrial capabilities.

However, a number of challenges remain. The financing of anti-infective innovation remains fragile, particularly for indications perceived as less profitable. Regulatory complexity, barriers to market access in low-income countries, and the large-scale production of innovative therapies all need to be addressed to ensure equitable access to treatments.

The future of infectious disease control will rely on an integrated approach: developing or improving prophylactic tools, combining treatments, rapid diagnosis, epidemiological surveillance, personalized care, and anticipating emerging threats. Digital technologies, artificial intelligence, and Industry 4.0 will also play a growing role in optimizing R&D, production, and therapeutic monitoring.

Ultimately, the convergence of chemistry, biotechnology, immunology, and data science paves the way for precision infectious disease, better equipped to tackle 21st-century pathogens—known and still unknown.

SCIENTIFIC ARTICLES

Read the different inputs from the scientific community on infectious diseases

MOPEVAC, A LIVE-ATTENUATED VIRAL VACCINE PLATFORM AGAINST ALL PATHOGENIC ARENAVIRUSES, AND BEYOND

Xavier Carnec[1,2], Mathieu Mateo[1,2,], Stéphanie Reynard[1,2,*], Clara Germain[1,2], Alexandra Journeaux[1,2], Sotiris Missailidis[3], Sylvain Baize[1,2]*

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Introduction

Emerging viral infections represent one of the major challenges for human health and vaccination remains the most effective way to deal with that plague. Among the different approaches available to immunize people, live-attenuated viral vaccines are among the most efficient ones because of the potent and long-lasting humoral and cellular immunity they induce. These vaccines have been behind major achievements in terms of disease prevention such as smallpox, measles, yellow fever, polyomyelitis. In the era of mRNA vaccines, live-attenuated vaccines remain very important and represent an efficient way to develop vaccines. Among emerging viral infections, viral hemorrhagic fevers induced by risk-group 4 viruses such as Ebola (EBOV), Marburg, Lassa (LASV), Junin (JUNV), Crimean-Congo hemorrhagic fever (CCHFV) viruses represent a major threat. Indeed, these zoonotic diseases, or vector borne disease in the case of CCHF, are responsible for sporadic outbreaks in Africa and South America with very high lethality rate (20-90%). After the initial transmission to humans from viral reservoirs (bats for filoviruses, rodents for arenaviruses) or after tick bite, these viruses can be transmitted from human to human after contact with infected body fluids. There is currently no effective antiviral treatment to control these diseases, except for the poorly efficient ribavirin (LASV, CCHFV) or for a mix of monoclonal antibodies against EBOV that allows to decrease lethality. Except for JUNV and EBOV, no vaccine is available against these viruses. As these viruses emerge in remote areas where health care structures are limited and often in low- and middle-income countries (LMIC), vaccine development has to target products able to induce a robust immunity and that do not need regular boost to maintain the protection. These specificities advocate for approaches based on live-attenuated viral vaccines.

Our fundamental research on the pathogenesis and immune responses induced by LASV compared to the closely related but nonpathogenic Mopeia virus (MOPV) has allowed the development of a new live-attenuated viral vaccine platform named MOPEVAC. We have used it to develop vaccines against all pathogenic arenaviruses and have recently extended its application to non-arenavirus targets.

Results

Live-attenuated vaccines remain very important and represent an efficient way to develop vaccines. Indeed, live-attenuated vaccines have allowed major breakthroughs in the fight against diseases, because of their low-cost and long-term efficacy. Mopeia virus (MOPV) is an old-world arenavirus isolated from Mastomys natalensis in Mozambic. It is closely related to Lassa virus (LASV) (75% of homologies) but was never detected in humans and is non-pathogenic for non-human primates. Using reverse genetics, we modified MOPV to generate a live-attenuated viral vaccine. To further attenuate MOPV, we introduced 6 amino acid changes into the catalytic site of the exonuclease domain encoded in the nucleoprotein (NP) to abrogate the ability of MOPV to digest double stranded RNA and to inhibit type I IFN production. Each of the 6 amino acid changes is sufficient to kill exonuclease, ensuring that no reversion toward wild-type phenotype can occur. To immunize against LASV, we swapped MOPV glycoprotein (GPC) by the LASV one (Fig 1) (1).

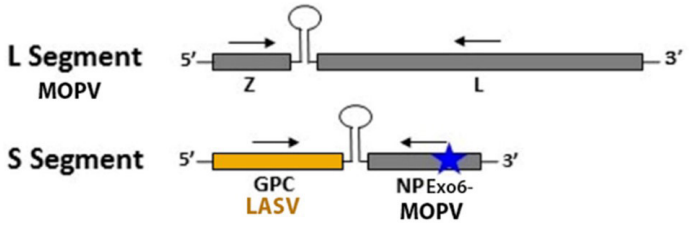

Figure 1: *Genomic structure of MOPEVAC$_{LAS}$*

MOPEVAC$_{LAS}$ replicates well in Vero cells, including in GMP grade cells cultured without animal product, and elevated viral yields are harvested in supernatants (Fig 2).

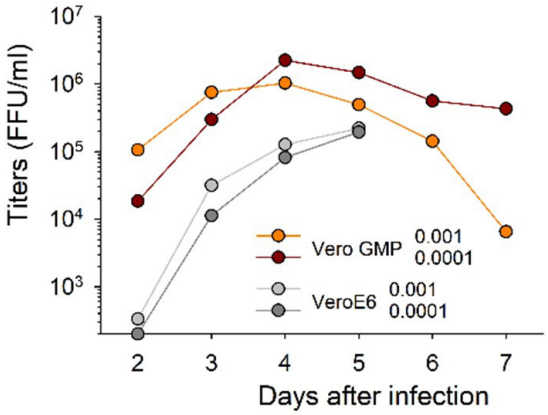

Figure 2: *Replication of MOPEVAC$_{LAS}$ in Vero E6 cells and in GMP Vero cells at MOI = 0.001 or 0.0001*

The candidate is genetically stable. No mutation was detected in the genome after 10 passages (1). To evaluate the efficacy of MOPEVAC$_{LAS}$ to protect against LASV, we immunized CMs with a single IM dose of 5 x 10^6 FFU. Neither clinical signs nor vaccine viremia/shedding were observed, showing a satisfactory safety profile (2). Robust and balanced humoral and cellular responses specific for GPC and NP of LASV were induced. IgG titers ranged from 1:250 to 1:1,000 and neutralizing antibodies (NAbs) measured with infectious LASV were present before the challenge. CD4+ and CD8+ T cells produced TNFα and IFNγ in response to LASV GPC and NP peptides after immunization, showing the induction of LASV-specific T cells. The homologies between MOPV and LASV NP explain why MOPEVACLAS can generate immunity against both LASV GPC and NP. One month after immunization, CMs were challenged with Josiah LASV, the reference strain used to generate the vaccine. While all control animals succumbed, the 4 vaccinees remained healthy and the only clinical sign observed was transient moderate-grade fever in 3 of them. Whereas elevated viremia was observed in controls, low and transient LASV viral loads were measured in 3 MOPEVAC$_{LAS}$ animals, the fourth one presenting sterilizing immunity (Fig 3).

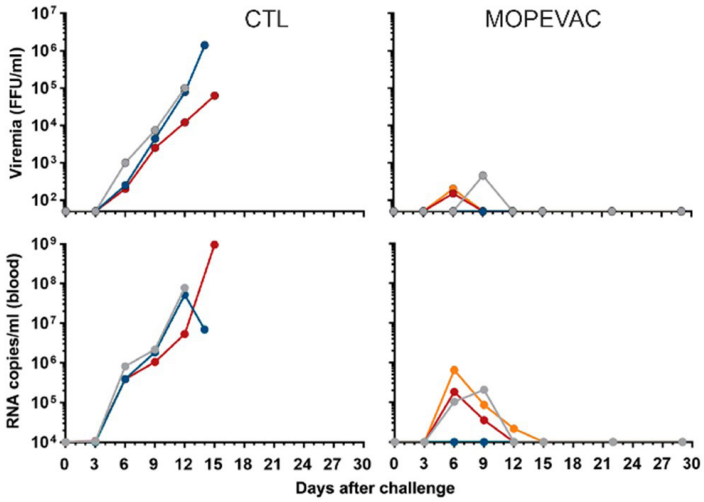

Figure 3: *Infectious viral titers and RNA viral loads in plasma of control (n=3) and MOPEVAC-immunized (n=4) cynomolgus monkeys after LASV challenge*

The challenge boosted humoral (Nabs, LASV IgGs) and cellular immunity, with IgGs mainly directed against NP and GP2 and titers ≥ 16,000 by day 9. From 6 days post infection (DPI), an elevated number of LASV GPC and NP-specific CD4+ and CD8+ T cells circulated in vaccinees but not in controls (Fig 4).

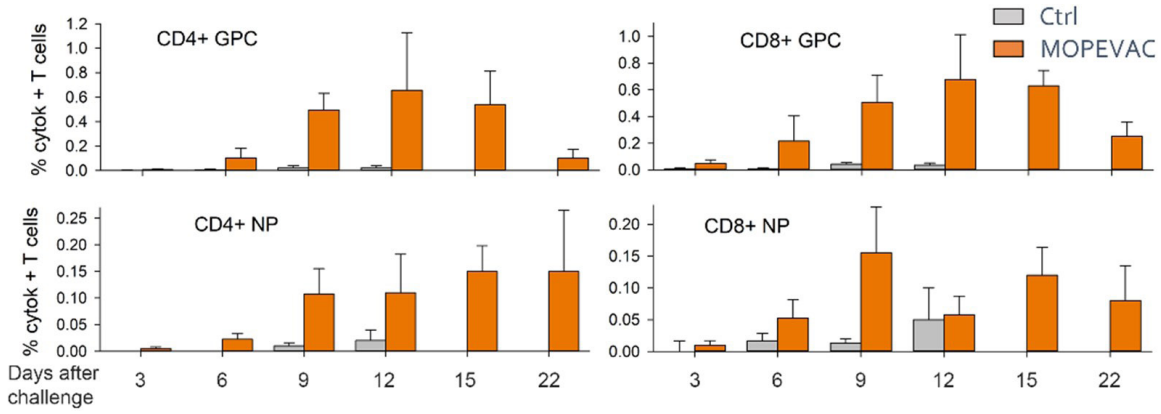

Figure 4: *Quantification of IFNγ and/or TNFα producing CD4+ and CD8+ T cells in response to LASV-derived peptides in LASV-infected control and MOPEVAC-immunized CMs*

Thus, a single shot of MOPEVAC$_{LAS}$ protects CMs against LASV thanks to balanced immune responses against GPC and NP. We have now achieved the preclinical development of this vaccine, by showing that protective immunity is rapidly generated, as CMs immunized 8 days before a LASV challenge were protected. Moreover, the protection induced by the vaccine is observed with the most divergent LASV strain compared to the one used as an antigen source. Finally, we have also shown that a prime-boost immunization with 10^6 FFU induces a long-term immunity, with animals still protected one year after immunization. Moreover, this experiment also allowed to demonstrate that the booster was highly immunogenic and therefore, that no anti-vector immunity is induced by the first immunization. The vaccine candidate has now entered clinical development. We have indeed set up the production process for MOPEVAC$_{LAS}$ thanks to a collaboration with a CRO and significant funding by the BPI France. A GMP MVSS batch has already been produced, then an engineering run that is currently used in a toxicological study performed in rabbits. The production of a GMP batch is currently in progress and a first-in-humans double blind placebo-controlled phase 1 clinical trial will be initiated in early 2026 (Fig. 5).

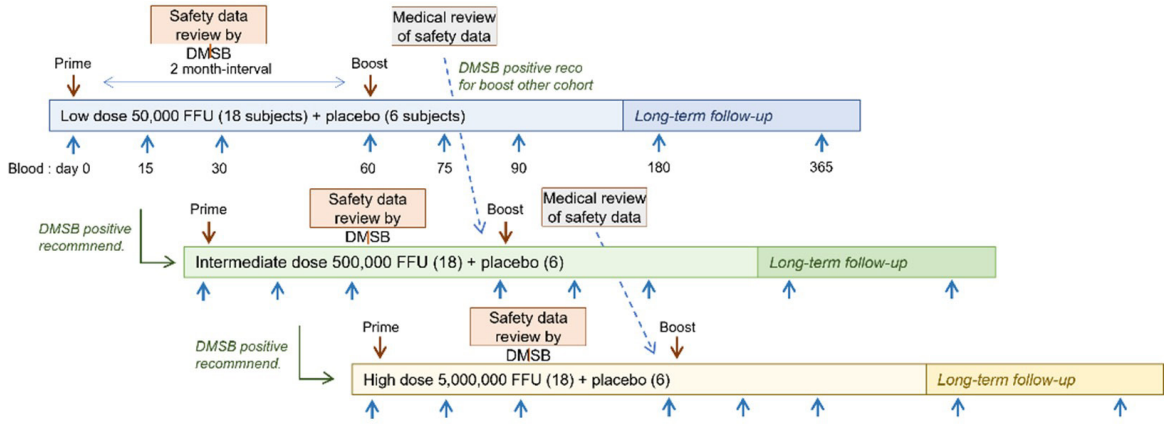

Figure 5: *Design of the first-in-human phase 1 trial planned in 2026 in France*

This assay will allow to demonstrate the safety of increasing dose of MOPEVAC$_{LAS}$ in humans, as well as to provide first insights into its cellular and humoral immunogenicity.

In addition to LASV, we have generated vaccines against other pathogenic arenaviruses. In particular, Junin (JUNV), Machupo (MACV), Guanarito (GTOV), Chapare (CHAV), and Sabia (SABV) are responsible for VHF in South America with a 25% lethality rate and no vaccines or treatments are available against these highly pathogenic viruses, except for a live-attenuated vaccine against JUNV available only in Argentina. As it would be unwise to try to develop vaccines against each of these viruses, we generated a pentavalent vaccine, named MOPEVAC$_{NEW}$, by mixing together the five monovalent MOPEVAC vaccines directed against these new world arenaviruses (NWA). The production of these five vaccines is facilitated by the similar replication kinetics in Vero cells. We immunized CM with MOPEVAC$_{NEW}$ and challenged them with MACV, GTOV, JUNV, CHAV, or SABV. A robust antibody response was induced in vaccinees, and the booster injection increased the humoral response substantially (Fig. 6).

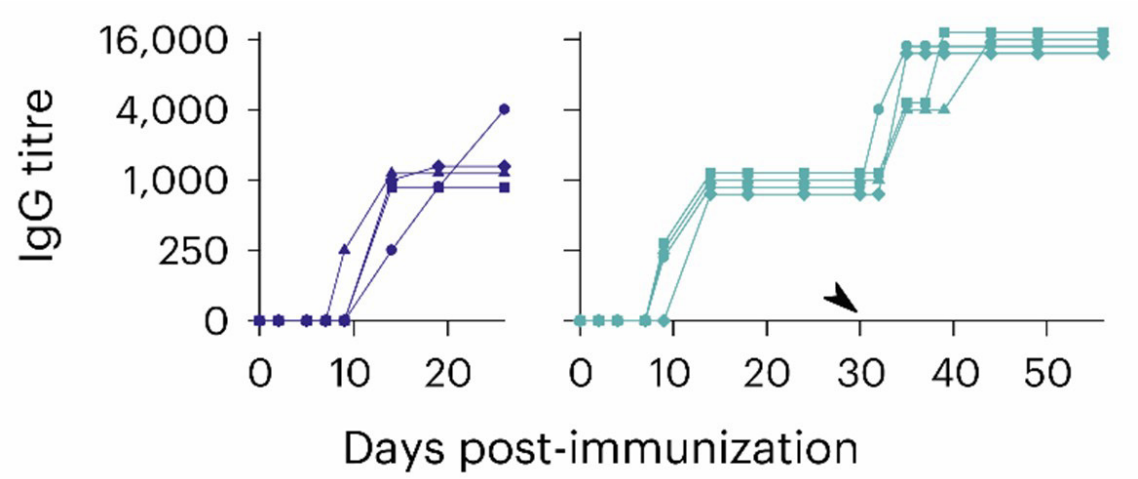

Figure 6: *MACV-specific IgGs induced in animals (n=4) immunized with prime (left graph) or prime-boost (right graph) injections of MOPEVAC. The arrow shows the day of the booster injection*

High titers of neutralizing antibodies (NAbs) specific for the five targeted viruses were detected in immunized cynomolgus monkeys. In contrast, we failed to detect antigen-specific T cells in the circulation of animals after immunization. We then challenged animals with each of the five viruses. Whereas control animals either died or were severely ill, all immunized animals were protected with sterilizing immunity (3). Thus, $MOPEVAC_{NEW}$ is efficient against all pathogenic NWA. Whereas $MOPEVAC_{LAS}$ induces a robust T cell response, the presence of a NWA GPC in the vector induces a different immunity, with huge NAbs and IgGs but no significant detection of specific-T cells in blood. These results indicate that intrinsic properties of the respective GPC are able to orientate the immunity induced by MOPEVAC. As for $MOPEVAC_{LAS}$, we demonstrated that the vaccine was efficient as soon as 10 days after a single shot and at least one year after a prime or a prime-boost immunization. The pre-clinical development of this vaccine candidate is now completed and the next step will be to adapt the $MOPEVAC_{LAS}$ process of production to $MOPEVAC_{NEW}$ and to initiate the clinical development. These results demonstrate that the MOPEVAC platform allows to easily generate efficient vaccines against all pathogenic arenaviruses. The advantage of this is the ability of the platform to accommodate the GPC of new emerging arenaviruses, such as Lujo virus, with a production process that will be very similar.

We recently moved the MOPEVAC platform towards a more universal vaccine platform that would allow to generate candidates directed against non-arenavirus pathogens. The MOPEVAC backbone has been modified to allow the expression of heterologous open-reading frames in each of the RNA genomic segments. To provide a proof-of-concept of the immunogenicity and efficacy of this new live-attenuated viral vaccine platform, we have generated vaccine candidates against Crimean-Congo hemorrhagic fever virus (CCHFV) based on the expression of Gc, Gn, N, and GP38 antigens. We evaluated the efficacy of three different vaccine combinations in the mouse IFNAR-/- model, a model in which CCHFV induces a uniformly lethal infection in about 4 days. All three candidates induced a protective immunity, associated with the induction of high levels of antibodies and of robust CD8+ and CD4+ T cell responses, mainly directed against Gc and N. These preliminary results demonstrate the efficacy of the MOPEVAC platform beyond arenaviruses. Other vaccine candidates are already in preparation and the characterization of the biological properties of this vaccine platform in terms of expression of heterologous antigens and genetic stability is in progress.

Altogether, MOPEVAC platform has allowed to generate efficient vaccines against all pathogenic arenaviruses, with one candidate in clinical evaluation. The vaccines have demonstrated safety in animal models, induce balanced immune responses adapted to the ones expected against the targeted pathogens and also induce a protective immunity at very early time after immunization and with expected high durability. The arenavirus vaccines present the advantage of being efficient as reactive vaccines in case of outbreaks to limit the viral dissemination and of being a vaccine approach adapted to the need of low and middle income countries, with high immunogenicity, early and long lasting protection with a small number of doses and good stability profiles, where these viruses circulate. The expected safety and immunogenicity results that should emerge from the first-in-human clinical trial with MOPEVAVLAS and the inclusion of the platform to the European Vaccine Hub (EVH) of pandemic preparedness, will pave the way for further development of other vaccine candidates using this novel and promising vaccine platform.

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VECTORS FOR GENE THERAPY: FOSTERING RESEARCH THROUGH NETWORKING

ThéGéVec Working Group – ANRS MIE

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The landscape of vectors for gene therapy

After more than 30 years of research investment, we now witness many validations of the therapeutic relevance for gene transfer proofs of concept (1). Therapeutic efficacy of gene therapy was first obtained in genetically engineered patients' cells, such as T lymphocytes for treating genetic diseases and later in cancer immunotherapy, and through the use of hematopoietic stem cells (HSC) to correct genetic defects by introducing a functional copy of a gene (or its cDNA) to compensate for host dysfunction (2). Viral vectors have been pivotal in these successes. More recently, non-viral vectors have started to offer clinically relevant methods that allow in vivo delivery (3).

While considered the most physiological approach, envisioning the direct execution of gene transfer or modification in specific cells within patients was long thought to be inaccessible. The field of gene therapy initially advanced through ex vivo delivery of gene-modified cells. As expected, it significantly broadened its scope once in vivo gene delivery, particularly via systemic vector administration, became feasible (4). Today, the field is reaching a stage where cell subsets can be targeted in vivo with some specificity, in particular for the precise genomic modifications that can be achieved through gene editing (5,6). These advancements were made possible by overcoming several challenges, including limited delivery, specificity in cell targeting, at the transcription or DNA level, lack of durability, and strong immune responses. Over the past 20 years these features have prompted major efforts in vector design, notably in France.is a quite simple for a virus but complicated as a therapeutic modality.

Today, novel gene therapy approaches sail with a tailwind. AAV vectors are widely used for gene delivery in living organisms (7). Recently, the range of applications for gene therapy has expanded considerably as a consequence of the growth and refinement of these innovative technologies' toolbox. Expanding vectors' ability to target specific cells or tissues could provide a straightforward and safe way to achieve therapeutic effects. In the following paragraphs, we provide a brief overview of the current state of the art and the future perspectives in development, particularly in the context of combating infectious diseases.

Viral vectors

Viruses co-evolved with their host in an arms race fashion. Extant viruses retain the ability to complete a productive replication cycle in their hosts. Beyond knowledge objectives, for containment and therapy, we need to learn to understand the molecular mode supporting the viral life cycle. Among the different stages of virus-cell interactions, entry and genome delivery are conventionally the first. Viruses evolved features that enable the transfer of their genetic material, which is then expressed within infected cells. Of note, some defective viruses carrying hijacked genomic sequences behave as natural gene transfer vectors. This meets one goal of gene therapy, explaining how viruses were the first biological tool researchers thought of for these applications.

Among the panoply of viruses that could be used, those with well-described vector strains or with biological amenities for manipulation initially supported vector development. In this regard, other main features sought were tolerance to genetic engineering, preserved infectivity, low immunogenicity, and allowing useful genetic material packaging while offering an elevated biosafety, in particular without the generation of replication competent contamination.

As a result, some viruses have considered more promising candidates for the elaboration of vectors for gene therapy purposes. Among these were adenoviruses, adeno-associated (AAV), herpes virus and retroviruses including lentiviruses.

Adeno-associated virus derived vectors

Development of AAV-derived vectors rose in the 90's thanks to their small size, ease of vector generation and production, and efficacy in mediating in vivo gene transfer (4). AAV also benefited from the immunological response observed against a leak in adenoviral protein expression, which led to short-term expression and significantly hindered their development, a setback further amplified by a fatal incident in a trial (8).

Moreover, the availability of multiple AAV serotypes with different tissue tropism has enabled preferential, though not exclusive, transduction of specific organs (9). Advances in redirecting AAV tropism—particularly to reduce liver targeting or to favor certain tissues — have since been achieved. These advances largely rely on capsid-structure determination, enabling the identification of insertion sites for targeting peptides (10). Alternatively, these sites are susceptible to host banks of random peptides for selection-driven molecular evolution to select tissue-specific vectors. Such developments of novel serotypes and rational capsid engineering have led to improved transduction efficiency, contributed to the reduction of the immunological response, and expanded the range of targetable tissues. Efforts to optimize AAV genome design — such as using self-complementary AAVs, synthetic promoters, or post-transcriptional regulatory elements— have enhanced gene expression levels and durability (11,12). The use of insulator elements and optimized expression cassettes —including tissue-specific promoters and codon optimization—has further improved expression precision and minimized epigenetic silencing, contributing to more robust and sustained therapeutic outcomes (13). However, the size limitation of recombinant genomes remains a major constraint for AAV vectors. Strategies are also being explored to promote episomal persistence, extending the therapeutic effect of non-integrating vectors like AAV. Of note, records accumulated from numerous clinical trials and the commercial developments of AAV vectors showed unexpected molecular traces of random-integration of small fragments of the vector-genome.

Lentivectors: HIV-derived vectors

The understanding of the molecular basis underlying oncoretroviruses mode of action, particularly their co-transmission of deficient and wild-type complementary strains, led to the design of gene transfer vectors and their packaging systems (14). Massive work confirming retroviral vectors efficacy and compliance with requirements to support efficient ex vivo gene transfer triggered a series of pioneering clinical trials in T cells or HSC, and offered the first success in terms of clinical effects.

However, enveloped viruses are somehow reluctant to concentration and more prone to complement inactivation following vascular delivery. This limits in vivo administration and targeting. Also, the wide use of pantropic envelopes (such as VSV-G) reduces the opportunity for specific targeting (15). The advent of the now widely used lentiviral vectors, derived from HIV-1, which are capable of infecting resting cells, has improved their efficacy for correcting phenotypes. Improvements in vector safety—such as the development of self-inactivating (SIN) vectors and splitting trans-complementation viral-sequences in multiple plasmids (split-genome design) have significantly reduced the risk of insertional mutagenesis and the formation of replication-competent virus (16).

Beyond gene transfer with viral vectors

Futhermore, advances in genome editing (e.g., CRISPR/Cas9, TALENs, site-specific recombinase) now enable the use of vector DNA, from AAV or LV, as a donor template for targeted insertion of therapeutic genes into specific or "safe harbor" loci, thereby improving safety through crontolled integration (17).

Concerning cell-specific targeting, the identification of VSV-G's cellular target and the determination of its 3D structure in complex with its receptor, allowed the identification of mutations abolishing receptor recognition while retaining its fusion activity. This paved the way for cell-specific targeting by engineering the envelope glycoproteins. These modifications of VSV-G as well as those of paramyxovirus glycoproteins (measles, Nipah virus) or Sindbis virus envelopes have opened new possibilities for in situ/in vivo targeted therapies (18,19).

Non-viral vectors

By leveraging insights from the strengths and limitations of viral approaches (targeting specificity, immune system evasion, and gene expression regulation), non-viral strategies are now being designed with enhanced composition and effectiveness. For example, lipid nanoparticles have proven their clinical value through their use in mRNA vaccines against COVID-19, offering rapid development cycles. Moreover, the results of several clinical trials in gene therapy demonstrate their flexibility to carry various nucleic acid cargos, including mRNA, siRNA, or CRISPR-Cas components. Non-viral vectors offer a high potential for reduced immunogenicity, scalable production, and ease of customization. They are increasingly seen as practical alternatives or complements to viral systems, especially for applications requiring repeat dosing or tailored delivery. Interestingly, advances in the field of non-viral vectors are also feeding back into viral vector development (20). Innovations in synthetic chemistry, nanoparticle design, and targeted delivery from non-viral platforms offer new strategies to improve viral vector formulations, through hybrid systems that combine viral efficiency with synthetic targeting or shielding components (21).

Barriers to overcome

In the context of clinical gene transfer, several structural features of the human organism must be considered, including some modifications more specifically linked to the targeted pathology. Anatomical barriers, which vary depending on , can be circumvented through vascular delivery or may require minimally to highly invasive surgical procedures. Once inside the body, histological barriers, including endothelial cells, the blood-brain barrier, and the extracellular matrices associated with supportive tissues limit access to target cells.

At a cellular level, depending on the platform, gene transfer must be directed to specific compartments such as the cytoplasm, nucleus, or, in some cases, the mitochondria, in accordance with the therapeutic goal. Beyond the plasma membrane — composed of lipids, proteins, and glycan chains closely associated with the extracellular matrix — the cytoplasm hosts several biological systems capable of detecting exogenous genetic information. Most of these systems are part of the innate immune system which contributes to the initiation of a cellular immune reaction that may reduce gene transfer and subsequently become systemic (22).

Researchers working on gene therapy using viral and non-viral vector approaches are struggling to reduce the burden posed by several of these challenges. This influences notably the structure of the delivered genetic material, its intracellular fate (whether in the cytoplasm, nucleus, or mitochondria), its persistence in integrated or episomal forms, and the mechanisms of its expression.the burden posed by several of these challenges.

Immunity

Immune responses remain a key limitation in gene therapy. Both innate and adaptive immunity against vector components (e.g. AAV capsids) or against the transgene can affect vector efficacy and prevent gene expression and limit the possibility of repeat dosing. A major challenge is the potential need for multiple injections, which may be less effective over time if neutralizing immune responses develop after the first dose.

To overcome this, researchers have developed platform-based adaptations. For AAV, synthetic capsids and alternative AAV serotypes with reduced immunogenicity or improved tropism are being studied (23). Strategies such as temporary immunosuppressive regimens, immune checkpoint blockades (e.g., anti-CD40L), or vector shielding through PEGylation and exosome-associated vectors (such as vexosomes) are under investigation to enhance tolerability and repeat dosing (24).

In addition, computational modeling and artificial intelligence (AI) are now being applied to predict vector-host interactions, guide capsid design, and accelerate high-throughput screening of engineered variants with enhanced safety and efficacy. These advances reflect the rapid evolution of viral vectorology into a robust platform adaptable to a wide range of clinical needs (25).

Intermediate conclusion

Finally, beyond issues related to gene expression, the question of cell targeting—its efficiency and specificity—is critical for several key aspects. (1) Specificity of modification: ensuring that genetic information is transferred mostly/only into the cells relevant to the targeted disease. (2) Ability to overcome histological barriers. (3) Mode of administration: while intravenous delivery is the most practical, it is not specific to any particular organ and leads to vector clearance by natural filters such as the liver, lungs, and spleen. (4) The need to produce large quantities of vectors to compensate for the rapid clearance mentioned above. Most of these features influence biosafety concerns that need preclinical testing before administration to humans.

Vectors for RNA vaccines

Traditional vaccines often struggle to provide lasting protection against rapidly evolving viruses such as HIV-1, HSV, RSV, and influenza. Moreover, the processes involved in their generation are typically slow to respond to emerging viral threats, as evidenced during the Ebola, Zika, and COVID-19 outbreaks. In contrast, genetic vaccines—particularly mRNA and viral vector-based platforms—offer a faster, flexible, and potentially more effective alternative.

Over the past few decades, mRNA and viral vector-based vaccines have seen remarkable progress, with the COVID-19 pandemic serving as a major catalyst. More than a billion doses of mRNA vaccines have been administered worldwide, underscoring the platform's scalability and effectiveness. This rapid advancement was driven by innovations in mRNA design and manufacturing, including optimized untranslated regions (UTRs), enhanced cap structures, codon optimization, improved purification processes, and the development of efficient delivery systems (26).

The success of mRNA vaccines against SARS-CoV-2—including robust protection against variants like Omicron after booster doses—validated the platform's potential(27). It also established scalable GMP manufacturing and global distribution infrastructure, paving the way for broader applications. Notably, mRNA vaccines targeting RSV (28) and combination RSV-influenza viruses have now been approved, while candidates for diseases such as Nipah virus, shingles, and CMV are advancing through clinical trials (27). Beyond conventional mRNA formats, self-amplifying mRNA (saRNA) vaccines represent a significant potential advancement. By incorporating genes encoding viral replicases, saRNA vaccines enable intracellular RNA amplification, leading to stronger antigen expression and hence improved immune responses. These vaccines, often based on alphavirus or vesicular stomatitis virus (VSV) backbones, leverage endogenous adjuvant effects through the production of double-stranded RNA and other pathogen-associated molecular patterns (PAMPs)(29). Despite their larger size, saRNA vaccines are progressing in clinical development for both infectious diseases and oncology. The recent approvals of KOSTAIVE® (ARCT-154) by the FDA and EMA, and GEMCOVAC-19/OM in India, underscore the promise of this modality.

Two additional innovations are emerging as next-generation mRNA platforms. The trans-amplifying RNA (taRNA) system separates replicase and antigen-encoding RNAs, offering modularity and dose flexibility. Meanwhile, circular RNA (circRNA) vaccines, characterized by a covalently closed-loop structure, exhibit enhanced stability against exonuclease degradation (30). Both taRNA and circRNA hold significant promise for the development of novel immunotherapies targeting cancer and infectious diseases.

Crucially, the success of these advanced RNA vaccine platforms hinges on efficient and targeted delivery systems, many of which rely on vector-based technologies. Lipid nanoparticles (LNPs) have emerged as a gold standard for mRNA delivery, improving cellular uptake by facilitating endocytosis and protecting the RNA cargo from enzymatic degradation. Modern LNP formulations have been optimized to target dendritic cells and other antigen-presenting cells while reducing accumulation in off-target organs such as the liver, thereby enhancing both safety and immunogenicity (31). Similarly, viral vectors—such as adenovirus or measles virus-based platforms—offer high transduction efficiency and inherent immunostimulatory properties that augment vaccine efficacy. These delivery vectors not only enable tissue-specific targeting and controlled antigen expression but also contribute significantly to dose sparing and scalability in manufacturing. As a result, delivery vector design has become a central pillar in the success of next-generation genetic vaccines.

Vectors against viral infections

In contrast, latent viral infections—such as HIV, hepatitis B virus (HBV), and herpes simplex virus (HSV)—remain among the most challenging to cure. These viruses establish reservoirs that are invisible to both the immune system and conventional antivirals: HIV and HBV by integrating into host DNA, and HSV by persisting in a dormant state within neurons (32–34). Current treatments can suppress viral replication and manage symptoms, but they do not eliminate the latent virus, necessitating lifelong therapy.

This persistence poses not only individual health risks but also a significant public health concern: if patients lose access to treatment, viral reactivation can lead to disease progression and renewed transmission, restarting the cycle of infection. Therefore, the development of curative strategies targeting latent reservoirs remains a critical unmet need.

Vector-based approaches are now being explored to address the persistent challenge of latent viral reservoirs, particularly in chronic infections such as HIV, hepatitis B virus (HBV), and herpes simplex virus (HSV). Gene therapy vectors—especially those delivering programmable gene-editing tools like CRISPR/Cas9—offer the potential to eradicate latent viral genomes from their cellular sanctuaries, marking a shift from lifelong disease management to curative strategies (35). However, the challenge is either to develop specific targeting of the reservoir or a broad transfer with antiviral activities showing specificity.

For HIV, recent gene-based technologies include CRISPR/Cas9 editing of integrated proviral DNA, delivery of broadly neutralizing antibodies via AAV or mRNA vectors, and in vivo generation of engineered immune cells (36). Clinical trials exemplify this momentum: Excision BioTherapeutics is conducting a Phase I trial using AAV vectors to deliver CRISPR/Cas9 designed to excise HIV DNA (37); Calimmune reached Phase II with a strategy using autologous hematopoietic stem cells modified to knock out the CCR5 co-receptor (38); and AGT Therapeutics is testing a Phase I approach combining CCR5 knockout with miRNA hairpins targeting HIV TAT and VIF genes (39,40).

Similarly, for HBV, therapeutic strategies are targeting the virus's persistent episomal form, covalently closed circular DNA (cccDNA)(41). One example is Precision BioSciences, which is developing an mRNA-LNP-delivered ARCUS nuclease to attack both cccDNA and integrated HBV DNA in a program currently in early Phase I (42). Another is Chroma Medicine's CRMA-1001, an epigenetic editor that combines a DNA-binding domain with an effector to induce durable transcriptional silencing of HBV genes; this LNP-delivered platform is in preclinical development.

Across both HIV and HBV, a major technical barrier is the effective targeting of viral reservoirs in hard-to-reach tissues such as the brain and lymphoid organs. To overcome this, advanced viral (e.g., AAV, lentivirus) and non-viral (e.g., lipid nanoparticles) vector platforms are being engineered for improved tissue tropism, specificity, and delivery efficiency (43,44).

Together, these emerging vector-based and gene-editing therapies represent a radical departure from traditional treatments. By directly targeting the genetic basis of viral persistence, they offer the potential for curative interventions in diseases once thought incurable, transforming not only prognosis but also the long-term quality of life for millions worldwide.

ThéGeVec

Considering that 2025's approaches in gene therapy remain amenable to needed improvements, ThéGéVec working group was created within the French research agency for emerging infectious diseases (ANRS MIE) in order to catalyze the development and transfer of cutting-edge innovative vector platforms.

To reach this goal, the working group intends to capitalize on existing recognised expertise in different fields of interest by favouring connections between academic research and the world of therapeutic applications at an early stage of research project conception. It aims to develop AI-integrated digital web platforms to identify synergies between research teams and looks to associate academic or industrial actors if needed.

One outcome could also be to match academic laboratories with private partners, ranging from biotech startups to larger pharmaceutical groups, all interested in co-developing technologies, accessing novel delivery methods or exploring early licensing opportunities in the field of infectious diseases.

Another key mission will be to engage with funding agencies and investment networks to streamline and secure financial support for promising projects. This includes advising on structuring grants, preparing for seed-stage fundraising, and organizing dedicated investor-researcher meetings. Furthermore, the group will aim to provide guidance to facilitate navigation through the complex regulatory landscape (clinical trial authorizations, EMA/ANSM procedures).

Altogether, these actions are expected to accelerate innovation timelines, help the emergence of groundbreaking vector engineering strategies and promote more rapid patient access to next-generation therapies.

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WHY DIAGNOSTICS MATTER IN THE FIGHT AGAINST ANTIMICROBIAL RESISTANCE

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Throughout the 20th century, public health has been transformed by innovations such as improved hygiene, vaccines, and antibiotics, which have had a profound impact on life expectancy and quality of life. Since their introduction in the early 1940s, antibiotics have played an indispensable role in modern medicine. They not only treat common bacterial infections but also support modern medicine by enabling complex medical procedures such as organ transplants, cancer treatments, immunotherapies, and intricate surgeries.

However, overuse and misuse of antibiotics in humans, animals, and agriculture have led to the rise of antimicrobial resistance (AMR). Currently, nearly 1.14 million people worldwide die directly from AMR and 4.71 million indirectly (1). Alarmingly, even the most powerful antibiotics are threatened as it is estimated that, by 2035, resistance to the so-called 'Reserve' antibiotics will increase by 45% and resistance to 'last-resort' antibiotics by 328% compared to 2005 (2). As a result, the entire disease management process is affected by AMR.

As this persistent and silent threat continues to grow, the current clinical pipeline of available antibiotics, including those recently approved, remains "insufficient to tackle the challenge of antimicrobial resistance" (3). Most major pharmaceutical companies have removed antibiotics from their R&D priorities, due to the lack of sufficient return on investment, unlike other therapeutic areas, such as oncology and chronic diseases, and despite the life-saving nature of antibiotics. Innovation in antibiotics is primarily driven by startups, which often lack the financial resources to fund large and costly clinical trials. Fortunately, new policy initiatives and business models are being developed to better support this crucial therapeutic area, like the "pull mechanism" used in the UK Antibiotic Subscription Model Pilot (4).

Antimicrobial Stewardship and the Role of Diagnostics

AMR has now escalated to the point where the WHO has identified it as one of the top 10 global public health threats to humanity (5).

In response to this challenge, antimicrobial stewardship (AMS) has emerged over the past two decades as one of the key strategies to combat AMR. AMS involves the careful and responsible use of antimicrobials (mostly antibiotics but also antivirals and antifungals) (6, 7).

AMS aims at achieving the prescription of the most appropriate antimicrobial therapy with a dual objective:

- Improve individual patient outcomes in the short term,
- Preserve antibiotic efficacy and thus modern medicine and public health in the long term.

However, antimicrobial prescription relies on essential information provided by clinical examination and in vitro diagnostics (IVD) to determine the nature of the causative pathogen and its susceptibility to antimicrobial agents, as well as the immunological status of the patient.

This information is used to best guide individual therapy all along the clinical pathway (Figure 1). Additionally, such tests also help to protect the hospital community (other patients and healthcare workers) by preventing and controlling transmissible infections caused, for example, by respiratory viruses or multidrug-resistant organisms (MDROs).

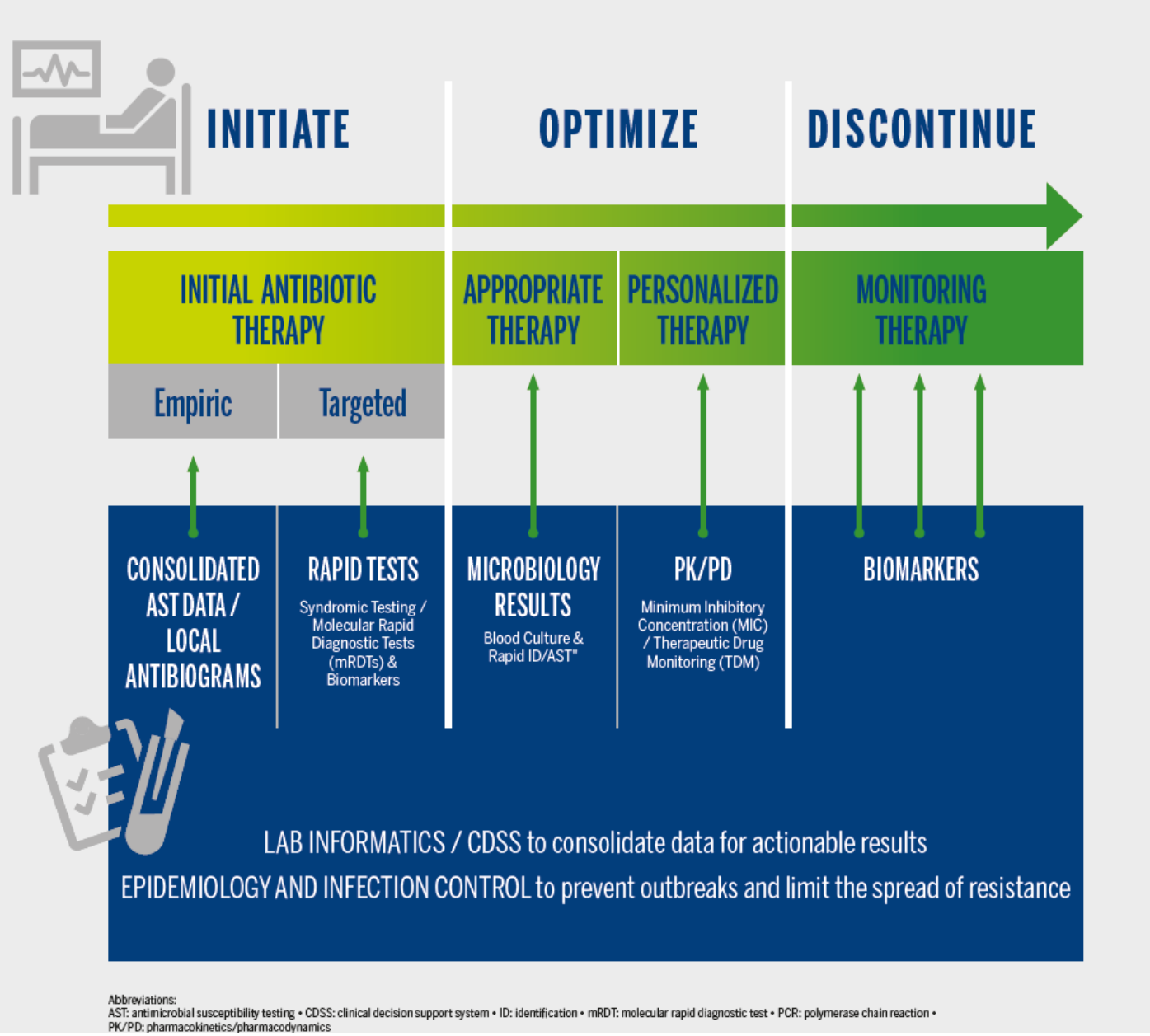

Figure 1: *Illustration of how diagnostics support the antibiotic prescribing process all along the patient care pathway*

Timely diagnostic test results enable tailored antibiotic therapies, optimizing patient health outcomes and generating healthcare cost-savings - this is the essence of medical value (8). Furthermore, using fewer antibiotics with a targeted spectrum reduces the pressure to select resistant organisms, thereby helping to maintain the efficacy of existing antibiotics.

Combatting AMR with innovative diagnostic solutions

Leading the charge in innovation and R&D, many diagnostics companies have invested over several decades to develop new technologies that can be utilized in laboratories or closer to patients, thereby enhancing AMS support.

- **Rapid Diagnostic Testing/Tests (RDTs)**

Automated antibiotic susceptibility tests (AST) help reduce time to microbial identification and resistance mechanism detection. Microbiology labs equipped with automated systems can now provide results from bacterial colony growth as well as direct from samples – including blood, cerebrospinal fluid, stool, and respiratory samples, and they can do it in hours rather than days. This rapid turnaround supports clinicians in personalizing patient therapy sooner and reducing unnecessary use of antibiotics.

More sophisticated technologies have also emerged in recent years that can rapidly identify infectious microorganisms and their antibiotic resistance determinants from culture isolates using mass-spectrometry techniques (MALDI-TOF) or directly from clinical samples using molecular techniques. A notable advancement is the syndromic panel approach, where user-friendly polymerase chain reaction systems can detect the genetic fingerprints of pathogens responsible for specific clinical conditions such as respiratory infections, pneumonia, meningitis, encephalitis, and bloodstream infections. With ever shorter result times (within 15 minutes), these technologies are becoming increasingly available at the point of care, such as hospital emergency departments or doctors' offices.

These technologies significantly expedite antimicrobial therapy, reducing the duration of antibiotic use and inpatient stays, lowering patient mortality and isolations times, and enhancing infection control when integrated with AMS programs (9). This not only results in cost savings but improves productivity and boosts patient and physician satisfaction. An increasing body of clinical utility data supports inclusion of these innovative technologies in clinical guidelines, as seen with severe community acquired pneumonia (10).

- **Biomarkers and Host Response Monitoring**

A complementary approach involves monitoring host biomarkers in the early stages of an infection to reflect the patient's bodily response. The COVID-19 pandemic highlighted the overprescription of antibiotics relative to actual bacterial co-infection rates (11). The potential role of biomarkers in excluding bacterial co-infection was demonstrated, showing that a negative procalcitonin (PCT) result upon admission correlates with shorter antimicrobial courses, earlier cessation of therapy, and predicts a lower frequency of intensive care unit (ICU) admission (12). Another study suggests that using PCT as a guide for de-escalation of antibiotics in ICU patients significantly reduced antibiotic usage by two days (13).

- **Information Technology and Decision Support**

Information technology (IT) capabilities and software solutions are crucial to the success of AMS programs. Companies continue to develop new IT solutions to optimize laboratory work and aggregate data from various hospital sources (e.g., patients' electronic health records, pharmacy, laboratory) into insightful information for real-time patient management. For instance, clinical decision-support software (CDSS) can help increase compliance with evidence-based care guidelines and antibiotic susceptibility test results, leading to optimized antibiotic prescribing decisions (14).

With advanced IT capabilities, healthcare systems can push real-time information and respond swiftly if an antibiotic treatment needs to be halted. Delaying action by even four to five days can mean the difference between preventing a misdiagnosis and, consequently, developing antibiotic resistance, which makes future treatments less effective.

- **Artificial Intelligence in Infectious Disease Management**

The field of data science is rapidly evolving from expert systems based on rigid rules to machine learning systems where algorithms learn from data and interpret unknown situations. In infectious diseases, this translates into earlier and more accurate prediction tools, such as (15):

- Prognostic scores for specific populations (e.g., patient severity, sepsis alerts)
- Prediction of resistance (e.g., risk of MDRO carriage)
- Interpretation of AST results
- Selection of antibiotic regimens
- Prediction of treatment responses

- **Public Authorities and Healthcare Involvement**

Although AMR has not attracted as much attention as COVID-19, leaders and international organizations (16) consider it to be a looming threat.

Many health agencies, including the ECDC, recognize the need to allocate a larger portion of healthcare expenditure towards innovations in diagnostics. Promoting the added value that diagnostics bring to the fight against AMR is essential. We must advance towards more integrated diagnostics, associating them with the prescription of specific drugs. It is crucial to establish the shared value in both diagnostics and treatment, moving towards personalized medicine in infectious disease management.

The holistic value of diagnostics to support infection management in general, and AMR in particular, is gradually being revealed through a growing body of evidence. This value extends from the direct and immediate impact on patient health to the indirect and long-lasting effects at the community and population/societal level (Figure 2).

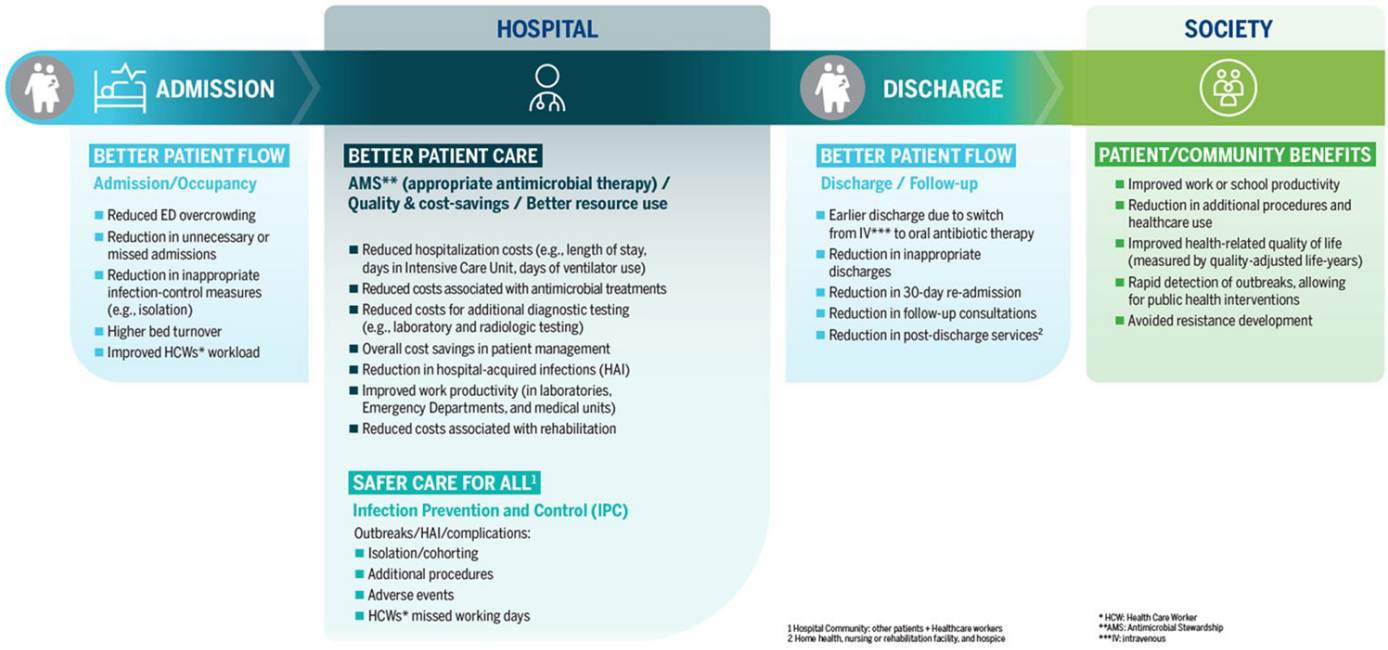

Figure 2: *Value framework of diagnostic supporting infection management in hospital*

The demand for developed AMS programs in healthcare is immense and cannot be overstated. This includes those within healthcare's pharmaceutical systems, which the ECDC considers a key element of AMS (16). It has become vital for healthcare systems and government organizations to incentivize AMS programs, ensuring prescriptions are limited to what is necessary. Often, diagnostic tools needed to distinguish between viral and bacterial infections are under-valued.

Healthcare stakeholders should invest in innovative diagnostic solutions to increase confidence in point-of-care decision-making, helping physicians manage patients more effectively. To provide faster, more accurate results, AMS must be advanced by more hospitals and physicians with diagnostic data management technologies.

Health authorities should also facilitate market access for innovative diagnostic solutions by defining and implementing new economic models to encourage innovation, and by accelerating and simplifying the regulatory approval process.

- **Perspectives for the future**

Diagnostics are essential in curbing AMR, by guiding the appropriate use of antimicrobials, informing infection control, and enabling better surveillance and treatment outcomes. Innovative diagnostic solutions bring game-changing improvements in terms of speed, usability and data accuracy. Yet, despite this, they remain undervalued and therefore underutilized in both policy and practice. The 'way forward' now lies in improved access to diagnostics, which requires reforming the processes for their regulation, approval, reimbursement and funding.

ABOUT BIOMERIEUX

Pioneering Diagnostics

A world world leader in the field of in vitro diagnostics since 1963, bioMérieux is present in 45 countries and serves more than 160 countries with the support of a large network of distributors. In 2024, revenues reached €4 billion, with over 93% of sales outside of France.

bioMérieux provides diagnostic solutions (systems, reagents, software and services) which determine the source of disease and contamination to improve patient health and ensure consumer safety. Its products are mainly used for diagnosing infectious diseases. They are also used for detecting microorganisms in agri-food, pharmaceutical and cosmetic products. www.biomerieux.com.

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MULTIPLEX REAL-TIME PCR DETECTION OF MONKEYPOX VIRUS

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INTRODUCTION

Monkeypox virus (MPXV) is a double-stranded DNA poxvirus that causes mpox (formerly known as monkeypox), which historically has been a rare disease that results in similar symptoms to smallpox (1). Prior to the 2022 outbreak, mpox was mainly found in several countries in Central and West Africa. There are two clades of mpox virus, Clade I and Clade II, and infections from the current outbreak stem from Clade II (2). As of October 3, 2022, monkeypox virus has spread to 106 countries with 68,874 cases, resulting in outbreaks in 100 non-endemic countries (3). The WHO Director-General declared the ongoing mpox outbreak a Public Health Emergency of International Concern on July 23, 2022. U.S. CDC testing guidance recommends hydrolysis probe-based (e.g., TaqMan®) qPCR for detection of viral targets in DNA purified from patient samples, owing to the high accuracy and sensitivity of qPCR (4,5).

To facilitate molecular diagnostic development efforts, here we demonstrate the detection of synthetic monkeypox viral DNA using Luna qPCR reagents. We show high specificity and sensitivity in the detection of monkeypox DNA using the Luna Universal Probe qPCR Master Mix (NEB #M3004). The limit of detection (LOD) on synthetic viral DNA is five copies per reaction. The exceptional performance in multiplex amplification allows users to detect control human DNA in the same reaction. Similar results were achieved with the Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019), which was evaluated due to the concurrent SARS-CoV-2 pandemic and allows users to rely on a single reagent for detection of both viruses, if desired.

MATERIALS

Reagents
The list of reagents used is available here.

Primers and probes
See Table 1

Template
ATCC® Quantitative Synthetic Monkeypox virus DNA (#VR-3270SD)

TABLE 1: Primers and Probes
Cycling conditions (NEB recommendation): Single-plex or 2-plex: 95°C for 1 min., 45 cycles of 95°C for 10 sec., 60°C for 30 sec. PCR instruments: Bio-Rad CFX Opus (Default mode), Applied Biosystems (ABI) 7500, Thermo Fisher Scientific QuantStudio 6

ASSAY	PRIMER/PROBE		REF.	NOTE	CONC. (µM)
	NAME	SEQUENCE			
CDC-Non-variola Orthopoxvirus	CDC-OP-F	5′-TCAACTGAAAAGGCCATCTATGA-3′	CDC	Probe quencher modified	Primer: 0.4 Probe: 0.2
	CDC-OP-R	5′-GAGTATAGAGCACTATTTCTAAATCCCA-3′			
	CDC-OP-FAM	5′-FAM-CCATGCAAT/ZEN/ATACGTACAAGATAGTAGCCAAC-3′IABkFQ			
RP-DNA	RP-DNA-F	5′-AGATTTGGACCTGCGAGCG-3′	CDC	Probe fluorescent dye and quencher modified	Primer: 0.4 Probe: 0.2
	RP-DNA-R	5′-GAGCGGCTGTCTCCACAAGT-3′			
	RP-DNA-Cy5	5′-Cy5-TTCTGACCT/TAO/GAAGGCTCTGCGCG-3′ IABRQ			
MP-Generic	MP-G-F	5′-GGAAAATGTAAAGACAACGAATACA-3′	CDC	F primer and probe quencher modified	Primer: 0.4 Probe: 0.2
	MP-G-R	5′-GCTATCACATAATCTGGAAGCGTA-3′			
	MP-G-FAM	5′-FAM-AAGCCGTAA/ZEN/TCTATGTTGTCTATCGTGTCC-3′IABkFQ			

RESULTS

Detecting Synthetic MPXV Using the Luna Universal Probe qPCR Master Mix

Both the non-variola orthopoxvirus generic and monkeypox virus generic single-plex test procedures outlined by the CDC were evaluated using the Luna Universal Probe qPCR Master Mix (NEB #M3004) on three different realtime PCR instruments: Bio-Rad® CFX Opus, Applied Biosystems® (ABI) 7500 and Thermo Fisher Scientific® QuantStudio® 6. Each CDC test procedure requires a minimum of two individual reactions per patient sample: one for nonvariola orthopoxvirus or monkeypox virus and a second reaction for human DNA as a control (e.g., RNase P). The non-variola orthopoxvirus single-plex reaction (CDC-OP) includes a pair of primers and a FAM labeled probe that targets a region of the monkeypox virus F8L gene (4). The CDC-OP primer/probe set also detects other orthopoxviruses (e.g., cowpox) except for smallpox, which is caused by variola virus. Although not specific for monkeypox virus, positive detection using the CDC-OP assay and clinical presentation is sufficient for treatment. The monkeypox virus generic test (MP-Generic) targets the tumor necrosis factor (TNF) receptor gene (J2L) (5,6). However, three recent cases in California have shown a significant deletion in this gene that may lead to false negative results, suggesting caution must be used when using this assay. In our evaluation of these two target designs, the quenchers of both probes were modified according to recommendations provided by the oligo vendor. Additionally, the forward primer for the MP-Generic test was truncated by a single base. Data was collected using our standard concentration recommendations for primer (0.4 µM) and probe (0.2 µM) with slightly modified cycling condition for NEB #M3004 (Table 1). For ABI instruments, the passive reference dye was set to ROX to enable data normalization.

The Luna Universal Probe qPCR Master Mix detected the F8L or J2L gene target from 53,000 copies down to 5.3 copies of synthetic viral DNA template (Synthetic Monkeypox Virus DNA from ATCC #VR-3270SD) on all three instruments with exceptional sensitivity, reproducibility, and qPCR performance using either the non-variola Orthopoxvirus primers/ probes or the monkeypox virus generic primers/probes (Figure 1). Similar performance was observed using the Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019, data not shown). Although this Luna one-step mix is typically intended for RNA detection, the reagent allows higher sample input volumes given its 4X concentration and includes thermolabile UDG for carryover prevention. Given the ongoing SARS-CoV-2 pandemic, this single mix can be used to detect either virus. Furthermore, the CDC's recommendations for primer/probe concentrations and cycling conditions can also be used with either Luna reagent with no observable impact to MPXV detection.

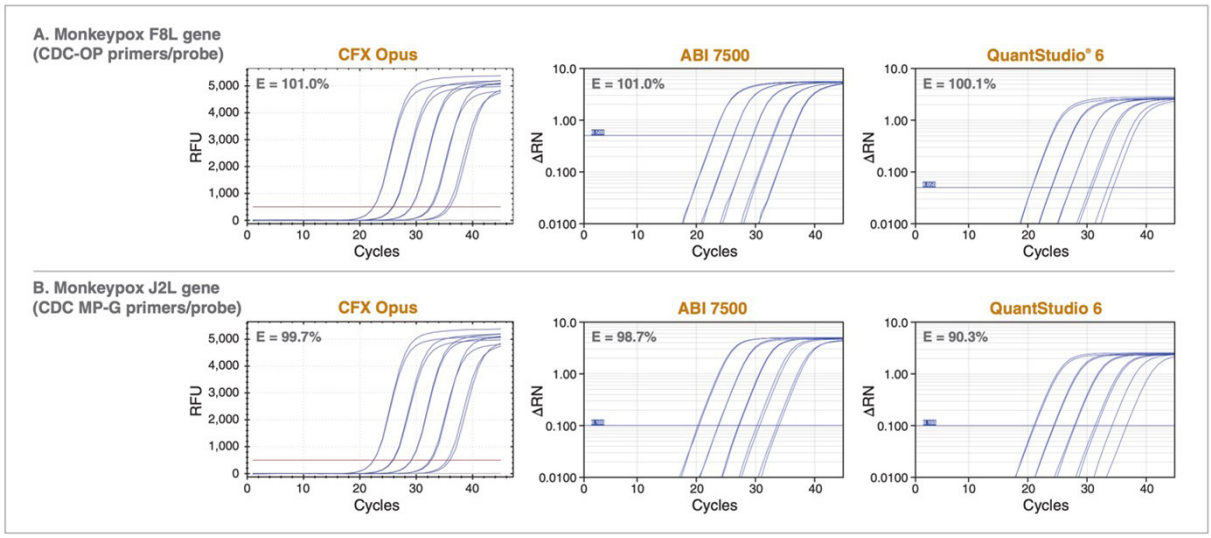

Figure 1: *The Luna Universal Probe qPCR Master Mix offers exceptional sensitivity, reproducibility and qPCR performance. Detection of the monkeypox F8L gene using the CDC-OP primers/probe (A) and the J2L gene using the CDC MP-G primers/probe (B) was performed using the Luna Universal Probe qPCR Master Mix over a 5-log range of input template (53,000 cp – 5.3 cp synthetic monkeypox virus DNA, ATCC #VR-3270SD) with 2 replicates at each dilution. Instruments: CFX Opus (Bio-Rad®), ABI 7500 and QuantStudio 6 (Thermo Fisher Scientific®).*

Sensitive Detection of Synthetic MPXV using the Luna Universal Probe qPCR Master Mix in 2-plex assay.

Multiplex assays offer a more efficient testing option in diagnostic settings, allowing a single sample to be interrogated for various targets simultaneously. Each MPXV CDC test design described above utilizes detection of RNase P in a second independent reaction as a control to confirm the presence of human DNA. We investigated the use of a 2-plex assay that allows for detection of human RNase P and the monkeypox virus F8L gene (CDC-OP) in a single reaction. The FAM fluorophore of the human RNase P probe was changed to Cy5™ to accommodate the multiplex design. This allows detection of the internal control in the Cy5 channel while non-variola Orthopoxvirus detection remains in the FAM channel.

The Luna Universal Probe qPCR Master Mix detected the monkeypox F8L target from 53,000 copies down to 5.3 copies of synthetic viral DNA template (ATCC #VR-3270SD) in the presence of 5 ng Jurkat DNA on all 3 instruments (Figure 2), consistent with the performance in the single-plex assay. To determine the LOD (95% confidence) of the monkeypox 2-plex assay, we evaluated the Luna reagent on all three real-time PCR instruments. The LOD for each instrument was established by testing 34 replicates of 5-copy synthetic viral DNA input and 2 non-template controls. The Luna Universal Probe qPCR Master Mix detected all 34 replicates on all three qPCR instruments, while the non-template control reactions lacked amplification. RNase P was also 100% detected simultaneously on Cy5 channel. Similar results were also observed using the Luna Probe One-Step RT-qPCR 4X Mix with UDG.

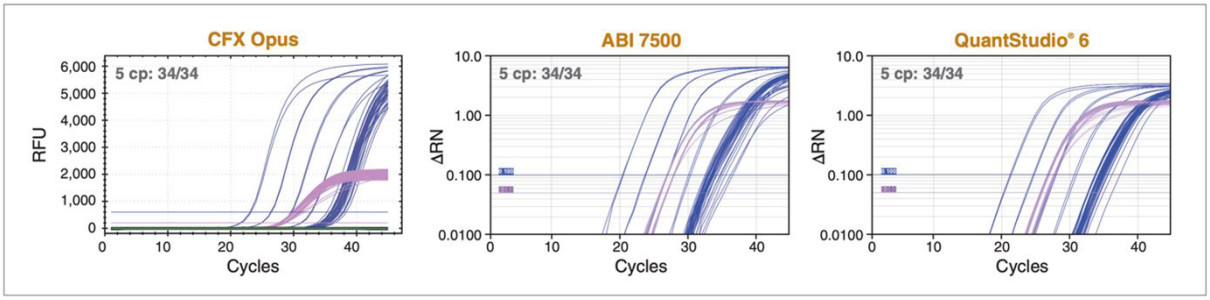

Figure 2: *The Luna Universal Probe qPCR Master Mix offers robust performance in 2-plex detection of synthetic monkeypox and human RNase P control DNA. 2-plex qPCR assays targeting the monkeypox F8L gene (CDC-OP, blue) and human RNase P DNA (RP-DNA, light purple) were performed using the Luna Universal Probe qPCR Master Mix over a 5-log range of input template (53,000 cp – 5.3 cp synthetic monkeypox virus DNA, ATCC #VR-3270SD, diluted in 5 ng/µl Jurkat genomic DNA) with 2 replicates at each dilution. To verify the limit of detection (LOD) of the Luna qPCR reagent, an additional 34 replicates of 5 cp input was tested in the 2-plex assay. Across all three instruments, 34 out of 34 replicates were detected. Instruments: CFX Opus (Bio-Rad), ABI 7500 and QuantStudio 6 (Thermo Fisher Scientific).*

Reagent Tolerance to Viral Transport Medium (VTM).

Currently, both the WHO and the CDC recommend specimen collection from skin lesion swabs and DNA extraction for diagnostic testing. However, extraction-free workflows may be possible. Given that swabs can be stored dry or in Viral Transport Medium (VTM), VTM tolerance is critical for development of direct detection workflows of monkeypox virus DNA in patient samples. We therefore tested the effects of VTM on the Luna Universal Probe qPCR Master Mix. The Luna reagent was highly tolerant of VTM, with no detectible effect on quantitation and LOD detection with 2 µl VTM presence per 20 µl reaction (10% v/v) (Figure 3). Detection of low input (5 copies/reaction) was impacted by 5 µl VTM per 20 µl reaction (25% v/v), but negligible effects were observed for high inputs.

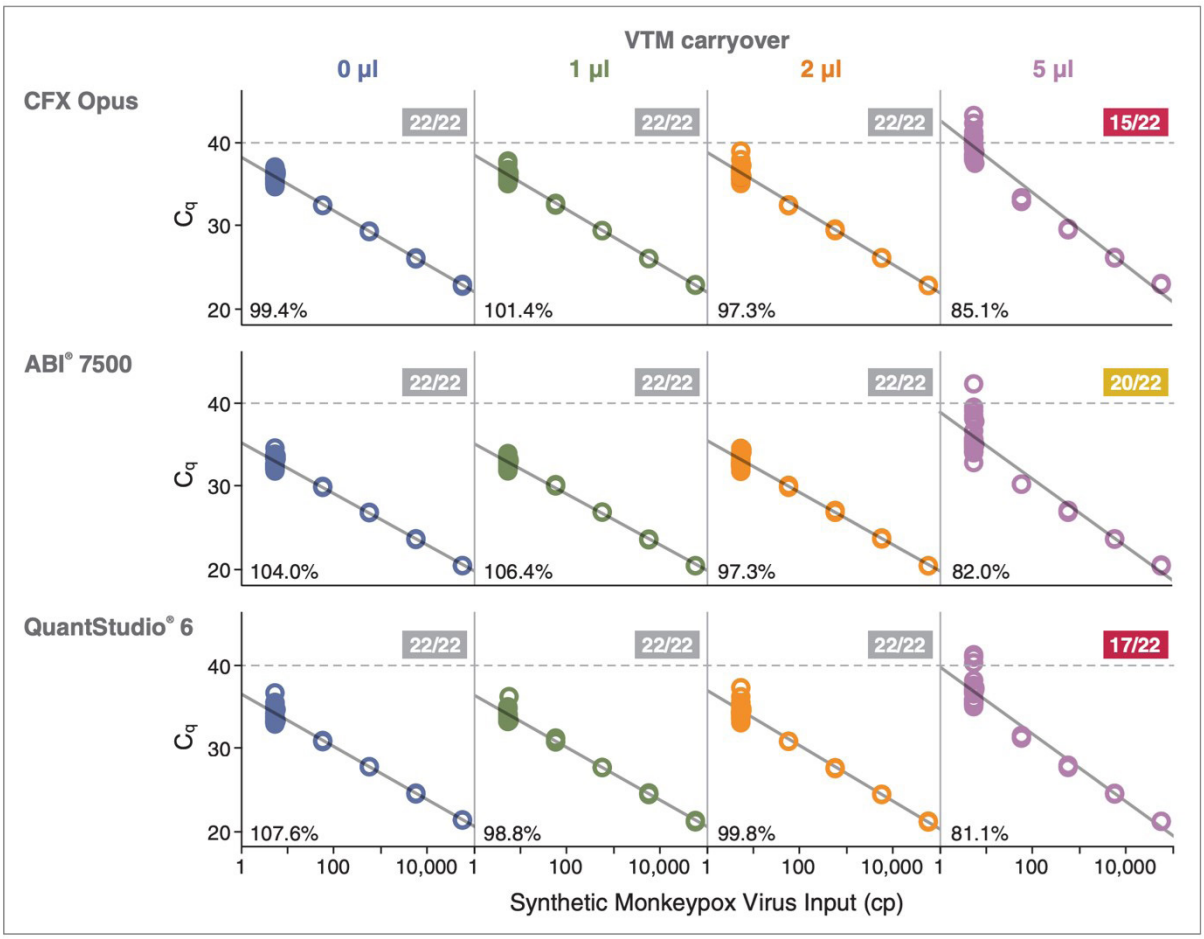

Figure 3: *Luna Universal Probe qPCR Master Mix tolerates up to 10% Viral Transport Medium. 2-plex qPCR assays targeting the monkeypox F8L gene (CDC-OP) and human RNase P (RP-DNA) were performed using the Luna Universal Probe qPCR Master Mix over a 5-log range of input template amount (53,000 cp – 5.3 cp synthetic monkeypox virus DNA, ATCC #VR-3270SD in 10 ng of Jurkat RNA) with 2 replicates at each dilution. To test any impact to LOD, an additional 22 replicates of 5 cp input was tested in the 2-plex assay. The assays were performed in the absence or presence of a gradient of Viral Transport Medium (VTM) up to 5 µl in a 20 µl reaction. Instruments: CFX Opus (Bio-Rad), ABI 7500 and QuantStudio 6 (Thermo Fisher Scientific).*

CONCLUSION

Molecular diagnostics continue to play a critical role in the detection and diagnosis of infectious diseases. The recent rise in mpox cases around the globe has sparked concern and many labs are turning to nucleic acid amplification tests to help prevent the spread of the disease. The details outlined herein showcase how the Luna reagents can be used in previously developed single-plex real-time PCR assays by the CDC for detection of monkeypox virus while achieving an LOD of 5 copies of synthetic DNA per reaction. Modification of the fluorophores and use of the Luna reagents allows these real-time PCR assays to be converted into multiplex tests. We hope the high specificity and sensitivity data and recommendations presented will help labs develop mpox assays that meet their specific needs.

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Founded in 1974 as a collective of scientists dedicated to developing innovative products for the life sciences industry, New England Biolabs® (NEB®) is proud to be a world leader in the discovery and production of enzymes for molecular biology applications – from cloning to advanced tools for NGS, genome editing, and synthetic biology to support among other key biotech markets incl. molecular diagnostics, biotherapy and vaccines development.

Since its establishment, NEB has remained committed to delivering high-quality, innovative tools that support both external research and its own scientific endeavors. The company places strong emphasis on scientific progress, environmental responsibility (B Corp-certified), and community engagement. NEB's profits have consistently funded an extensive research program, driving continuous innovation and ensuring close alignment with the evolving needs of the scientific community.

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TRANSFORMING THE RESPONSE TO INFECTIOUS DISEASES WITH ARTIFICIAL INTELLIGENCE

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MAbSilico

Introduction

Global changes like population growth, urbanization, ecosystem disruption, climate change, and extensive travel are spurring the emergence of new diseases, necessitating innovative solutions beyond traditional public health and clinical methodologies. Artificial Intelligence (AI) has emerged as a transformative force, poised to redefine our capacity to predict, detect, manage, and control infectious diseases. AI's strength lies in its unparalleled ability to process vast, complex datasets at unmatched speeds, unlocking new paradigms in surveillance, diagnostics, therapeutics, and public health intervention strategies. While traditional methods face delays, AI offers real-time analysis of diverse data from clinical, environmental, and societal spheres. It enables sophisticated predictive models for epidemic trajectories and automation of tasks like medical image interpretation and pathogen genome deciphering. AI's potential to personalize treatments and dramatically speed up drug and vaccine discovery marks a significant leap in combating infectious threats, fundamentally reshaping infectious disease management towards enhanced preparedness and precision. This paper analyzes AI's multifaceted role across the spectrum of disease management, from early warning and surveillance to diagnostics, therapeutics, vaccine development, and public health response optimization.

I. AI-powered foresight: early warning, surveillance, and outbreak prediction

AI is revolutionizing epidemic intelligence and public health responses by enabling predictive analytics, enhancing real-time surveillance, and providing advanced geospatial insights (1,2). For early warning, AI leverages diverse and unconventional data sources (internet signals, environmental data, electronic health records, pathogen genomics) to detect outbreak signals before they become clinically apparent. The Canadian platform BlueDot, for example, identified pre-clinical outbreak signals of Ebola, Zika, and COVID-19 (https://bluedot.global/). Geospatial AI is used for visualizing disease spread, identifying hotspots, and mapping high-risk areas for diseases like malaria or dengue by analyzing environmental and climate data, and subsequent vectors spread (3).

AI also significantly improves real-time epidemiological monitoring by automating rapid analysis of vast pathogen genomic sequences to identify mutations, track variant spread, and predict characteristics like transmissibility or immune evasion, allowing effective responses such as adapting vaccine formulations, as seen with SARS-CoV-2. These models are facilitated by large data-collection platforms like GISAID (https://gisaid.org/). Machine learning models further forecast outbreak likelihood and zoonotic spillover by monitoring wildlife migration, changes in farming landscapes, population density, and genomic characteristics (4).

II.Enhancing diagnostic precision and speed with AI

Rapid and accurate diagnosis is fundamental to effectively control infectious diseases. AI-powered diagnostic tools for both imaging and non-imaging data hold immense potential for filling diagnostic gaps in resource-limited settings.

II.1. Intelligent analysis of medical imagery (X-rays, CT, Microscopy)

AI in medical imaging can identify subtle disease features sub-perceptible to the human eye, leading to earlier or more accurate diagnoses. CNN architectures automatically learn hierarchical features from imaging modalities like X-rays, CT scans, and microscopy slides. These computer-aided diagnosis (CAD) systems were successfully used in several infectious diseases. During the pandemic, architectures like COVID-Net and ResNet50 demonstrated high accuracy (i.e., 91%) in identifying early signs of COVID-19 pneumonia from chest X-rays (CXR) and CT scans (5). Similar approaches for tuberculosis detection on CXR showed such sensitivity and specificity that the WHO recommended CAD as an alternative to human readers for CXR interpretation in 2021 ((6) and for review: (7)). CNN models also analyze microscopy images for malaria diagnosis, crucial in developing countries where trained personnel are lacking. Go et al. developed an automated method reaching 97.50% accuracy for detecting infected unstained blood cells from holographic microscopy images (8). A classification model of Giemsa-stained blood smears showed 99.51% accuracy in discriminating malaria parasite types, i.e. Plasmodium falciparum or P. vivax (9). This is of the utmost importance, as the therapeutic strategy against malaria varies according to the parasite. Stained biopsies can also be analyzed automatically; Aiforia's platform successfully discriminated markers profiles in kidney biopsies from Hantavirus-induced HFRS (Hemorrhagic Fever with Renal Syndrome) vs. other kidney diseases (10).

But beyond pathogen diagnosis, diagnosing antimicrobial sensitivity is critical. Antimicrobial resistance (AMR) is a global health threat associated with millions of deaths in 2021 (11). Weis et al. developed an AMR-prediction model from 300,000 MALDI-TOF spectra and resistance phenotypes, reaching 0.74 to 0.8 accuracy for pathogens like Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae (12).

II.2. Leveraging non-imaging data (EHRs, Lab Results, Symptomatology)

AI algorithms skillfully process complex non-imaging patient data, including structured (lab values) and unstructured data (clinical notes, symptoms) from electronic health records. NLP (Natural Language Processing) models extracts information from textual sources, while ML (Machine Learning) models identify patterns and support diagnostic reasoning. Tools like Bio+ClinicalBERT, XGBoost, Hathr AI, and IBM Watson Health solutions aim to apply AI in healthcare, alleviating burdens on medical professionals.

III. Accelerating the development of therapeutics and vaccines

Traditional drug and vaccine development is a lengthy and expensive process with a high attrition rate. AI tools, grouped under the umbrella of Model-Informed Drug Development (MIDD), significantly accelerate and optimize multiple stages, from target identification (including variant monitoring) to clinical trial design.

III.1. Early discovery of small antimicrobial compounds

The discovery of antibiotics is undoubtedly one of the greatest discoveries of humankind in the 20th century. However, their intensive use for the past 75 years led to the increasing development of resistant bacterial pathogens, and the constant need for new antimicrobial drugs. AI has shown success in selecting and designing antimicrobial molecules (for review: (13,14)). Wong et al. used deep learning to identify substructures linked to antibiotic activity from 12 million chemical compounds, validating 283 predictions and discovering new antibiotic classes effective against methicillin-resistant S. aureus and vancomycin-resistant enterococci in mouse models (15). Stokes et al. developed a DNN (Deep Neuronal Network) for drug repurposing, identifying halicin from 6000 compounds from the Drug Repurposing Hub database (16), which showed broad-spectrum bactericidal activity against pathogens including Mycobacterium tuberculosis, Clostridioides difficile, Acinetobacter baumannii and carbapenem-resistant Enterobacteriaceae (17). Peptide spaces are also examined; Wan et al. used a "molecular de-extinction" strategy with a deep-learning model on extinct organism proteomes, predicting 37,000 peptides with antimicrobial activity, some validated in mice (18).

Further than selection in large collection, AI can design new compounds ab initio. SyntheMol, a generative model, designed 6 novel, validated antibacterial drugs effective against A. baumannii and other phylogenetically-distant pathogens (19). Das et al.'s de novo peptide design identified two peptides potent against multidrug-resistant K. pneumoniae bacteria with low toxicity (20). Porto et al. designed novel guavanin peptides from guava, offering a strategy for developing effective peptide antibiotics from natural products (21).

III.2. Early discovery of large biological antimicrobial compounds

Monoclonal antibodies (mAbs) are prophylactic and therapeutic tools for cancers, autoimmune disorders, and infectious diseases, acting by direct neutralization and indirect immune clearance. Currently, 17 mAbs are marketed for infectious diseases (13 viral, 4 bacterial) (www.antibodysociety.org/resources/approved-antibodies). Antibody AI design is nascent but has seen recent progress in optimization. Zhu et al.'s GUIDE (Generative Unconstrained Intelligent Drug Engineering) engine optimized AstraZeneca's mAb AZD3152 against 24 contemporary and previous SARS-CoV-2 variants, and 20 potential future escape variants (22). Shanker et al. used a structure-informed language model to significantly increase the affinity of 2 anti-SARS-CoV-2 mAbs (i.e. LY-CoV-1404 and SA58) against escape mutants (23). These studies highlight AI's value in modifying existing therapies and enabling novel antibody discovery.

III.3. AI-driven vaccine design

Vaccines are fundamental to public health. AI is increasingly important in the development process of vaccines (for review: (24)). For antigen selection, AI analyzes pathogen genomic data and protein structures to predict B- and T- cells epitopes, their exposure on MHC molecules and immunogenicity. Deep learning identified novel antigens for COVID-19 (25,26) and malaria vaccines (27), particularly useful for adapting to mutating pathogens. For formulation, AI helps discover and refine adjuvants using virtual screening and modeling to enhance immune response (28). AI also predicts and optimizes vaccine stability, critical for mRNA vaccines notably (29). Zhang et al.'s LinearDesign method for mRNA codon and structure optimization significantly enhanced stability and immunogenicity for COVID-19 and varicella-zoster virus vaccines, boosting antibody titers (30).

III.4. Clinical trials optimization and analysis

AI helps design and execute clinical trials more efficiently, by identifying suitable patient cohorts (e.g., by genetic markers) notably. Real-time AI analysis of trial data allows agile decision-making, improved safety monitoring, and early efficacy signal detection, potentially shortening trial durations. "In silico" trials using virtual patients or digital twins are emerging (31). They are computational models simulating human responses trained from disease biology, pathophysiology, and known pharmacology. They offer potential for virtual control arms, reducing placebo needs, and accelerating timelines, especially for rare diseases. These technologies aid in predicting drug efficacy/safety (like, e.g., QSP (Quantitative Systems Pharmacology) models simulating drug metabolism and effects), evaluating devices, and identifying patient subgroups for personalized medicine. However, the field needs robust validation, high-quality data, and evolving regulatory frameworks. Currently, virtual patients only complement traditional trials but hold immense potential for more efficient, ethical, and patient-centric research.

IV.Optimizing public health interventions and healthcare operations

AI's utility extends to public health responses and healthcare management, providing tools for intelligent resource allocation, enhanced containment, and effective public communication, ensuring timely and impactful interventions.

IV.1. Intelligent resource allocation, patient triage and healthcare system preparedness

Epidemics strain healthcare systems. AI predicts healthcare demand and optimizes resource allocation (beds, ICU capacity, ventilators, staff, medicines) by analyzing historical trends, epidemiological forecasts, and real-time infection patterns. This allows a proactive stance in preparing for surges. AI also shows promise in patient triage during mass casualty events or pandemics, prioritizing care by assessing severity and other factors to optimize patient flow (32). Digital contact tracing applications augmented manual efforts in countries like South Korea and Singapore during COVID-19 (33,34). Such AI-driven foresight was invaluable during COVID-19, helping prevent healthcare systems from being overwhelmed.

IV.2. AI for public health communication and combating misinformation

Outbreaks often trigger an "infodemic" of misinformation. During COVID-19, AI-powered chatbots like the French CovidBot (https://info.covidbot.fr/) provided accurate health information, encouraged compliance with guidelines, offered symptom assessments, and mental health support, offering scalable 24/7 support. AI is critical in this "infodemic counter-offensive". NLP algorithms monitor social media and news to understand public concerns, and track misinformation. For example, Dominique, an AI assistant in Brazil (https://autoaipandemics.icmc.usp.br/), opposed COVID-19 disinformation. This real-time analysis enables officials to tailor communication and counter false narratives, making AI essential for modern public health communication during crises.

Conclusion

AI integration in clinical medicine, particularly infectious diseases, significantly augments capabilities in surveillance, diagnostics, therapeutic innovations, and public health operations. A comparative table of AI applications in key infectious diseases in provided in Table1. However, challenges including lack of disease specificity, data scarcity, and spectrum bias (gender, racial) in AI models, underscore needs for refinement and regulation. Ethical issues of patient privacy, data protection, and health equity require careful consideration to prevent exacerbating disparities. The European Commission's AI Act (https://digital-strategy.ec.europa.eu/en/policies/regulatory-framework-ai) is the first comprehensive law addressing these issues. Legal implications of AI-related errors and liability need clear frameworks. Addressing these limitations by professionals, policymakers, and researchers is crucial for realizing AI's full benefits. Despite challenges, AI's transformative potential is undeniable, with evidence of improved patient outcomes and optimized healthcare delivery. The accuracy, reliability and interpretability of AI tools are expected to increase even further in the future. It holds immense potential for a qualitative leap forward in our ability to combat infectious threats.

Infectious Disease	Primary AI Use Cases	Notable AI Tools/Platforms/Techniques	Documented Impact/Successes	Specific Challenges Encountered
COVID-19	Early warning, CXR/CT diagnosis, forecasting, resource allocation, contact tracing, public health messaging, vaccine research input	BlueDot, ResNet50, COVID-Net, various ML/DL forecasting models, NLP for sentiment analysis, AI chatbots	Rapid detection, expedited diagnosis, informed resource planning, enhanced public communication	Data heterogeneity, model generalizability, rapid viral evolution, infodemic management.
Influenza	Forecasting seasonal activity, syndromic surveillance, risk prediction from EHRs	LSTMs, CNN-LSTM hybrids, ML classifiers, NLP for social media analysis	Improved short-term forecast accuracy, identification of at-risk individuals	Model interpretability, external validation, data sparsity, overfitting.
Tuberculosis (TB)	CXR-based detection/screening, CAD software, potential for treatment outcome prediction	CNNs for image analysis, SVMs, various CAD platforms (e.g., via TB REACH)	Increased case detection, accelerated diagnosis, screening in remote/vulnerable populations	Access to quality X-ray equipment in LMICs, integration into workflows, training personnel.
Malaria	Parasite detection in blood smears (microscopy), vector surveillance (image/acoustic), risk mapping	CNNs for image/acoustic analysis (VectorCam, HumBug), citizen science platforms (Mosquitodashboard.org)	Faster/more accurate parasite detection, enhanced mosquito surveillance, community engagement	Scalability in low-resource settings, data for diverse mosquito species, user adoption of new tools.
Ebola Virus Disease	Outbreak prediction, real-time surveillance, contact tracing support, resource allocation	NLP for news/report analysis (e.g., BlueDot), predictive modeling	Early warnings of potential spread, support for response coordination	Severe data limitations during acute outbreaks, infrastructure challenges in affected regions, real-time data integration.
Hantavirus	Research into pathogenesis via image analysis of kidney biopsies (quantifying immune markers)	AI image analysis platforms (e.g., Aiforia) for quantitative histology	Enhanced understanding of immune responses in HFRS, standardized analysis of tissue samples	Primarily research-focused currently, translation to routine diagnostics or prediction.
Zika Virus	Early outbreak detection, forecasting research, misinformation control	NLP for signal detection (BlueDot, HealthMap), forecasting models, AI chatbots for information	Early warnings, improved understanding of spread dynamics	Limited data during initial emergence, complexity of vector-borne transmission modeling.

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FROM RESEARCH TO CLINICAL TRIALS: BUILDING A NASAL VACCINE PLATFORM FOR THE FUTURE

Lovaltech, BioMAP, Olon France, Biodyssey

Introduction

A nasal subunit vaccine against COVID-19 has been developed by the BioMAP research team from the "Infectiology and Public Health" unit (UMR ISP 1282) a joint research unit between INRAE and the University of Tours supported by a broad academic and industrial collaborative network.

This vaccine combines a unique multivalent fusion protein with biocompatible mucosal nanocarriers. In relevant preclinical models, it has demonstrated its ability to induce both systemic and mucosal immune responses, particularly at the respiratory tract, the primary site of viral entry. This protective immune response blocks viral replication at an early stage, providing : 1/ protection against symptomatic forms of COVID-19; 2/ a reduction in contagiousness, thereby reducing virus circulation and the emergence of new variants. Thanks to its innovative protein design, this vaccine offers cross-protection against both current and future SARS-CoV-2 variants.

Today, this vaccine claims to be the only one in the world capable of providing total protection against SARS-CoV-2 infection.

To transfer this academic innovation into a viable product, Lovaltech, a biotech company based in Tours and labeled as a DeepTech by BPI France, was created in January 2022. Lovaltech is leading the industrial development and clinical progression of the nasal vaccine until its commercialization.

With initial financial support from MESRI and ANRS-MIE, Lovaltech led the GMP development phase of the vaccine protein and mucosal excipient (production and pharmaceutical release) through partnerships with the CDMOs Olon France and Stanipharm.

Winner of the i-Lab 2022 National Contest for Innovative Business Creation, the start-up accelerated the development of its vaccine by launching regulatory toxicity and immunogenicity studies to demonstrate the safety and efficacy of the vaccine candidate in animals before. These studies, conducted from October 2022 by C-Ris Pharma (a French CRO specializing in non-clinical studies), confirmed a favorable safety profile of the vaccine.

Regarding the medical device – of unprecedented design – it is being developed according to specifications established by Lovaltech in collaboration with Aptar Pharma, a global leader in the manufacturing of sprays and aerosols for the pharmaceutical market. Aptar Pharma is committed to developing a production line for this device for Lovaltech vaccines on French territory as part of the #France2030 call for projects, for which Lovaltech is a laureate.

To date, the company has completed the main phases of pharmaceutical development for its first vaccine candidate:

- CMC development and analytical methods have been finalized for the mucosal excipient and nearing completion for both for the drug substance (DS) and drug product (DP), with a potency test validating the dual valency of the active ingredient against Spike and Nucleoprotein.
- The nasal delivery medical device has also been validated throught in vitro nasal deposition models and in vivo studies (pilot study in rabbits and regulatory toxicity studies) with the DP.
- GMP production batches for the DS and DP are finalized and certified.
- Clinical Trial Authorization (CTA) has been obtained by French authorities to start the Phase 1 clinical trials.

Lovaltech is now conducting Phase I/II clinical trials for this first 100% French vaccine developed on its platform, with the first patients already vaccinated.

The design of Phase I/II clinical trials, aiming to confirm the safety and immunogenicity of the vaccine, has been carried out in recent months with the support of ANRS | Emerging Infectious Diseases, co-sponsor of the upcoming clinical trials with the CHRU of Tours as the lead investigator and the AP-HP Cochin hospital. This project, winner of the RecH-MIE 2023 program, focuses on a randomized, controlled, multicenter Phase I/II study comparing the safety and immunogenicity of a booster dose of the intranasal COVID-19 vaccine (expressing SARS-CoV-2 N/S recombinant proteins) with a booster dose of the COVID-19 mRNA vaccine in healthy adult volunteers. This trial represents a global First-in-Human milestone.

Lovaltech aims to become a global leader in vaccination by developing a vaccine platform designed to accelerate the creation of new protein-based nasal vaccines targeting infectious diseases that are poorly or not covered by current vaccines and to prevent future pandemics. This platform, relying on the expertise of the BioMAP team, a member of the MAbImprove LabEx and UMR ISP 1282, enables the design and development of vaccine candidates within months up to preclinical proof of concept.

Current pipeline indications include:

1. Influenza: The design of a vaccine protein combining two hemagglutinins from different seasonal variants and the nucleoprotein has been validated, with the objective of a universal vaccine capable of inducing a protective immune response against all influenza strains (TRL 2-3). The INRAE Experimental Infectiology Platform (PFIE) will validate its efficacy in a ferret model.

2. Malaria: Development of a multimeric protein vaccine targeting several Plasmodium antigens specific to different infectious stages (hepatic, blood, and sexual). Lovaltech leverages advances in artificial intelligence to predict complex target protein structures with high precision and develop an effective vaccine. Recent proof-of-concept studies based on nasal administration of total antigens have shown strong immune responses in the spleen and liver after two nasal vaccinations.

3. COVID-Long: Lovaltech has developed an innovative preclinical animal model to study Long COVID, addressing a major unmet medical need. This model replicates the persistent immune dysfunction and inflammation observed in human cases, providing a robust framework to evaluate therapeutic interventions. Building on this breakthrough, Lovaltech is preparing to test the therapeutic efficacy of its intranasal vaccine in this model. The goal is to demonstrate that the vaccine can alleviate symptoms of Long COVID by targeting viral reservoirs and modulating immune responses, paving the way for the development of a novel treatment option for affected patients. These studies will further consolidate the vaccine's potential as a dual-purpose solution, both preventive and therapeutic.

Intranasal spike and nucleoprotein fusion protein-based vaccine provides cross-protection and reduced transmission against SARS-CoV-2 variants

The development of second-generation vaccines, able to induce mucosal immunity at the primary site of viral infection, is imperative to limit virus contagiousness and control the COVID-19 transmission and SARSCoV-2 variation. Nasal vaccines offer an attractive alternative to injectable vaccines and offer promise for respiratory infectious diseases with unmet medical needs and potential future pandemics. They have the capacity to elicit both humoral and cellular-mediated immune responses at systemic and mucosal levels. Nasal vaccines can stimulate the production of neutralizing IgA antibodies in the respiratory tract and promote lung-resident T and B memory cells allowing long-term protection against viral replication and transmission [1,2,3]. Moreover, mucosal IgA exhibits multivalency and cross-protective activity across different SARS-CoV-2 variants [1,4,5]. Concurrently, considerable efforts have been directed towards developing mucoadhesive and biocompatible delivery systems to prolong nasal retention and enhance antigen uptake by antigen-presenting cells (APCs) in the respiratory tract [6,7]. Maltodextrin mucoadhesive nanocarriers (Nc) provide a suitable approach to stimulate mucosal and systemic immunity by improving the stability, absorption, and retention time of antigenic molecules in the nasal mucosa [6,7].

BioMAP research team engineered a nasal subunit vaccine composed of an original heteromultimeric fusion protein consisting of 6 spike proteins and 2 nucleoproteins combined with biocompatible mucosal Ncs. Our findings demonstrate that Nc-SwFN eliciting a robust protective immunity characterized by (1) systemic and mucosal neutralizing antibodies targeting the spike protein, (2) spike and nucleoprotein-specific T-cell responses against SARS-CoV-2 variants, and (3) lung-resident memory T cells involved in the limiting the spread of SARS-CoV-2 spread.

Challenge studies conducted in mouse and hamster models have revealed that Nc-SwFN confers substantial protection against COVID-19 morbidity, mortality, virus dissemination in the lungs, nasal turbinates and brain. Notably, the Nc-SwFN vaccine not only prevents nasal virus shedding but also block virus transmission from vaccinated to unvaccinated animals. Our findings suggest that the nasal Nc-SwFN vaccine approach may hold promise as a broad-spectrum vaccine candidate against current and emerging SARS-CoV-2 variants.

- SwFN protein was associated with the mucosal nanocarrier surface and could mimic the native virion morphological features

The vaccine SwFN fusion protein was designed with two different chains (Fig. 1). The heavy chain (SwFN-hc) consists of the Wuhan ectodomain of the spike protein with a trimerization sequence, the hinge-CH2-CH3 assembly and then the nucleoprotein. Thus, the heavy chain will be dimerized via the hinge-CH2-CH3. In order to have a trimeric spike, a light chain (Sw-lc), consisting of the ectodomain of the spike protein and the trimerization sequence, was added. Several light chains can be self-assembled with the heavy chain dimer. Thereby, the fusion protein represented a heteromultimeric fusion protein, consisting of 6 spike proteins (2 trimer bouquets) and 2 nucleoproteins (Fig. 1). Despite the considerable size of the SwFN protein (~1000 kDa), its satisfying production yield was reached, and its purification was facilitated by the use of protein A affinity chromatography which allowed the recovery of only SwFN fusion protein (with SwFN-hc and Sw-lc) without soluble trimeric spike protein (Sw).

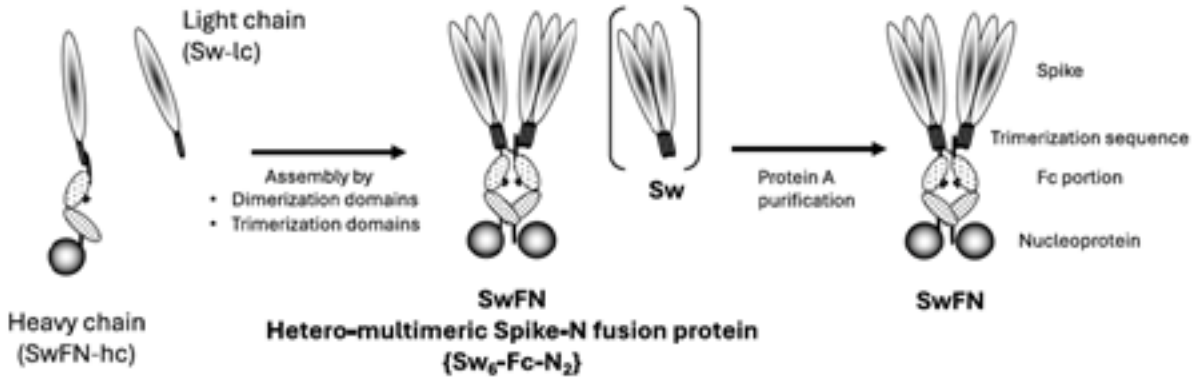

Fig. 1: *Characterization of SwFN protein and Nc-SwFN vaccine. A Schematic representation of the heteromultimeric fusion protein. The two polypeptide chains constituting the fusion protein were presented at the far left, followed by the self-assembly thanks to the dimerization domain/ trimerization sequence block of the different chains. A schematic presentation of the isolated protein was mentioned on the right.*

- Complexation of the vaccine protein with mucosal Ncs was a prerequisite to induce humoral immunogenicity at mucosal level

Proteins were complexed with mucosal Ncs with a ratio of 3:1 (Nanocarriers: Protein). Analysis of the vaccine preparation by Native Page with silver nitrate sensitive staining did not reveal free proteins confirming total protein association to Ncs. Protein complexation with Ncs was visualized with transmission electronic microscopy (TEM) (Fig. 2).

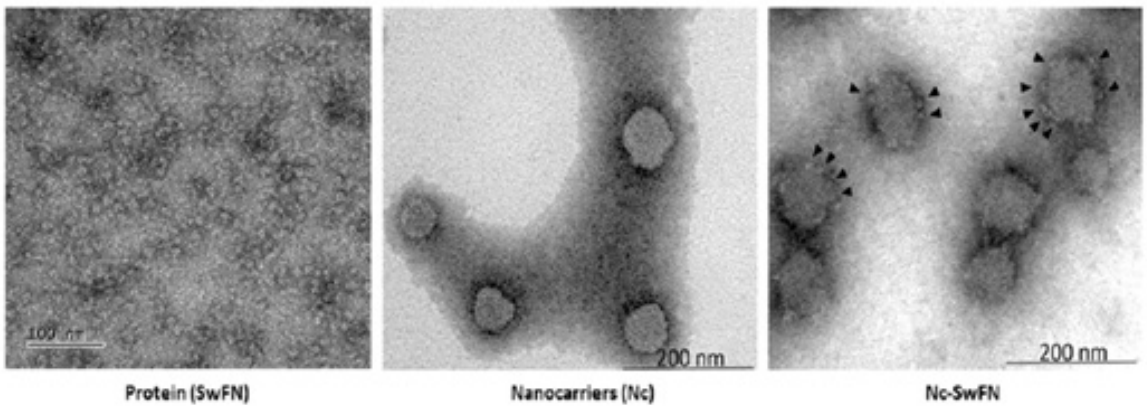

Fig. 2: *Transmission electronic microscopy analysis of the SwFN (left), nanocarriers alone (medium), and the SwFN complexation with the nanocarriers (right). Arrowheads show examples of fusion proteins associated with the surface of the nanocarriers. Scale bar is 200 nm.*

The analysis of humoral immunogenicity, especially in mucosal compartments, is a key feature to confirm the importance of using Ncs as a nasal vaccine delivery system. To prove the added value of maltodextrin mucosal Ncs, female Balb/c mice were immunized twice at three weeks of intervals by the intranasal route with the Nc alone, the SwFN soluble fusion protein (SwFN) or the SwFN complexed protein with the nanocarriers (Nc-SwFN). Humoral immune response was studied 7 days after the last immunization against the spike from the Wuhan variant and nucleoprotein by analyzing IgG and IgA antibodies in serum, nasal, and bronchoalveolar secretions. Only anti-spike antibodies were detected. Compared to the Nc control mice France, Nc-SwFN immunized mice produced significantly higher amounts of serum anti-spike IgG and IgA antibodies. Vaccination with SwFN soluble protein induced only the production of anti-spike IgG antibodies in serum but at a lower level (Fig. 3A, B). The ability of these antibodies to neutralize SARS-CoV-2 virus has been studied. Polymerase chain reaction-based live virus neutralization assays were used to quantify the titer of neutralizing antibodies in serum.

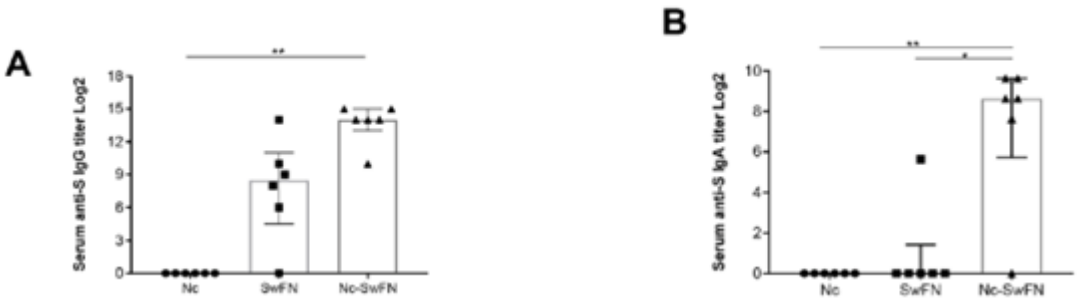

Fig. 3 (A,B): *Humoral immune response against spike protein after SwFN and Nc- SwFN immunizations. Female Balb/c mice were immunized twice at 3-week interval by intranasal route with nanocarriers alone (Nc) (n = 6 animals), SwFN (n = 6 animals), and Nc-SwFN complexation (n = 6 animals). Serum IgG and IgA antibodies were analyzed by specific anti-spike ELISA 7 days after the last immunization and presented respectively in (A, B).*

All data generated or analyzed during this study are available in the specific publication: https://doi.org/10.1038/s41541-025-01123-y

Overcoming CMC Challenges to Deliver a Nasal Vaccine Candidate to the Clinic

Transitioning the Lovaltech vaccine from research-grade material to clinical-grade drug product (DP) required overcoming significant Chemistry, Manufacturing, and Controls (CMC) challenges. Lovaltech, supported by a network of experienced CDMOs including Stanipharm and Olon France and a team of CMC experts from Biodyssey, adopted a phased CMC development strategy to meet regulatory and quality requirements, ultimately enabling the delivery of a compliant regulatory dossier and the production of clinical-grade batches.

Early efforts focused on generating sufficient quantities of protein through transient expression in CHO cells, which served as a starting point for purification feasibility and analytical method screening. In parallel, two plasmid vectors encoding the heavy and light chains of the SwFN fusion protein were constructed, enabling the development of a stable clonal CHO cell line. Selection of the top-producing clone was based on productivity, stability, and quality attributes of the expressed protein.

Once the cell line was established, Lovaltech advanced upstream and downstream process development to meet the purity and viral clearance criteria necessary for clinical-grade material. Olon France played a pivotal role in upstream process optimization and the development of scalable, GMP-compliant production protocols, while also supporting analytical method validation and formulation development.

On the analytical side, Lovaltech designed a comprehensive strategy to characterize both the drug substance (DS) and drug product (DP), including identity testing, purity assessment, concentration analysis, and aggregation monitoring. Analytical methods were progressively refined, validated, and transferred to GMP settings in accordance with ICH guidelines. A key milestone was the development of a robust potency assay demonstrating the dual valency of the antigen against both SARS-CoV-2 Spike and Nucleoprotein, aligned with the vaccine's intended mechanism of action.

For the DP, complexation of the antigen with maltodextrin-based nanocarriers was scaled up and evaluated through in vitro and in vivo testing to confirm nasal deposition, mucosal retention, and immunogenicity. This complexation step, carried out under sterile conditions, added a layer of formulation and fill-finish complexity. Lovaltech also addressed the unique regulatory environment associated with this tripartite product—combining biological DS, chemical excipient, and intranasal medical device—by aligning its CMC strategy with EMA guidelines for combination products.

Throughout 2023 and 2024, Lovaltech manufactured a series of non-GMP and GMP batches at increasing scale to support toxicological studies, DS/DP stability assessment, and clinical trial readiness. GMP-grade clinical batches of DS and DP were successfully released in early 2025, following the approval of the Investigational Medicinal Product Dossier (IMPD), marking the completion of early CMC development. This achievement was made possible thanks to Lovaltech's strategic planning, the close support of Biodyssey, a consulting firm specializing in CMC development for complex biologics. Biodyssey provided operational guidance on analytical development, process scale-up, regulatory directives, and risk mitigation—critical areas where emerging biotechs often face the most significant challenges. Their contribution ensured continuity across the various phases of CMC development and facilitated alignment with regulatory expectations.

The Lovaltech case highlights the importance of early CMC integration, expert guidance, and cross-functional coordination when developing innovative vaccine platforms. The company's success in establishing a robust and regulatory-compliant manufacturing process for its nasal COVID-19 vaccine positions it as a credible player in the field of next-generation mucosal vaccines.

Today, Lovaltech continues its journey with the launch of its Phase I/II clinical trial in France and the inclusion of the first patients, marking a major step toward demonstrating the clinical potential of its nasal vaccine platform.

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ABOUT LOVALTECH

Lovaltech is a innovative leading biotechnological company dedicated to developing innovative health solutions to address global health emergencies. With a strong focus on breakthrough innovation and cutting-edge scientific expertise, Lovaltech strives to improve the quality of life for people worldwide. Lovaltech aims to become a global leader in vaccination through its vaccine platform, with the goal of accelerating the development of new protein vaccines administered nasally to target current infectious diseases poorly or not covered by vaccination, and to counter future pandemics in a One-Health context.

https://www.lovaltechnology.com

ABOUT BIOMAP

BioMAP (Biopharmaceuticals & Microorganisms Against Pathologies) is a research team within the ISP 1282 joint unit (INRAE – University of Tours), specialized in the development of innovative mucosal vaccines and immunotherapies. BioMAP has built a strong expertise in mucosal immunology, protein engineering, and nanoparticle-based delivery systems. The team collaborates with academic and industrial partners to design next-generation vaccines against infectious diseases and cancer. BioMAP played a central role in the conception and preclinical validation of the nasal COVID-19 vaccine candidate developed with Lovaltech.

ABOUT OLON FRANCE

Olon France, part of the Olon Group, is a Contract Development and Manufacturing Organization (CDMO) providing end-to-end manufacturing services for biopharmaceutical companies—from preclinical trials through to full commercial production. As Olon Group's France hub, we leverage global-scale capabilities and strategic synergies to support both clinical- and commercial-scale manufacturing. Our expertise spans industrial process development and the production of biological molecules using mammalian and microbial hosts, as well as the manufacture of bioconjugates and nanoformulated drugs. Olon France is one of the few CDMOs capable of mastering the entire biotherapeutics value chain, offering integrated services from cell-line and strain development to aseptic filling.

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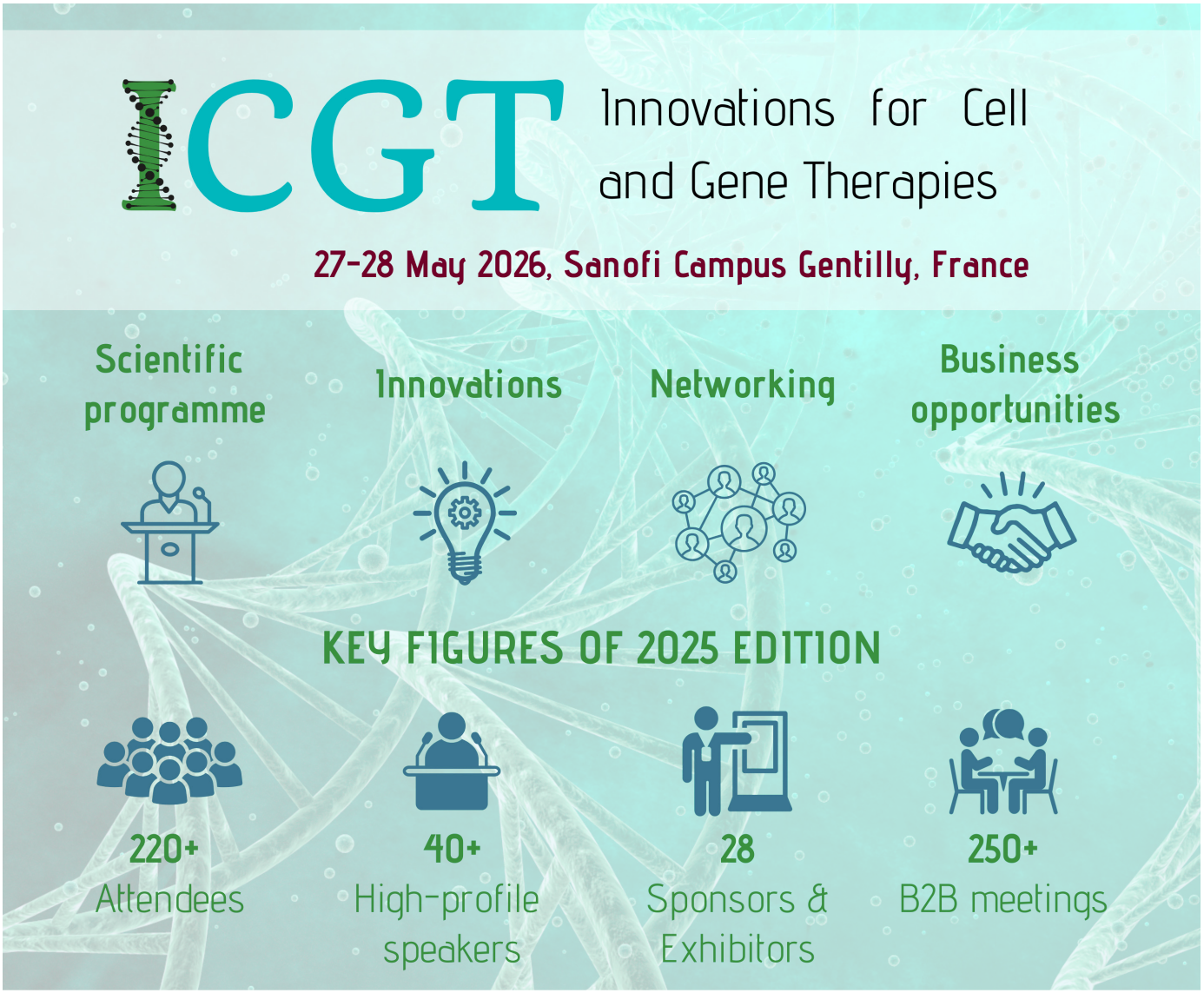
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