Assegno dal titolo “Saggio di immunita’ contro Streptococcus pneumoniae indotta da vaccini a RNA” – Tutor: Prof. Marco R Oggioni, come da piano formativo allegato.

Progetto Ricerca Finalizzata RF-2021-12375437 UO3 dal titolo “Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models” finanziato dal Ministero della Salute - CUP J35E22000760005.

Piano Formativo

- Nel primo mese la/il ricercatrice/ore vincitrice di questo assegno sara’ tenuta ad effettuare il corso e sostenere l’esame colleagto del IZSLER di Biologia e gestione degli animali da laboratorio, moduli 3.1, 4, 5, 6.1, 7. DM 5 agosto 2021 roditori e lagomorfi.

- Nei mesi 2-6 del assegno la/il ricercatrice/ore dovra validare nei laboratori FaBiT modelli di infezione endovenosa con Streptococcus pneumoniae.

- Nei mesi 7-12 del assegno la/il ricercatrice/ore dovra effettuare esperimenti di immunizzazione con mRNA vaccine seguiti da infezione con S. pneumoniae. Dovra’ inoltre valutare sia l’immunita’ dei topi prima del challenge che la protezione conferita.

Ministero della Salute
Direzione generale della ricerca e dell'innovazione in sanità
BANDO RICERCA FINALIZZATA 2021
esercizio finanziario anni 2020-2021 - Progetto Completo

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

MDC primary: Malattie Infettive

MDC secondary: Ematologia e Immunologia

Project Classification IRG: Immunology

Project Classification SS: Vaccines Against Microbial Diseases - VMD

Project Keyword 1: Vaccine development: adjuvants, conjugates, immunomodulators, platforms, DNA vaccine, peptide and protein vaccine, subunit vaccine, live-attenuated vaccine, plant based vaccine, optimization of vaccine delivery using vectors, plasmids and virus-like particles, nanoparticles, needle or needleless technology.

Project Keyword 2: mRNA vaccines

Project Keyword 3: Mycobacterium tuberculosis/streptococcus pneumoniae

Project Request: **Animals:** [X] **Humans:** [ ] **Clinical trial:** [ ]

The object/s of this application is/are under patent copyright Y/N: [ ]

Operative Units

	INSTITUTION	Department/Division/Laboratory	Role in the project
1	Istituto Superiore di Sanita'	Department of Infectious Diseases/Immunology Unit	Coordination, mRNA synthesis, mice immunization and evaluation of humoral and cellular immune responses to mRNA immunization
2	Istituto Superiore di Sanita'	Department of Infectious Diseases/Antibiotic Resistance and special pathogens Unit	Genetic characterization of S. pneumoniae strains, opsonophagocytosis assay on clinical isolates, M. tuberculosis culture
3	University of Bologna	Pharmacy and Biotechnology	Testing host response to pneumococcal vaccine

Operative Unit not SSN: OU3

Investigators, Institution and Role in the Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	MONACO MONICA	2 - Istituto Superiore di Sanita'	Genetic characterization of S. pneumoniae strains, opsonophagocytosis assay on clinical isolates, M. tuberculosis culture	04/08/1970
2	Oggioni Marco Rinaldo	3 - University of Bologna	Testing host response to pneumococcal vaccine	01/01/1965
3	MARIOTTI SABRINA	1 - Istituto Superiore di Sanita'	Mice immunization, humoral and cellular immune responses to M. Tuberculosis	04/07/1975
4	Sgarbanti Marco	1 - Istituto Superiore di Sanita'	DNA vector design for in vitro mRNA synthesis; mRNA synthesis, purification and quality controls; testing of mRNA-driven protein expression in human cells; cellular immunity	03/11/1968

Co-PI: Sgarbanti Marco

Person in charge for the animal experiment: MARIOTTI SABRINA

Ministero della Salute Direzione generale della ricerca e dell'innovazione in sanità BANDO RICERCA FINALIZZATA 2021 esercizio finanziario anni 2020-2021 - Progetto Completo	**Project Title:** Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models **Project duration (months):** 36
Project Code: RF-2021-12375437	**Principal Investigator:** NISINI ROBERTO
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Retired personnel: None within three years of project start

Overall Summary

We developed an immunization system based on in vitro transcribed (IVT) messenger (m)RNA vehiculated by different delivery systems including asymmetric liposomes (AL) which, exposing phosphatidylserine (PS) on their external leaflet, selectively target macrophages (Mø) and dendritic cells. We plan to use this technology to selectively deliver IVT mRNA coding for M. tuberculosis (Mtb) or S. pneumoniae (Spn) proteins together with suitable adjuvants. The mRNA-dependent in situ expression of antigens is expected to generate both cellular and humoral specific immune responses against these bacteria. We will deliver mRNA coding for: i) ESAT-6 and Ag85b, 2 immunogenic and protective Mtb antigens or ii) PspA (family 1 and 2 variants) and Ply (as toxoid), 2 immunogenic Spn proteins. The efficacy of murine immunization will be evaluated by measuring specific antibody titers by ELISA and serum opsono-phagocytosis and cellular immunity by ELISPOT and testing tissue Mø in murine and human models

Background / State of Art

mRNA vaccine technology has demonstrated an unprecedented translational potential characterized by high efficacy and safety during the ongoing SARS-CoV-2 pandemic. mRNA vaccines circumvent the challenges posed by pre-existing or post- vaccination immunity against viral vectors and overcome the need for delivery devices required for DNA vaccines. mRNA does not integrate into chromosomes avoiding the risks of oncogenesis and insertional mutagenesis. In addition to the capacity to elicit high protective antibody titers against translated antigen, mRNA vaccines were shown to induce antigen specific T cell responses. In addition to SARS-CoV-2, mRNA platforms also showed strong potential as vaccines against other viral pathogens, such as Zika or influenza virus, but applications against bacterial diseases have been sporadically approached so far. Lipid nanoparticles (LNPs) were successfully employed for mRNA SARS-CoV-2 vaccination, however other delivery systems are available to be used with the purpose of avoiding the side effects recorded with LNPs. Mtb and Spn are prototypic intracellular and extracellular bacteria, respectively. Prevention of diseases caused by these bacteria relies on available vaccines: BCG for tuberculosis (TB) and polysaccharide vaccine (PPV) or protein-conjugated polysaccharide vaccines (PCVs) for Spn diseases, but several limitations call for improving the strategy for vaccinations against these diseases

It's available a Systematic Review on this topic? Si

It's available as publication please report bibliographic data? Si

Bibliographic data (DOI): 10.1016/S0140-6736(22)00152-0

Hyphotesis and Specific AIMS

Hyphotesis and Significance

We hypothesize that immunization with mRNA represents a valuable tool to confer protective immunity against infectious diseases caused by bacteria. We plan to evaluate the efficiency of mRNA immunization against infection caused by Mtb and Spn that represent prototypical bacteria of preferential intracellular or extracellular growth, respectively. The defense against intracellular bacteria mainly relies on the expansion of antigen-specific T cells, while the production of high affinity specific antibodies with opsono-phagocytosis or toxin neutralization functions protects from extracellular bacteria that often show an intracellular phase that correlates with the invasiveness potential.

Vaccines for the prevention of diseases caused by Mtb and Spn are commercially available: BCG for tuberculosis (TB) and polysaccharide vaccine (PPV) or protein-conjugated polysaccharide vaccines (PCVs) for Spn diseases, but these vaccines have numerous limitations.
For the prevention of TB, several vaccines are being developed to ameliorate the low BCG efficacy, but even the most advanced vaccine candidate failed to demonstrate superiority to BCG in a phase III trial.
For the prevention of diseases caused by Spn, the large number of Spn serotypes (>90), the occurrence of the replacement phenomenon associated with selective pressure due to the PCVs and the variable immunogenicity of the Spn polysaccharides limit the efficacy of polysaccharide-based vaccines for Spn, so a universal vaccine promoting anti-Spn protein antibodies cross-reactive among the serotypes is highly desirable.
We plan to in vitro-transcribe mRNA coding for i) ESAT-6 and Ag85b, two well established immunogenic Mtb antigens in the anti-TB model, or ii) PspA variants and Ply (as toxoid forms), two immunogenic pneumococcal proteins. Different adjuvants, already approved for human use, will be used based on experience with ongoing SARS-CoV-2 vaccine development.
For the delivery of mRNA, we will test different delivery formulations, including the newly developed AL exposing PS on their external leaflet that are targeted to antigen presenting cells. In fact, due to PS exposure, AL were proven to selectively deliver their cargo to Mø and DC, enhancing the innate immunity functions of these cells through bioactive lipids reducing inflammation
The successful validation of our hypotheses will represent a proof of concept for the validity of mRNA based vaccination tailored for diseases caused by extracellular or intracellular bacteria.
In the Mtb model our approach will permit to validate the possibility to induce cellular responses, including CD8 T cells, against in vivo synthesized proteins for intracellular pathogens.
In the Spn vaccination model, we propose to investigate the role of two major Spn proteins as vaccine antigens to circumvent the limits of PPV and PCVs with the theoretic capacity to counteract different Spn serotypes and suitable for immunization of infants and adults. The innovation will include both the first application of a liposome delivered mRNA vaccine to prevention of Spn caused diseases, and the test of a pneumococcal vaccine formulation that includes surface exposed protein and a toxoid, in analogy to the anti-pertussis acellular vaccine.
The experience with the COVID-19 vaccine demonstrates that mRNA vaccination has a rapid translational potential to the SSN.

Preliminary Data

We approached mRNA vaccine technology [1] since it circumvents the challenges posed by pre- or post-vaccination immunity against viral vectors and overcome the need for delivery devices of DNA vaccines [2,3]. mRNA does not integrate into chromosomes avoiding risks of oncogenesis and insertional mutagenesis [4]. mRNA vaccines demonstrated an unprecedented translational capacity during the SARS-CoV-2 pandemic [5] to elicit specific antibody and cellular responses [6,7]. In addition to COVID-19, mRNA platforms showed strong potential as vaccines against other viral diseases [2,3,7], but uses in the protection against bacterial diseases have been occasionally approached [8]. Cationic liposomes (CL) are a delivery system for mRNA vaccines [9] in addition to lipid nanoparticles (LNPs) containing PEGylated lipids [10] and asymmetric liposomes (AL) [11,13].
BCG is the current vaccine for TB [14,15] and the polysaccharide (PP) vaccine (PPV) or protein-conjugated PPV (PCVs) for Spn diseases [16]. New vaccines are being tested to ameliorate the low BCG efficacy [17], but the most advanced vaccine candidate failed to demonstrate superiority to BCG in a phase III trial [18].
The large number of Spn serotypes, the replacement phenomenon associated with PCVs [19] and the variable immunogenicity of the Spn PPs limit the efficacy of PP-based vaccines, thus a universal anti-protein vaccine promoting

antibodies cross-reactive among all serotypes is highly desirable [16,20]. In addition, the induction of an immune response to a conserved non-PP antigen was proposed to increase efficacy of meningitis prevention [21]. As a preliminary approach, AL, with or without the TLR4 stimulator Lipid A (MPLA), were loaded with mRNA coding for: i) ESAT-6 or Ag85b, two Mtb antigens in the anti-TB model, or ii) the SARS-CoV-2 Spike protein (Pfizer-Biontech, published mRNA sequence), respectively. In both cases, results indicated the efficacy of immunization strategy in the mouse model. We also designed mRNA constructs coding for Spn PspA sequences of the proline rich domain (PRD) [22]. Moreover, we designed pneumolysin mRNA constructs by selecting two sequences including known toxoid variations in the synthetic mRNA [23,24]

LEGEND TO FIGURE
Fig.1A schematics of DNA plasmid constructs and preparation for in vitro synthesis of mRNAs and subsequent in vitro transcription, are presented. Asymmetric liposome (AL), and lipid nanoparticle (LNP) structure is depicted. ALs, and LNPs are used and proposed here for mRNA encapsulation, respectively.
Fig.1B-1 shows the integrity and size of synthesized mRNAs analyzed by denaturing agarose RNA gel (left), their specificity by RT-PCR upon amplification of the coding sequences derived from retrotranscribed cDNAs (center), and the expressed Ag85b protein, upon mRNA transfection, in HEK 293 cell supernatant, as an example (right).
Fig. 1B-2 shows THP1 cells treated in vitro with ALs containing GFP mRNA and observed by fluorescence microscopy after a 24h incubation. The majority of cells were fluorescent suggesting the effective delivery of mRNA and the consequent synthesis of the GFP protein.
Fig.1C-1 shows the efficacy of mRNA vaccination, demonstrated by the quantification of specific Ig in sera of immunized mice by ELISA. ALs, with encapsulated synthetic Lipid A, containing mRNA coding for the SARS-CoV-2 Spike protein or a control mRNA were i.m. administered to Balb/C mice at the indicated intervals. The efficacy of mRNA vaccination was demonstrated by the quantification of specific Ig in sera of immunized mice by ELISA (Fig.1C-2).
Fig.1D shows the immunogenic region selected to synthesize mRNA fragments from prolin-rich domain (PRD) of PspA families and selected mutations in native Ply to obtain Ply toxoid (T)1 and PlyT2 toxoids.

Picture to support preliminary data
preliminary_results.pdf

Specific Aim 1

Production of vaccines: i) to synthetize IVT mRNA coding for selected immunogenic proteins important to induce an effective defense against Mtb or Spn; ii) to test the most effective liposome formulation for mRNA delivery; iii) to evaluate the efficacy of adjuvants, selected among those approved for human use, based on their ability to induce both humoral and cellular immune responses in the mouse model

Specific Aim 2

To evaluate the immune response against intracellular bacteria, using Mtb as a model, following liposome-delivered adjuvanted mRNA as a vaccine. The humoral immune response against the Mtb ESAT-6 and Ag85b known to represent protective antigens will be measured. The cellular immune response will also be measured by ELISPOT and by the capacity of specific lymphocytes to kill Mtb after in vitro infection of peritoneal Mø obtained from non-immunized mice.

Specific Aim 3

Production of vaccines: i) to synthetize IVT mRNA coding for selected immunogenic proteins important to induce an effective defense against Mtb or Spn; ii) to test the most effective liposome formulation for mRNA delivery; iii) to evaluate

the efficacy of adjuvants, selected among those approved for human use, based on their ability to induce both humoral and cellular immune responses in the mouse model inhibition test. In addition, it will be tested by the ability of sera from immunized mice to reduce intracellular pneumococcal replication in tissue Mø in mice, recently recognized by us as the hallmark of pneumococcal invasive disease and proposed as a correlate of protection. In addition, we have the opportunity to test the capacity of sera to reduce infection and replication of Spn in CD169+ human splenic tissue macrophages in human spleen ex vivo perfusion and infection models.

Experimental Design Aim 1

The pUC57 plasmid is used as a backbone to contain the T7 RNA polymerase promoter placed upstream the 5' and 3' untranslated regions (UTRs) of highly stable cellular transcripts, containing the GFP, ESAT- 6, Ag85b, PspA variants or Ply toxoid (PlyT) gene sequences (codon optimized for optimal mammalian cell expression), followed by a PolyA tail. BspQI linearized plasmids are purified and in vitro transcription performed with 5'capping (ARCA) of mRNAs, modified bases addition (5-methyl-CTP and Pseudo-UTP) to increase mRNA stability and to reduce the activation of innate immune responses potentially able to further reduce mRNA stability and protein synthesis, followed by DNase treatment to remove the DNA template and mRNA purification by a commercially available kit. Pure 5'-capped, base modified, and poly adenylated mRNA will be ready for insertion in liposomes (AL or CL). AL characterized by the presence of PS at the outer membrane leaflet , DOPE (1,2-dioleoyl- sn-glycero-3- phosphoethanolamine) with or without alpha-Galactosylceramide (alpha-GalCer) or mono phosphoryl Lipid A (MPLA) are generated as described [12]. CL are prepared as previously described [4]. Moreover, in order to test LNP formulations containing both MPLA and PS at the outer membrane leaflet (see Fig. 1A) a LNP manufacturing service will be provided by CordenPharma. This company, that provides global commercial supply, including LNPs for commercially available anti COVID-19 mRNA-based vaccines, is also involved in early-stage drug development and can move the effective manufactured LNPs from GLP to GMP formulations. The avoidance of PEGylated lipids within newly generated LNPs aims at reducing potential PEG-related post-vaccination side effects [25]. Selected AL, CL, and LNPs, will be used for mRNA delivery throughout the in vivo studies.

Experimental Design Aim 2

Mtb will be used as a model of intracellular bacteria and mRNA coding for ESAT-6 or Ag85b have been chosen as immunogens, since these individual antigens have been shown to induce strong immune responses in a number of animal models and are believed to contribute to Mtb control, particularly if specific CD8 T cells are expanded [26, 27]. Mice will be divided in control groups (inoculated with empty liposomes/LNPs) and experimental groups immunized with AL, CL, (for comparison), or LNPs carrying mRNA coding for the selected Mtb proteins and boosted after 15 days. 15 days later, mice will be sacrificed, blood and spleens collected and used to obtain sera and cells. Ig classes and IgG subclasses will be measured by ELISA and specific CD4 and CD8 T cells by ELISPOT after magnetic sorting. Cells will also be used to measure the capacity to kill Mtb after in vitro infection of peritoneal Mø obtained from non-immunized mice.

Experimental Design Aim 3

Spn will be used as a model of extracellular bacteria and mRNA coding for PspA variants and for toxoid forms of Ply have been chosen as immunogens. PspA is a major protective antigen exposed on the surface of all Spn strains [22]. We will synthesize mRNAs coding for selected regions of the proline-rich domains (PRD) of PspA, which were described to elicit cross-protective antibodies against Spn strains [22, 28]. Since native Ply is toxic, we will synthesize mRNAs corresponding to 2 mutant versions of Ply (PlyT1 and 2) [23, 29, 30] which resulted protective in animal models or safe in phase I clinical trials in humans [29]. Mice will be treated as reported above with AL, CL, or LNPs carrying mRNA coding for the selected Spn proteins. Mouse sera positive for anti-PspA variants antibodies will then be tested by OPKA using a collection of Spn isolates. Sera will also be tested in modified OPKA, tailored to primary tissue macrophages, that will be set up as spleen and liver macrophages were recognized as major players in clearing Spn during infection, as recently shown by us [31-33].

Sera specific for anti-PlyTs antibodies will be tested for Ply neutralization in vitro. In vivo protection will be tested by intranasal and intravenous infection taking care to monitor possible variation of Spn clearance by tissue macrophages [31-33]. In vivo protection will be tested as described [34].

Metodologies and statistical analyses:

Methodologies (describe all measures taken to minimize / avoid bias)

We plan to evaluate the efficiency of mRNA immunization against infection caused by Mtb and Spn that represent prototypical bacteria at preferential intracellular or extracellular growth, respectively. The defense against intracellular bacteria mainly relies on the expansion of antigen specific T cells, while the production of high affinity specific antibodies with opsono-phagocytosis or toxin neutralization functions protects from extracellular bacteria [25]. We plan to encapsulate the in vitro-synthesized mRNA coding for ESAT-6 or Ag85b, two immunogenic Mtb specific proteins within liposomes or LNPs. For Spn, we plan to encapsulate the in vitro-synthesized mRNA coding for the proline-rich domain of PspA and mRNA coding for two Ply toxoid forms, within liposomes or LNPs. Mice will be immunized with mRNA loaded AL, CL (used for comparison), or LNPs, and sera will be tested to measure antigen specific antibodies by ELISA, OPKA and Ply neutralization assay, while cellular immunity by ELISPOT, macrophage-tailored OPKA and cytometry. Spleens of mice producing specific antibodies will be used to produce anti-PspA or anti-PlyT monoclonal antibodies (moAb) [26]. DNA plasmids for GFP, Flag-ESAT-6, Flag-Ag85b, Flag-PspA, and Flag-PlyT1/2 mRNAs are designed and generated by de novo gene synthesis and codon optimization for optimal mammalian cell expression and linearized with the BspQI restriction enzyme. Linearized DNAs are purified and submitted to in vitro transcription, with 5'capping (ARCA) and modified bases (5-methyl-CTP and Pseudo-UTP), followed by purification; the obtained mRNAs are ready for insertion [4]. Lipoplexes will be formed via incubation of mRNA with liposomes at room temperature for 20 min, as described [35]. AL will be generated by using PS (with or without stearylated octaarginine), DOPE (with or without alpha-GalCer or MPLA) to generate outer membrane leaflet, as previously described [11]. mRNA encapsulation will be allowed by suspending mRNA in TRIS buffer during the generation of the inner monolayer. Liposome size and their number will be assessed by flow cytometry as described [11]. Encapsulation efficiency will be assessed by Quanti-iT RiboGreen assay, as described [36]. LNPs, containing, or not, the above mentioned mRNA, will be generated at CordenPharma. Mice will be immunized by intramuscular (i.m.) or subcutaneous (s.c) injection of empty AL, CL, or LNPs (as negative control) or by liposomes/LNPs carrying mRNA coding for antigens and boosted two weeks later. Two weeks after the last boost, spleens will be homogenized and CD4 and CD8 T cells will be separated by immune-magnetic sorting. Sera, obtained from collected blood, will be used at serial dilution for specific antibodies determination. ELISA and ELISPOT will be performed using recombinant protein to coat plates as previously described [37, 38]. Cytokines release by antigen stimulated splenocytes will be measured by ELISA. Killing capacity of splenocytes will be performed using mouse peritoneal Mø infected with H37Rv Mtb and measured as reduction of colony forming unit (CFU) after 21 days of culture on agar plates [39]. MoAb will be produced as described [40]. A standardized OPKA to reproduce estimates of the functional phagocytic activity of sera for Spn will be used according to the CDC protocol. (https://www.vaccine.uab.edu/uploads/mdocs/cdc-ops3.pdf). In addition, we will perform a macrophage-tailored OPKA as we identified tissue macrophages as the prime cellular defense against Spn both in spleen and liver [31-33]. The inhibition tests for anti-Ply antibody will be performed according to [34]. Protection by Spn infection will be measured with a challenge test by intranasal and intravenous injection of Spn in mice immunized or not.

Methods of data collection (Indicate the data that will be collected, the tools used)
The proposal does not foreseen to collect data from patients or healthy individuals.

Statistic plan (calculation of statistical data)
Sample size for animal studies will be estimated considering a 95% confidence level and a level of precision of 10%

Statistical analysis (describe the main statistical analysis)
Kolmogorov-Smirnov test will be used to assess the normality of the data distributions and then the appropriate parametric or nonparametric test will be utilized using the fourth version of GraphPad Prism Software. The p values<0.05 will be considered significant.

Timing of analysis data (indicate duration of study: duration of enrollment, of therapy, follow-up etc)
The duration of the study is 36 months.
The analysis of the in vitro capacity of nanoparticles to deliver mRNA and permit in loco Mtb protein synthesis in human Mø and DC will be performed within the 18th month;
The analysis of the cellular and humoral responses induced against Mtb proteins by mice immunization with mRNA will be performed within the 30th month;
The analysis of the humoral and cellular responses induced against Spn proteins by mice immunization with mRNA and challenge experiments will be performed within the 34th month;
Final analysis will be performed at the 30th-36th month.

Expected outcomes

Our study proposes a win-win scenario for the field of vaccinology against bacterial diseases and for the society as a whole. In particular we expect to validate the mRNA vaccine technology as a novel tool to induce both the cellular and the humoral immune responses against intracellular and extracellular bacteria by comparing different liposome formulations for the optimal response. The results, in the field of TB prevention, could be useful in future attempts to empower the existing BCG vaccine by promoting a cellular response, including both CD4 and CD8 T lymphocytes specific for selected proteins and to tune host immune response acting on antigen presentation with lipid delivered by AL or LNPs. In the field of the prevention of invasive infections caused by Spn, the results could indicate a novel promising strategy for development of a universal vaccine overcoming the limits of PPV and PCVs, in particularly with respect to meningitis, considering that the induction of an immune response to conserved non-carbohydrate antigens was proposed to increase efficacy of meningitis prevention [21].

Risk analysis, possible problems and solutions

AIM 1: No major problems are foreseen, since mRNA coding for GFP, ESAT-6 and Ag85b have already been produced and validated. The same technology will be used to produce mRNA for Spn antigens. The produced liposomes have been shown to deliver GFP mRNA in vitro to Mø, and to deliver mRNAs coding for Mtb or SARS-CoV-2 antigens in mice, indicating the effectiveness of the system in vivo. AIMs 2 and 3: No major problems are foreseen, since all the techniques needed for the development of this project have been already fully established in the participating laboratories. In case of low Ig responses, different vaccination schedules and adjuvants (alpha-GalCer or synthetic Lipid A, an adjuvant already approved for human use and easy to incorporate in liposomes) will be tested. An important advantage of this proposal is

that the technology of RNA vaccines allows to re-explore well known vaccine candidates, as the patents on these antigens do not cover vaccination with mRNA.

Significance and Innovation

The validation of our hypotheses will represent a proof of concept for a mRNA based vaccination tailored for diseases caused by extra- or intracellular bacteria and a preclinical phase for a future trial. In the Mtb model our approach will permit to validate the possibility to induce CD8 T cell against proteins of intracellular pathogens. In the Spn vaccination model, we will investigate the role of two major Spn antigens as RNA vaccine candidates to circumvent the limits of PPV and PCVs with the theoretic capacity to counteract different Spn serotypes and suitable for immunization of infants and adults. The innovation will include both the first application of a liposome/LNPs delivered mRNA vaccine to prevention of Spn caused diseases, and a specific analysis of involvement of tissue macrophages in antibody dependent protection from invasive disease. The development of a tissue macrophage tailored OPKA may also provide a new model to detect correlates of vaccine protection for Spn.

Description of the complementary and sinergy research team

This integrated cellular and humoral mRNA vaccination strategy against diseases caused by prototypic extracellular or intracellular bacteria has high chances to succeed thanks to the multidisciplinary collaboration between our laboratories, whose high standard research in molecular biology, liposome-based strategies, Mtb, Spn, anti-mycobacterial and anti-pneumococcus immunity is internationally recognized. The research activity of Dr. Nisini group is aimed at investigating the humoral and cellular immune response to mRNA immunization in in vitro and in vivo models. Dr. Sgarbanti team will be responsible for the plasmid-based mRNA design and synthesis and for the design of the optimal composition of nanoparticles for the delivery of mRNAs. Dr. Monaco group has a long-lasting leading research activity on pneumococcal diseases. Through the national surveillance of invasive pneumococcal diseases (IPD) in Italy gathered a huge Spn clinical isolates collection spanning the majority of serotypes and a deep knowledge of the impact of Spn vaccination on IPD.
The group leaded by Prof. Oggioni has in the past few years uncovered significant aspects of Spn invasive disease describing the important contribution of tissue macrophages in murine, porcine and human models. This novel point of view will allow to have a revised look at the immunity generated by well-known pneumococcal antigens and their role in protection from infection

Training and tutorial activities

Key personnel and collaborators of this proposal are planning to participate to different international and national meetings and/or courses related to the project objectives.
The participants to the project will organize an ECM (Continuous Education in Medicine) training courses for MD and Biologists of the health care system based on novel vaccine strategies aimed at increasing efficacy and reducing side effects.
In the first months of the project, PI and collaborators will also organize intensive training courses for the young investigators hired in the project on TB and Spn immunology and microbiology, and mice immunization.

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Bibliography

1 Brito, L. A. Adv Genet 89, 179 (2015).
2 Hekele, A. Emerg Microbes Infect 2, e52 (2013).
3 Stanton, M. G. Nucleic Acid Ther 28 (2018).
4 Pardi, N. Methods Mol Biol 1499 (2017).
5 Liu X, Lancet. 398 (2021)
6 Liang, F. Mol Ther 25 (2017).
7 Richner, J. M. Cell 168 (2017).
8 Maruggi, G. Vaccine 35 (2017).
9 Desmet, C. J. Nat Rev Immunol 12 (2012)
10 Turner JS Nature. 596 (2021)
11 Greco, E. Proc Natl Acad Sci U S A 109 (2012).
12 Poerio, N. Sci Rep 7 (2017).
13 Nisini, R. Front Immunol 9 (2018).
14 Roy, A. BMJ 349 (2014).
15 Mangtani, P. Clin Infect Dis 58 (2014).
16 Principi, N. Expert Opin Biol Ther 18 (2018).
17 Macleod, M. BMJ 360 (2018).
18 Tameris, M. D. Lancet 381 (2013).
19 Balsells, E. PLoS One 12 (2017).
20 Ginsburg, A. S. Expert Rev Vaccines 11 (2012).
21 Mukerji, R. The Ped.Infec. Dis. Journ. 39 (2020)
22 Mukerji R, Vaccine. 36 (2018)
23 Kirkham, L. A. Infect Immun 74 (2006).
24 Oloo EO. Journal Biol Chem 286 (2011)
25 Garvey LH, Br J Anaesth. 26 (2021)
26 Brandt, L. Infect Immun 68 (2000).
27 Kariyone, A. Int Immunol 15 (2003).
28 Yuki Y, Mol Pharm. 18 (2021)
29 Kamtchoua, T. Vaccine 31 (2013)
30 Chen, A. Clin Vaccine Immunol 22 (2015).
31 Ercoli G, Nat Microbiol. 3 (2018)
32 Carreno D, EBioMedicine 72 (2021)
33 An H, J Exp Med. 219(2022)
34 Salha, D. Infect Immun 80 (2012)
35 Michel, T. Mol Ther Nucleic Acids 8 (2017).
36 Chen, S. J Control Release 235 (2016)
37 Santodonato, L. J Immunol 170 (2003).
38 von Hunolstein, C. Vaccine 19 (2001).
39 Mariotti, S. J Immunol 191 (2013)
40 Mariotti S. Front. Immunol 12 (2021)

	Project Title: Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models **Project duration (months):** 36
Project Code: RF-2021-12375437	**Principal Investigator:** NISINI ROBERTO
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Timeline / Deliverables / Payable Milestones

D1_Ethical Committee approval (UO1-2)
D2_Design and preparation of CL, AL (UO1) and LNPs (CordenPharma)
D3_Definition of the sequences of Spn proteins to be used for mRNA synthesis (UO2)
D4_Syntesis of mRNA coding for ESAT-6, Ag85b and Spn proteins, with modified bases, i.e. 5-methyl-CTP and Pseudo-UTP (UO1)
D5_In vitro evaluation of the capacity of CL, AL, or LNPs to deliver mRNAs and allow Mtb protein synthesis (UO1-2)
D6_ Mice immunization with mRNAs coding for Mtb proteins (UO1)
D7_Set up of OPKA using Spn isolates from collection and human collected serum samples (UO2-3)
D8_Evaluation of cellular and humoral responses induced against Mtb proteins (UO1-2)
D9_ Immunization of mice with mRNA containing modified bases, coding for Spn proteins delivered by CL LNPs or AL (UO1)
D10_ Evaluation of humoral and cellular responses induced against Spn antigens by macrophage-tailored OPKA and challenge experiments (UO1-2)
D11_ Data analysis and manuscripts preparation (All)

Milestones 18 month

1: Preparation of CL, selected AL, and LNPs and identification of the more efficient liposome/LNPs formulation for optimal delivery of mRNA in Mø and DC;
2: Synthesis of different mRNA coding for Mtb or Spn proteins
3: Evaluation of the safety and immunogenicity of mRNA coding for Mtb proteins in mice;

Milestones 36 month

4: Description of cellular and humoral responses induced against ESAT-6 and Ag85b by mice immunization with mRNA; 5: Description of humoral and cellular responses induced against Spn proteins by mice immunization with mRNA;
6: Definition of the most effective and safe form of mRNA delivery by liposomes/LNPs for immunization against infectious diseases caused by intracellular or extracellular bacteria for vaccine development

Gantt chart

gantt.pdf

Equipment and resources available

Facilities Available

The Department of Infectious Diseases (DMI) at the Istituto Superiore di Sanità (ISS) is fully equipped with high-standard equipment for microbiology, cell biology, molecular biology, cellular and humoral immunology and tissue culture studies in both a biosafety laboratory level (BSL) 2 and a BSL3 laboratory for in vitro research with live Mtb. In addition, an animal house (classified as specific pathogen free), a flow cytometry facility, transmission electron confocal and fluorescence microscopy, refrigerated centrifuges, incubators, a fluorescence microscope with camera, spectrophotometer microplate reader as well as instruments needed for the proposed project (including, ELISA and ELISPOT plate readers, PCR and real time PCR machines, western-blot system) Luminex and other instruments needed for the immunological characterization of the cellular and immune responses are readily available at ISS. The laboratories of Prof Oggioni at the University of Bologna have access to animal facilities and to confocal microscopy facilities to perform the work for in vivo and in vitro

testing of RNA induced vaccine immunity.

Subcontract

None

Translational relevance and impact for the National Health System (SSN)

New vaccines against bacterial diseases are expected to have wide public health applicability and strategic impact on the NHS policies in a global health approach, also considering the difficulties to treat diseases caused by emerging antibiotic resistant bacteria. The mRNA-based vaccine safety, efficacy and unprecedent translation capacity were evident during the SARS-CoV-2 pandemic. The results of this proposal will represent a preclinical phase for the development of vaccines to prevent/treat Spn infections and TB, but also a model of a successful strategy appliable to other bacterial infections. mRNA vaccines may represent a complementary approach to BCG vaccination in TB prevention and a contribution to definitively increase the coverage of PCVs contrasting the phenomenon of Spn serotype replacement. A mRNA-based vaccine against the Spn surface protein PspA and Ply could represent a winning strategy to reduce the impact of Spn related diseases, including the meningitis sequelae

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Principal Investigator (PI) Profile

NISINI ROBERTO

Birth date: 26/01/1959

Institution: Istituto Superiore di Sanita'
Department/Unit: Department of Infectious Diseases/Immunology Unit
Position Title: Principal investigator

Education and training

Institution and Location	Degree	Year(s)	Field of study
University Hospital Basel, Center for teaching and research, Basel-Switzerland	On Job training	1995	Cellular and molecular immunology of infectious diseases
Basel Institute for Immunology, Basel-Switzerland	On Job Training	1989	Cellular Immunology with particular regard to T cell functions and charachterization
National Institutes of Health, National Cancer Institute, Bethesda, Maryland, US.	Fellowship	1985	Immunology
University of Rome " La Sapienza" Rome-Italy	Specialty in Allergology and Clinical Immunology	1993	Allergology and Clinical Immunology
University of Rome " La Sapienza" Rome-Italy	Specialty in Internal medicine	1988	Internal Medicine-Infecious Diseases
University of Rome " La Sapienza" Rome-Italy	Degree MD	1983	Degree in Medicine and Surgery

Personal Statement:

Dr. Nisini will coordinate the interdisciplinary team and will be responsible for mice immunization schedules and analyses of induced humoral and cellular immunity. He will organize progress report meetings to discuss gathered data and plan the next experimental procedures to optimize the team work, identify possible problems and find solutions

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Istituto Superiore di Sanità	Department of Infectious Diseases	Roma, Italy	Director of the Immunology Unit	2017	2022
Istituto Superiore di Sanità	Department of Infectious, Parasitic and Immunomediated Diseases	Roma, Italy	Director Anti-infectious Disease Unit	2010	2017
Istituto Superiore di Sanità	Laboratory of Bacteriology and Medical Mycology	Roma, Italy	senior researcher	1997	2010
Italian Air Force	Division of Research and Experimentation	Pratica di Mare, Roma, Italy	Medical Officer (Major) Head of the Hygiene and Immunology unit	1986	1997

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Official H index: 31.0 (autocertificated)

Scopus Author Id:7004131053 **ORCID ID:**0000-0003-1077-423X **RESEARCH ID:**M-4363-2015

Other awards and honors
Fogarty Research Fellowship. NIH, USA. 1984

Other CV informations
Patent n.102017000074060 dated 30.09.2019
Coordinator (Co) or Principal Investigator (PI) of 29 national and international proposals (1999-2021), including:
-Co, FP6 EC proposal: MILD-TB. 2006
-PI, FP7 EC proposal:NewTBVAC. 2009
-PI, NIH-IMH proposal: Rapid detection of Mycobacterium tuberculosis specific biomarkers by core-shell hydrogel particles: 2010
-Co, Italian Ministry of Health: Nanotechnology for the multiplex diagnosis of infectious diseases. 2013
-PI, Horizon 2020 EC proposal: TBVI-2020. 2015
-PI, Italian Ministry of Defense proposal: Safety and efficacy of multiple vaccinations in military recruits. 2017
-Co, NPS-NATO proosal :New and validated tools for the diagnosis and follow-up of SARS-CoV-2 infected individuals 2020

Selected peer-reviewed publications of the PI valid for minimum expertise level

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
The first, holistic immunological model of COVID-19: Implications for prevention, diagnosis, and public health measures	Article	454	31	2020	10.1111/pai.13271	32359201	6.377	95	L
Human CD4+ T-cell response to hepatitis delta virus: Identification of multiple epitopes and characterization of T-helper cytokine profiles	Article	2242	71	1997	10.1128/JVI.71.3.2241-2251.1997	9032359	5.821	87	F
The multirole of liposomes in therapy and prevention of infectious diseases	Review	-	9	2018	10.3389/fimmu.2018.00155	29459867	4.716	80	F
Endogenous PGE2 promotes the induction of human Th17 responses by fungal ß-glucan	Article	947	88	2010	10.1189/jlb.0310139	20807707	4.626	32	L
Mycobacterium tuberculosis subverts the differentiation of human monocytes into dendritic cells	Article	3050	32	2002	10.1002/1521-4141(200211)32:11<3050::AID-IMMU3050>3.0.CO;2-K	12385024	4.832	81	L
Detection of interleukin-2 in addition to interferon-gamma discriminates active tuberculosis patients, latently infected individuals, and controls	Article	1282	16	2010	10.1111/j.1469-0691.2009.03104.x	19886902	4.784	82	L
Cytometric detection of antigen-specific IFN-gamma/IL-2 secreting cells in the diagnosis of tuberculosis	Article	-	9	2009	10.1186/1471-2334-9-99	19549330	2.55	68	L

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
ß-Glucan of Candida albicans cell wall causes the subversion of human monocyte differentiation into dendritic cells	Article	1136	82	2007	10.1189/jlb.0307160	17656653	4.3	30	L
Cell wall-associated alpha-glucan is instrumental for Mycobacterium tuberculosis to block CD1 molecule expression and disable the function of dendritic cell derived from infected monocyte	Article	2081	9	2007	10.1111/j.1462-5822.2007.00940.x	17441985	5.293	68	L
Antigenic properties and processing requirements of 65-Kilodalton mannoprotein, a major antigen target of anti-Candida human T-cell response, as disclosed by specific human T-cell clones	Article	3728	69	2001	10.1128/IAI.69.6.3728-3736.2001	11349037	4.212	47	F
Mycobacterium bovis Bacillus Calmette-Guérin infects DC-SIGN - dendritic cell and causes the inhibition of IL-12 and the enhancement of IL-10 production	Article	106	78	2005	10.1189/jlb.0105037	15845642	4.627	43	L
Clinical and immunological response to typhoid vaccination with parenteral or oral vaccines in two groups of 30 recruits	Article	582	11	1993	10.1016/0264-410x(93)90237-r	8488716	2.8	32	F
Mycobacterium tuberculosis diverts alpha interferon-induced monocyte differentiation from dendritic cells into immunoprivileged macrophage-like host cells	Article	4385	72	2004	10.1128/IAI.72.8.4385-4392.2004	15271894	4.033	42	L
Candida albicans Yeast and Germ Tube Forms Interfere Differently with Human Monocyte Differentiation into Dendritic Cells: A Novel Dimorphism-Dependent Mechanism to Escape the Host's Immune Response	Article	833	72	2004	10.1128/IAI.72.2.833-843.2004	14742527	4.033	43	L
Bacillus Calmette-Guérin shares with virulent Mycobacterium tuberculosis the capacity to subvert monocyte differentiation into dendritic cell: Implication for its efficacy as a vaccine preventing tuberculosis	Article	3848	22	2004	10.1016/j.vaccine.2004.07.009	15364431	2.824	28	L
The adjuvant effect of synthetic oligodeoxynucleotide containing CpG motif converts the anti-Haemophilus influenzae type b glycoconjugates into efficient anti-polysaccharide and anti-carrier polyvalent vaccines	Article	3058	19	2001	10.1016/s0264-410x(01)00048-2	11312000	2.943	27	L
Synthetic oligodeoxynucleotide containing CpG motif induces an anti-polysaccharide type 1-like immune response after immunization of mice with Haemophilus influenzae type b conjugate vaccine	Article	295	12	2000	10.1093/intimm/12.3.295	10700464	3.13	23	L
Dormant Mycobacterium tuberculosis fails to block phagosome maturation and shows unexpected capacity to stimulate specific human T lymphocytes	Article	274	191	2013	10.4049/jimmunol.1202900	23733870	5.362	22	L

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
Immunogenicity of anti-Haemophilus influenzae type b CRM197 conjugate following mucosal vaccination with oligodeoxynucleotide containing immunostimulatory sequences as adjuvant	Article	2229	20	2002	10.1016/s0264-410x(02)00113-5	12009277	2.811	26	L
Presentation of superantigen by human T cell clones: A model of T-T cell interaction	Article	2033	22	1992	10.1002/eji.1830220812	1353448	4.9	22	F

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

Selected peer-reviewed publications of the PI for the evaluation CV

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
The first, holistic immunological model of COVID-19: Implications for prevention, diagnosis, and public health measures	Article	454	31	2020	10.1111/pai.13271	32359201	6.377	95	L
Beneficial Role of Phytochemicals on Oxidative Stress and Age-Related Diseases.	Review	-	8748253	2019	10.1155/2019/8748253	31080832	2.276	123	O
The Multirole of Liposomes in Therapy and Prevention of Infectious Diseases	Review	155	9	2018	10.3389/fimmu.2018.00155	29459867	4.716	80	F
Urine lipoarabinomannan glycan in HIV-negative patients with pulmonary tuberculosis correlates with disease severity.	Article	-	9	2017	10.1126/scitranslmed.aal2807	29237757	16.71	58	O
Mycobacterium tuberculosis gene expression at different stages of hypoxia-induced dormancy and upon resuscitation	Article	565	54	2016	10.1007/s12275-016-6150-4	27480637	1.924	23	O
Candida albicans targets a lipid raft/dectin-1 platform to enter human monocytes and induce antigen specific T cell responses	Article	e0142531	10	2015	10.1371/journal.pone.0142531	26562838	3.057	13	O
Safety and immunogenicity of co-administered MF59-adjuvanted 2009 pandemic and plain 2009-10 seasonal influenza vaccines in rheumatoid arthritis patients on biologicals	Article	287	177	2014	10.1111/cei.12292	24666311	3.037	21	O
Dormant Mycobacterium tuberculosis fails to block phagosome maturation and shows unexpected capacity to stimulate specific human T lymphocytes	Article	274	191	2013	10.4049/jimmunol.1202900	23733870	5.362	22	L
Mycobacterium tuberculosis may escape helper T cell recognition by infecting human fibroblasts	Article	722	74	2013	10.1016/j.humimm.2013.02.005	23459076	2.282	15	L
Janus-faced liposomes enhance antimicrobial innate immune response in Mycobacterium tuberculosis infection	Article	-	109	2012	10.1073/pnas.1200484109	22538807	9.737	41	O

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

CO-PI Profile

Sgarbanti Marco

Birth date: 03/11/1968

Institution: Istituto Superiore di Sanita'
Department/Unit: Department of Infectious Diseases/Immunology Unit
Position Title: DNA vector design for in vitro mRNA synthesis; mRNA synthesis, purification and quality controls; testing of mRNA-driven protein expression in human cells; cellular immunity

Education and training

Institution and Location	Degree	Year(s)	Field of study
University of Rome "Tor Vergata"	PhD	2008	Immunology/Transcription and regulation of the HIV-1 Long Terminal Repeat promoter by cellular factors in CD4+ T cells
University of Rome "La Sapienza"	Biological Sciences	1998	Molecular and cellular Biology: Role of Interferon Regulatory transcription Factors in the regulation of Interferon-induced gene expression

Personal Statement:

Dr Sgarbanti will apply his know-how in transfection of mammalian cells with DNA expression vectors, siRNA, and very recently in vitro transcribed mRNA within this project to design, synthesize, and check for quality and expression in mammalian cells, IVT mRNA coding for ESAT-6 and Ag85b proteins of Mtb, and PspA and Ply proteins of Spn.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Istituto Superiore di Sanità	Department of Infectious Diseases/ Immunology Unit	Rome	Researcher	2017	2022
Istituto Superiore di Sanità	Department of Infectious, Parasitic and Immune-Mediated Diseases/ Molecular Pathogenesis Unit	Rome	Researcher	2003	2017
Lady Davis Institute for Medical Research/McGill University	Molecular Oncology Group	Montreal, Québec, Canada	Post Doctoral Fellow	2000	2003

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Official H index: 15.0 (autocertificated)

Scopus Author Id:6506227463 **ORCID ID:**0000-0002-0433-5110 **RESEARCH ID:**C-2962-2016

Other awards and honors

Winner of the Grand Challeges Explorations in Global Health 2011 (Round 6) in the HIV-1 research field, a grant awarded by the Bill & Melinda Gates Foundation

Other CV informations

During the last ten years Dr. Sgarbanti was involved in DNA vector building taking advantages of de novo gene synthesis technology, to produce shRNA, viral and cellular proteins, and very recently mRNAs, applyied to antiviral research. Most of his research work involved transfection of mammalian cells with DNA expression vectors, siRNA, and very recently in vitro transcribed mRNA.

Selected peer-reviewed publications of the Co-PI valid for minimum expertise level

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
Modulation of human immunodeficiency virus 1 replication by interferon regulatory factors	Article	1359	195	2002	10.1084/jem.20010753	12021315	15.837	61	F
A requirement for NF-kappa B induction in the production of replication-competent HHV-8 virions	Article	770	23	2004	10.1038/sj.onc.1207707	15235582	6.318	34	F
IRF-7: New role in the regulation of genes involved in adaptive immunity	Article	325	1095	2007	10.1196/annals.1397.036	17404045	1.731	19	F
HIV-1 latency: An update of molecular mechanisms and therapeutic strategies	Review	1715	6	2014	10.3390/v6041715	24736215	3.353	43	L
IkappaB kinase ¿ targets interferon regulatory factor 1 in activated T lymphocytes	Article	1054	34	2014	10.1128/MCB.01161-13	24396068	4.777	18	F
Therapeutics for HIV-1 reactivation from latency	Review	394	3	2013	10.1016/j.coviro.2013.06.001	23810462	6.298	26	F
IRF-1 is required for full NF-kappa B transcriptional activity at the human immunodeficiency virus type 1 long terminal repeat enhancer	Article	3632	82	2008	10.1128/JVI.00599-07	18216101	5.308	56	F
Interferon regulatory factor-1 acts as a powerful adjuvant in tat DNA based vaccination	Article	702	224	2010	10.1002/jcp.22169	20432465	3.986	20	F
The design of optimal therapeutic small interfering RNA molecules targeting diverse strains of influenza a virus	Article	3364	27	2011	10.1093/bioinformatics/btr555	21994230	5.468	12	L
The development of immune-modulating compounds to disrupt HIV latency	Review	159	23	2012	10.1016/j.cytogfr.2012.05.003	22766356	8.831	11	L
Alternate NF-kB-independent signaling reactivation of latent HIV-1 provirus	Article	e00495	93	2019	10.1128/JVI.00495-19	31243131	4.501	3	L
Analysis of the signal transduction pathway leading to human immunodeficiency virus-1-induced interferon regulatory factor-1 upregulation	Article	187	1030	2004	10.1196/annals.1329.024	15659797	1.789	6	F

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
I¿B kinase-¿-mediated phosphorylation triggers IRF-1 degradation in breast cancer cells	Article	459	22	2020	10.1016/j.neo.2020.07.004	32784074	5.715	1	F
Fighting HIV-1 Persistence: At the Crossroads of "Shoc-K and B-Lock"	Review	1517	10	2021	10.3390/pathogens10111517	34832672	3.492	0	L

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Biographical Sketch Contributors. N. 2

MONACO MONICA

Birth date: 04/08/1970

Institution: Istituto Superiore di Sanita'
Department/Unit: Department of Infectious Diseases/Antibiotic Resistance and special pathogens Unit
Position Title: Genetic characterization of S. pneumoniae strains, opsonophagocytosis assay on clinical isolates, M. tuberculosis culture

Education and training

Institution and Location	Degree	Year(s)	Field of study
University of Rome "Tor Vergata"	PhD in Medical Microbiology and Immunology	2007	CA-MRSA human infections in the community
University of Rome " La Sapienza" Rome-Italy	Postgraduate Specialization in Clinical Pathology, with honors	2001	Study of colonization and infection due to Candida spp in patients with lymphoma HIV related
University of Rome " La Sapienza" Rome-Italy	Licence to practice as Biologist	1998	biology
University of Rome " La Sapienza" Rome-Italy	Degree in Biological Sciences	1996	VR-Enterococci causing invasive infections in patients with leukemia

Personal Statement:

Dr Monaco will be in charge for the genetic characterization of S. pneumoniae strains of the ISS collection and for surpervise, opsonophagocytosis assay on clinical Spn isolates and M.tuberculosis culture

Positions and honors

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Positions

Institution	Division / Research group	Location	Position	From year	To year
Istituto Superiore di Sanità	Department of Infectious Disease	Rome	Staff researcher	2007	2022
Istituto Superiore di Sanità	Department of Infectious parasitic and immunomediated Diseases	Rome	Temporary Reseacher	2002	2007
Istituto Superiore di Sanità	Department of Infectious parasitic and immunomediated diseases	rome	PhD fellow	2003	2007
Istituto Superiore di Sanità	Department of Infectious, Parasitic and immunomediated Disease	Rome	Reseacher fellow	2000	2002

Official H index: 28.0 (autocertificated)

Scopus Author Id:8973597500 **ORCID ID:**0000-0002-6628-1621 **RESEARCH ID:**C-8819-2016

Other awards and honors

Grant of the Eur. Soc. of Clinical Microbiology and Infectious Diseases 2004
Nat.Operational Contact Point (NOCP) for Diphtheria at ECDC (2010-)
NOCP for AMR at ECDC (2018-)
Nat.Tech. Coordinator for the European Antimicrobial Resistance Genes Surveillance Network at ECDC (2019-)
Member of the Coordinating Committee for the strategy to contrast AMR at the Italian MoH (2021)
NOCP for the European Antimicrobial Resistance Genes Reference Laboratory Capacity project at ECDC (2021-)

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Biographical Sketch Contributors. N. 3

Oggioni Marco Rinaldo

Birth date: 01/01/1965

Institution: University of Bologna
Department/Unit: Pharmacy and Biotechnology
Position Title: Testing host response to pneumococcal vaccine

Education and training

Institution and Location	Degree	Year(s)	Field of study
University of Siena, Italy	Clinical Microbiology Specialisation	1994	Thesis of heterologous gene expression in bacteria
University of Verona, Italy	Medical Degree	1990	Thesis on conjugative transposons

Personal Statement:
Prof Oggioni will be in charge for the evaluation of the specific immune response to pneumococcal vaccine based on mRNA technology. In particular, based on his recent discovery that many bacterial species have an intracellular phase early during infection, that represents a crucial check-point for the onset of invasive diseases, he will test the efficacy of vaccination in mice and porcine organ ex-vivo perfusion assay. These assays will help identifying correlates of protection

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
University of Bologna	Department Pharmacy and Biotechnology. Farmacia e Biotecnologie	Bologna, Italy	Professor	2020	2022
University of Leicester	Dept. Genetics and Genome Biology	Leicester,UK	Professor	2013	2022
Azienda Ospedaliera Senes	UOC Batteriologia	Siena	Dirigente Medico - Consultant	1993	2013

Official H index: 44.0 (autocertificated)

Scopus Author Id:57203558449 **ORCID ID:**0000-0003-4117-793X **RESEARCH ID:**K-3839-2016

Other awards and honors
2022 Steering Committee SIMGBM (Societa¿ Italiana di Microbiologia Generale e Biotecnologie) 2019 Fellow of the Royal Society of Biology FRSB

2019 Fellow of the International Society of Antimicrobial Chemotherapy FISAC 2016 Affiliate member Royal College of Pathologists

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

2015 Fellow of the UK Higher Education Academy FHEA

2013-19 Chair of the ESCMID study group of infections of the brain ESGIB

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Biographical Sketch Contributors. N. 4

MARIOTTI SABRINA

Birth date: 04/07/1975

Institution: Istituto Superiore di Sanita'
Department/Unit: Department of Infectious Diseases/Immunology Unit
Position Title: Mice immunization, humoral and cellular immune responses to M. Tuberculosis

Education and training

Institution and Location	Degree	Year(s)	Field of study
La Sapienza University of Rome	PhD in Medical Microbiology and Immunology	2007	Immunology: study of the role of CD1e molecule in lipid antigen processing
La Sapienza University of Rome	Specialization course in Health Statistics	2003	Statistics of diagnostic tests
La Sapienza University of Rome	Master¿s Degree in Biological Sciences summa cum laude	2000	Immunology: efficacy and safety of new vaccine formulation against Haemophilus influenzae using polysaccharides and synthetic oligonucleotides containing CpG

Personal Statement:

Dr Mariotti will be responsible for the preparation and control of lipid nanoparticles, including AL and she will be in charge for mouse immunization and for the evaluation of humoral and cellular immune response to Mycobacterium tuberculosis following vaccination with mRNA technology

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Istituto Superiore di Sanità	Department of Infectious Diseases, Immunitology Unit	Rome	Reseasch Scientist	2005	2022
University Hospital of Basel	Laboratory of Experimental Immunology	Basel (Switzerland)	Research Scientist	2002	2005
Istituto Superiore di Sanità	Laboratory of Bacteriology and Medical Mycology	Rome	Research Scientist	2001	2002
Istituto Superiore di Sanità	Laboratory of Bacteriology and Medical Mycology	Rome	Visitor student for the diploma thesys	1998	2000

Official H index: 21.0 (autocertificated)

Scopus Author Id:7005679665 **ORCID ID:**0000-0002-7009-7616 **RESEARCH ID:**M-4361-2015

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Other awards and honors

Winner of ImmunoTools special AWARD 2014.
Patent n. 102017000074060 accepted the 30/09/2019, title "Method for the multi-parametric analysis of programmed cell death by flow cytometry".
Partecipation, understanting and completation of "LTK Module 1E: Introductory course in laboratory animal science at University of Zurig from 17th to 21st March 2003.

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Expertise Research Collaborators

Selected peer-reviewed publications of the Research Group / Collaborators

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
Oggioni Marco Rinaldo	The impact of ethnicity on clinical outcomes in COVID-19	Review	-	23	2020	10.1016/j.eclinm.2020.100404	32632416	3.5	333	O
MONACO MONICA	Epidemic of carbapenem-resistant Klebsiella pneumoniae in Europe is driven by nosocomial spread.	Article	1919	4	2019	10.1038/s41564-019-0492-8	31358985	15.54	172	O
Oggioni Marco Rinaldo	Intracellular replication of Streptococcus pneumoniae inside splenic macrophages serves as a reservoir for septicaemia	Article	600	3	2018	10.1038/s41564-018-0147-1	29662129	17.8	65	L
Sgarbanti Marco	Development and validation of a novel dual luciferase reporter gene assay to quantify ebola virus VP24 inhibition of IFN signaling	Article	98	10	2018	10.3390/v10020098	29495311	3.811	10	O
MONACO MONICA	Occurrence of carbapenemase-producing Klebsiella pneumoniae and Escherichia coli in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study.	Article	153	17	2017	10.1016/S1473-3099(16)30257-2	27866944	25.14	308	O
Oggioni Marco Rinaldo	Phase-variable methylation and epigenetic regulation by type I restriction-modification systems	Article	S3	41	2017	10.1093/femsre/fux025	28830092	16.4	56	L
MARIOTTI SABRINA	A method permissive to fixation and permeabilization for the multiparametric analysis of apoptotic and necrotic cell phenotype by flow cytometry	Article	1115	91	2017	10.1002/cyto.a.23268	29072808	3.26	10	F
MARIOTTI SABRINA	Liposomes loaded with bioactive lipids enhance antibacterial innate immunity irrespective of drug resistance	Article	-	7	2017	10.1038/srep45120	28345623	4.122	13	O

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
MONACO MONICA	Whole-Genome Sequencing for Routine Pathogen Surveillance in Public Health: a Population Snapshot of Invasive Staphylococcus aureus in Europe	Article	e00444-16	7	2016	10.1128/mBio.00444-16	27150362	6.956	123	O
Sgarbanti Marco	Type I IFN - A blunt spear in fighting HIV-1 infection	Review	143	26	2015	10.1016/j.cytogfr.2014.10.004	25466629	7.294	19	O
MARIOTTI SABRINA	Aloe-emodin exerts a potent anticancer and immunomodulatory activity on BRAF-mutated human melanoma cells	Article	283	762	2015	10.1016/j.ejphar.2015.05.057	26048310	2.73	31	O
MARIOTTI SABRINA	Presepsin as a potential marker for bacterial infection relapse in critical care patients. A preliminary study	Article	567	53	2015	10.1515/cclm-2014-0119	24897401	3.017	23	O
Oggioni Marco Rinaldo	A random six-phase switch regulates pneumococcal virulence via global epigenetic changes	Article	5055	5	2014	10.1038/ncomms6055	25268848	14.9	179	L
MONACO MONICA	Colistin resistance superimposed to endemic carbapenem-resistant Klebsiella pneumoniae: a rapidly evolving problem in Italy, November 2013 to April 2014	Article	-	19	2014	10.2807/1560-7917.es2014.19.42.2093	25358041	4.659	160	F
Sgarbanti Marco	HIV-1 latency: An update of molecular mechanisms and therapeutic strategies	Review	1715	6	2014	10.3390/v6041715	24736215	3.359	43	L
Sgarbanti Marco	IkB kinase e targets interferon regulatory factor 1 in activated T lymphocytes	Article	1054	34	2014	10.1128/MCB.01161-13	24396068	5.256	18	F

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

Ministero della Salute
Direzione generale della ricerca e dell'innovazione in sanità
BANDO RICERCA FINALIZZATA 2021
esercizio finanziario anni 2020-2021 - Progetto Completo

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Total proposed budget (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1a Staff Salary	240.000,00	240.000,00	not permitted	0,00
1b Researchers' Contracts	419.612,00	223.439,00	196.173,00	43,61
2 Equipment (Leasing - Rent)	145.000,00	145.000,00	0,00	0,00
3a Supplies	309.012,00	128.212,00	180.800,00	40,19
3b Model Costs	34.500,00	15.000,00	19.500,00	4,33
3c Subcontracts *	0,00	0,00	0,00	0,00
3d Patient Costs	0,00	0,00	0,00	0,00
4 IT Services and Data Bases	0,00	0,00	0,00	0,00
5 Publication Costs	11.500,00	8.000,00	3.500,00	0,78
6 Convegni	11.000,00	7.000,00	4.000,00	0,89
7 Travels	7.000,00	3.000,00	4.000,00	0,89
8 Overheads *	40.897,00	0,00	40.897,00	9,09
9 Coordination Costs	1.000,00	0,00	1.000,00	0,22
Total	1.219.521,00	769.651,00	449.870,00	100,00

* percentage calculated as average value between all the Operating Units.

Report the Co-Funding Contributor:

Co-funding will be provided by Istituto Superiore di Sanità and Università di Bologna

Ministero della Salute Direzione generale della ricerca e dell'innovazione in sanità **BANDO RICERCA FINALIZZATA 2021** **esercizio finanziario anni 2020-2021 - Progetto Completo**	**Project Title:** Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models **Project duration (months):** 36
Project Code: RF-2021-12375437	**Principal Investigator:** NISINI ROBERTO
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Budget Justification	
1a Staff Salary	-UO1: Senior Investigator/PI (1st-2nd year(y): 3 man-months (m/m); 3rd/y: 4 m/m); Investigator/co-PI (3 m/m /y); & collaborators in the group -UO2: Senior Investigator (1st/y: 3 m/m; 2nd &3rd/y: 4 m/m) & collaborators in the group; -UO3: -UO3: Professor 3
1b Researchers' Contracts	-UO1: 1 researcher contract (2 years); 1 fellowship (1 year) -UO2: 1 researcher contract (2 years); -UO3: contract for a post-doctoral fellow (15 months).
2 Equipment (Leasing - Rent)	Co-funding through available equipment in the laboratories of the three Units
3a Supplies	Molecular biology reagents and kits, cell and bacterial culture media, chemicals, reagents for liposomess, disposable plastic material (plates, flasks, tubes, pipettes), antibodies, ELISA and ELISPOT kits
3b Model Costs	Mice and associated costs (maintenance and handling); BSL3 facility usage for cellular infection.
3c Subcontracts	none
3d Patient Costs	none
4 IT Services and Data Bases	none
5 Publication Costs	Costs for publications of short or full-length articles on peer-reviewed international scientific journals (oper access).
6 Convegni	Costs to attend international scientific meetings (registration fees) and report up-dates on results as emerged by intermediate or conclusive data evaluation
7 Travels	Travel costs to attend international scientific meetings and report up-dates on results as emerged by intermediate or conclusive data evaluation.
8 Overheads	Institution bench fees
9 Coordination Costs	Costs to organize starting or conclusive meetings with all the collaborators

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Proposed total budget UO1 Institution: Istituto Superiore di Sanita' (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1a Staff Salary	100.000,00	100.000,00	not permitted	0,00
1b Researchers' Contracts	128.000,00	30.827,00	97.173,00	35,71
2 Equipment (Leasing - Rent)	65.000,00	65.000,00	0,00	0,00
3a Supplies	162.000,00	32.000,00	130.000,00	47,77
3b Model Costs	30.000,00	15.000,00	15.000,00	5,51
3c Subcontracts	0,00	0,00	0,00	0,00
3d Patient Costs	0,00	0,00	0,00	0,00
4 IT Services and Data Bases	0,00	0,00	0,00	0,00
5 Publication Costs	4.000,00	3.000,00	1.000,00	0,37
6 Convegni	3.200,00	2.000,00	1.200,00	0,44
7 Travels	3.000,00	1.000,00	2.000,00	0,73
8 Overheads	24.737,00	0,00	24.737,00	9,09
9 Coordination Costs	1.000,00	0,00	1.000,00	0,37
Total	520.937,00	248.827,00	272.110,00	100,00

Report the Co-Funding Contributor:

Ministero della Salute Direzione generale della ricerca e dell'innovazione in sanità **BANDO RICERCA FINALIZZATA 2021** **esercizio finanziario anni 2020-2021 - Progetto Completo**	**Project Title:** Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models **Project duration (months):** 36
Project Code: RF-2021-12375437	**Principal Investigator:** NISINI ROBERTO
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Budget Justification	
1a Staff Salary	-UO1: Senior Investigator/PI (1st-2nd year(y): 3 man-months (m/m); 3rd/y: 4 m/m); Investigator/co-PI (3 m/m /y); & collaborators in the group
1b Researchers' Contracts	-UO1: 1 researcher contract (2 years); 1 fellowship (1 year)
2 Equipment (Leasing - Rent)	Co-funding through available equipment in the laboratories of the UO1
3a Supplies	Molecular biology reagents and kits, cell and bacterial culture media, chemicals, disposable plastic material (plates, flasks, tubes, pipettes), antibodies, ELISA and ELISPOT kits
3b Model Costs	Mice and associated costs (maintenance and handling); BSL3 facility usage for cellular infection
3c Subcontracts	none
3d Patient Costs	none
4 IT Services and Data Bases	none
5 Publication Costs	costs for publications of short or full-lenght articles on peer-reviewed international scientific journals (opoen access)
6 Convegni	Cost to attend international scientific meetings (registration fees) and report updates on results as emerged by intermediate or conclusive data evaluation.
7 Travels	Travel costs to attend international scientific meetings and report updates on results as emerged by intermediate or conclusive data evaluation
8 Overheads	Institution bench fees
9 Coordination Costs	Costs to organize starting or conclusive meetings with all the collaborators

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Proposed total budget UO2 Institution: Istituto Superiore di Sanita' (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1a Staff Salary	85.000,00	85.000,00	not permitted	0,00
1b Researchers' Contracts	176.000,00	110.000,00	66.000,00	59,64
2 Equipment (Leasing - Rent)	35.000,00	35.000,00	0,00	0,00
3a Supplies	93.812,00	63.012,00	30.800,00	27,83
3b Model Costs	0,00	0,00	0,00	0,00
3c Subcontracts	0,00	0,00	0,00	0,00
3d Patient Costs	0,00	0,00	0,00	0,00
4 IT Services and Data Bases	0,00	0,00	0,00	0,00
5 Publication Costs	4.000,00	3.000,00	1.000,00	0,90
6 Convegni	3.800,00	2.000,00	1.800,00	1,63
7 Travels	2.000,00	1.000,00	1.000,00	0,90
8 Overheads	10.060,00	0,00	10.060,00	9,09
9 Coordination Costs	not permitted	not permitted	not permitted	0,00
Total	409.672,00	299.012,00	110.660,00	100,00

Report the Co-Funding Contributor:

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Budget Justification

1a Staff Salary	-UO2: Senior Investigator (1st/y: 3 m/m; 2nd &3rd/y: 4 m/m) and collaborators
1b Researchers' Contracts	-UO2: 1 researcher contract (2 years);
2 Equipment (Leasing - Rent)	none
3a Supplies	bacterial culture media, chemicals, disposable plastic material (plates, flasks, tubes, pipettes), antibodies,
3b Model Costs	none
3c Subcontracts	none
3d Patient Costs	none
4 IT Services and Data Bases	none
5 Publication Costs	sts for publications of short or full-length articles on peer-reviewed international scientific journals (open access)
6 Convegni	Costs to attend international scientific meetings (registration fees) and report up-dates on results as emerged by intermediate or conclusive data evaluation.
7 Travels	Travel costs to attend international scientific meetings and report up-dates on results as emerged by intermediate or conclusive data evaluation
8 Overheads	Institution bench fees
9 Coordination Costs	none

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Proposed total budget UO3 Institution: University of Bologna (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1a Staff Salary	55.000,00	55.000,00	not permitted	0,00
1b Researchers' Contracts	115.612,00	82.612,00	33.000,00	49,18
2 Equipment (Leasing - Rent)	45.000,00	45.000,00	0,00	0,00
3a Supplies	53.200,00	33.200,00	20.000,00	29,81
3b Model Costs	4.500,00	0,00	4.500,00	6,71
3c Subcontracts	0,00	0,00	0,00	0,00
3d Patient Costs	0,00	0,00	0,00	0,00
4 IT Services and Data Bases	0,00	0,00	0,00	0,00
5 Publication Costs	3.500,00	2.000,00	1.500,00	2,24
6 Convegni	4.000,00	3.000,00	1.000,00	1,49
7 Travels	2.000,00	1.000,00	1.000,00	1,49
8 Overheads	6.100,00	0,00	6.100,00	9,09
9 Coordination Costs	not permitted	not permitted	not permitted	0,00
Total	288.912,00	221.812,00	67.100,00	100,00

Report the Co-Funding Contributor:

Project Title:
Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models

Project duration (months): 36

Project Code: RF-2021-12375437

Principal Investigator: NISINI ROBERTO

Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare

Applicant Institution: Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Budget Justification

1a Staff Salary	-UO3: Professor 3 PM (class 4, part time), researcher 3 PM (class 6, full time), researcher 3PM (class 3, full time)
1b Researchers' Contracts	-UO3: 1 contract for a post-doctoral fellow (15 months).
2 Equipment (Leasing - Rent)	none
3a Supplies	cell culture media, chemicals, disposable plastic material (plates, flasks, tubes, pipettes), primary and secondary, antibodies, microscopy materials
3b Model Costs	Animal purchase and facility access charges
3c Subcontracts	none
3d Patient Costs	none
4 IT Services and Data Bases	none
5 Publication Costs	Costs for publications of short or full-length articles on peer-reviewed international scientific journals (open access)
6 Convegni	Costs to attend international scientific meetings (registration fees) and report up-dates on results as emerged by intermediate or conclusive data evaluation.
7 Travels	Travel costs to attend international scientific meetings and report up-dates on results as emerged by intermediate or conclusive data evaluation.
8 Overheads	Institution bench fees
9 Coordination Costs	none

Ministero della Salute Direzione generale della ricerca e dell'innovazione in sanità **BANDO RICERCA FINALIZZATA 2021** **esercizio finanziario anni 2020-2021 - Progetto Completo**	**Project Title:** Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models **Project duration (months):** 36
Project Code: RF-2021-12375437	**Principal Investigator:** NISINI ROBERTO
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Principal Investigator Data

Cognome: NISINI
Nome: ROBERTO
Codice fiscale: NSNRRT59A26H501G
Documento: Patente, Numero: U1Y107824P
Data di nascita: 26/01/1959
Luogo di nascita: Roma
Provincia di nascita: RM
Indirizzo lavorativo: Viale Regina Elena 299
Città: Roma
CAP: 00161
Provincia: RM
Email: roberto.nisini@gmail.com
Altra email: roberto.nisini@iss.it
Telefono: +393496921421
Altro telefono: 3496921421
Qualifica: Primo <ricercatore
Struttura: Dipartimento Malattie Infettive
Istituzione: Istituto Superiore di Sanità
Datore/ente di lavoro? Si
Datore/ente di lavoro SSN? Si
Nome datore/ente di lavoro non SSN:
Nome istituzione SSN: Istituto Superiore di Sanità
Tipo contratto: Lavoro Subordinato a Tempo Indeterminato

Con l'invio della presente proposta si dichiara che la stessa o parti significative di essa non sono oggetto di altri finanziamenti pubblici o privati e che di conseguenza vi è assenza del c.d. doppio finanziamento ai sensi dell'art. 9 del Regolamento (UE) 2021/241, ossia che non ci sia una duplicazione del finanziamento degli stessi costi da parte di altri programmi dell'Unione, nonché con risorse ordinarie da Bilancio statale.

By submitting this proposal, I declare that no significant part or parts of it are recipient of any other public or private funding and that consequently there isn't any so-called double financing pursuant to art. 9 of Regulation (EU) 2021/241, i.e. that there is no duplication in the financing of the same costs by other Euopean Union programs or any other ordinary resources from the State budget.

Ministero della Salute Direzione generale della ricerca e dell'innovazione in sanità **BANDO RICERCA FINALIZZATA 2021** **esercizio finanziario anni 2020-2021 - Progetto Completo**	**Project Title:** Asymmetric liposomes carrying mRNA as a promising novel vaccine approach against bacterial diseases: the Mycobacterium tuberculosis and Streptococcus pneumoniae models **Project duration (months):** 36
Project Code: RF-2021-12375437	**Principal Investigator:** NISINI ROBERTO
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Istituto Superiore di Sanita'

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Project validation result