Interferon gamma-1b for the prevention of hospital-acquired pneumonia in critically ill patients: a phase 2, placebo-controlled randomized clinical trial

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.

2. Original statistical analysis plan, final statistical analysis plan, summary of changes.

Protocol PREV-HAP

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"Human recombinant interferon gamma-1b for the prevention of hospital-acquired pneumonia in critically ill patients: a double-blind, international, phase 2, randomized, placebo-controlled trial - the PREV-HAP study"

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SIGNATURE PAGE

SPONSOR SIGNATURE

The sponsor agrees to comply with the laws and regulations on clinical trials for the conduct of the above-mentioned study and agrees to abide by all provisions set forth therein.		
Name and capacity of the signatory representative: **For the Sponsor and by delegation of the Managing Director, the Director of Research and Innovation**	**Date:** 8/02/2021	**Signature:**

INVESTIGATOR'S SIGNATURE

I have read all the pages of the clinical trial protocol sponsored by Nantes University Hospital. I confirm that this protocol contains all the information necessary for the conduct of the trial. I agree to conduct the trial according to the protocol and to abide by all provisions set forth therein. I agree to conduct the trial in compliance with:

- the principles of the "Declaration of Helsinki",
- international (ICH) and National good clinical practice regulations and guidelines
- European regulations and national laws and regulations relating to clinical trials,

I also agree for the investigators and other qualified members of my staff to have access to the copies of this protocol and documents concerning the conduct of the study so that they abide by all provisions set forth therein.

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Principal investigator	**Name and institution:**	**Date:**	**Signature:**

LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
AEMPS	Agencia Espanola de Medicamentos y Productos Sanitarios
ANSM	Agence Nationale de Sécurité du Médicament et des produits de santé
ALT	Alanine transaminase
AST	Aspartate transaminase
BAL	Bronchoalveolar lavage
CEA	Cost Effectiveness Analysis
CRA	Clinical Research Associate (monitor)
CTCL	Cutaneous T Cell Lymphoma
D#	Day number
DDD	Defined Daily Doses
DLT	Dose-Limiting Toxicity
DP	Drug Product
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
EQ-5D-3L	EuroQol 5 dimensions 3 Levels
ESICM	European Society of Intensive Care Medicine
FIH	First-in-Human
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HADS	Hospital Anxiety and Depression Scale
HAP	Hospital-Acquired Pneumonia
HLA-DR	Human Leukocyte Antigen
HRQoL	Health-Related Quality of Life
ICER	Incremental cost-effectiveness ratio
IMPD	Investigational Medicinal Product Dossier
MA	Marketing Authorisation
NCA	National Competent Authority
QALY	Quality-Adjusted Life Year
rHuIFNγ	recombinant human interferon gamma
ROSALI	RespOnse Shift ALgorithm at Item-level
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SC	Subcutaneous
SF-36	Short Form (36)
SFAR	Société Française d'Anesthésie Réanimation
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
SWLS	Satisfaction With Life Scale
VAP	Ventilator-Associated Pneumonia

CONTENTS

INTRODUCTION

Hospital-acquired Pneumonia (HAP) is an infectious disease of major concern in the world, and the most frequent cause of hospital-acquired infections with 500,000 episodes being treated every year in Europe. Despite the development of European recommendations, the incidence remains high, with dramatic medical consequences: existing therapies and preventive measures do not result in the expected favourable outcome (clinical cure and survival) for 30% of patients. HAP are moreover the main cause of antibiotic consumption in European hospitals and are increasingly induced by drug-resistant pathogens. New, alternative and more effective host-targeted strategies are therefore urgently needed to fight antibiotic resistance.

PREV-HAP study is part of a larger project entitled 'Host-targeted Approaches for the Prevention and the treatment of Hospital-Acquired Pneumonia' (HAP^2), funded by the European Union's H2020 research and innovation programme under grant agreement N°847782. HAP^2 aims to develop stratified host-directed drugs and biomarkers to enhance the prevention and the treatment of HAP and develop precision medicine in infectious diseases. Its ambition is to revolutionize the management of HAP: capitalising on the novel concept of critical-illness related immunosuppression altering the host-pathogens interactions, the aim is to propose a complete reappraisal of the physiopathology of HAP based on the concept of respiratory dysbiosis. "The HAP^2" project will reach two ground-breaking objectives in the field of bacterial infections: first the development of host-targeted approaches for the prevention and the treatment of a severe bacterial infection through the supplementation of the IFN-γ whose production is defective in patients at risk of pneumonia ; second the development of a clinico-biological score based on an integrative assessment of the host-pathogen interactions and genetic variation, to predict the course of HAP and the response to treatment. Our interdisciplinary consortium, bringing together 10 partners from academia and industry with expertise in clinical trials, immunology, microbiome analysis, omics and social sciences is uniquely placed to achieve this ambition within a 5-year project.

The main hypothesis of the PREV-HAP study is that **human recombinant Interferon gamma 1b (rHuIFN-γ, Imukin) treatment can restore immunity in critically ill patients and prevent Hospital-Acquired Pneumonia.**

We also hypothesize that the in vivo investigations of the host-pathogens interactions can be used for the stratification of patients into high/low risk and responders/non-responders to host-targeted prevention of hospital-acquired infections.

The involvement of a state of critical-illness related immunosuppression in the susceptibility to hospital-acquired pneumonia is widely accepted, and an emerging trend is that the development of drugs for the treatment of this acquired immunosuppression will prevent infection and enhance outcomes of hospitalized patients.

It has been demonstrated that the productions of IFN-γ by immune cells are decreased in critically ill patients, and that these defects are associated with the susceptibility to HAP. rHuIFN-γ has neither been tested nor is recommended as adjunctive treatment of patients with HAP. **Based on these specific factors identified in the host response**, we propose to use rHuIFN-γ as **novel preventive approach for HAP**.

1.JUSTIFICATION OF THE STUDY

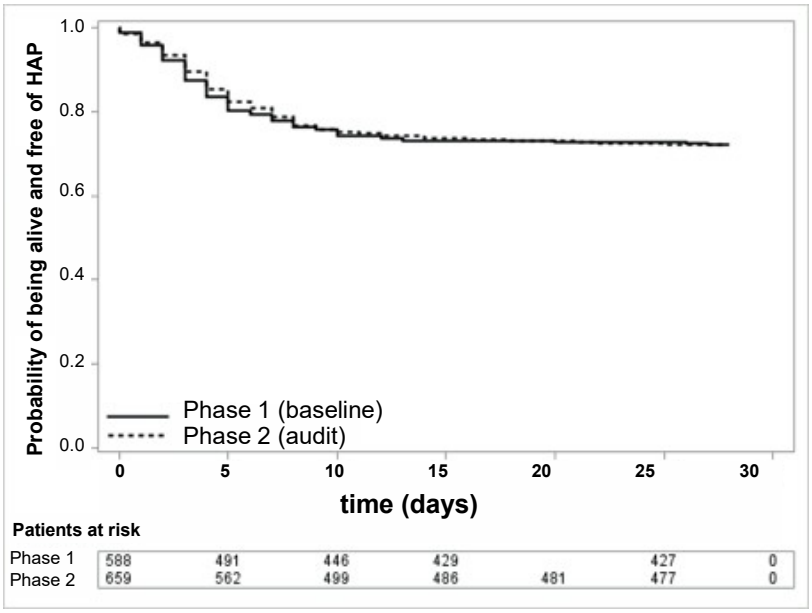
1.1. Positioning of the study

1.1.1. Epidemiology of Hospital-Acquired Pneumonia (HAP) in critical ill patients

Hospital-acquired pneumonia (HAP) **is the most frequent cause of hospital-acquired infections, with 500,000 episodes of HAP being treated every year in Europe(7)**, and accounting for 22% of all hospital acquired infections in a multistate point-prevalence survey(8). The incidence of HAP has barely decreased over the last decades and still routinely exceeds 10 cases /100 hospitalisations in critically ill patients(9).

The medical consequences of HAP are dramatic with prolonged hospitalization, long-term asthenia and depression, and increased risk of death (10)(11). The economic burden of ICU-acquired pneumonia, particularly VAP, is important. The patients often require longer periods of ventilatory assistance and have significantly longer ICU and hospital stays. On a per-case basis case VAP is associated with additional unadjusted hospital costs ranging between 40 000 and 49 000 USD in the USA (12,13).In France the average cost for each day in intensive care unit (ICU) is 2000 euros/day, and the cost of each episode of HAP is 40.000 euros (14)(15).

European, French and American society of intensive care have recently published guidelines in order to prevent hospital acquired pneumonia (16–18). These strategies aim at reducing oro-pharyngeal bacterial load in order to minimize germs aspiration. Yet, except for selective digestive decontamination which reduce mortality of critically patients (19), other interventions didn't improve significantly patient outcomes (9). We evaluated in the Pneumocare study (clinicaltrial.gov: NCT03348579) the impact of the French 2017 guidelines on the risk of HAP. We observed in 1300 patients included in 35 French ICUs that the risk of HAP remained unchanged around 25% of patients hospitalized 3 days or more in ICUs. This result demonstrated that new therapies are needed to further enhance the prevention of HAP in critically ill patients.



Probability of HAP before (phase 1, n=630 patients) or after (phase 2, n=650 patients) the application of the SFAR/SRLF recommendations (Roquilly et al. Clin Infect Dis 2020)

1.1.2. Rational for immunotherapy in critical ill patients

i. Risk of HAP and decreased production of IFN-γ during critical-illness related immunosuppression

During infections, activated monocytes and dendritic cells (DCs) stimulated by bacterial and viral antigens release IL-12 which induces the production of IFN-γ by innate-like lymphocytes (notably Natural Killer (NK) cells).Patients with inherited deficiency in IFN-γ production are highly susceptible to respiratory infections[1]. In mice model mimicking HAP, the lung response to secondary pneumonia is characterized by a decreased production of IFN-γ by NK cells as compared to normal response to pneumonia.

We have tested the hypothesis that treatments with rHu-IFNγ can restore immune resistance to bacteria. We have notably been demonstrated that IFN-γ restores the metabolic activity and the functions of monocytes[2], reversing a major feature of critical-illness related immunosuppression. Several case reports on the use of rHuIFN-γ in septic patients have shown promising effects[3].

In conclusion, our consortium has demonstrated that the susceptibility to HAP is a consequence of the limited stimulation of NK cells by monocytes and DCs, and that IFN-γ supplementation restores immune competence during HAP (Figure 2). These data strongly support that rHuIFN-γ treatment, as a compensatory therapy to overcome critical-illness related immunosuppression, can restore immunity and enhance the treatment of HAP.

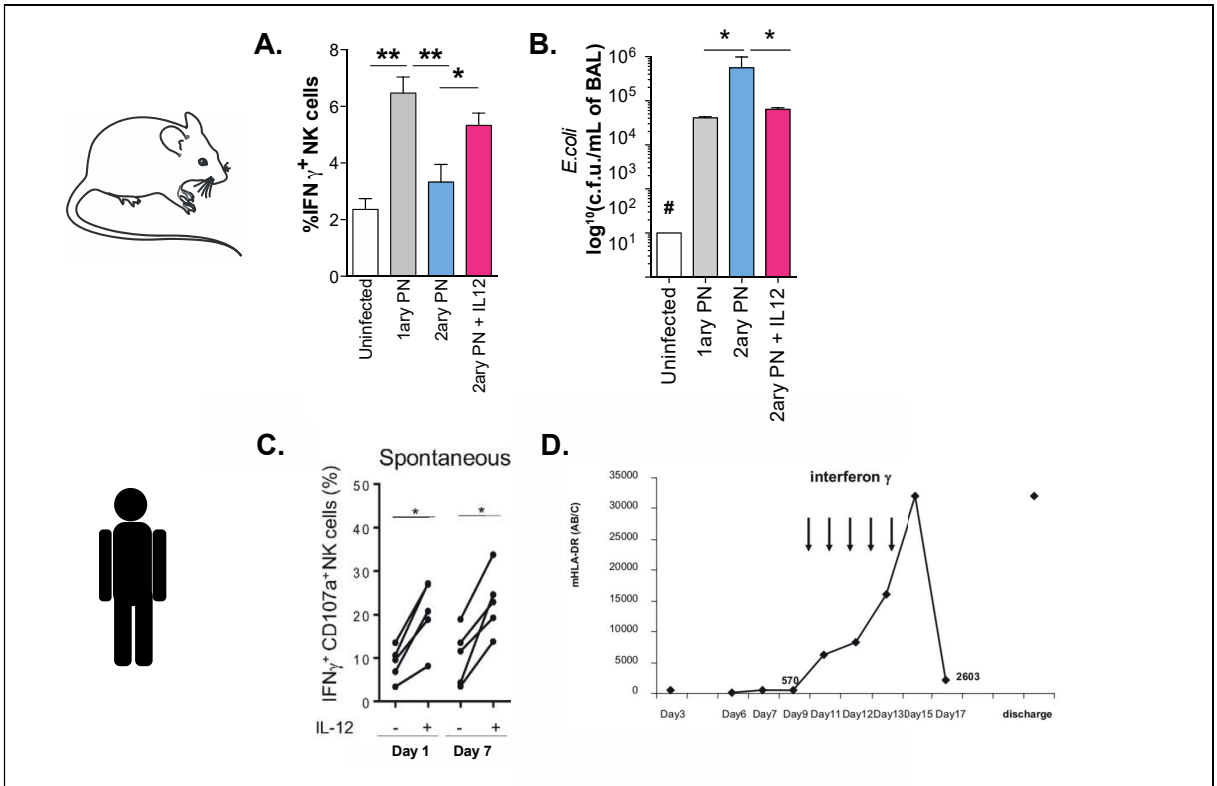


Figure 2. Proof of concept for the treatment of HAP with IFN-γ

[1] *Hambleton et al. NEJM 2011, Bogunovic Science 2012, Picard Am J Hum Gen 2002.*

[2] *Chen et al. Nature Immunol 2016*

[3] *Luckasewicz et al. Crit Care Med 2009, Docke et al. Nature Med 1997.*

(A-C) Mice are infected and spontaneously recover from a bacterial pneumonia. Infection-cured mice are challenged by a secondary bacterial pneumonia mimicking HAP. **(A)** Decreased production of IFNγ by NK cells, and restoration by IL-12 treatment during *Staphylococcus aureus* HAP in mice. **(B)** Restoration of IFN-γ production by NK cells is associated with increased the bacterial clearance during HAP in mice. **(C)** Decreased production of IFNγ by NK cells collected 1 day or 7 days after hospitalisation, and restoration by IL-12 treatment, during in vitro stimulation. **(D)** Evolution of the paralysis of monocytes (mHLA-DR) in human treated with rHuIFN-γ for HAP (Issue from Lukaszewicz. Crit Care Med 2009). .*p<0.05, **p<0.01

ii. Status of development of rHuIFN-γ (Imukin®) for randomized clinical trials

rHuIFN-γ (Imukin®) is commercialized by Clinigen and **already approved in Europe** for the treatment of infections in patients with chronic granulomatous disease. It can thus be sourced easily, ensuring feasibility. Administrations of rHuIFN-γ as a rescue therapy have moreover been reported in several case reports of protracted hospital-acquired infections[4], reinforcing the timeliness of the clinical trials proposed by our consortium.

iii. Dose regimen justification

The recommended dose of **rHuIFN-γ (Imukin®)** is 50 μg/m^2 of body surface area in patients treated for chronic granulomatous disease. Dose adaptation in intensive care units has been intensely investigated notably for antimicrobial treatments, and it is now recognized that dose-adaption to the weight of patients is not accurate in critically ill patients (Tangden et al. Intensive Care Med 2017). Body Surface Area-based dose adaption can be biased by the extreme and rapid variations of body weight frequently observed in critically ill patients (You et al. J Crit Care 2013). In the published series of critically ill patients, recombinant human rHuIFNγ was used at a fixed dose of 100 μg/48 hours (Payen, BMC Infectious Diseases, 2019; Docke, Nature Med 1997). We thus decided to test a fixed dose of 100 μg/48 hours. The immunological follow-up planned in the study (samples collected at day 3 and day 7) will be used to develop formula to predict pharmacokinetic for **rHuIFN-γ (Imukin®)** in critically ill patients.

The timing of treatment is also critical to consider. While it was suggested that rHuIFNγ treatment can be more effective when administrated beyond day 7 of hospitalization (Payen, BMC Infectious Diseases, 2019), we aim to prevent HAP which can develop from the second day of hospitalization, and most frequently before day 7 (Roquilly et al. Clin Infect Dis 2020). Since we have reported that the production of IFNγ by lymphocytes is decreased from the first day of hospitalization (Roquilly et al. Clin Immunol 2017), we proposed that early (from day 2) but prolonged (5 injections every 48 hours, so up to day 9 after inclusion) treatment is the best period of therapy to prevent HAP with rHuIFNγ.

iv. Biomarkers for the prediction of HAP course and for the response to treatment

Individuals might be responding differently to immune interventions, thus the validation of biomarkers for patient stratification is an asset to immune interventions. Several biomarkers have been associated with HAP in critically ill patients, but none is recommended for clinical practice. The main reason is that bulk-omics approaches largely fail to capture the complexity of HAP. The new gold standard is to use large cohorts of patients, bar coding of the samples, high-throughput analysis followed by unbiased algorithm guided analysis. **We will thus combine cutting-edge high-throughput investigations to capture the complexity of the host-pathogens interactions and to clinically validate biomarkers for the stratification patients into low/high risk of poor outcomes of HAP and into responders/non-responders to immunotherapy.**

[4] *Docke et al. Nature Med 1997, Lukaszewicz et al. Crit Care Med 2009*

Host background. Dr. Li has demonstrated that the inter-individual variation of cytokine responses to pathogens is explained by **genome-wide single-nucleotide polymorphism (SNP) genotypes. Dr Li has identified six cytokine quantitative trait loci (QTLs) playing** a critical role in the variability in cytokine production by human immune cells in response to pathogens[5]. **Genetic variations, as assessed by these SNP**, are thus probably associated with the defect of the IFN-γ axis in hospitalized patients. The level of blood cytokines levels, notably IFN-γ dependent chemokines, are also associated with the risk of HAP in trauma patients[6].

Host status. Prof. Becher has developed **high-dimensional single-cell mass cytometry** and a bioinformatics pipeline for the in-depth characterization of immune cell subsets in peripheral blood mononuclear cells (PBMCs) isolated from liquid biopsies of patients[7]. This approach is a powerful tool for characterization of the myeloid system and lymphocyte compartment which can permit the prediction of the response to immunotherapy in cancer patients[22]. Prof. Netea has demonstrated the role of **the epigenetic reprogramming of monocytes** in the modifications of their **transcriptomic** activity and their ability to produce cytokine in response to pathogens[8]. This phenomenon of trained immunity is associated with exacerbated inflammatory response during secondary infections.

Microbiome composition. Prof. Dickson has shown that **respiratory microbiome alterations** play an important role in the development of lung inflammation during HAP and reflect variation in baseline lung innate immunity[9]. The lower respiratory tract harbors a highly diverse microbiome made of large numbers of commensal bacteria species and viruses. In critically ill patients, the biomass of the lung bacterial component of the microbiome increases over time, whereas its diversity decreases, and the diagnosis of HAP has been correlated with these alterations. Dr Josset has developed a method to investigate the respiratory virome based on metagenomics next-generation sequencing[10]. The **human virome** includes diverse commensal and pathogenic viruses that evoke a broad range of immune responses from the host. In organ transplant recipients, immunosuppressants strongly affect the structure of the virome in plasma, and the total viral load increases with immunosuppression[11]. The investigation of the respiratory microbiome composition (bacteria and virus) is thus proposed as a surrogate marker of immunocompetence.

Integration of high throughput analyses of the host and of the microbiome. We aim to build clinico-biological scores taking into consideration demographic values (**gender, age, genetic variations**) and high throughput analyses of biomarkers[12]. This approach will be employed to deeply characterize the host-pathogens interactions before the treatment, to investigate the temporal immune response to rHuIFN-γ and finally to stratify patients as responders and non-responders.

[5] *Li et al. Nature Med 2016.*
[6] *Roquilly et al. Crit Care Med 2014.*
[7] *Becher et al. Nature Immunol 2014, Nature Med 2018*
[8] *Netea et al. Science 2014, Cell 2016 & 2018*
[9] *Dickson et al. Lancet 2014, Am J RespirCrit Care Med 2015&2018, Lancet Respir Med 2015, Nature Microbiol 2016*
[10] *Bal, BMC Inf Dis, 2018*
[11] *De Vlaminck et al. Cell 2013.*
[12] *Goris et al. Brain 2015.*

The ground-breaking concept underpinning the development of biomarkers in the "PREV-HAP" study is to have access to cutting-edge high throughput analyses of the host and of microbiome composition and to be able to combine all these data to achieve a full understanding of the host-pathogen interactions *in vivo* (Figure 3).

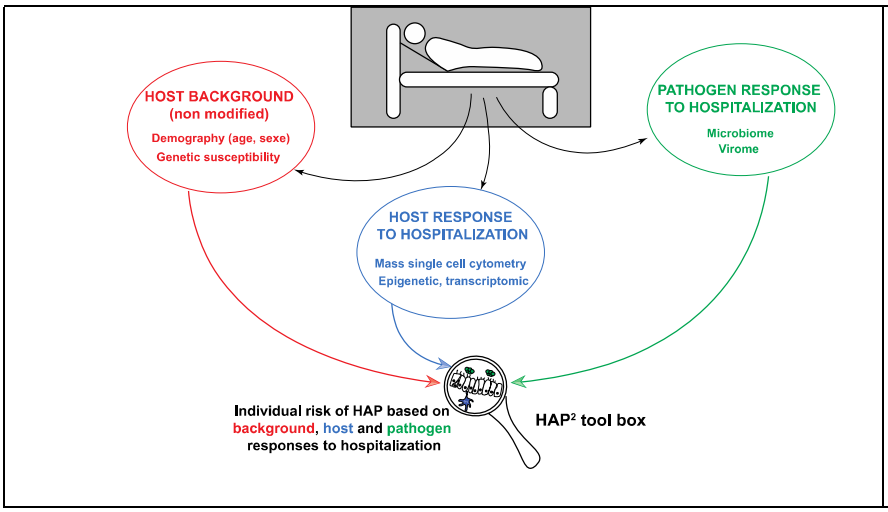


Figure 3. The "**HAP²" toolbox** is the integration of the patient background, of the host adaptation and of the pathogen modifications during hospitalization to develop biomarker and predict individual response to treatment of HAP.

v. "HAP2" project: a timely step to develop a stratified immune therapy for HAP

After a decade of continuous progress in the knowledge and the comprehension of the mechanisms of HAP by the partners, the interdisciplinary "HAP²" project is particularly timely (**Figure 4**), and PREV-HAP trial will help to reach two outcomes:

1/ **host-targeted drugs** (rHuIFN-γ will be brought from "bench to bedside", i.e. from a technology readiness level (TRL) 4-5 (technology validated in significant environment) to TRL7 (e.g. demonstration in clinical environment);

2/ **biomarkers** for the prediction of HAP outcomes from TRL2 (characteristic proof-of-concept) to TRL4 (validation in laboratory environment).

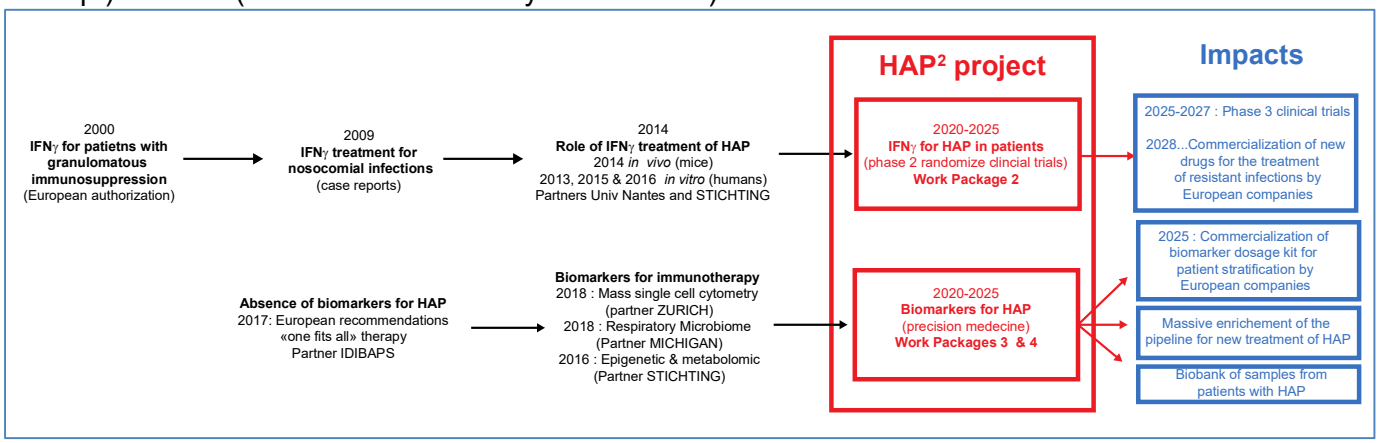


Figure 4 - Positioning of the "HAP²" project: bringing HAP prevention/treatment drugs from TRL 4-5 to TRL7 and biomarkers from TRL2 to TRL4

vi. Conclusions

The rates of HAP observed with current strategies underline the limits of current approaches of HAP prevention. **We have thus proposed that the prevention of HAP should aim to restore mucosal immunity and respect the diversity of the microbiome, rather than to sterilize airways with antibiotics (Figure 5)[13].** The development and validation of such strategies able to restore the mucosal immunity will probably minimize, or even replace, antibiotics - which are currently the sole therapies to date - for the management of HAP. The development of rHu-IFNγ is well advanced and the implementation of phase 2 randomized clinical trial is timely.

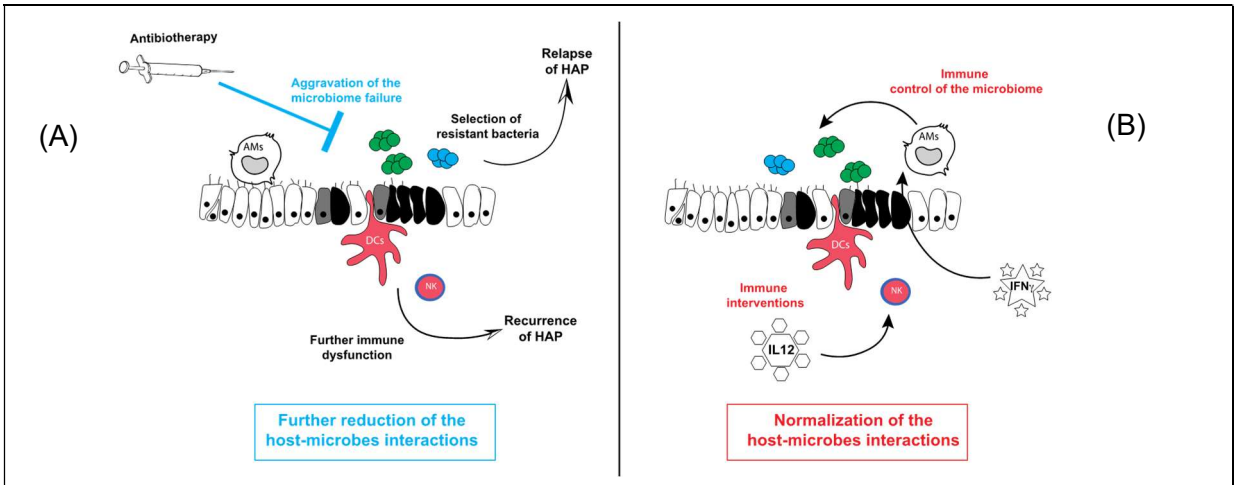


Figure 5. Current and proposed approaches to prevent hospital-acquired pneumonia
(A) Current approach of HAP: antibiotherapy alters the metabolomic functions of the microbiome, further reduces its diversity and selects resistant bacteria. The host remains susceptible to recurrence of relapse of HAP due to persisting immune dysfunction.
(B) HAP[2]. Immune interventions have the potential to clear virulent pathogens and to normalize the immune control of the microbiome (DCs: dendritic cells, NK: NK cells, AMs: Alveolar macrophages)

Current strategies for the prevention of HAP are "one-fits all patients" approaches which lead to a large proportion of treatment failures. Although each individual patient likely responds differently to therapeutic intervention, there are currently no reliable biomarkers for the stratification of patients predicting therapy success/failure in a given individual. Several biomarkers have been associated with HAP in critically ill patients, but none has been widely implemented in clinical practice[14]. Notably, the investigation of the host, or of the microbiome, fails to diagnose pneumonia when they are conducted separately[15]. **We propose to realize a biocollection of samples (blood and respiratory fluids) to combine develop biomarkers for the stratification of patients and the development of a precision medicine (theranostic).**

[13] *Roquilly et al. Lancet Respir Med 2019.*
[14] *Torres et al. EurRespir J 2017.*
[15] *Man et al. Lancet Respir Med 2019.*

1.2. Benefits and risks for subjects taking part in the study

1.2.1. Benefits

Individual benefit

The expected individual benefit of a treatment that improves the prevention of respiratory complications in critically ill patients is to decrease the risk of death, to reduce the duration of mechanical ventilation, and to increase the long-term quality of life. The individual benefit will thus be directly observed by the patient himself, and demonstrated by the result of the statistical analysis. These data will be measured at D28 and at D90

Collective benefit

The morbidity and mortality after hospital acquired pneumonia remain high, and also considering the number of episodes of infections, the burden is very important for the society (improvement of medical care). **The cost of hospitalization could be diminished, and the economic benefit could be high.** In a long-term approach, prevention of HAP could also fasten the return to work (economic benefit). If rHuIFN-γ reduce even slightly the morbidity/mortality induced by pneumonia, this study could deeply modify the medical care of patients all over the world.

On the other hand, diminishing the incidence of pneumonia would considerably reduce antibiotic consumption, producing a significant ecologic benefit concerning bacterial resistance. Indeed, up to 70% of ICU patients receive empirical or definite antimicrobial therapy on a given day, and the average volume of antibiotic consumption in this ICU patients is estimated as 1,563 defined daily doses (DDD) per 1000 patient-days (95% confidence interval 1,472–1,653) — that is, almost three times higher than in ward patients 16. rHuIFN-γ, which will reduce by 20% the risk of HAP has thus the potential to decrease the mean duration of antibiotherapy by 2 days in hospitalized patients. **The antibiotic selection pressure on resistant bacteria will thus be significantly reduced, increasing Europe's capacity to reduce the emergence of resistant bacteria.**

1.2.2. Risks

Individual risk

- Physical risks and constraints

No physical constraint is to be reported. Subcutaneous injections of rHuIFN-γ will occur during ICU hospitalization for a maximal duration of 9 days. Injections could be slightly painful, but are usually very well tolerated. Skin reaction such as local inflammation may also happen. Moreover, it is likely that the critically ill patients won't feel the puncture due to the sedation which is commonly used during the ICU stay.

Biological samples will be collected after the inclusion in the study immediately before study treatment injection, then before the 2nd injection at day3 (Visit 2), and before the 4th injection at day 7 (Visit 4).

Liver cytolysis has been described in children treated with rHuIFN-*γ* for months. This side effect, which resolves without sequelae upon treatment discontinuation, should not be observed in this trial evaluating short course of rHuIFN-*γ* in adults. Biological surveillance of

[16] *Bitterman et al. Clin Microbil Infect 2016.*

the transaminases will be performed but it should add no extra puncture, as ICU patients usually have daily biological tests at this stage.

Patients and relatives will receive a phone call at 1 and 3 months to ensure proper completion of the quality of life questionnaires (and the patient notebook for the patient). If the questionnaires haven't been completed and returned by post, the patient and the relative will answer to them during 10 to 15 minutes directly by phone.

A psychologist interview will be conducted by a researcher in psychology at M3 for some patients (and their relatives) included in Nantes. No additional appointment is set.
All in all physical risks and constraints are negligible.

- Disease-related risks

The risks of natural progression of hospital acquired pneumonia are:

- Pleural empyema, lung abscess
- Acute respiratory distress syndrome
- Relapse, recurrence of pneumonia
- Prolonged mechanical ventilation
- Death

- IMP risks

rHuIFN-γ. The drug has marketing authorization for the reduction of the frequency of serious infections in patients with chronic granulomatous disease or malignant osteopetrosis.

In these indications, as listed in 4.8 of IMUKIN SmPC, the most common adverse reactions are flu-like symptoms characterized by fever, headache, chills, myalgia or fatigue (with sometimes incomplete symptomatology). Hypersensitivity to the active substance (interferon gamma-1b) or to any of the excipients can't be excluded. Because Interferon gamma-1b is an exogenous protein, it may lead to the occurrence of antibodies during the course of treatment.

For the PREV-HAP study, a Reference Safety Information (RSI) adapted to the study indication is provided by the sponsor. No SAR is expected and all serious adverse effects are considered as SUSARs..

Placebo for rHuIFN-γ (NaCl). NaCl is commonly administered subcutaneously for hydration in vulnerable populations. Adverse reactions are most related to an overdose, with manifestation due to hypernatremia as nausea, confusion, but remain very unlikely with a subcutaneous injection, regarding low administered dosage. Local reaction may also occur.

- Concomitant treatment-related risks

Antibiotic therapy (eg. beta-lactamin ...) will be the most frequent concomitant treatment in the study, however, other concomitant drugs may be used as painkillers, hypnotics, bronchodilatators, steroids…. Medical devices may also be used for ventilation (tracheal tubes) and other current cares (such as for instance urinary catheter).

According to previous experiences, expected major adverse reactions with concomitant treatments are often related to antibiotics with allergic reaction to antimicrobial therapy, digestive disorders with Colitis and diarrhea including clostridium difficile colitis.

- Psychological risks and constraints

Patients are blinded to the study arm adjudication. The situation may induce anxiety.

The study drug administration needs an additional puncture site. Despite few local complications expected, it may cause little pain or apprehension. However, due to neurological injury, and/or sedation, patients won't probably feel the puncture. The patients included should experience no distress or feeling of dependence. The psychological constraints related with the pathology itself could be important but without relationship with the protocol.

For 20 patients included in Nantes (and their relatives who signed their own ICF), unpleasant emotions may be experienced during consultation with a researcher in psychology at M3 (unpleasant emotions such as fear or sadness for instance might be elicited by the recall of the ICU stay experience).

- Socio-economic risks

ICU hospitalizations cause dramatic changes in life of patients, including alteration of the social status and job loss. It may also have consequence on insurance and credit. Hospital acquired pneumonia increase the durations of hospitalization and of rehabilitation, worsening these consequences.

However, participating to the PREV-HAP study won't cause any change of social status and/or job; no consequence on insurance and credit; no devaluation of confidence in the attending physician; no change of relationship with others. There is no socio-economic risk resulting from the study.

Collective risk

The treatment management didn't induce increased risk (eg ecologic.) regarding standard care. The collective risk is limited.

1.2.3. Benefit / risk balance

Individually, the outcome of patients could be directly improved by the treatment (reduction of the risk of treatment failure, diminution of the duration of hospitalization and of the risk of death), while the risk of adverse effects is limited (IMUKIN® is approved for human use in Europe since 1992). The individual benefit/risk balance of the study protocol is therefore highly favorable.

Collectively, the study will develop new treatment which will decrease the burden of hospital-acquired infection, limit the antibiotic selection pressure of resistant bacteria and become new alternative for the treatment of highly resistant bacteria. Such outcomes will drastically decrease the cost for the society of carrying for hospitalized patients. The collective benefit/risk balance of the study protocol is therefore highly favorable.

2. OBJECTIVES AND ENDPOINTS

2.1. Primary objective and endpoint

2.1.1. Primary objective

The primary objective is to determine if rHu-IFNγ, as compared with placebo, could reduce the rate of **hospital-acquired pneumonia** and improve outcomes in patients admitted to intensive care unit and requiring mechanical ventilation.

2.1.2. Primary endpoint

To demonstrate the efficiency of rHuIFN-γ for the prevention of hospital-acquired pneumonia**, the primary endpoint is the composite outcome of all-cause mortality at day 28 and/or the occurrence of hospital-acquired pneumonia within 28 days after randomization**.

Hospital acquired-pneumonia is diagnosed after the 48th hour of hospitalization according to European and French guidelines (Torres et al. Eur Respir J 2017; Leone et al. ACCPM 2018):

- at least two of the following criteria: body temperature >38°C; leukocytosis>12000 cells per mL, leucopenia <4000 cells per mL, or purulent pulmonary secretions,
- appearance of a new infiltrate or change in an existing infiltrate on chest radiography,
- positive culture of a respiratory tract samples from mechanically ventilated patients with quantitative culture (for patients with antibiotics < 48h) (thresholds of 10^4 colony-forming units (CFU) per mL for a bronchoalveolar lavage, 10^5 CFU/mL for a blind BAL (mini BAL) sample, and ≥10^5 CFU/mL for a tracheal sample). A semi-quantitative is acceptable, notably for patients with antibiotics >48h. Respiratory samples are obtained before starting any new antibiotic treatment.

An adjudication committee, composed of 1 investigator by country who will be blinded to the trial-group assignments, will review the medical charts of patients with respiratory tract infections of the 2 other participating countries, in order to review the diagnosis. Guidelines will be provided to the members by the sponsor. The primary endpoint concerning the occurrence of HAP will be based on the re-reading and review of each diagnosis by two adjudication committee members. In case of disagreement between the 2 adjudication committee members, the clinician's diagnostic will prevail.

In case of medical history of CAP, the diagnosis of HAP will be confirmed if one or more bacteria was not present at the time of CAP.

2.2. Secondary objectives and endpoints

2.2.1. Secondary objectives

The secondary objectives are:

- to demonstrate the **efficiency** of rHuIFN-γ, on pneumonia-associated morbidity and mortality reduction
- to demonstrate the **efficiency** of rHuIFN-γ on antimicrobial therapy utilization reduction
- To describe the **safety and tolerability** of rHu-IFNγ
- To assess the **suitability, acceptability, and adaptability** of rHu-IFNγ
- To assess the **economic efficiency** of rHuIFN-γ for the prevention of pneumonia
- To develop **biomarkers** for the stratification of patients into responders and non-responders of rHuIFN-γ
- To develop a **biobank** of blood and respiratory samples collected in humans at risk of hospital-acquired pneumonia

2.2.2. Secondary endpoints

The secondary outcomes to determine the efficiency of rHu-IFNγ, on pneumonia-associated morbidity and mortality reduction are:

- All-cause mortality at D28 and D90
- Hospital acquired Pneumonia at D28
- Bacterial ecology of the 1st episode of HAP (respiratory fluids)
- Rate of ventilator-associated tracheobronchitis at D28 defined as at least two of the following criteria: body temperature >38°C; leukocytosis>12000 cells per mL, leucopenia <4000 cells per mL, or purulent pulmonary secretions and a positive culture of a respiratory tract samples, **without** appearance of a new infiltrate or change in an existing infiltrate on chest radiography (Martin-Loeches et al. Lancet Respir Med 2015)
- Acute Respiratory Distress Syndrome within 28 days after randomization
- Duration of antimicrobial therapy at D28, antibiotic free days at D28 (the number of antibiotic free days is defined as the number of days between D1 and D28 for which living patients don't receive antibiotics. Dead patients will be ascribed 0 antibiotic free days).
- Duration of mechanical ventilation at D90, mechanical ventilation free days at D90 defined as the number of days between D1 and D90 for which living patients breath spontaneously. Dead patients will be ascribed 0 mechanical ventilation free days.
- Duration of ICU hospitalization at D90, Duration of hospitalization at D90, hospital free days at D90 defined as the number of days between D1 and D90 for which living patients is outside of hospital. Dead patients will be ascribed 0 mechanical ventilation free days.

The secondary outcomes to determine **the tolerance** of rHu-IFNγ are:

- Rate of serious adverse effects and suspected unexpected serious adverse reaction (SUSAR) at D15.
- Rate of leukocytosis, neutropenia, lymphopenia, thrombopenia at D15.
- Rate of liver cytolysis (Increases in AST and/or ALT) at D15.
- Rate of pancreatitis (Increase in Lipase) at D15.
- Fever, headache, nausea at D15.
- Allergic reaction at D15.
- Injection site reaction at D15.
- Myalgia, arthralgia, back pain at D15

The secondary outcomes to determine the **economic efficiency** of rHu-IFNγ in the prevention of pneumonia are:

- Economic endpoint at 3 months: **Incremental cost effectiveness ratio (ICER)**.

We will assess the economic efficiency of the rHuIFN-γ compared to the placebo by performing a cost-effectiveness analysis (CEA) using QALYs (Quality-Adjusted Life-Years) as a measure of effectiveness. QALYs are a measure of effectiveness specifically designed for economic evaluations. Their main advantage is to combine information about the length and the quality of life of patients into a single index measure. Specifically, the CEA will consist in estimating an incremental cost-effectiveness ratio (ICER) as follows:

$$ICER = \frac{\left(Costs_{\text{rHuIFN}-\gamma} - Costs_{\text{comparator}}\right)}{\left(Effectiveness_{\text{rHuIFN}-\gamma} - Effectiveness_{\text{comparator}}\right)}$$

Where 'comparator' corresponds to the placebo. Effectiveness will be assessed in terms of QALYs. QALYs are constructed by weighting each period of time, typically one year, by a health-related quality of life index (called a utility score) ranging from 0, that represents "being dead", to 1, that corresponds to a state of "perfect health". The utility scores will be determined by asking the patients to fill in the EQ-5D-3L health-related quality of life questionnaire.

The results of the cost-effectiveness analysis (i.e. the ICER) will be expressed in terms of costs per QALY gained. See paragraph 6.2.8. for statistical analysis.

The secondary outcomes to determine the **suitability and acceptability** of rHu-IFNγ **from the patients' and relatives' perspectives** are:

<u>Suitability</u>

- Changes in health-related quality of life (HRQoL) from one (M1) to three months (M3) after randomization measured with the Short Form **(SF)-36 scale** validated in French, Greek, and Spanish
- Changes in anxiety and depression from M1 to M3 measured with the **HADS scale** validated in French, Greek, and Spanish
- Changes in subjective well-being from M1 to M3 measured with the **Satisfaction With Life Scale (SWLS)** validated in French, Greek, and Spanish

At M1 and M3, these questionnaires will be filled in by the patient (patient's perspective) and by one relative (relative's perspective). The relatives could be the legal representative who

have signed the consent for the inclusion of their relative, or another relative; the aim being that it should be the person closest to the patient emotionally, and that it is the same person who answers the questionnaires at M1 and M3, if they consent to. If the patient is discharged from hospital before M1, the questionnaires will be given to the patients at discharge, or sent by post, to be returned to the clinical team, and the patient and his/her relative will be contacted by phone at M1 and M3, to ensure the good completion of the questionnaires. If his/her condition does not allow him/her to answer the questionnaires, only the questionnaire of the relative will be collected (from the relative's perspective).

Acceptability

Adaptation of the patients to their health state and its evolution from M1 to M3 using differential item functioning and **response shift analyses** for HRQoL, anxiety and depression.

2.3. Objective and endpoints for ancillary studies

We hypothesized that if rHu-IFNγ will reduce the risk of HAP, thus potentially reducing of hospitalization and sequalae. Although this treatment should be highly accepted by patients and relatives assessing its suitability and acceptability from a quantitative and a qualitative perspective are important information to assess before making recommendations of clinical use.

This ancillary study thus aims to to provide additional evidence on the suitability and the acceptability (in terms of HRQoL and mental health) of rHu-IFNγ from the patients' and relatives' perspectives

To test the suitability and the acceptability of rHu-IFNγ, we will scrutinize and provide more insight, from a qualitative perspective in psychology, into the effects of the treatment on the patients' and relatives' adaptation (response shift between M1 and M3) to their health state and its changes. Using qualitative methods will enable to explore the ways in which patients and relatives respond to their circumstances in the contexts of their lives, and contextualize the ways in which they understand and define their experience following ICU stay. Allowing participants time to freely explain cognitions and experiences will provide a more thorough understanding on how patients and relatives approach their experience.

At Day 90, the qualitative study will be proposed to 20 consecutive patients from one center (Nantes Hospital) and 20 relatives (1 per patient), who will thus be distributed in a balanced way between each group. The relatives may be the legal representative who has signed the consent for the inclusion of the patient, or another relative; the aim being that it should be the person closest to the patient emotionally, and that it is the same person who has answered the questionnaires for the quantitative study at M1 and M3, provided they consent to do so.

Semi-structured interviews will be conducted by a researcher in psychology with patients and relatives (dyads) to gain more insight into the understanding and the interpretation of quantitative data, as recommended by literature on human sciences[17]. An interview guide will be developed on the basis of the literature on the psychological consequences of the immune intervention for patients. The interviews will be recorded with the participant's consent to allow

[17] Bioy A., Castillo M-C., Koenig M. (2021). Les méthodes qualitatives en psychologie clinique et psychopathologie. Paris:Dunod.

full transcription while respecting their anonymity and confidentiality. This interviews are highly specialized and thus can only be performed in one center of the study.

Qualitative data will be analyzed using a lexical analysis to describe what patients have told, together with a content analysis (thematic categorical classification, Bardin, 2003; Blanchet & Gotman, 2007) to highlight the topics of the corpus and interpret data[18]. More specifically, the interview guide will include 5 areas: 1/ Current life story and changes caused by the intervention in terms of HRQoL and mental health, 2/ Management of emotions such as distress and well-being, 3/ Management of fundamental cognitive schemas such as beliefs and goals, 4/ Behavioral management and social (inter)actions, 5/ Reassessment of self and HRQoL (i.e. response shift).

The discourses of the patients and their relatives will be analyzed in the following manner to answer the three following questions: “What are the topics addressed by the patients and relatives?”, “What is being said?", and “How is this verbalized?". The transcribed text of the interviews will be divided into segments allowing for categorical classification according to the topics that were addressed by patients and relatives. Numerical analysis of the lexicon will enable to identify what was being said, and numerical analysis of linguistics will explore how things were expressed.

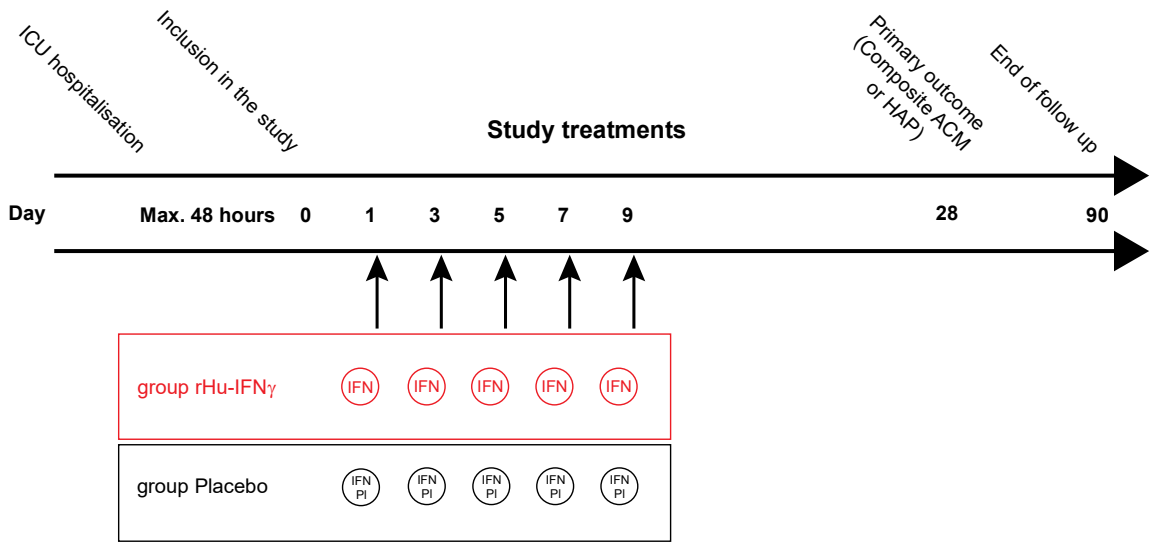
Briefly, the division of the discourses will be based on the proximities between the words used. Using open-source softwares “Iramuteq” and/or “Tropes” speech will be divided into units based on frequency which will then be classified into categories according to thematic groupings. The topics will be identified according to the study of co-occurrences and recurrences in discourse. Using open-source software “Sonal” a lexicometric analysis will enable to extract meaning from the structure and organization of discourse. The linguistic discourse analysis will assign words in lexical categories and subcategories to perform a content analysis, a cognitive-emotional discursive analysis, and diagnose the structure of the speech and the intention of the respondent (e.g. style (argumentative, narrative...), detection of doubts, removal of ambiguities, words occurrence).

We will use a mixed method design combining this qualitative approach with the quantitative approaches used for response shift analyses by drawing a parallel between them in order to provide narrative contexts to numerical data, providing stories behind the numbers and the comparsion of the HRQoL and mental health between the two study groups.

This mixed method approach will provide more insight into the suitability and acceptability of rHu-IFNγ from the patients’ and relatives’ perspectives, in terms of changes in HRQoL and in anxiety and depressive disorders after ICU stay. It will expand the knowledge on the patients’ and relative’s journey during this recovery period which remains understudied. Combing qualitative and quantitative approaches will allow painting a more complete picture of the recovery process which is a complex phenomenon encompassing many dimensions of physical, emotional, economic, and social health, and having different meanings to different individuals.

[18] Blanchet, A., &Gotman, A. (2007). L’enquête et ses méthodes: L’entretien. Paris: Armand Colin, et Bardin, L. (2003). L’analyse de contenu. Paris: PUF.

3. STUDY TREATMENTS



The patient will receive the following Investigational Medicinal Products (IMPs), depending on his/her randomization arm:

- **Arm 1 (Recombinant Interferon gamma 1b):**
  - **Recombinant Interferon gamma 1b** (IMUKIN®, from Clinigen®): 5 subcutaneous injections (100 µg/0,5ml) from day 1 to day 9 (i.e. 1 injection every 48h)
- **Arm 2 (Placebo):**
  - Recombinant Interferon gamma 1b placebo (IFN Pl): 5 subcutaneous injections (0,5ml) from day 1 to day 9, i.e. 1 injection every 48h

3.1. Description and mode of administration

3.1.1. IMP1: IMUKIN® (recombinant interferon gamma-1b – rHu-IFNγ)

Molecule name: IMUKIN® (recombinant interferon gamma-1b – rHu-IFNγ)

Qualitative and quantitative Product composition:

Each vial (0.5 ml) contains 2 x 10^6 IU (0.1 mg) recombinant human interferongamma-1b. Interferon gamma-1b is produced in an E. coli expression system.
List of excipients:
D-Mannitol
Disodium succinate hexahydrate
Polysorbate 20
Succinic acid
Water for injections

Manufacturer:

CLINIGEN HEALTHCARE B.V.
SCHIPHOL BOULEVARD 359
WTC SCHIPHOL AIRPORT, D TOWER 11TH FLOOR
1118BJ SCHIPHOL
NETHERLANDS

The drug has marketing authorization for the reduction of the frequency of serious infections in patients with chronic granulomatous disease or severe, malignant osteopetrosis.

Pharmaceutical form and packaging used:

IMUKIN® 2 x 10^6 IU (0.1 mg) is a clear, colourless solution for injection (subcutaneous use).
2 ml glass vials (Type I borosilicate glass) which are stoppered with grey butyl rubber stoppers with aluminium/polypropylene flip-off type caps.
Pack sizes: 1 vial in one folding box.

Storage and Handling

The vials need to be stored in a refrigerator (2-8°C). The vials must not be frozen.

Administration
IMUKIN® will only be used in the randomization arm 1:

- **Recombinant Interferon gamma 1b** (IMUKIN®, from Clinigen®): 100µg/0,5ml subcutaneous injections from day 1 to day 9 (5 injections, i.e. 1 injection every 48h,),

Dose adjustment
No dose adjustment is foreseen, but a drug discontinuation car occur in case of liver cytolysis (AST and/or ALT > 5N).

Reference documents
The reference document for this IMP is the current version of the Reference Safety Information provided by the sponsor.

3.1.2. IMP2: IMUKIN® Placebo

Molecule name: IMUKIN® placebo

Qualitative and quantitative product composition:

Clear and colourless solution of NaCl 0.9%.

Manufacturer:

PPRIGO – Rennes University Hospital
Pharmacy Department – Hôpital Sud
Pharmacotechnology Unit
16 Boulevard de Bulgarie
35200 Rennes
France

Pharmaceutical form and packaging used:

IMUKIN® placebo (NaCl 0.9%) is a clear, colourless solution for injection (subcutaneous use).

2 ml glass vials (Type I borosilicate glass) which are stoppered with grey butylrubber stoppers with aluminium/polypropylene flip-off type caps.

Storage and Handling
The vials need to be stored in a refrigerator (2-8°C). The vials must not be frozen.

Administration
IMUKIN® placebo is to be administered by subcutaneous injection, in randomization arm 2:

- Recombinant Interferon gamma 1b placebo (IFN PI): 0,5ml subcutaneous injections from day 1 to day 9 (5 injections, i.e. 1 injection every 48h).

Dose adjustment
No dose adjustment is foreseen, but a drug discontinuation car occur in case of liver cytolysis (AST and/or ALT > 5N).

Reference documents
The Reference Safety Information is in the current version of NaCl SmPC (section 4.8).

3.1.3. Other study treatments

Not applicable

3.2. Treatment compliance follow-up

For the compliance to the rHuIFN-γ and placebo treatments, all vials will be stored after use, for counting and auditing. All processing units (used or not) will be stored in the ICU and sent for destruction to the site pharmacy (when applicable) after use. The administrations will be recorded in the medical file of the patients, and these pieces of information will be collected in the eCRF.

A CRA of the sponsor will verify the returned units during monitoring visits, and the destruction may be carried out after each monitoring visit, after receipt of authorization for destruction from the sponsor.

3.3. Experimental drug circuit

3.3.1. General circuit

The sponsor will provide the clinical sites with the IMUKIN® and placebos vials:

- IMUKIN® vials will be purchased by the Sponsor
- IMUKIN® placebo vials will be manufactured by a subcontractor mandated by the sponsor, and provided to the French coordinating center (CHU Nantes).

The supplying will be adjusted according to the rhythm of the inclusions, of the study progress and of the expiry dates of IMPs.

All vials of IMUKIN® and placebo will be labelled, packed and stored under supervision of the pharmacist of the French coordinating center (CHU Nantes). IMPs will be delivered to each specific study site. Only the pharmacy of the French study's coordinating center will be aware of vials' composition (coding list).

At each study site, local investigators (surgeons, anesthetists), nurses and the patient will be blinded to the allocation group, and each site will be responsible of the storage, delivery and accounting of the treatments.

3.3.2. Experimental drug storage conditions

Description of dispensary storage

Upon receipt at each site, IMPs are kept in a cool dry place, protected from light, where the temperature stays between 2-8°C. As of reception by the pharmacy (or directly in the ICU, depending on local SOP) of the institution, the vials will be stored in a secured place whose temperature is monitored.

Description of department storage

The IMPs dispensing will be made starting from the medical prescriptions, by the pharmacy of the institution and according to local SOPs or by the local investigators' team of the institution according to the local SOPs.

Upon receipt in each department, IMPs are kept in a cool dry place, protected from light, where the temperature stays between 2-8°C. The vials will be stored in a secured place whose temperature is monitored.

Description of storage at patient's home

Not applicable

3.3.3. Unblinding procedure

Unblinding procedure can be requested by the sponsor :

- where knowledge of this information is necessary for the management of SUSAR and for the quality of the information transmitted to the DSMB, the competent authorities and Ethics Committees. An unblinding procedure is then carried out in agreement with the Safety Unit of the Sponsor;
- when drafting the Annual Safety Report if the analysis of the listing of the SAEs or AEs reveals a significant difference between the encoded randomization arms (up to the discretion of the Safety Unit of the Sponsor, and the DSMB).

Unblinding procedure only takes place under the conditions described in the protocol, and in compliance with the Sponsor's internal procedure (0062-PR-049_PROM-COORD-Blind lifting procedure). The Sponsor's Vigilance Unit is duly authorised to ask the sponsor's data manager to lift the blind according to this procedure : the Vigilance Unit analyses the SAEs for all the IMPs. It assesses causality and uses the reference information identified in the protocol to define the expected or unexpected character. Only in the event of SUSAR or a New Safety Information occurring in the patients, the data-manager lifts the blind for this event only, at the request of the vigilance officer.

Unblinding is also required at the end of the research by the biostatistician in charge of statistical analysis and by the Safety Unit of the Sponsor for the recoding of all SAEs in the Safety database of the Sponsor and for the drafting of the Final Safety Report.

Only data managers and clinical trials pharmacists at the University Hospital of Nantes can be informed of the treatments given to patients included (via the list or individual notifications sent by email).

3.4. Authorised and unauthorised treatments

3.4.1. Authorised treatments

All treatments usually used to prevent and treat hospital-acquired pneumonia in critically ill patients are authorized, notably (not exhaustive) Antimicrobial therapy (all molecules): antibiotics, antiviral therapy; selective oropharyngeal decontamination, selective digestive decontamination.

3.4.2. Unauthorised treatments

No treatment is forbidden in this protocol with the exception of open-labelled treatment with rHuIFN-γ during the first 28 days of the trial. The participation to another drug clinical trial is not authorized during the trial's participation, but the participation to a clinical trial with minimal risks and constraints (such as taking blood for example) is authorized.

3.4.3. Emergency treatments

Not applicable.

4.STUDY POPULATION

4.1. Description of the population

We will include 200 adult patients hospitalized in intensive care units, under mechanical ventilation in three European countries (see Statistic section for justification of the statistical power). The investigators working in Intensive Care Units will be responsible for the screening and the inclusion of critically ill patients. Patients under guardianship or trusteeship, pregnant women, minors won't be included in the trial.

The participation to another drug clinical trial is not authorized during the trial's participation, but the participation to a clinical trial with minimal risks and constraints (such as taking blood for example) is authorized.

In this study, inclusions will be realized in immediate vital emergency situation. Indeed, the treatment of hospital-acquired pneumonia is an emergency and it is recommended to start the treatment within the first hour after the diagnosis (Torres et al. European Guidelines for hospital-acquired pneumonia. Eur Respir. J 2017). Given the inclusion criteria (critically ill patients suffering acute pneumonia, or at risk of pneumonia due to critical illness), patient won't be able to express their consent before the inclusion in the study. Thus, an emergency consent procedure is needed and justified.

This procedure will allow some sites (depending on national regulatory approvals) to include the patients without having the patient's consent in a first time, nor the legal representative consent (but the consent will be sought as soon as possible).

Patients will be recruited in **3 European countries** during a 2-year period. Available data from these centers indicate that 10 to 20 patients fulfil the inclusion criteria monthly. A mean number of patients meeting non-inclusion criteria (including refusal to participate) of no more than 50% has been anticipated (worse scenario). The recruitment is competitive between the centers. The population recruitment will be stopped as soon as 200 patients are included in the study.

The criteria for site selection are:

- Potential recruitment (>10 patients/month with inclusion criteria)
- Previous experience of investigation in at less one randomized clinical trial in the last 5 years.
- Capacity to include patients 7 days a week.
- Capacity to manage the collection and the storage of the biological samples, ideally access to a dedicated facility.
- Capacity to guarantee the access to the study treatments 7 days a week to the investigators.

To ensure the feasibility of the study, we have taken the following decisions:

- Inclusion/non-inclusion criteria are consistent with routine care. A mean number of patients meeting non-inclusion criteria (including refusal to participate) of no more than 50% has been anticipated (worse scenario).
- Decisions about most aspects of patient care will be performed according to the expertise and routine clinical practice at each center. Little differences with standard practice set the stage for good adherence to the study protocol.

- A steering committee will insure the supervision of the trial. This committee will be composed of Pr Roquilly (HAP² coordinator), Pr Torres (National Coordinator for Spain and leader of HAP² WP4) and Dr Koulenti (Scientific Coordinator for Greece and leader of HAP² WP6), of Pr Sébille (leader of HAP² WP5 and methodologist for the 2 clinical trials of this European project, University Nantes) and of Dr Flet (Dept. of pharmacy, CHU Nantes). Regular meetings will be planned to evaluate the progress of the trial and adherence to the protocol.
- Dedicated clinical research associates on sites and/or study nurses will be made available at each center for follow-up and data registration.
- The 28-days and 90-days follow-up will be realized by the investigating center.
- The members of the steering committee, and the participating centers, are all experienced in the conduct of multi-center randomized clinical trials published in the field of hospital-acquired pneumonia: *JAMA (Torres et al. 2015, Roquilly et al. 2011)*, Lancet Infectious Diseases (Torres et al. 2018) *Lancet Resp Med (Roquilly et al. 2014), Am J RespirCrit Care Med (Roquilly et al. 2013) and Intensive Care Medicine (Roquilly 2017), Critical Care Med (Koulenti et al. 2009, national coordinator of the SAATELLITE trial and of the* COMBACTE network*)*.

4.2. Inclusion criteria

- **Adult** patients (18yr to 85yr).
- Hospitalized in intensive care unit for less than 48 hours**.**
- Receiving **invasive mechanical ventilation** at the time of inclusion.
- **One or more acute organ failure** at the time of inclusion **among**: neurological (Glasgow coma scale <13 before sedation), hemodynamic (norepinephrine, epinephrine, or any other vasopressor at a dose of ≥ 0.1 µg per kilogram of body weight per minute or ≥0.5 mg per hour for at least 6 hours), respiratory (PaO_2 / FiO_2< 200) and/or renal (creatininemia > 2 fold higher than the basal value and/or oliguria < 0.5 mL/kg/hour for at less 12 hours).
- **Informed consent** from a legal representative, or emergency procedure (when possible according to national regulation, see below). As is not possible to obtain the patient consent prior the inclusion (comatose patients), patient consent for the study continuation will be obtained as soon as deemed possible.
- Person insured under a health insurance scheme.

4.3. Non-inclusion criteria

These criteria define the characteristics meaning that the subject is not eligible for inclusion in the study:

- Pregnant women (serum or urine test), breastfeeding women
- Patient under legal protection (incl. under guardianship or trusteeship)
- **Hypersensitivity** to the active substance (interferon gamma-1b) or known hypersensitivity to related products, such as another interferon, or to any of the following excipients: Mannitol, Disodium succinate hexahydrate, Succinic acid, Polysorbate 20
- Severe **hepatic insufficiency** (Child Pugh score B or C)
- Liver cytolysis with hepatic enzymes (AST and/or ALT) > 5N

- **Severe chronic renal insufficiency** (MDRD Creatinine Clearance < 10 ml/min/1.73m^2)
- **Immunosuppression** (hematologic cancer, aplasia, chemotherapy/radiotherapy for cancer within 3 months prior to the inclusion, known infection Human immunodeficiency virus, concomitant use of any anti-graft rejection drug).
- **Coma after resuscitated cardiac arrest**
- **Cervical spinal cord injury**
- Participation to a drug interventional study within 1 month prior to the inclusion
- Hospital-acquired pneumonia before inclusion in the study during the current hospitalization.
- Sustained hyperlactatemia > 5 mmol/L.

5. STUDY DESIGN AND CONDUCT

5.1. Study schedule

Activities	V0 Inclusion visit within 6h before 1st injection	V1 Day 1	V2 Day 3	V3 Day 5	V4 Day 7	V5 Day 9	V6 Day 15	V7 Day 28	V8 Day 90
Inclusion and non-inclusion criteria verification + Patient and/or legal representative information, and consent (+ relative's consent for the questionnaires at M1 and M3)	X (legal representative) *and patient and relative as soon as deemed possible*								
Pregnancy test – urine or blood	X								
Randomisation	X								
Clinical examination	X	X	X	X	X	X	X	X	
IMP administration (IMUKIN® or placebo)		X	X	X	X	X			
Collection of the respiratory fluid and of blood (peripheral blood mononuclear cells)	X		X		X				
Liver Function Test (AST, ALT, bilirubin) + Blood count test	X		X		X		X		
Lipase test	X				X		X		
Compliance		X	X	X	X	X			
Adverse events		X	X	X	X	X	X	X	X
Concomitant medications		X	X	X	X	X	X		
Patient notebook (+ extraction of hospital database if applicable) for consumption of pharmaceuticals, consultations...									X
Patient's and relative's perspective**: Health-related quality of life (SF-36), anxiety/depression (HADS), subjective well-being (SWLS) ; Patients perspective only: EQ-5D-3L	X *(EQ-5D-3L only)****							X	X
Interview with a researcher in psychology (20 patients and their relative in Nantes)									X

** These data will be measured at D28 and at D90 using quality of life questionnaires, sent by post (or given directly at the patient's discharge), or collected directly during the phone call.
These questionnaires will be filled by the patient (patient's perspective) and by one relative (relative's perspective), except for EQ-5D-3L, filled in by the patient only. If the patient is discharged from hospital, he will receive the questionnaires by post, to be returned to the clinical team, and he and his/her relative will be contacted by phone, to ensure the good completion of the questionnaires. If the patient is still hospitalized and his/her condition does not allow him to answer the questionnaires, only the questionnaire of the relative will be collected (from the relative's perspective). The phone call will ensure proper completion of the quality of life questionnaires (and the patient notebook for the patient). If the questionnaires haven't been completed and returned by post, the patient and the relative will answer to them during 10 to 15 minutes directly by phone.

*** At baseline, given that all patients will be unable to answer to a questionnaire, we will assume an equal level of quality of life for all. This level will be determined by a group of expert physicians before the inclusion in the trial. This score will be applied to all patients in the trial, at the baseline.

- **Screening (<48 hours after admission in intensive care unit).**

- **Inclusion/non-inclusion criteria:** all consecutive adult patients under mechanical ventilation will be assessed for eligibility. The clinicians will verify inclusion and exclusion criteria.

- **Informed Consent Form (ICF).** We anticipate that most patients will be unable to give consent before their inclusion (e.g. patients will be under invasive ventilatory support, sedated, or unconscious). In accordance with EU and national regulations, an emergency procedure for obtaining written consent from a legal representative will be submitted for approval to the relevant authorities and Ethics Committees. This procedure will allow some sites (depending on national regulatory approvals) to include the patients without having the patient's consent in a first time, nor the legal representative consent if he's not present (but the consent will be sought as soon as possible).

Apart from this emergency procedure, prior to the patient's inclusion in the trial, written consent from a legal representative will be obtained. The investigator informs the legal representative and answers all questions about the objective, the nature of the constraints, the foreseeable risks, the expected benefits of the research. He/she also specifies the patient's rights in the research and provides a copy of the information sheet and consent to the patient (or the legal representative). The legal representative will be invited to take time to reflect on the information provided and ask further questions. If the legal representative agrees to participate, the legal representative and the investigator record their full names, date and sign the consent form. A copy of this document is given to the legal representative and the original is kept by the investigator. The investigator will subsequently obtain patient consent to continue the research **as soon as the patient becomes able to consent** and clearly explain the study's aim and requirements to the patient, using the informed consent form, to be dated and signed.

Some patients may still be unable to give their consent before discharge from hospital, because of cognitive impairment either due to the initial pathology causing their hospitalization (for example: traumatic brain injury, stroke) or due to complications arising during their stay in ICU. If no legal representative is present to consent, best efforts will be made to obtain consent from a representative for the study. If the patient's representatives remain unreachable at the end of the study, patient's data will be analyzed. This procedure will be followed only in countries allowing such procedure, after ethics committee approval.

Information will be given in both **oral and written form** in the native language and non-medical terms so it can be fully understood. The trial will be described truthfully with regards to the purpose, nature, scope and possible consequences of the study. Patients and/or legal representative will be ensured that whatever their choice may be, there will be no consequences on the standard medical care received by the patient. It will be explained that agreement to participate must be made freely and willingly. Participants will be invited to take time to consider the information, to ask questions and to make further enquiries.

The trial information sheet will also contain detailed explanations about biological samples collected during the trials: type, quantity, destinations. Patients will also be required to consent to secondary use of their samples for further research after the end of the project (specific **biobank consent form**).

- The relative consent will also be obtained before asking them to complete the 1 month and 3 months questionnaires (SF-36, HADS, SWLS)

- In any case, the sponsor will comply with the regulations in force in each country regarding the collection of consent from persons unable to give consent

- For **women in child bearing age**, a urinary or a blood pregnancy test will be performed to rule out any ongoing pregnancy. As soon as patients are able to express their consent to continue the research, they will be asked to take effective contraception for up to 28 days after

the end of treatment.

- **Inclusion visit (within 48 hours after admission in intensive care unit).**
  The local investigator will perform a clinical examination of the patient.
  After the signature of the informed consent by the legal representative if available (emergency procedure for inclusion without patient or legal representative signed consent will be possible according national regulations), enrolled patients will be randomized by local investigators using a dedicated, password-protected, SSL-encrypted website (eCRF, Ennov Clinical) to allow immediate and concealed allocation. Each patient will be given a unique patient-number and a randomization number. Randomization sequence will be generated by blocks, and will be stratified according to cause of admission in ICU (sepsis or no), and according to the country (France, Spain or Greece). Patients will be randomized to:
    - **Arm 1: Recombinant Interferon gamma 1b** (IMUKIN®, from Clinigen®)
    - **Or arm 2: Placebo**.

  The investigator will provide the pharmacist, or the delegated ICU personnel (when applicable, depending on national SOP) with a prescription including the randomization number.
  EQ-5D-3L: given that all patients will be unable to answer to a questionnaire at the baseline, we will assume an equal level of quality of life for all. This level will be determined by a group of expert physicians.

Blinding

All vials of IMUKIN® and placebo will be labelled, and packed under supervision of the pharmacist of the French coordinating center (CHU Nantes). IMPs will be delivered to each specific study site. Only the pharmacy of the French study's coordinating center will be aware of vials' composition (coding list). At each study site, local investigators (surgeons, anesthetists), nurses and the patient will be blinded to the allocation group.

The computer program will assign kit numbers to the patient. Each study site will have sufficient IMPs to be allocated to include patients. This will ensure that the patient will receive only the treatment of the arm in which he was randomized. The IMUKIN® and placebo vials are manufactured, labelled and packaged to maintain the blind.

At each participating center, data will be collected and entered into the electronic web-based case report form (eCRF) by trial or clinical trained personal (clinical research associate), blinded to the allocation group, under the supervision of the trial site investigators.

Moreover, double blinding and the use of well-defined and pre-specified primary/secondary outcome measures will control for the risk of evaluation and reporting bias, respectively.

- **Recombinant Interferon gamma 1b or placebo administrations (day 1 to day 9).**

  The first injection of the study treatments (IMUKIN® or its placebo) is performed in the 6 hours following the randomization, followed by 4 injections of IMUKIN® or its placebo (1 injection every 48 hours +/- 2 hours until day 9).

  The pharmacist (or the investigator, depending on local SOP) will provide the allocated treatment (identified only by its identifying number) to the nurses of the intensive care unit. All members of sites pharmacy and intensive care unit including the doctor and the nurses will remain blinded to the allocated treatment group. The patient will receive the following Investigational Medicinal Products (IMPs), depending on his/her randomization arm:

Arm 1 (rHu-IFNγ):

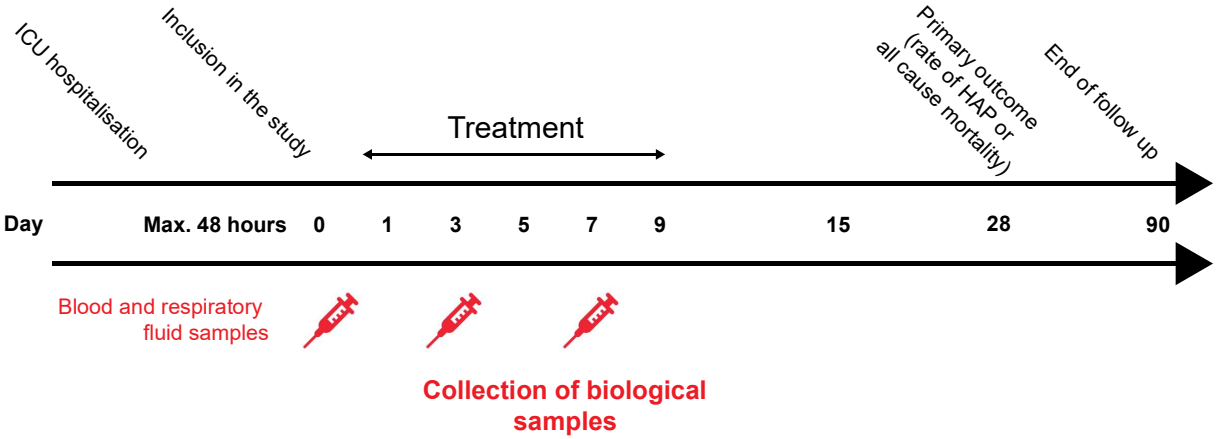
- **Recombinant Interferon gamma 1b** (IMUKIN®, from Clinigen®): 100 µg/0,5ml subcutaneous injections from day 1 to day 9 (5 injections, i.e. 1 injection of 100 µg every 48h, regimen adapted from SmPC),

Arm 2 (Placebo):

- **Recombinant Interferon gamma 1b placebo**: 5 subcutaneous injections from day 1 to day 9 (i.e. 1 injection of 0,5ml every 48h).

- **Collection of human samples**

  - Blood is collected at inclusion visit (between the randomization and the first administration of the study treatment), day 3 (before the 2nd injection), day 7 (before the 4th injection).
  - Tracheal aspirates are collected with Fibro mucus aspirators (provided to the sites by the sponsor) in patients with tracheal intubation at day inclusion (immediately before the first administration of the study treatment), day 3 (before the 2nd injection), day 7 (before the 4th injection). If the patient is extubated, no respiratory sample is to be collected.
  - In case of impossibility to collect the biological samples (blood and or respiratory fluid), the patients continue to receive the study treatment and is clinically followed according to the protocol.



- **Follow-up**

Standard of cares for the prevention of hospital-acquired pneumonia will comply with the international guidelines[19].

In each group, patients will be assessed:

- During ICU hospitalization:

- Daily clinical evaluation for the diagnosis of hospital-acquired pneumonia; follow-up of any AE/SAR/SAE;
- Liver function tests and blood count tests at Visit 0, Visit 2 (day 3), Visit 4 (day 7), Visit 6 (day 15)
- Lipase test at Visit 0 (inclusion), Visit 4 (day 7) and Visit 6 (day 15)

[19]Kallil et al. Clin Infect Dis 2016 (American guidelines); Torres et al. Eur Respir J 2017 (European guidelines); Leone et al. ACCPM 2018 (French guidelines)

- Compliance and concomitant medication follow-up

- Day 28: collection of the primary outcome. If the patient is discharged before day 28, the patient notebook will be given to the patient at discharge, and the investigator team will contact the patient and the relative at D28 to collect the outcome and to ensure a good completion of the questionnaires (EQ-5D-3L for patient and Health-related quality of life (SF-36) questionnaire, anxiety/depression (HADS) questionnaire and subjective well-being (SWLS) questionnaire for both patient and relative), and to check the correct completion of the patient notebook. The questionnaires will be completed by the patient and by the relative from their own perspectives (except for EQ-5D-3L, from patient's perspective only). The method for completion of the questionnaires will be recorded in the eCRF (patient alone; assistance required (identification of the person who provided assistance, e.g. relative, formal caregiver…); completion by the clinical team during the phone contact).

- Day 90: the study nurse or a local investigator will call the patient and his/her relative or the patient's family doctor to find out their vital status. Data for utility scores is collected with the EQ-5D-3L, quality of life is assessed using the SF36 questionnaire. HADS and SWLS will be filled for anxiety and depression signs, and for subjective well-being respectively. The 4 questionnaires (EQ-5D-3L, SF-36, HADS and SWLS) will be completed by the patient, and the questionnaires SF-36, HADS and SWLS by the relative from their own perspectives, and the method of completing the questionnaires will be recorded in the eCRF (patient alone; assistance required (identification of the person who provided assistance, e.g. relative, formal caregiver…; completion by the clinical team during the phone contact). As for the patient notebook, the study nurse or the investigator will verify the correct completion during the phone call, and it will be asked to the patient to send the notebook and the questionnaires back to the team.

- Day 90 (An ancillary study) will concern 20 patients included in Nantes and their relative, if they consent to. Semi-structured interviews will be conducted by a researcher in psychology to gain more insight into the understanding and the interpretation of quantitative data, as recommended by literature on human sciences.

- **Differences with routine clinical practice**

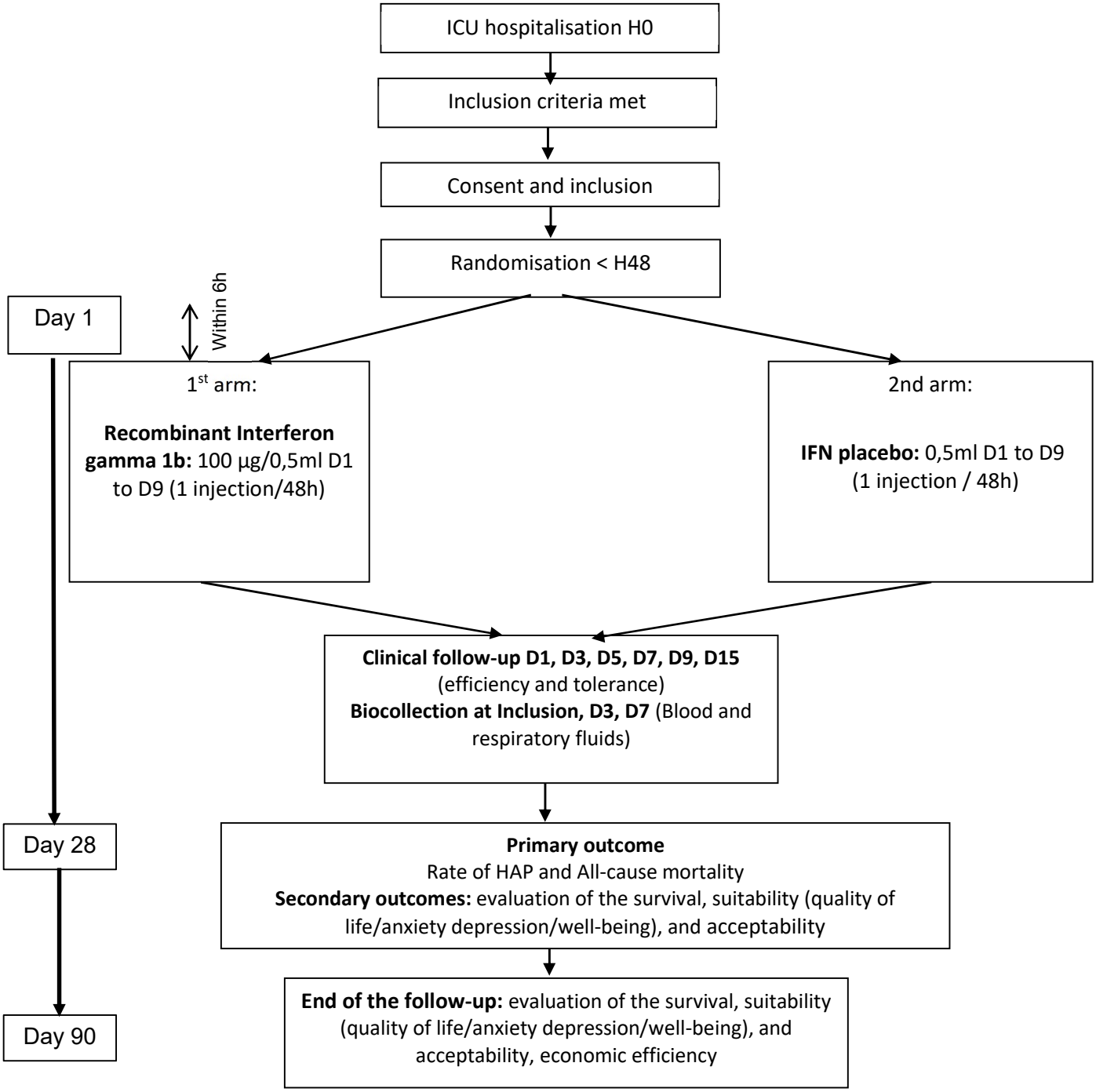
  The differences with routine clinical practice include:
  - the administration of IMUKIN® or of the placebo,
  - the liver blood tests at inclusion, day 3, day 7, day 15
  - the lipase test at Visit 0 (inclusion), Visit 4 (day 7) and Visit 6 (day 15)
  - the collection of blood samples (a maximum of 19 blood tubes by catheter or direct vessel puncture) and respiratory fluid samples will be collected by the ICU nurses for each patient during the study (3 visits). It will represent a maximum amount of 183 ml of collected blood per patient.

5.2. General study methodology

- Type of study: drug (**phase II**)
- **Multi-centre** international study
- **Placebo-Controlled, superiority study**
- **Randomised, stratified on the cause of hospitalization (sepsis or other) and country (France, Greece, Spain).**
- **Double-blind**
- **Parallel groups** study.

5.3. Study diagram

Recruitment duration: 24 months.
Follow-up duration / patient: 3 months
Duration of the entire trial: 27 months



5.4. Description and justification of the treatment plan

5.4.1. Cumulative Safety Information

IMUKIN® (recombinant interferon gamma-1b* – rHu-IFNγ*)
The drug has marketing authorization for the reduction of the frequency of serious infections in patients with chronic granulomatous disease or malignant osteopetrosis.

The real mechanism of action of interferon gamma-1b in such indications remained still unknown. Interferons are a family of functionally related proteins synthesized by eukaryotic cells in response to viruses and a variety of natural and synthetic stimuli. Findings related to superoxide anion production remain unequivocal. However, it is presumed that interferon gamma-1b increases macrophage cytotoxicity by enhancing the respiratory burst via generation of toxic oxygen metabolites capable of mediating the killing of intracellular micro-organisms. It increases HLA-DR expression on macrophages and augments Fc receptor expression, which results in increased antibody-dependent cell-mediated cytotoxicity.

In its MA indications, expected AE are detailed in 4.8 section of the SmPC. The most common adverse events are flu-like symptoms characterized by fever, headache, chills, myalgia or fatigue (with sometimes incomplete symptomatology). Hypersensitivity to the active substance (interferon gamma-1b) or to any of the excipients can't be excluded. Because Interferon gamma-1b is an exogenous protein, it may lead to the occurrence of antibodies during the course of treatment.

Regarding the study population, a Reference Safety Information (RSI) adapted to the study indication is provided by the sponsor. No SAR is expected and all serious adverse effects are considered as SUSARs.

Caution should be exercised when treating patients with known seizure disorders and/or compromised central nervous system function, cardiac disease, serious hepatic insufficiency and patients with severe renal insufficiency, because possible other adverse reactions, including those arising in special conditions.

Indeed, nausea, vomiting, abdominal pain seems to be common as well as depressive mood, reversible neutropenia and thrombocytopenia that can be severe and may be dose related have been observed. Liver enzyme increased that has been noted, especially in young children.

At high dosage or in case of overdose, reversible central nervous system adverse reactions including decreased mental status, gait disturbance and dizziness have been observed. Blood disorders including reversible neutropenia and thrombocytopenia as well as the onset of increased hepatic enzymes and of triglycerides have also been observed. Patients with pre-existing cardiac disease may experience an acute, self-limited exacerbation of their cardiac condition.

As pancreatitis (including fatal outcome) has also been reported as adverse effect (frequency not known), the sponsor will pay particular attention to lipase monitoring.

Interactions:

Interaction studies have only been performed in adults.

The interferon gamma 1-b does not reduce the efficacy of antibiotics or glucocorticoids; However, caution should be exercised when interferon gamma 1-b shall be associated with concomitant drugs, because It's also theoretically possible that hepatotoxic and/or nephrotoxic

drugs might have effects on the clearance of interferon gamma 1-b or that interferon gamma 1-b potentially can prolong the half-lives of simultaneously administered drugs, which are metabolized by the cytochrome P-450 system.

So, concurrent use of drugs having neurotoxic (including effects on the central nervous system), haemotoxic, myelosuppressive or cardiotoxic effects may increase the toxicity of interferons in these systems.

The concomitant administration of heterologous serum protein preparations or immunological preparations (e.g. vaccines) may increase the immunogenicity of interferon gamma 1-gamma.

In conclusion

No SAR is expected and all serious adverse effects are considered as SUSARs Moreover, the sponsor will also pay particular attention to lipase monitoring, in order to prevent any risk of pancreatitis.

Placebo:

IMUKIN® placebo is a clear and colorless solution of NaCl 0.9%, to be administered by subcutaneous injection.

Regarding the composition, the secured process of preparation and the route of administration, only local AEs with pain, erythema, and irritation are expected. The amount of NaCl does not suggest systemic hydro electrolytic or blood pressure adverse effects, nor infection.

5.4.2. Cumulative Efficacy Information

Animals

In mice models of secondary pneumonia, treatment with IL-12 restores the production of IFN-γ by natural killer cells, increases the bacterial clearance and decrease mice weight loss.

Humans cells

In vitro treatment of PBMCs with rHuIL-12 restores the production of IFN-γ by natural killer cells collected in hospitalized patients. *In vitro* treatment of PBMCs with rHu-IFNγrestores the metabolic function of lymphocytes collected in hospitalized patients. Treatment of severe septic patients with rHu-IFNγ restores phagocytosis and antigen presentation by monocytes.

Humans patients

Case series of critically ill patients treated with rHu-IFNγ has confirmed the properties of immune-stimulation (intermediate outcomes). rHu-IFNγ was associated in clinical cure of hospital-acquire infections.

5.5. Samples management

Each center will prepare and freeze biological samples according to procedures imposed by the sponsor (centrifugation, aliquoting, freezing) and in accordance with the needs of the analytical laboratories. All the sites will use the reagents and consumables described in the SOP provided by the sponsor in order to limit preparation bias.

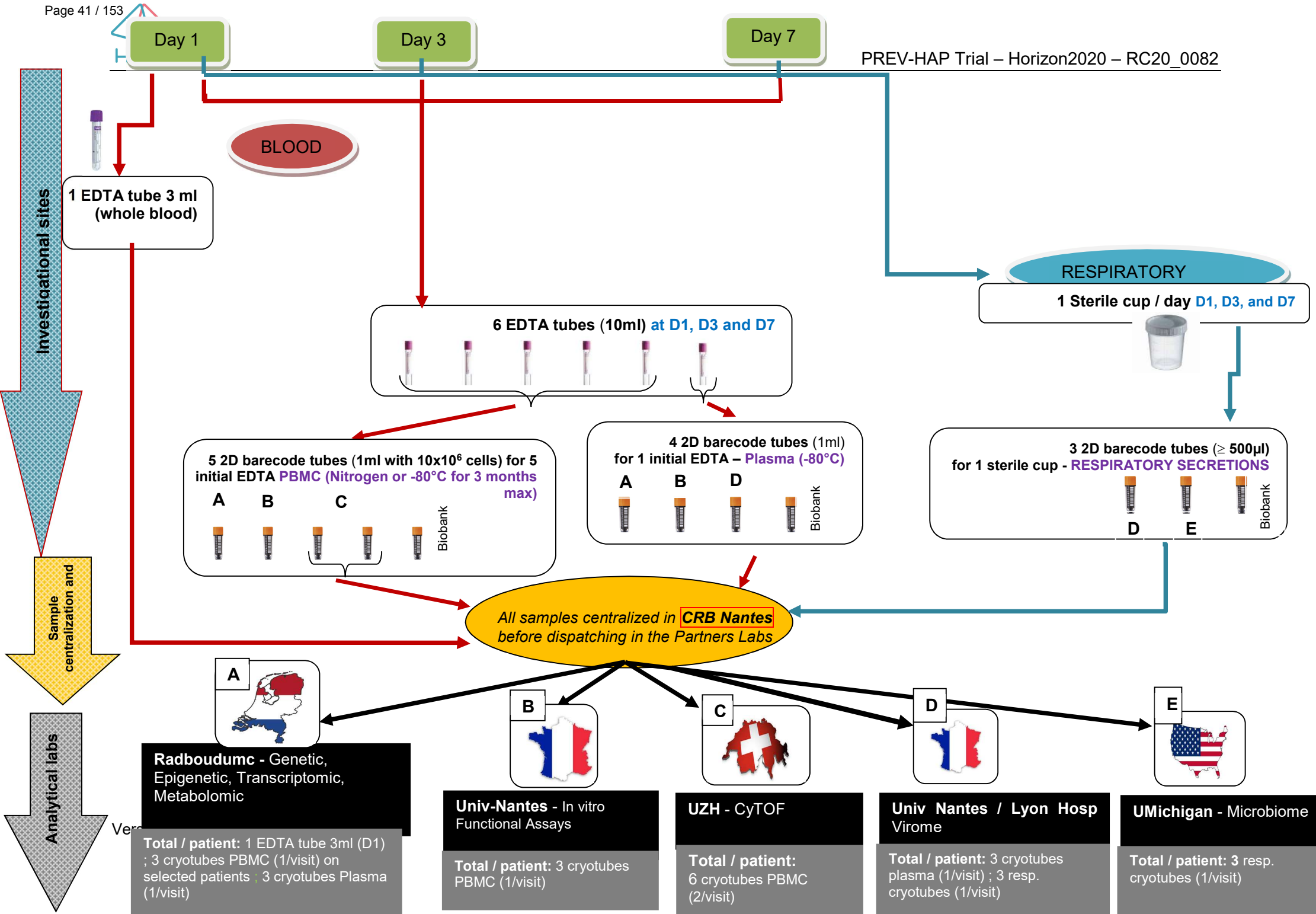
Samples will be stored in different boxes, depending on the nature of the samples.

A unique ID will be assigned to each aliquot. This ID will be reported on the label of each aliquot. At each visit, the samples will be recorded in the eCRF.

Each site will send every 3 months the prepared samples to the Biological Resources Centre of the Nantes University Hospital (CRB Nantes), whose follows the OCDE guidelines for biobanks and which is certified according to ISO 9001:2015 and to the French Afnor quality standard NF S96-900.

These samples will be transported in the presence of dry ice in order not to thaw the samples by a carrier authorized for transport classified UN3373. The list of sent samples will be exported from the eCRF.

At each reception, the CRB will carry out an inventory of each sample and check its labelling. PBMC samples will be stored in liquid nitrogen tanks (-196°C) and other types of samples will be stored in freezers at -80°C. The CRB's storage tanks and freezers are monitored and under automatic surveillance 24 hours a day to ensure the safety of the samples.
Finally, the samples will be sent to the various analytical laboratories twice: at mid-term and at the end of the studies. The transports will be carried out in dry ice by a carrier authorized for transports classified UN3373.


Day 1
Day 3
Day 7
BLOOD
1 EDTA tube 3 ml (whole blood)
Investigational sites
6 EDTA tubes (10ml) at D1, D3 and D7
RESPIRATORY
1 Sterile cup / day D1, D3, and D7
5 2D barecode tubes (1ml with 10x10⁶ cells) for 5 initial EDTA PBMC (Nitrogen or -80°C for 3 months max)
A
B
C
Biobank
4 2D barecode tubes (1ml) for 1 initial EDTA – Plasma (-80°C)
A
B
D
Biobank
3 2D barecode tubes (≥ 500µl) for 1 sterile cup - RESPIRATORY SECRETIONS
D
E
Biobank
Sample centralization and
All samples centralized in CRB Nantes before dispatching in the Partners Labs
Analytical labs
A
Radboudumc - Genetic, Epigenetic, Transcriptomic, Metabolomic
Total / patient: 1 EDTA tube 3ml (D1) ; 3 cryotubes PBMC (1/visit) on selected patients ; 3 cryotubes Plasma (1/visit)
B
Univ-Nantes - In vitro Functional Assays
Total / patient: 3 cryotubes PBMC (1/visit)
C
UZH - CyTOF
Total / patient: 6 cryotubes PBMC (2/visit)
D
Univ Nantes / Lyon Hosp Virome
Total / patient: 3 cryotubes plasma (1/visit) ; 3 resp. cryotubes (1/visit)
E
UMichigan - Microbiome
Total / patient: 3 resp. cryotubes (1/visit)

Samples collection details per patient

	Blood			Respiratory secretions
Inclusion – Day 1	✖		✖	✖
V2 – Day 3	✖			✖
V4 – Day 7	✖			✖
Samples collected:				
	EDTA tube 10ml		EDTA tube 3 ml	Sterile cup
Collected per visit	**6**		**1**	**1**
To prepare :				
Aliquots : 2D barcode microtubes	**4 x** 1ml  (1ml tubes)	**5 x** 1ml with $10x10^6$  (2ml tubes)	No aliquoting	**3 x <** 500µl  (0.5ml tubes)
Aliquot type	Plasma	PBMC	Whole blood	Respiratory secretions (transparent)
Sample requirements :				
Equipment & reagents needed for sample preparation	Centrifugation (1000xg, 10 min at RT) and aliquoting of plasma	Equipment: • safety cabinet II (sterile conditions) • centrifuge 1200xg • microscope & Malassez slide or equivalent Reagents: • Sterile Centrifuge Tube Falcon (50ml & 15 ml) • UNISEP tubes (provided by the Sponsor); • PBS 1x • hemolytic buffer • 4% Human albumin • DMSO	Freezing for DNA extraction	Aliquoting upon sterile conditions
Storage	-80°	Liquid nitrogen Or: -80°C for up to **3 months max**: →*Samples to be sent every 3 months to Nantes BRC for storage in liquid nitrogen*	-80°C	-80°C
Red flags	N/A	SOP provided by Sponsor - Personnel has to be trained	N/A	No centrifugation & and no additive

5.6. Identification of all data sources not included in the medical record

All the data sources will be included in the medical record of the patient including the quality of life questionnaires (EQ-5D-3L, Quality of life (SF-36), anxiety/depression (HADS), subjective well-being (SWLS)) at D28 and D90 and the patient's notebook, which will be then reported in the CRF.

5.7. Rules for discontinuing subject participation

5.7.1. Criteria in respect of early withdrawal of a subject from the study

Withdrawals from the study can only be effective after confirmation by the investigator and the sponsor. These withdrawals are always definitive.

These criteria should be clearly defined and validated by the study methodology expert.

In case of early withdrawal from the study, patients will be followed up until hospital discharge, according to routine clinical practice in each participating center.

Patients will be withdrawn **from the study** if:

- the patient, or his/her legal representative, withdraws consent,
- in case of legal criterion of non-inclusion not known at the time of inclusion due to the emergency inclusion procedure (patient under guardianship, curatorship, etc.)

Clinical data obtained before the consent withdrawal will be kept for the analyses. According to analysis populations, the patient will be excluded from the analyses or data will be imputed for the primary endpoint. These patients will not be replaced. In case of withdrawal after a SAR, patients will be followed up to the resolution or to the consolidation of this event.

A patient will be withdrawn from the **study treatment** for any of the following reasons (but are not limited to):

- Death
- Patient's request to withdraw his/her consent
- Patient becomes pregnant during the study period
- Liver Cytolysis (AST and/or ALT > 5N)
- Lipase > 5N
- Investigator's request to consider a change of therapy would be in the best interest of the patient
- Early termination of the study by the sponsor or a competent authority (safety reasons)

Patients should however remain in the trial for the purposes of follow-up and data analysis (with exception of patients who withdrew their consent).

5.7.2. Procedures in respect of early withdrawal of a subject from the study

For the data processing procedures in respect of subjects withdrawn early from the study, refer to the statistical section.

These patients will not be replaced.

In case of early withdrawal from the study treatment, patients should however remain in the trial for the purposes of follow-up and data analysis (with exception of patients who withdrew their consent). In case of withdrawal after a SAR, patients will be followed up to the resolution or to the consolidation of this event

In case of early withdrawal from the study, Clinical data obtained before the consent withdrawal will be kept for the analyses.

5.7.3. Criteria in respect of discontinuation of all or part of the study (excluding biostatistical considerations)

The end of the study is considered as the date of collection of all biomarker analyses.

This clinical trial will be followed by an independent Data and Safety Monitoring Board (DSMB); by a Steering Committee, as well as the entire consortium members of the HAP^2 project, in order to provide recommendations to the Sponsor regarding the safety of subjects, the conduct of the study and potential premature termination of the study.

An early, definitive or temporary discontinuation from part or all of the study can be done by Competent Authorities, Ethics committees, Sponsor or Data Safety Monitoring Board (DSMB)

In case of early discontinuation of the study on Sponsor's decision or DSMB, Ref-NCA, National Competent Authorities and Ethics Committees will be informed less than 15 days by mail.

In any case:

- A written confirmation of this early discontinuation of the study will be sent to Coordinator of this study and to each Principal Investigator of each center.
- All the patients included in the study will be informed and should realize the premature withdrawal visit.

The same applies to any investigator wanting to discontinue his/her participation to the study. The investigator must immediately inform the Sponsor in writing of this decision.

5.8. Patient medical care at the end of the study

No medical care related to the study will be continued after the end of the study.

The investigator will propose the best medical care to the patient, depending on his or her state of health at the end of the study

6. DATA MANAGEMENT AND STATISTICS

6.1. Data entry and data collection

6.1.1. Data entry, processing and circulation

Data collection for each person participating in the trial will be done with an electronic case report form (eCRF), created by the sponsor's data-management team, using a specialist software solution specifically designed for holding, auditing and checking trial data (Ennov Clinical Software).

Each person responsible for the filling of the eCRF (investigator, CRA...):

- will have to be identified in the table of delegations of responsibilities of each center (see investigator's file).
- Will have a "user" account with specific computer rights linked to his role (right to enter or modify a data, right to lock, monitor or sign a page of eCRF...)

Entering, viewing or modifying data will only be possible via the eCRF pages, on https://nantes-lrsy.hugo-online.fr/CSonline.

The data will be stored directly from the eCRF into the database hosted on a dedicated server, with controlled access (account/password) according to the user role. Any addition, modification or deletion of data will be recorded in a non-editable electronic file (the audit trail).

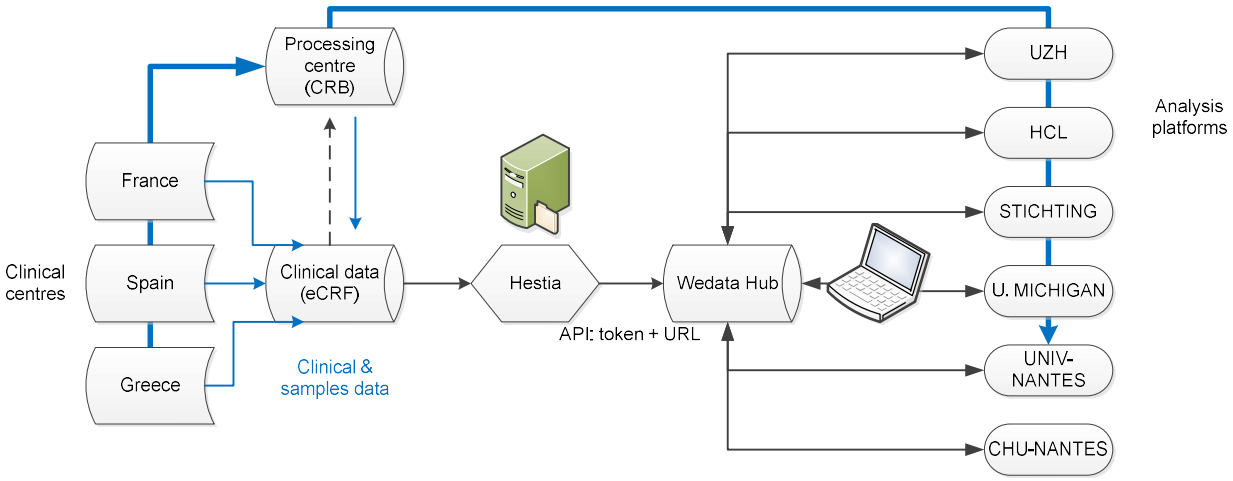
As for the health-economic analyse, extractions from the French hospitals database from the various participating centres will be sent by the investigation teams to the sponsor in a secure manner and stored on a secure server at the Nantes University Hospital accessible to the people responsible for the analysis.

6.1.2. Patient identification

The principal investigator and all co-investigators undertake to keep the identities of the persons who participate in the study confidential by assigning them a code (pseudonymisation). This code will be used for all the eCRF and all the attached documents (reports of imaging exams, biology, etc.). It will be the only information which will make it possible to make the connection with the patient retrospectively. The coding rule is the following: **month and year of birth, Inclusion number.**

6.1.3. Data Flow

The coded clinical data from the eCRF will be encrypted and automatically transferred to a different server of CHU Nantes, where it will be combined with the phenotypical, immunological, biomarker and multiomic's data generated in the context of HAP^2 WP3 for further analysis. A secure access to this second server will be created for the consortium's partner WeData (http://wedata.science/) for analysis, via a specific URL and token encrypted.



6.2. Statistics

6.2.1. Description of planned statistical methods, including planned intermediate analysis schedule

All analyses will be performed with the use of SAS software (version 9.4, NC, USA) before the breaking of the randomization code, according to International Conference on Harmonization-Good Clinical Practice guidelines.

Continuous variables will be presented as mean and standard deviations (as median and quartiles, otherwise). Categorical data will be presented as frequency and percentages.

All statistical analyses will take into account stratified randomization (cause of hospitalization (sepsis or not) and country (France, Greece, Spain)) as recommended in the CONSORT 2010 statement[20].

PRIMARY ANALYSIS

We will assess the efficiency of rHu-IFNγ for the prevention of hospital-acquired pneumonia with a Cox regression model (primary composite outcome: all-cause mortality at day 28 or the occurrence of hospital-acquired pneumonia within 28 days after randomization). Such an analysis combining the primary (occurrence of hospital-acquired pneumonia) and competing event (all-cause mortality) into a composite event has been recommended[21].

Crude and adjusted estimations on stratification factors will be given. The primary analysis will be adjusted on the stratification criteria and on center as a random effect.

SECONDARY ANALYSIS

To explore the risk of HAP in sub-populations (primary outcome), interaction terms between treatment arm and the following covariates will be tested in the Cox regression models (primary adjusted outcome):

[20] *CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials* BMJ *2010;340:c332*
[21] *Troendle JF et al.Stat Med.2018.*

- Randomization strata: Cause of hospitalization (sepsis or others), and country (France, Greece, Spain)
- Age (< or > 65 years)
- Severity upon ICU admission (Apache II 15-30, 30-45 or > 45)
- Time between the ICU admission and the first treatment injection (<24 hours; 24 to 36 hours, and 36-48 hours).
- Administration of glucocorticoid in ICU prior to the inclusion (yes/no)
- Analysis of the effect of treatment according to the COVID+ or COVID- status of the patients included in the study

All-cause mortality at day 90 will be analyzed with a Cox regression model, adjusted for stratification factors and on center as a random effect.

Categorical data (ventilator-associated tracheobronchitis at day 28, acute respiratory distress syndrome at day 28) will be analyzed with logistic regression model, adjusted for stratification factors and on center as a random effect.

Censored data (duration of antimicrobial therapy at day 28, duration of mechanical ventilation at day 90, duration of ICU hospitalization at day 90, duration of hospitalization at day 90) will be analyzed using Fine and Gray competing risks models to take into account the informative censoring and the competing risk due to death. The cumulative incidence functions (CIFs) of each competing event (death/end of antimicrobial therapy or death/extubation or death/end of hospitalization) will be estimated.

“Free-days” outcomes (antibiotic free days at day 28, mechanical ventilation free days at day 90, hospital free days at day 90) will be analyzed with a Mann-Whitney U.

Tolerance outcomes will be analyzed using logistic regression models.

PATIENT-REPORTED OUTCOMES DATA (SUITABILITY AND ACCEPTABILITY OF RHU-IFNγ)

Change in patient-reported outcomes data (quality of life, anxiety, depression) will be analyzed using longitudinal Rasch Measurement Theory (RMT)[22] models from the family of generalized random effects models. The RespOnse Shift ALgorithm at Item-level (ROSALI)[23] based on these models which has been showed to have good performance in a recently published simulation study[24] will be used. ROSALI will be developed and validated in WP5 using simulation studies to enable the use of RMT models as latent regression models to include covariates such as treatment, gender, and country. The development of ROSALI will allow investigating covariates’ effects on PRO (e.g. health-related quality of life) changes over time as well as on patients’ adaptation through response shift analyses (see WP5).

Patients' adaptation to their condition will also be investigated using regression analyses to test for the possibility of changes in the relationship between the patients' subjective well-being and their health-related quality of life[25]. Investigating this form of adaptation is important to assess the validity of one of the assumptions of the QALY (Quality-Adjusted Life-Years) measure that is commonly used to represent the effectiveness part of cost-effectiveness analyses (see the part describing the cost-effectiveness analysis). Various models will be estimated and compared to take into account factors such as unobserved individual heterogeneity for instance.

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22 Fischer GH, Molenaar IW, eds. Rasch Models: Foundations, Recent Developments, and Applications. New York: Springer-Verlag; 1995

23Guilleux A et al.. Qual Life Res.

[24] *Comparison of structural equation modelling, item response theory and Rasch measurement theory-based methods for response shift detection at item level: A simulation study.Blanchin M, Guilleux A, Hardouin JB, Sébille V.Stat Methods Med Res. 2019 Oct 30:962280219884574. doi: 10.1177/0962280219884574.*

[25] *Tessier P, Blanchin M, Sébille V. Does the relationship between health-related quality of life and subjective well-being change over time? An exploratory study among breast cancer patients. Soc Sci Med. 2017 Feb;174:96-103. doi: 10.1016/j.socscimed.2016.12.021.*

Semi-structured interviews will be conducted with patients and caregivers to gain more insight into the understanding and the interpretation of quantitative data [26]. An interview guide will be developed on the basis of the literature on the psychological consequences of the immune intervention for patients. Interviews will be audiotaped and transcribed verbatim by the interviewer with the patients' consent. Qualitative data will be analyzed using a lexical analysis to describe what patients have told, together with a content analysis (thematic categorial classification, Bardin, 2003 ; Blanchet &Gotman, 2007) to highlight the themes of the corpus and interpret data[27]. *See 2.3. Objective and endpoints for ancillary studies.*

6.2.2. Statistical justification of the number of inclusions

We will include 200 patients (100 patients receiving placebo, 100 patients receiving rHu-IFNγ). The rate of non survivors and/or hospital-acquired pneumonia in the placebo group is expected to reach 35%[28]. In this phase II clinical trial, the size of the effects with the studied treatment can not be estimated from current knowledge about the effect of these therapeutic strategies. We thus decided to rely on the recruitment capacity of the European centers allowing the inclusion of 100 patients / group over 24 months. This sample size will allow detecting a hazard ratio of 0.625 as compared to placebo with a 90% of statistical power and a double-sided type I error α at 5%.

6.2.3. Expected level of statistical significance

A two-sided P value of less than 0.05 will be considered for all analyses.

6.2.4. Statistical criteria for discontinuation of study

No interim analysis is planned for the efficiency.

6.2.5. Consideration method for missing, unused or invalid data

Lost to follow-up and missing data

There should be neither missing data nor lost to follow-up for the primary outcome which will be recorded in intensive care unit. Missing data will be described by treatment arm. According to the rate of missing data (over 5%) sensitivity analyses will be performed using multiple imputation methods.

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26 *Paillé, P., & Muchielli, A. (2005). L'analyse qualitative en sciences humaines et sociales. Paris: Armand Colin.*

27 *Blanchet, A., &Gotman, A. (2007). L'enquête et ses méthodes: L'entretien. Paris: Armand Colin, et Bardin, L. (2003). L'analyse de contenu. Paris: PUF.*

28Koulenti et al. Crit Care Med 2009 ; Asehnoune et al. Intensive Care Med 2017, Alvarez-Lerma et al. Crit Care Med 2018, and unpublished data from the Pneumocare study (1800 patients in 34 ICUs in France, 2018, numberclinical trial: NCT03348579)

Early withdrawals,
Withdrawals from the study can only be effective after confirmation by the investigator and the sponsor. These withdrawals are always definitive. In case of withdrawal from the study, patients will be followed up until hospital discharge, according to routine clinical practice in each participating center. Clinical data obtained before the consent withdrawal will be kept for the analyses. According to analysis populations, the patient will be excluded from the analyses or data will be imputed for the primary endpoint. These patients will not be replaced.
In case of withdrawal after a SAR, patients will be followed up to the resolution or to the consolidation of this event.

Non-compliance with the protocol
In case of non-compliance to the treatment regimen and/or to the collection of biological samples, the patients will be followed up to the end of the study, and the data will be kept for the analyze in intention to treat.

6.2.6. Management of changes made to the initial analytical strategy

An early, definitive or temporary discontinuation from part or all of the study can be done by Competent Authorities, Ethics committees, Sponsor or Data Safety Monitoring Board (DSMB)
In case of early discontinuation of the study on Sponsor's decision or DSMB, Ref-NCA, National Competent Authorities and Ethics Committees will be informed less than 15 days by mail.
In any case:

- A written confirmation of this early discontinuation of the study will be sent to Coordinator of this study and to each Principal Investigator of each center.
- All the patients included in the study will be informed and should realize the premature withdrawal visit.

The same applies to any investigator wanting to discontinue his/her participation to the study. The investigator must immediately inform the Sponsor in writing of this decision.

6.2.7. Choice of subjects to be included in analysis

Analyzes will be conducted, first, on data from the intention-to-treat (ITT) population, second, in the modified intention-to-treat (mITT) population as well as in the per-protocol population (PP).

- **Intention-to treat (ITT):** All randomized patients in the group in which they were randomised, regardless of the medical device/treatment received and breaches of the protocol. In case of missing data, the analysis of the ITT population will be performed by multiple imputation methods using demographic data (age, gender), stratification factors, IGS-II and cause of admission.
- **Modified intention to treat (mITT):** Randomized patients who have an assessable clinical outcome within the assessment window, fulfilling the major inclusion criteria, without major non-inclusion criteria, without consent withdrawal and who received at least one dose of treatmentare analyzed in the group in which they were randomised, regardless of the medical device/treatment received and other breaches of the protocol.

- **Per protocol (PP):** Randomized subjects who were treated in full compliance with the protocol (exclusion of the patients of the rHu-IFNγ group who have not received the complete drug regimen)

6.2.8. Economic evaluation

The cost-effectiveness analysis will be conducted from the perspective of the society with a three-month time horizon.

Assessment of costs

For all patients in the study the use of resources at the hospital and outside will be collected prospectively. Two modes of data retrieval will be combined: i) clinical research associates will record the consumption of resources in the hospital in combination with a database extraction of hospital information (outpatient consultations and procedures, hospitalizations) and ii) we will distribute diaries to patients to collect information about resources consumption after the initial hospitalization.

Patient pathways after discharge from initial hospitalization are defined as either follow-up within an after-care and rehabilitation structure, a return home, or a move to another care unit within the same hospital or one closer to the patient's home, cf. Figure 1.

We will retrieve within the hospital only resources related to pneumonia, for data retrieved outside the hospital we will be unable to differentiate what is due to pneumonia from other care so we will ask patients to record all care resources used over the period up to day 90. This will include, most notably, ICU length of stay, pharmaceuticals and consultations. We will also collect information about the time from caregivers (whether professional or informal) and about day out of work to value production losses.

To estimate costs, unit costs per each type of resource consumed will be estimated using accounting information, NHI official tariffs, and the hospitals' prices charged in different countries (France, Spain and Greece). The time of caregivers devoted to monitoring and assisting patients will be valued by applying a salary valorization equivalent to a home help job (proxy good method), and, we will calculate productivity losses using work stoppages.

Concerning the French patients, in case the return of patients' diaries on resource use would be deemed insufficient (less than 65% of the total) to carry out our study, we would use the database of the French Health Insurance in order to retrieve the consumption of ambulatory and hospital healthcare.

Resource use and resource unit costs will thus be collected and estimated for each country.

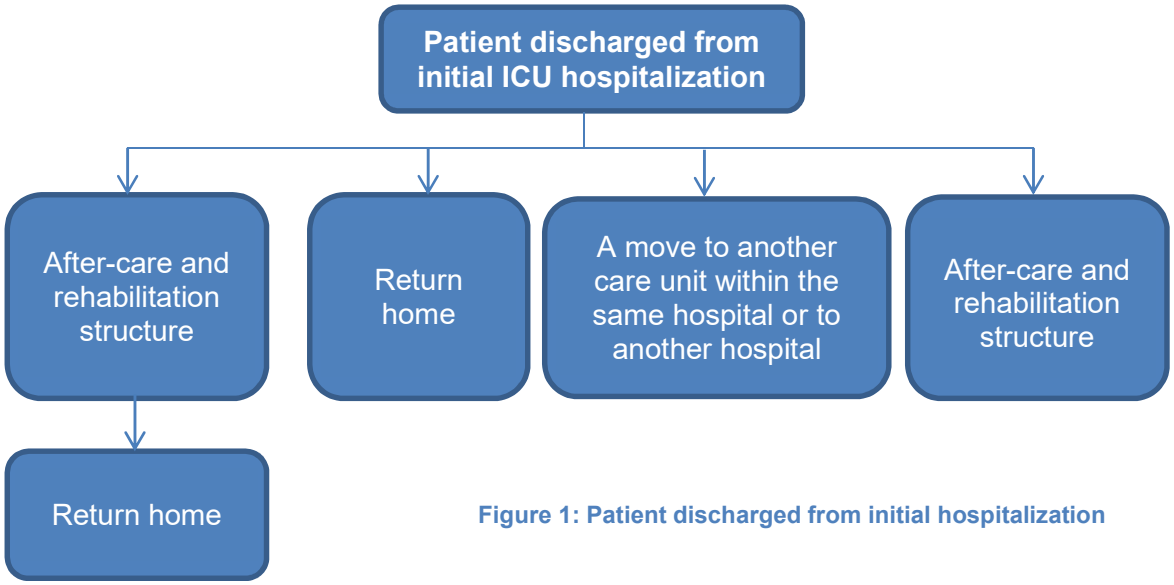


Figure 1: Patient discharged from initial hospitalization

Measures of effectiveness

The measure of effectiveness for the economic evaluation will be the number of QALYs. QALYs represent a measure of survival (life-years) weighted by health-related quality of life factors such that a weight of 0 represents death and a weight of 1 represents the best imaginable health state. An advantage of QALYs is that they allow to combine information about the length and the quality of life in a single index measure. In the study, QALYs will be estimated from answers to the EuroQol EQ-5D-3L quality of life questionnaire at the baseline, at day 28 and at three months after inclusion. Given that all patients will be unable to answer to a questionnaire at the baseline, we will assume an equal level of quality of life for all. This level will be determined by a group of expert physicians. If the patient is unable to complete the questionnaire at day 28 and at day 90, we will use the proxy version of the EQ-5D that will be completed by a physician.

To allow for the comparison between countries, we will use the European harmonized tariffs[29] to convert the EQ-5D answers into utility scores as was recently done in a multinational European cost-effectiveness analysis[30].

Results analysis

The cost-effectiveness analysis will be conducted on an intention to treat basis. Missing data about costs and QALYs will be imputed using multiple imputation methods.

Mean costs per type of resource used, mean total costs and mean QALYs per patient and their corresponding standard deviations will be presented. Differences in costs, in QALYs and the ICER will be estimated as well as the corresponding acceptability curve, i.e. the curve indicating the probability for an intervention to be cost-effective given the society's willingness to pay an additional unit of effectiveness (i.e. an additional QALY gained). We will also perform sensitivity analyses to assess the robustness of the results to the main assumptions of the analysis such as

[29] *Eur J Health Econ. 2003 Sep;4(3):222-31. A single European currency for EQ-5D health states. Results from a six-country study. Greiner W(1), Weijnen T,Nieuwenhuizen M, Oppe S, Badia X, Busschbach J, Buxton M, Dolan P, Kind P, Krabbe P, Ohinmaa A, Parkin D, Roset M, Sintonen H, Tsuchiya A, de Charro F*

[30] *J Antimicrob Chemother. 2018 Nov 1;73(11):3189-3198. doi: 10.1093/jac/dky309.*
Cost-effectiveness of internet-based training for primary care clinicians on antibiotic prescribing for acute respiratory tract infections in Europe.
Oppong R1, Smith RD2, Little P3, Verheij T4, Butler CC5, Goossens H6, Coenen S6,7, Jowett S1, Roberts TE1, Achana F8, Stuart B3, Coast J9

the methods to manage missing data, the methods to value units costs or the inclusion/exclusion of production losses for instance.

We will perform overall and country-specific cost-effectiveness analyses: incremental cost-effectiveness ratios (ICER) will be estimated for the whole sample (pooling together the data from the participating countries) and per country. Given the variety of approaches that may be followed to determine the cost-effectiveness ratios in multinational trials[31] [32], we will explore various approaches in sensibility analysis to assess the robustness of the results.

6.2.9. Randomisation

This is a **centralized randomization** performed directly on the eCRF with stratification on the cause of hospitalization (sepsis or not) and country.

The eCRF will be developed by CHU-Nantes, using a specialist software solution specifically designed for holding, auditing and checking trial data (Ennov Clinical Software - https://nantes-lrsy.hugo-online.fr/CSonline).

After the informed consent of patient's legal representative will be obtained, enrolled patients will be randomized by local investigators using this dedicated, password-protected, SSL-encrypted website to allow immediate and concealed allocation. Each patient will be given a unique patient-number and a randomization number. Randomization is performed in the first 48 hours following the admission in the intensive care unit.

[31] *Health Econ. 1998 Sep;7(6):481-93. Estimating country-specific cost-effectiveness from multinational clinical trials. Willke RJ1, Glick HA, Polsky D, Schulman K.*

[32] *Health Econ. 1998 Sep;7(6):481-93. Estimating country-specific cost-effectiveness from multinational clinical trials. Willke RJ1, Glick HA, Polsky D, Schulman K.*

7. PHARMACOVIGILANCE AND ADVERSE EVENT MANAGEMENT

7.1. Definitions

Pharmacovigilance	Science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem.
Adverse events (AE)	Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment An adverse event can therefore be any unfavourable and unintended sign (e.g. an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
Adverse reactions (AR)	A response to a medicinal product which is noxious and unintended. Response in this context means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility. An adverse reaction, in contrast to an adverse event, is characterised by the fact that a causal relationship between a medicinal product and an occurrence is suspected
Adverse reaction of an experimental medicinal product – Adverse Drug Reaction (ADR)	All untoward and unintended responses to an investigational medicinal product related to any dose administered.
Adverse reaction/event Intensity	Rated according to the CTCAE v.5.0 (excerpts in appendix XX) Any event not rated in the selected classification should be rated as follows: Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*. Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**. Grade 4 Life-threatening consequences; urgent intervention indicated. Grade 5 Death related to AE. *Activities of Daily Living (ADL)* **Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.* ***Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.*

Serious adverse events (SAE)	Any untoward medical occurrence or effect that: - results in death, - is life-threatening, - results in persistent or significant disability or incapacity, - requires hospitalisation or prolongation of existing hospitalisation, - is a congenital anomaly or birth defect. - is medically significant)
Unexpected adverse reactions	An adverse reaction, the nature or severity of which is not consistent with the applicable product information.
Suspected Unexpected Serious Adverse Reactions (SUSAR)	An untoward and unintended response to an investigational medicinal product, which is not listed is the applicable product information, and meets one of the serious criteria.
Emerging safety issue	Any new safety issue considered by the sponsor to require urgent attention by the competent authorities because of the potential major impact on the risk-benefit balance of the medicinal product and/or on patients' or public health and the potential need for prompt regulatory action and communication to patients and healthcare professionals.: ➢ Induce new evaluation of benefit/ risk ratio of the study or of the product object of the study, ➢ modify product utilization, the conduct of the study or documents related to the study ➢ Suspend or terminate the protocol under research or similar researches.
Causality	The Investigator must determine the relationship between the administration of IMP and the occurrence of an AE/SAE as Not Suspected or Suspected as defined below: *Not suspected:* A causal relationship of the adverse event to IMP administration is unlikely or remote, or other medications, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event. *Suspected:* There is a reasonable possibility that the administration of IMP caused the adverse event. 'Reasonable possibility' means there is evidence to suggest a causal relationship between the IMP and the adverse event. **Causality should be assessed and provided for every AE/SAE based on currently available information. Causality is to be reassessed and provided as additional information becomes available.**
Abuse	This corresponds to the persistent or sporadic, intentional excessive use of a medicinal product, which is accompanied by harmful physical or psychological effects.
Overdose	This refers to the administration of a quantity of a medicinal product given per administration or cumulatively, which is above the maximum recommended dose according to the authorized product information. Clinical judgement should always be applied.

	(Real overdose: due to a brut excessive amount / relative overdose: due to patient predisposal factors as renal insufficiency, hypo-albuminuria…)
Misuse	This refers to situations where the medicinal product is intentionally and inappropriately used not in accordance with the authorised product information.
Quality defect	Non conformity to the specifications described in the marketing authorisation file / CE marking / technical documentation or deviation against good manufacturing practices / good distribution/storage/labelling practices.
Medication Error	Medication errors are unintended failure (proved or potential) during the drug treatment process(from manufacturing to administration) that leads to, or has the potential to lead to, harm to the patient (risk or an adverse event for the patient).The risk of error or potential error concerns situations where the error did not happen, was intercepted but could have happen.

7.2. Safety evaluation parameters

7.2.1. Specific safety-related evaluation criteria

According to regulation, each AE/AR reported by the patient or identified by the investigator must be collected and reported to sponsor, as soon as he is aware, if it meets to seriousness criteria from inclusion of the subject, to the end of the participation. In addition, liver blood test will be carried out during the treatment period followed.

7.2.2. Methods and schedule envisaged to measure, compile and analyse safety evaluation parameters

Any AR/AE whether expected or unexpected, serious or not, must be real-time collected in the study eCRF.
Liver blood test will be followed at day 3, day 7 and day 15, and lipase test at day 7 and Day 15.

7.3. Expected ARs

In this protocol, the expected Adverse Event and Reactions are associated with the drug under study (IMUKIN®) and its comparator (IMUKIN® Placebo), concomitant drugs (antibiotics, painkillers, bronchodilatators, hypnotics…), protocol and disease.

The IMUKIN® Reference Safety Information, containing the exhaustive expected is provided by the sponsor.,
For the IMUKIN® placebo, the Reference Safety Information is in the current version of NaCl SmPC (section 4.8). Even if the administration route is different in PREV-HAP study than in the MA indication, this is a common practice to inject NaCl subcutaneously, with much larger volumes in geriatrics and palliative care. There is no particular risk due to the very limited volume, the

infectious risk of any subcutaneous injection being managed by classic non-specific barrier measures

Expected ARs for IMP are:

IMUKIN®(recombinant interferon gamma-1b – rHu-IFNγ): No SAR is expected and all serious adverse effects are considered as SUSARs.

As pancreatitis (including fatal outcome) has also been reported as adverse effect (frequency not known), the sponsor will pay particular attention to lipase monitoring.

IMUKIN® placebo:

Regarding the composition, the secured process of preparation and the route of administration, only local AEs with pain, erythema, irritation are expected for the placebo ; the amount of NaCl does not suggest systemic hydro electrolytic or blood pressure adverse effects, nor infection.

The expected ARs for concomitant drugs are not different of those observed in standard care and are listed in current version of each product's SmPC (section 4.8).

Concerning the protocol, conventional medical examination/evaluation in the overall care of patients, the excess of risk identified protocol dependent should be un-frequent.
Indeed, current procedures will induce the most frequent expected disorders: all patients receiving mechanical ventilation and suspected of hospital-acquired pneumonia have an intravenous device for the realization of intravenous injections during several days, and daily biological monitoring is a standard of cares.

- Local complications of subcutaneous administration, inflammation, infections.
- Local complications of blood sampling (hematoma, moderate pain)
- Complication of ventilation and intensive care unit standard care (as MD for ventilation, urinary catheter…)
- Complication of prolonged hospitalization in ICU (nervous and musculoskeletal disorders…)

Concerning the disease, the most frequent expected AEs in patients hospitalized in ICU and requiring mechanical ventilations are (non exhaustive list):

- Death
- Hospital acquired infections (pneumonia, septicaemia, urinary tract infections, surgical site infection)
- Organ failure (Respiratory distress syndrome, Acute Kidney Injury, Liver insufficiency, Hemodynamic shock)
- Haemorrhage
- Gastric ulcer
- Venous Thrombosis, pulmonary embolism
- Stroke
- Neuromyopathy
- Bed sores
- Prolonged mechanical ventilation

7.4. Adverse Events of Special Interest

Regarding the specificity of the study, these Adverse Events/Reactions should be considered and reported as SAEs:

- all acute respiratory distress syndrome (ARDS) developed after the first IMP administration related or not to an IMP
- seizure related to Interferon-γ/NaCL

7.5. Adverse event management

7.5.1. AR/AEcollection

Any AR/AE (unless specify otherwise below), whether expected or unexpected, serious or not, must be real-time collected in the study eCRF.

7.5.2. SAR/SAE reporting

All SARs/SAEs initial and follow-up information (except those specified below), whether expected or unexpected, must be reported without delay, and at the latest within 24 hours to the sponsor from the day the investigator becoming aware of the event, using the eCRF (in case of unavailability, the SAE/SAR notification should be sent to the sponsor by e-mail to recherche-pv@chu-nantes.fr).

The investigator documents the event and the medical diagnosis as well as possible: the information on this SAE/SAR form and on the attached documents must be complete, precise, clear (no use of abbreviations...) and coded.

The Investigator will report the action taken with IMPs as a result of an AE or SAE, as applicable (e.g., discontinuation or reduction of IMP, as appropriate) and report if concomitant and/or additional treatments were given for the event.

The investigator must establish a causal link between the adverse events.

The occurrence of a new safety event should be reported to the sponsor.

Pregnancy:

If a woman becomes pregnant as part of the research or if her partner is participating in the research, the pregnancy must be notified immediately to the sponsor.
The investigator informs the sponsor using the Pregnancy Form.
The investigator should follow the patient until the term of pregnancy or its interruption and notify the sponsor of the outcome using the Pregnancy Follow-up Form.
In the case of paternal exposure, the investigator should obtain the consent of the parturient to collect the pregnancy information.

Special Situation:
Overdose, misuse, errors or potential errors, quality defects are also reported to the sponsor, even if there is no associated adverse event, using the Special Situation Report (SSR) form.

7.5.3. Exclusion from reporting/notification

Regarding the specificity of the study, some adverse events have not to be collected in safety section of the eCRF:
AEs related to the ICU management or related to the medical history (notably the normal course of cause of ICU admission) and which are those classically observed in this context will not be collected as part of this protocol, with the exception of those related to the medicinal products under study or its comparators and placebo, which will be properly collected and notified if necessary.

7.5.4. Reporting period

All SAE/SAR must be reported to the sponsor if it happens for a research participant:

- Since the consent signature date,
- During all the participant follow up period scheduled by the study up to the collection of the primary outcome (day 28)

After the end of the patient follow-up and without any time limit if the investigator becomes aware of a SADR possibly linked to one of IMP, including Placebo.

7.5.5. Data and Safety Monitoring Board (DSMB)

The sponsor is responsible for setting up a Data and Safety Monitoring Board (DSMB), and for providing all operating support. The DSMB is an independent advisory committee which will review the safety data and provide recommendations to the Sponsor regarding the safety of subjects, the conduct of the study and potential premature termination.
Its 5 members, well-versed in the field of clinical trials (pathology, safety, ethic and methodology), are not involved in the study. They are appointed for the period of the study and undertake to participate and to respect the data confidentiality. The members of the DSMB are selected collectively by the coordinator and the sponsor.
The DSMB will be the recipient of all suspected unexpected severe adverse reaction (SUSAR), the annual safety reports and may be consulted by the sponsor if a SUSAR or SADR involves a specific analytical problem or in the event of doubt on the risk benefit arising in the course of the study. Usually blinded data will be submitted, but in case of difficulty or of suspected unbalanced risk, unblinded data could be discussed and, if required, provided exclusively to the DSMB members by the statisticians.

A meeting will be planned at the beginning of the study, to present the protocol to the DSMB members, and to plan the next steps and meetings of the DSMB. There will be at least one annual meeting, and the frequency of other meetings will be determined during the first meeting

The list of members of the DSMB is attached in appendix 8.

The working group on Pneumonia of the **European Society of Intensive Care Medicine (ESICM)** and the “Comité Reanimation” from the **French Society of Anesthesiology and Reanimation (SFAR)** have reviewed the study protocol before the grant application, and they will receive regularly a summary of the safety reports, for informationyearly the safety reports and provide scientific advice.

7.5.6. Responsibilities of the sponsor

The sponsor is responsible for the ongoing assessment of the safety of the research, both in terms of the procedures performed and the treatments used.

In accordance with the applicable regulations, the sponsor will report any suspicion of SUSAR to the competent authorities within the regulatory timeframe (European and national's regulations).

The sponsor shall report relevant additional information regarding unexpected serious adverse reactions in a follow-up report to EMA and National Competent Authorities (ANSM, AEMPS, EOF).

7.6. Follow-up procedure and period for subjects following the onset of adverse events

7.6.1. Procedure to follow for the patient concerned by the SAE

All events/reaction must be followed up until recovery, consolidation or death (event closed).

Pregnancy occurring during the study should be followed up at least until birth or even until the child reaches adulthood.
Delayed adverse reactions must be reported to the sponsor (if known to the investigator) even after the end of the study.

8. ADMINISTRATIVE AND REGULATORY ASPECTS

8.1. Source data and document access rights

Each patient's medical data shall only be provided to the sponsor or any person duly authorised by the sponsor, and, where applicable, to authorised health authorities, in confidential conditions. The sponsor and the competent authorities may request direct access to medical records for the purposes of verification of the procedures and/or data in respect of the clinical trial, within the limits authorised by the legislation and regulations.
The data compiled during the trial may be processed electronically in compliance with GDPR requirements.

8.2. Trial monitoring

Monitoring will be carried out by the Sponsor in France, by the National Coordinating Center in Spain and a CRO in Greece. A Clinical Research Associate (CRA) shall visit each site (investigator and pharmacy) regularly to conduct quality control on the data reported in the case report forms. All the CRAs will work with the same procedures whatever the country. The Sponsor will provide the SOPs and the monitoring manual.

The protocol has been classified according to the estimated level of risk for the patient taking part in the study. It shall be monitored as follows:

Risk C: high foreseeable risk

The monitoring frequency and intensity is dependent on the risk associated with the study: 100% of data from 100% of the patients.
A monitoring plan, validated by the investigator, project manager and monitoring CRA defines the data to be monitored and the frequency of visits.

The on-site monitoring visits shall be organised after making arrangements with the investigator. The CRAs should be able to consult on each site:
- the enrolled patients' data compilation records,
- the patients' medical and nursing files,
- the investigator file.
- the treatment storage and dispensation place

The CRA will submit regular visit reports to the Sponsor’s Project Manager.

Each patient's medical data shall only be provided to the sponsor or any person duly authorised by the sponsor, and, where applicable, to authorised health authorities, in confidential conditions. The sponsor and the competent authorities may request direct access to medical records for the purposes of verification of the procedures and/or data in respect of the clinical trial, within the limits authorised by the legislation and regulations.
The data compiled during the trial may be processed electronically in compliance with data protection regulatory requirements of each country. Patient anonymity will be finally conserved.

8.3. Inspection / Audit

Within the scope of this study, an inspection or audit may be conducted. The sponsor and/or participating centres should be able to provide inspectors or auditors with access to the data.

8.4. Written informed consent/Emergency consent form collection

In the study's context, inclusions will be realized in immediate vital emergency situation. Indeed, the treatment of hospital-acquired pneumonia is an emergency and it is recommended to start the treatment within the first hour after the diagnosis (Torres et al. European Guidelines for hospital-acquired pneumonia. EurRespi J 2017). Given the inclusion criteria (critically ill patients suffering acute pneumonia, or at risk of pneumonia due to critical illness), patient won't be able to express their consent before the inclusion in the study. Thus, an emergency consent procedure is needed and justified.

Moreover, the exclusion of patients unable to provide informed consent before the inclusion of the HAP^2 trials would induce a major bias, jeopardizing the scientific quality of the project.

The procedure of acquisition of the consent of the legal representatives will be described as follows, and will comply the national regulations in force, after Ethics Committees approval:

In case of patients able to provide consent at the time of inclusion:

Patients will be informed in complete and faithful terms and in understandable language of the objectives and constraints of the study, the potential risks, the required observation and safety measures, and their right to refuse to participate in the study or to revoke their consent at any time. The investigator must also inform the subjects of the Ethics Committee opinion. All information appears in an information notice and consent form given to the patient. Written informed consent will be obtained by the investigator. These documents will be approved by the competent Ethics Committee. Two original copies will be co-signed by both the investigator and the patient. The second copy is to be kept in the patient's medical record.

In case of patients unable to provide consent at the time of inclusion:

1. **First, the authorisation will be given by the legal representative** (see description below 1.a**)** or failing that an emergency procedure will be applied, in countries where local regulations allow this procedure (see description below 1.b).

    a. The investigator agrees to provide the **legal representative** with clear and precise information about the protocol and request from him/her a written and signed consent form (information form and consent form appended). The **legal representative** will sign and date the consent form, after taking time to reflect on the matter. The investigator shall also sign and date the consent form.

    The investigator's original shall be placed in the investigator file. The consent form is signed in duplicate: the investigator keeps the original and gives the copy to the support person.

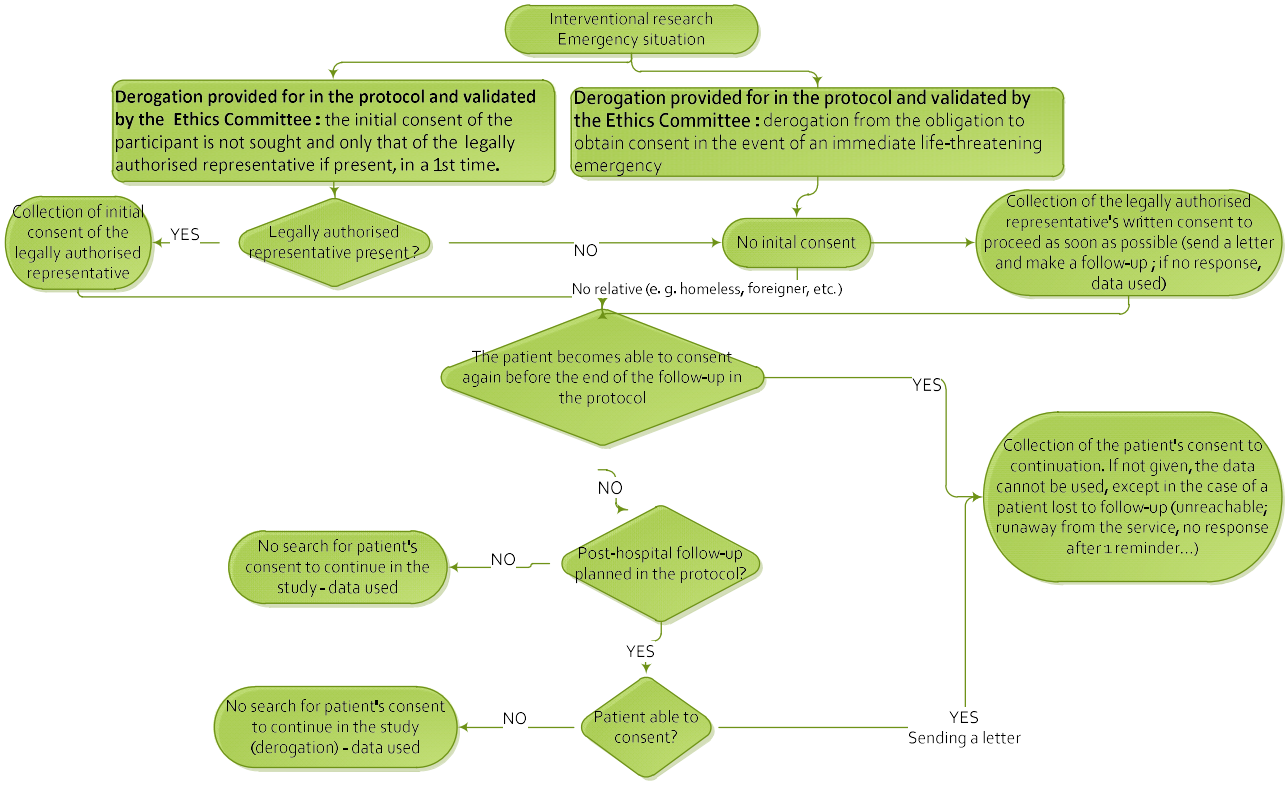
    b. Inclusions in the HAP^2 trials will be realized in situations of immediate vital emergency. Indeed, it is recommended to start the empiric antimicrobial therapy immediately after the collection of the respiratory fluids for patients with suspected hospital-acquired pneumonia (Torres et al. 2017). It will thus be possible to derogate to the consent collection obligation at the time of inclusion if the conditions for a fair information of the support person are not gathered. In this setting, the investigator will justify this procedure in the medical file of the patients,

and will seek the consent of the legal representative (if the patient is still unable to consent) to continue the study as soon as possible.

2. The **retrospective consent of the patient** will be collected by the investigator, or a physician who represents him. The participant will be informed as soon as deemed possible.

   Patient consent will be asked for the potential continuing of the research, and the utilization of his data, if he retrieves his ability to consent. The investigator agrees to provide the subject with clear and precise information about the protocol and request from him/her a written and signed consent form (information form and consent form appended) Patient will sign and date the consent form, after taking time to reflect on the matter. The investigator shall also sign and date the consent form. The investigator's original shall be placed in the investigator file. The consent form is signed in duplicate: the investigator keeps the original and gives the copy to the subject.

The emergency procedure is detailed below, and will be submitted to national regulatory approvals:



Patients can decide in the consent form if they want to be informed in case of incidental findings on samples analyzes or during the trials follow up.

In case of incidental findings, and if the patient has indicated that he/she wants to be informed, a specific consultation will be set up by the Medical Doctor in charge to propose adequate medical care, outside of the study.

8.5. Regulatory / ethics status

This clinical trial is conducted in 3 European countries and will comply with European Union clinical-trial and ethics legislation.

The sponsor is responsible for obtaining regulatory approvals of the clinical study before the initiation of the study:

- Competent Authorities: ANSM (Agence nationale de sécurité du médicament et des produits de santé) in France, AEMPS (Agencia Española de Medicamentos y Productos Sanitarios) in Spain and EOF in Greece ;
- National Ethics Committees ;
- Data protection regulation (GDPR + national regulations) ;

The sponsor will maintain close contact with regulatory authorities and independent ethics committees throughout the duration of the clinical trial and until the end of the study.

In any case, CHU Nantes, as sponsor, will ensure that all national and international regulatory and ethical requirements will have been met before initiating the clinical trial. The sponsor will rely on a coordinating structure in each country to independently submit to each NCA and EC, and on their expertise in the regulatory field specific to each country in terms of vigilance, data and personal protection, etc.

8.6. Amendments to the protocol

Requests for substantial modifications should be addressed by the sponsor for approval or notification to National competent authority and Ethics Committees concerned in compliance with the law and its implementing decrees.
The amended protocol should be a dated updated version.
The patient information and consent forms should be amended if required.

8.7. Study funding and insurance

This study is financed by the H2020 Programme of the European Union, under grant agreement number 847782.
The sponsor will take out an insurance policy covering the financial consequences of its civil liability in compliance with the regulations.

8.8. Publication rules

All trial sites including patients will be acknowledged, and all investigators at these sites will appear with their names under 'the HAP^2 investigators' in an Appendix to the final manuscript. The Steering Committee will grant authorship depending on personal involvement according to the Vancouver definitions.
If a trial site investigator is to gain authorship, the site has to include 10 patients or more. If the site includes 25 patients or more, two authorships will be granted.
The listing of authors will be as follows: A Roquilly (coordinator) will be responsible for the writing of the manuscript and the first author (and corresponding author), and the next authors (from the 2nd place in the list of authors) will be the other investigators according to the number of included patients per study site (for center with > 10 patients), and finally, D. Koulenti, A. Torres and K. Asehnoune will appear as the last three authors with equal participation to this work.

The study will be registered in clinicaltrials.gov database.

The sponsor will enter the study results in the European Union database as soon as the main publication from the research is released, in order to preserve intellectual property.

8.9. Outcome of biological samples

At the end of the study, biological samples resulting from sampling (blood and tracheal aspirates) shall be kept and the subject's written consent should be collected and the samples stored in one of Nantes University Hospital biocollections: biocollection section "IBIS - immunology " under the responsibility of Pr. Asehnoune. This biocollection and consent procedure have been registered under number DC-2012-1555 with the approval of CPP OUEST IV dated 30/06/2014. The patient's written consent (or legal representative consent, if applicable) will be collected by a specific consent form. **In case the patient has been included with Emergency consent form and no legal representative can be found, the biological samples will not be kept in this biocollection.**

8.10. Source data archiving

The investigator should archive all study data for at least 15 years after the end of the study.
At the end of the study, the investigator shall also receive a copy of the data for each patient in the investigator's centre sent by the sponsor.
Archiving procedure will be performed according to the relevant European / local regulations in place.

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APPENDIX 1: INVESTIGATOR LIST

Name	Department	Title	institution	Address	Telephone, fax and e-mail	Membership number
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Name	Area of medicine	Title	Name of institution	Name and address of affiliated department	Telephone, fax and e-mail	Membership number Order of Physicians
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Protocol PREV-HAP

Eudract: 2020-000620-18
Ref: RC20_0082

"Human recombinant interferon gamma-1b for the prevention of hospital-acquired pneumonia in critically ill patients: a double-blind, international, phase 2, randomized, placebo-controlled trial - the PREV-HAP study"

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SIGNATURE PAGE

SPONSOR SIGNATURE

The sponsor agrees to comply with the laws and regulations on clinical trials for the conduct of the above-mentioned study and agrees to abide by all provisions set forth therein.

Name and capacity of the signatory representative:	Date:	Signature:
For the Sponsor and by delegation of the Managing Director, the Director of Research and Innovation	21 JUIN 2021	La Directrice Adjointe Recherche Mme Anne ROYER-MOËS

INVESTIGATOR'S SIGNATURE

I have read all the pages of the clinical trial protocol sponsored by Nantes University Hospital. I confirm that this protocol contains all the information necessary for the conduct of the trial. I agree to conduct the trial according to the protocol and to abide by all provisions set forth therein. I agree to conduct the trial in compliance with:

- the principles of the "Declaration of Helsinki",
- International (ICH) and National good clinical practice regulations and guidelines
- European regulations and national laws and regulations relating to clinical trials,

I also agree for the investigators and other qualified members of my staff to have access to the copies of this protocol and documents concerning the conduct of the study so that they abide by all provisions set forth therein.

	Name:	Date:	Signature:
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Principal investigator	Name and institution:	Date:	Signature:

LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
AEMPS	Agencia Espanola de Medicamentos y Productos Sanitarios
ANSM	Agence Nationale de Sécurité du Médicament et des produits de santé
ALT	Alanine transaminase
AST	Aspartate transaminase
BAL	Bronchoalveolar lavage
CEA	Cost Effectiveness Analysis
CRA	Clinical Research Associate (monitor)
CTCL	Cutaneous T Cell Lymphoma
D#	Day number
DDD	Defined Daily Doses
DLT	Dose-Limiting Toxicity
DP	Drug Product
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
EQ-5D-3L	EuroQol 5 dimensions 3 Levels
ESICM	European Society of Intensive Care Medicine
FIH	First-in-Human
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HADS	Hospital Anxiety and Depression Scale
HAP	Hospital-Acquired Pneumonia
HLA-DR	Human Leukocyte Antigen
HRQoL	Health-Related Quality of Life
ICER	Incremental cost-effectiveness ratio
IMPD	Investigational Medicinal Product Dossier
MA	Marketing Authorisation
NCA	National Competent Authority
QALY	Quality-Adjusted Life Year
rHuIFNγ	recombinant human interferon gamma
ROSALI	RespOnse Shift ALgorithm at Item-level
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SC	Subcutaneous
SF-36	Short Form (36)
SFAR	Société Française d'Anesthésie Réanimation
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
SWLS	Satisfaction With Life Scale
VAP	Ventilator-Associated Pneumonia

CONTENTS

INTRODUCTION

Hospital-acquired Pneumonia (HAP) is an infectious disease of major concern in the world, and the most frequent cause of hospital-acquired infections with 500,000 episodes being treated every year in Europe. Despite the development of European recommendations, the incidence remains high, with dramatic medical consequences: existing therapies and preventive measures do not result in the expected favourable outcome (clinical cure and survival) for 30% of patients. HAP are moreover the main cause of antibiotic consumption in European hospitals and are increasingly induced by drug-resistant pathogens. New, alternative and more effective host-targeted strategies are therefore urgently needed to fight antibiotic resistance.

PREV-HAP study is part of a larger project entitled 'Host-targeted Approaches for the Prevention and the treatment of Hospital-Acquired Pneumonia' (HAP^2), funded by the European Union's H2020 research and innovation programme under grant agreement N°847782. HAP^2 aims to develop stratified host-directed drugs and biomarkers to enhance the prevention and the treatment of HAP and develop precision medicine in infectious diseases. Its ambition is to revolutionize the management of HAP: capitalising on the novel concept of critical-illness related immunosuppression altering the host-pathogens interactions, the aim is to propose a complete reappraisal of the physiopathology of HAP based on the concept of respiratory dysbiosis. "The HAP^2" project will reach two ground-breaking objectives in the field of bacterial infections: first the development of host-targeted approaches for the prevention and the treatment of a severe bacterial infection through the supplementation of the IFN-γ whose production is defective in patients at risk of pneumonia ; second the development of a clinico-biological score based on an integrative assessment of the host-pathogen interactions and genetic variation, to predict the course of HAP and the response to treatment. Our interdisciplinary consortium, bringing together 10 partners from academia and industry with expertise in clinical trials, immunology, microbiome analysis, omics and social sciences is uniquely placed to achieve this ambition within a 5-year project.

The main hypothesis of the PREV-HAP study is that **human recombinant Interferon gamma 1b (rHuIFN-γ, Imukin) treatment can restore immunity in critically ill patients and prevent Hospital-Acquired Pneumonia.**

We also hypothesize that the in vivo investigations of the host-pathogens interactions can be used for the stratification of patients into high/low risk and responders/non-responders to host-targeted prevention of hospital-acquired infections.

The involvement of a state of critical-illness related immunosuppression in the susceptibility to hospital-acquired pneumonia is widely accepted, and an emerging trend is that the development of drugs for the treatment of this acquired immunosuppression will prevent infection and enhance outcomes of hospitalized patients.

It has been demonstrated that the productions of IFN-γ by immune cells are decreased in critically ill patients, and that these defects are associated with the susceptibility to HAP. rHuIFN-γ has neither been tested nor is recommended as adjunctive treatment of patients with HAP. **Based on these specific factors identified in the host response**, we propose to use rHuIFN-γ as **novel preventive approach for HAP**.

1.JUSTIFICATION OF THE STUDY

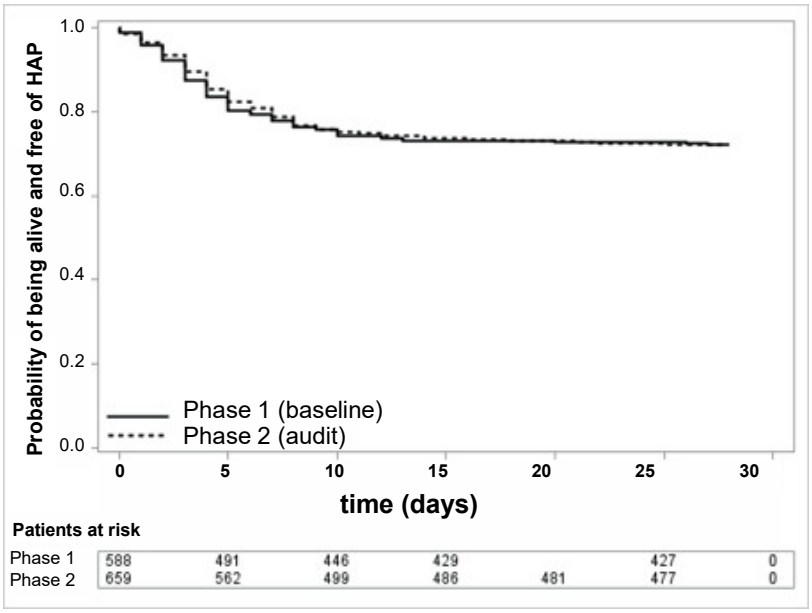
1.1. Positioning of the study

1.1.1. Epidemiology of Hospital-Acquired Pneumonia (HAP) in critical ill patients

Hospital-acquired pneumonia (HAP) **is the most frequent cause of hospital-acquired infections, with 500,000 episodes of HAP being treated every year in Europe(7)**, and accounting for 22% of all hospital acquired infections in a multistate point-prevalence survey(8). The incidence of HAP has barely decreased over the last decades and still routinely exceeds 10 cases /100 hospitalisations in critically ill patients(9).

The medical consequences of HAP are dramatic with prolonged hospitalization, long-term asthenia and depression, and increased risk of death (10)(11). The economic burden of ICU-acquired pneumonia, particularly VAP, is important. The patients often require longer periods of ventilatory assistance and have significantly longer ICU and hospital stays. On a per-case basis case VAP is associated with additional unadjusted hospital costs ranging between 40 000 and 49 000 USD in the USA (12,13).In France the average cost for each day in intensive care unit (ICU) is 2000 euros/day, and the cost of each episode of HAP is 40.000 euros (14)(15).

European, French and American society of intensive care have recently published guidelines in order to prevent hospital acquired pneumonia (16–18). These strategies aim at reducing oro-pharyngeal bacterial load in order to minimize germs aspiration. Yet, except for selective digestive decontamination which reduce mortality of critically patients (19), other interventions didn't improve significantly patient outcomes (9). We evaluated in the Pneumocare study (clinicaltrial.gov: NCT03348579) the impact of the French 2017 guidelines on the risk of HAP. We observed in 1300 patients included in 5 French ICUs that the risk of HAP remained unchanged around 25% of patients hospitalized 3 days or more in ICUs. This result demonstrated that new therapies are needed to further enhance the prevention of HAP in critically ill patients.



Probability of HAP before (phase 1, n=630 patients) or after (phase 2, n=650 patients) the application of the SFAR/SRLF recommendations (Roquilly et al. Clin Infect Dis 2020)

1.1.2. Rational for immunotherapy in critical ill patients

i. Risk of HAP and decreased production of IFN-γ during critical-illness related immunosuppression

During infections, activated monocytes and dendritic cells (DCs) stimulated by bacterial and viral antigens release IL-12 which induces the production of IFN-γ by innate-like lymphocytes (notably Natural Killer (NK) cells).Patients with inherited deficiency in IFN-γ production are highly susceptible to respiratory infections[1]. In mice model mimicking HAP, the lung response to secondary pneumonia is characterized by a decreased production of IFN-γ by NK cells as compared to normal response to pneumonia.

We have tested the hypothesis that treatments with rHu-IFNγ can restore immune resistance to bacteria. We have notably been demonstrated that IFN-γ restores the metabolic activity and the functions of monocytes[2], reversing a major feature of critical-illness related immunosuppression. Several case reports on the use of rHuIFN-γ in septic patients have shown promising effects[3].

In conclusion, our consortium has demonstrated that the susceptibility to HAP is a consequence of the limited stimulation of NK cells by monocytes and DCs, and that IFN-γ supplementation restores immune competence during HAP (Figure 2). These data strongly support that rHuIFN-γ treatment, as a compensatory therapy to overcome critical-illness related immunosuppression, can restore immunity and enhance the treatment of HAP.

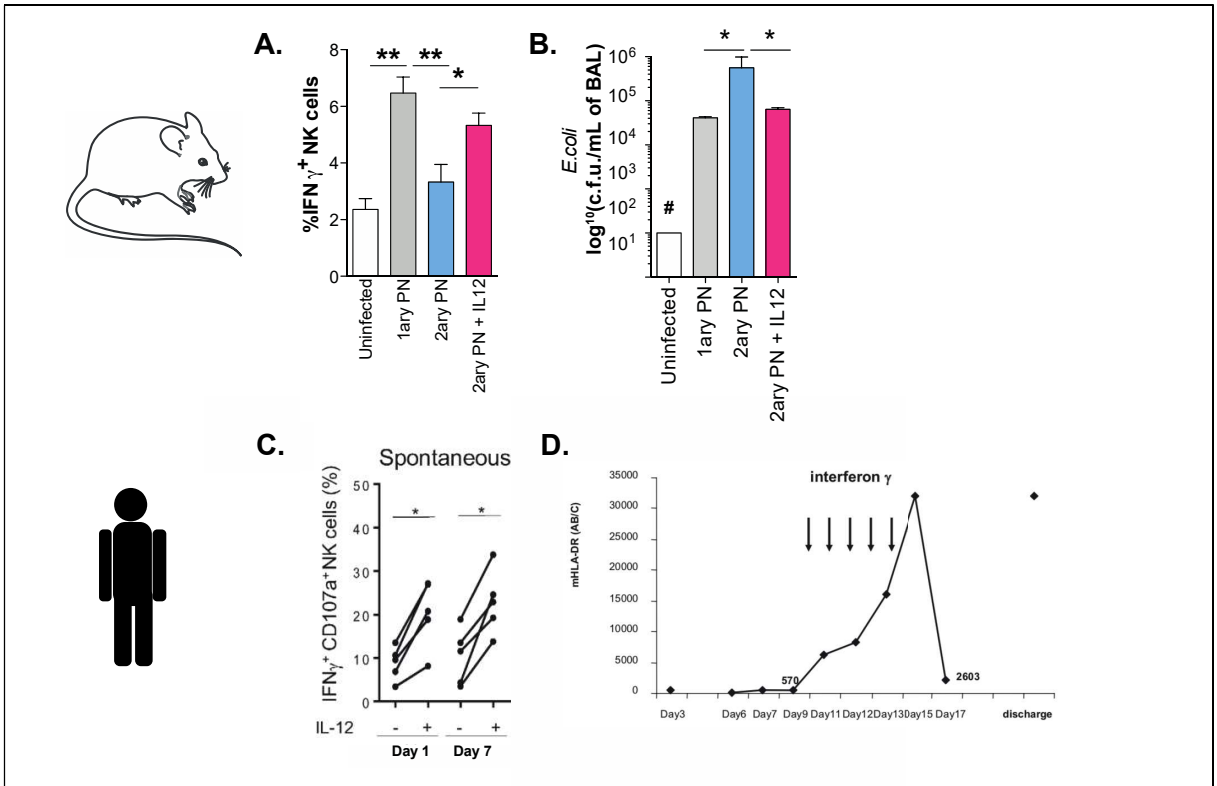


Figure 2. Proof of concept for the treatment of HAP with IFN-γ

[1] *Hambleton et al. NEJM 2011, Bogunovic Science 2012, Picard Am J Hum Gen 2002.*

[2] *Chen et al. Nature Immunol 2016*

[3] *Luckasewicz et al. Crit Care Med 2009, Docke et al. Nature Med 1997.*

(A-C) Mice are infected and spontaneously recover from a bacterial pneumonia. Infection-cured mice are challenged by a secondary bacterial pneumonia mimicking HAP. **(A)** Decreased production of IFNγ by NK cells, and restoration by IL-12 treatment during *Staphylococcus aureus* HAP in mice. **(B)** Restoration of IFN-γ production by NK cells is associated with increased the bacterial clearance during HAP in mice. **(C)** Decreased production of IFNγ by NK cells collected 1 day or 7 days after hospitalisation, and restoration by IL-12 treatment, during in vitro stimulation. **(D)** Evolution of the paralysis of monocytes (mHLA-DR) in human treated with rHuIFN-γ for HAP (Issue from Lukaszewicz. Crit Care Med 2009). .*p<0.05, **p<0.01

ii. Status of development of rHuIFN-γ (Imukin®) for randomized clinical trials

rHuIFN-γ (Imukin®) is commercialized by Clinigen and **already approved in Europe** for the treatment of infections in patients with chronic granulomatous disease. It can thus be sourced easily, ensuring feasibility. Administrations of rHuIFN-γ as a rescue therapy have moreover been reported in several case reports of protracted hospital-acquired infections[4], reinforcing the timeliness of the clinical trials proposed by our consortium.

iii. Dose regimen justification

The recommended dose of **rHuIFN-γ (Imukin®)** is 50 μg/m^2 of body surface area in patients treated for chronic granulomatous disease. Dose adaptation in intensive care units has been intensely investigated notably for antimicrobial treatments, and it is now recognized that dose-adaption to the weight of patients is not accurate in critically ill patients (Tangden et al. Intensive Care Med 2017). Body Surface Area-based dose adaption can be biased by the extreme and rapid variations of body weight frequently observed in critically ill patients (You et al. J Crit Care 2013). In the published series of critically ill patients, recombinant human rHuIFNγ was used at a fixed dose of 100 μg/48 hours (Payen, BMC Infectious Diseases, 2019; Docke, Nature Med 1997). We thus decided to test a fixed dose of 100 μg/48 hours. The immunological follow-up planned in the study (samples collected at day 3 and day 7) will be used to develop formula to predict pharmacokinetic for **rHuIFN-γ (Imukin®)** in critically ill patients.

The timing of treatment is also critical to consider. While it was suggested that rHuIFNγ treatment can be more effective when administrated beyond day 7 of hospitalization (Payen, BMC Infectious Diseases, 2019), we aim to prevent HAP which can develop from the second day of hospitalization, and most frequently before day 7 (Roquilly et al. Clin Infect Dis 2020). Since we have reported that the production of IFNγ by lymphocytes is decreased from the first day of hospitalization (Roquilly et al. Clin Immunol 2017), we proposed that early (from day 2) but prolonged (5 injections every 48 hours, so up to day 9 after inclusion) treatment is the best period of therapy to prevent HAP with rHuIFNγ.

iv. Biomarkers for the prediction of HAP course and for the response to treatment

Individuals might be responding differently to immune interventions, thus the validation of biomarkers for patient stratification is an asset to immune interventions. Several biomarkers have been associated with HAP in critically ill patients, but none is recommended for clinical practice. The main reason is that bulk-omics approaches largely fail to capture the complexity of HAP. The new gold standard is to use large cohorts of patients, bar coding of the samples, high-throughput analysis followed by unbiased algorithm guided analysis. **We will thus combine cutting-edge high-throughput investigations to capture the complexity of the host-pathogens interactions and to clinically validate biomarkers for the stratification patients into low/high risk of poor outcomes of HAP and into responders/non-responders to immunotherapy.**

[4] *Docke et al. Nature Med 1997, Lukaszewicz et al. Crit Care Med 2009*

Host background. Dr. Li has demonstrated that the inter-individual variation of cytokine responses to pathogens is explained by **genome-wide single-nucleotide polymorphism (SNP) genotypes. Dr Li has identified six cytokine quantitative trait loci (QTLs) playing** a critical role in the variability in cytokine production by human immune cells in response to pathogens[5]. **Genetic variations, as assessed by these SNP**, are thus probably associated with the defect of the IFN-γ axis in hospitalized patients. The level of blood cytokines levels, notably IFN-γ dependent chemokines, are also associated with the risk of HAP in trauma patients[6].

Host status. Prof. Becher has developed **high-dimensional single-cell mass cytometry** and a bioinformatics pipeline for the in-depth characterization of immune cell subsets in peripheral blood mononuclear cells (PBMCs) isolated from liquid biopsies of patients[7]. This approach is a powerful tool for characterization of the myeloid system and lymphocyte compartment which can permit the prediction of the response to immunotherapy in cancer patients[22]. Prof. Netea has demonstrated the role of **the epigenetic reprogramming of monocytes** in the modifications of their **transcriptomic** activity and their ability to produce cytokine in response to pathogens[8]. This phenomenon of trained immunity is associated with exacerbated inflammatory response during secondary infections.

Microbiome composition. Prof. Dickson has shown that **respiratory microbiome alterations** play an important role in the development of lung inflammation during HAP and reflect variation in baseline lung innate immunity[9]. The lower respiratory tract harbors a highly diverse microbiome made of large numbers of commensal bacteria species and viruses. In critically ill patients, the biomass of the lung bacterial component of the microbiome increases over time, whereas its diversity decreases, and the diagnosis of HAP has been correlated with these alterations. Dr Josset has developed a method to investigate the respiratory virome based on metagenomics next-generation sequencing[10]. The **human virome** includes diverse commensal and pathogenic viruses that evoke a broad range of immune responses from the host. In organ transplant recipients, immunosuppressants strongly affect the structure of the virome in plasma, and the total viral load increases with immunosuppression[11]. The investigation of the respiratory microbiome composition (bacteria and virus) is thus proposed as a surrogate marker of immunocompetence.

Integration of high throughput analyses of the host and of the microbiome. We aim to build clinico-biological scores taking into consideration demographic values (**gender, age, genetic variations**) and high throughput analyses of biomarkers[12]. This approach will be employed to deeply characterize the host-pathogens interactions before the treatment, to investigate the temporal immune response to rHuIFN-γ and finally to stratify patients as responders and non-responders.

[5] *Li et al. Nature Med 2016.*
[6] *Roquilly et al. Crit Care Med 2014.*
[7] *Becher et al. Nature Immunol 2014, Nature Med 2018*
[8] *Netea et al. Science 2014, Cell 2016 & 2018*
[9] *Dickson et al. Lancet 2014, Am J RespirCrit Care Med 2015&2018, Lancet Respir Med 2015, Nature Microbiol 2016*
[10] *Bal, BMC Inf Dis, 2018*
[11] *De Vlaminck et al. Cell 2013.*
[12] *Goris et al. Brain 2015.*

The ground-breaking concept underpinning the development of biomarkers in the "PREV-HAP" study is to have access to cutting-edge high throughput analyses of the host and of microbiome composition and to be able to combine all these data to achieve a full understanding of the host-pathogen interactions *in vivo* (Figure 3).

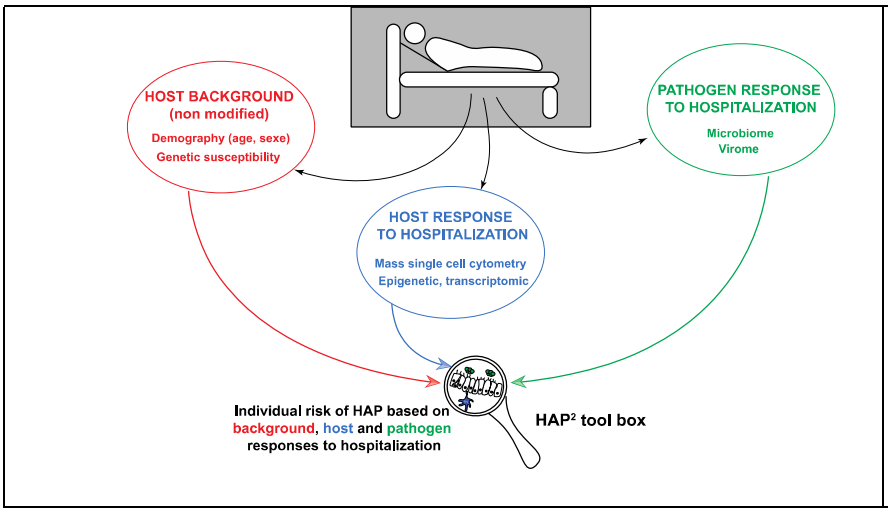


Figure 3. The "**HAP²" toolbox** is the integration of the patient background, of the host adaptation and of the pathogen modifications during hospitalization to develop biomarker and predict individual response to treatment of HAP.

v. "HAP2" project: a timely step to develop a stratified immune therapy for HAP

After a decade of continuous progress in the knowledge and the comprehension of the mechanisms of HAP by the partners, the interdisciplinary "HAP²" project is particularly timely (**Figure 4**), and PREV-HAP trial will help to reach two outcomes:

1/ **host-targeted drugs** (rHuIFN-γ will be brought from "bench to bedside", i.e. from a technology readiness level (TRL) 4-5 (technology validated in significant environment) to TRL7 (e.g. demonstration in clinical environment);

2/ **biomarkers** for the prediction of HAP outcomes from TRL2 (characteristic proof-of-concept) to TRL4 (validation in laboratory environment).

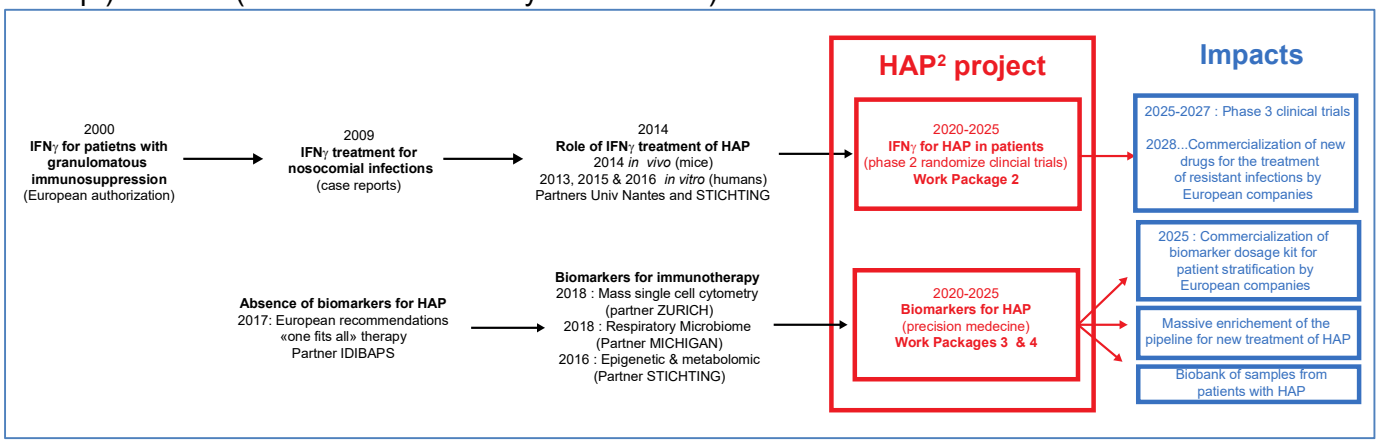


Figure 4 - Positioning of the "HAP²" project: bringing HAP prevention/treatment drugs from TRL 4-5 to TRL7 and biomarkers from TRL2 to TRL4

vi. Conclusions

The rates of HAP observed with current strategies underline the limits of current approaches of HAP prevention. **We have thus proposed that the prevention of HAP should aim to restore mucosal immunity and respect the diversity of the microbiome, rather than to sterilize airways with antibiotics (Figure 5)[13].** The development and validation of such strategies able to restore the mucosal immunity will probably minimize, or even replace, antibiotics - which are currently the sole therapies to date - for the management of HAP. The development of rHu-IFNγ is well advanced and the implementation of phase 2 randomized clinical trial is timely.

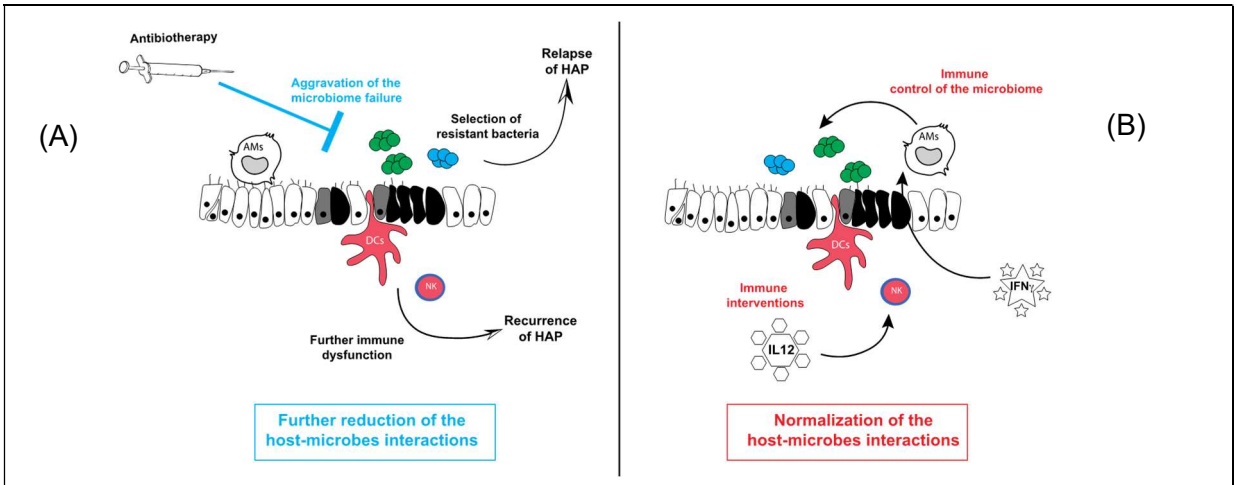


Figure 5. Current and proposed approaches to prevent hospital-acquired pneumonia
(A) Current approach of HAP: antibiotherapy alters the metabolomic functions of the microbiome, further reduces its diversity and selects resistant bacteria. The host remains susceptible to recurrence of relapse of HAP due to persisting immune dysfunction.
(B) HAP[2]. Immune interventions have the potential to clear virulent pathogens and to normalize the immune control of the microbiome (DCs: dendritic cells, NK: NK cells, AMs: Alveolar macrophages)

Current strategies for the prevention of HAP are "one-fits all patients" approaches which lead to a large proportion of treatment failures. Although each individual patient likely responds differently to therapeutic intervention, there are currently no reliable biomarkers for the stratification of patients predicting therapy success/failure in a given individual. Several biomarkers have been associated with HAP in critically ill patients, but none has been widely implemented in clinical practice[14]. Notably, the investigation of the host, or of the microbiome, fails to diagnose pneumonia when they are conducted separately[15]. **We propose to realize a biocollection of samples (blood and respiratory fluids) to combine develop biomarkers for the stratification of patients and the development of a precision medicine (theranostic).**

[13] *Roquilly et al. Lancet Respir Med 2019.*
[14] *Torres et al. EurRespir J 2017.*
[15] *Man et al. Lancet Respir Med 2019.*

1.2. Benefits and risks for subjects taking part in the study

1.2.1. Benefits

Individual benefit

The expected individual benefit of a treatment that improves the prevention of respiratory complications in critically ill patients is to decrease the risk of death, to reduce the duration of mechanical ventilation, and to increase the long-term quality of life. The individual benefit will thus be directly observed by the patient himself, and demonstrated by the result of the statistical analysis. These data will be measured at D28 and at D90

Collective benefit

The morbidity and mortality after hospital acquired pneumonia remain high, and also considering the number of episodes of infections, the burden is very important for the society (improvement of medical care). **The cost of hospitalization could be diminished, and the economic benefit could be high.** In a long-term approach, prevention of HAP could also fasten the return to work (economic benefit). If rHuIFN-γ reduce even slightly the morbidity/mortality induced by pneumonia, this study could deeply modify the medical care of patients all over the world.

On the other hand, diminishing the incidence of pneumonia would considerably reduce antibiotic consumption, producing a significant ecologic benefit concerning bacterial resistance. Indeed, up to 70% of ICU patients receive empirical or definite antimicrobial therapy on a given day, and the average volume of antibiotic consumption in this ICU patients is estimated as 1,563 defined daily doses (DDD) per 1000 patient-days (95% confidence interval 1,472–1,653) — that is, almost three times higher than in ward patients 16. rHuIFN-γ, which will reduce by 20% the risk of HAP has thus the potential to decrease the mean duration of antibiotherapy by 2 days in hospitalized patients. **The antibiotic selection pressure on resistant bacteria will thus be significantly reduced, increasing Europe's capacity to reduce the emergence of resistant bacteria.**

1.2.2. Risks

Individual risk

- Physical risks and constraints

No physical constraint is to be reported. Subcutaneous injections of rHuIFN-γ will occur during ICU hospitalization for a maximal duration of 9 days. Injections could be slightly painful, but are usually very well tolerated. Skin reaction such as local inflammation may also happen. Moreover, it is likely that the critically ill patients won't feel the puncture due to the sedation which is commonly used during the ICU stay.

Biological samples will be collected after the inclusion in the study immediately before study treatment injection, then before the 2nd injection at day3 (Visit 2), and before the 4th injection at day 7 (Visit 4).

Liver cytolysis has been described in children treated with rHuIFN-*γ* for months. This side effect, which resolves without sequelae upon treatment discontinuation, should not be observed in this trial evaluating short course of rHuIFN-*γ* in adults. Biological surveillance of

[16] *Bitterman et al. Clin Microbil Infect 2016.*

the transaminases will be performed but it should add no extra puncture, as ICU patients usually have daily biological tests at this stage.

Patients and relatives will receive a phone call at 1 and 3 months to ensure proper completion of the quality of life questionnaires (and the patient notebook for the patient). If the questionnaires haven't been completed and returned by post, the patient and the relative will answer to them during 10 to 15 minutes directly by phone.

A psychologist interview will be conducted by a researcher in psychology at M3 for some patients (and their relatives) included in Nantes. No additional appointment is set.
All in all physical risks and constraints are negligible.

- Disease-related risks

The risks of natural progression of hospital acquired pneumonia are:

- Pleural empyema, lung abscess
- Acute respiratory distress syndrome
- Relapse, recurrence of pneumonia
- Prolonged mechanical ventilation
- Death

- IMP risks

rHuIFN-γ. The drug has marketing authorization for the reduction of the frequency of serious infections in patients with chronic granulomatous disease or malignant osteopetrosis.

In these indications, as listed in 4.8 of IMUKIN SmPC, the most common adverse reactions are flu-like symptoms characterized by fever, headache, chills, myalgia or fatigue (with sometimes incomplete symptomatology). Hypersensitivity to the active substance (interferon gamma-1b) or to any of the excipients can't be excluded. Because Interferon gamma-1b is an exogenous protein, it may lead to the occurrence of antibodies during the course of treatment.

For the PREV-HAP study, a Reference Safety Information (RSI) adapted to the study indication is provided by the sponsor. No SAR is expected and all serious adverse effects are considered as SUSARs..

Placebo for rHuIFN-γ (NaCl). NaCl is commonly administered subcutaneously for hydration in vulnerable populations. Adverse reactions are most related to an overdose, with manifestation due to hypernatremia as nausea, confusion, but remain very unlikely with a subcutaneous injection, regarding low administered dosage. Local reaction may also occur.

- Concomitant treatment-related risks

Antibiotic therapy (eg. beta-lactamin ...) will be the most frequent concomitant treatment in the study, however, other concomitant drugs may be used as painkillers, hypnotics, bronchodilatators, steroids…. Medical devices may also be used for ventilation (tracheal tubes) and other current cares (such as for instance urinary catheter).

According to previous experiences, expected major adverse reactions with concomitant treatments are often related to antibiotics with allergic reaction to antimicrobial therapy, digestive disorders with Colitis and diarrhea including clostridium difficile colitis.

- Psychological risks and constraints

Patients are blinded to the study arm adjudication. The situation may induce anxiety.

The study drug administration needs an additional puncture site. Despite few local complications expected, it may cause little pain or apprehension. However, due to neurological injury, and/or sedation, patients won't probably feel the puncture. The patients included should experience no distress or feeling of dependence. The psychological constraints related with the pathology itself could be important but without relationship with the protocol.

For 20 patients included in Nantes (and their relatives who signed their own ICF), unpleasant emotions may be experienced during consultation with a researcher in psychology at M3 (unpleasant emotions such as fear or sadness for instance might be elicited by the recall of the ICU stay experience).

- Socio-economic risks

ICU hospitalizations cause dramatic changes in life of patients, including alteration of the social status and job loss. It may also have consequence on insurance and credit. Hospital acquired pneumonia increase the durations of hospitalization and of rehabilitation, worsening these consequences.

However, participating to the PREV-HAP study won't cause any change of social status and/or job; no consequence on insurance and credit; no devaluation of confidence in the attending physician; no change of relationship with others. There is no socio-economic risk resulting from the study.

Collective risk

The treatment management didn't induce increased risk (eg ecologic.) regarding standard care. The collective risk is limited.

1.2.3. Benefit / risk balance

Individually, the outcome of patients could be directly improved by the treatment (reduction of the risk of treatment failure, diminution of the duration of hospitalization and of the risk of death), while the risk of adverse effects is limited (IMUKIN® is approved for human use in Europe since 1992). The individual benefit/risk balance of the study protocol is therefore highly favorable.

Collectively, the study will develop new treatment which will decrease the burden of hospital-acquired infection, limit the antibiotic selection pressure of resistant bacteria and become new alternative for the treatment of highly resistant bacteria. Such outcomes will drastically decrease the cost for the society of carrying for hospitalized patients. The collective benefit/risk balance of the study protocol is therefore highly favorable.

2. OBJECTIVES AND ENDPOINTS

2.1. Primary objective and endpoint

2.1.1. Primary objective

The primary objective is to determine if rHu-IFNγ, as compared with placebo, could reduce the rate of **hospital-acquired pneumonia** and improve outcomes in patients admitted to intensive care unit and requiring mechanical ventilation.

2.1.2. Primary endpoint

To demonstrate the efficiency of rHuIFN-γ for the prevention of hospital-acquired pneumonia**, the primary endpoint is the composite outcome of all-cause mortality at day 28 and/or the occurrence of hospital-acquired pneumonia within 28 days after randomization**.

Hospital acquired-pneumonia is diagnosed after the 48th hour of hospitalization according to European and French guidelines (Torres et al. Eur Respir J 2017; Leone et al. ACCPM 2018):

- at least two of the following criteria: body temperature >38°C; leukocytosis>12000 cells per mL, leucopenia <4000 cells per mL, or purulent pulmonary secretions,
- appearance of a new infiltrate or change in an existing infiltrate on chest radiography,
- positive culture of a respiratory tract samples from mechanically ventilated patients with quantitative culture (for patients with antibiotics < 48h) (thresholds of 10^4 colony-forming units (CFU) per mL for a bronchoalveolar lavage, 10^5 CFU/mL for a blind BAL (mini BAL) sample, and ≥10^5 CFU/mL for a tracheal sample). A semi-quantitative is acceptable, notably for patients with antibiotics >48h. Respiratory samples are obtained before starting any new antibiotic treatment.

An adjudication committee, composed of 1 investigator by country who will be blinded to the trial-group assignments, will review the medical charts of patients with respiratory tract infections of the 2 other participating countries, in order to review the diagnosis. Guidelines will be provided to the members by the sponsor. The primary endpoint concerning the occurrence of HAP will be based on the re-reading and review of each diagnosis by two adjudication committee members. In case of disagreement between the 2 adjudication committee members, a consultation meeting will be organised in the presence of an expert radiologist..

In case of medical history of CAP, the diagnosis of HAP will be confirmed if one or more bacteria was not present at the time of CAP.

2.2. Secondary objectives and endpoints

2.2.1. Secondary objectives

The secondary objectives are:

- to demonstrate the **efficiency** of rHuIFN-γ, on pneumonia-associated morbidity and mortality reduction
- to demonstrate the **efficiency** of rHuIFN-γ on antimicrobial therapy utilization reduction
- To describe the **safety and tolerability** of rHu-IFNγ
- To assess the **suitability, acceptability, and adaptability** of rHu-IFNγ
- To assess the **economic efficiency** of rHuIFN-γ for the prevention of pneumonia
- To develop **biomarkers** for the stratification of patients into responders and non-responders of rHuIFN-γ
- To develop a **biobank** of blood and respiratory samples collected in humans at risk of hospital-acquired pneumonia

2.2.2. Secondary endpoints

The secondary outcomes to determine the efficiency of rHu-IFNγ, on pneumonia-associated morbidity and mortality reduction are:

- All-cause mortality at D28 and D90
- Hospital acquired Pneumonia at D28
- Bacterial ecology of the 1st episode of HAP (respiratory fluids)
- Rate of ventilator-associated tracheobronchitis at D28 defined as at least two of the following criteria: body temperature >38°C; leukocytosis>12000 cells per mL, leucopenia <4000 cells per mL, or purulent pulmonary secretions and a positive culture of a respiratory tract samples, **without** appearance of a new infiltrate or change in an existing infiltrate on chest radiography (Martin-Loeches et al. Lancet Respir Med 2015)
- Acute Respiratory Distress Syndrome within 28 days after randomization
- Duration of antimicrobial therapy at D28, antibiotic free days at D28 (the number of antibiotic free days is defined as the number of days between D1 and D28 for which living patients don't receive antibiotics. Dead patients will be ascribed 0 antibiotic free days).
- Duration of mechanical ventilation at D90, mechanical ventilation free days at D90 defined as the number of days between D1 and D90 for which living patients breath spontaneously. Dead patients will be ascribed 0 mechanical ventilation free days.
- Duration of ICU hospitalization at D90, Duration of hospitalization at D90, hospital free days at D90 defined as the number of days between D1 and D90 for which living patients is outside of hospital. Dead patients will be ascribed 0 mechanical ventilation free days.

The secondary outcomes to determine **the tolerance** of rHu-IFNγ are:

- Rate of serious adverse effects and suspected unexpected serious adverse reaction (SUSAR) at D15.
- Rate of leukocytosis, neutropenia, lymphopenia, thrombopenia at D15.
- Rate of liver cytolysis (Increases in AST and/or ALT) at D15.
- Rate of pancreatitis (Increase in Lipase or amylase if the lipase test cannot be carried out, according to the usual practice of the centres) at D15.
- Fever, headache, nausea at D15.
- Allergic reaction at D15.
- Injection site reaction at D15.
- Myalgia, arthralgia, back pain at D15

The secondary outcomes to determine the **economic efficiency** of rHu-IFNγ in the prevention of pneumonia are:

- Economic endpoint at 3 months: **Incremental cost effectiveness ratio (ICER)**.

We will assess the economic efficiency of the rHuIFN-γ compared to the placebo by performing a cost-effectiveness analysis (CEA) using QALYs (Quality-Adjusted Life-Years) as a measure of effectiveness. QALYs are a measure of effectiveness specifically designed for economic evaluations. Their main advantage is to combine information about the length and the quality of life of patients into a single index measure. Specifically, the CEA will consist in estimating an incremental cost-effectiveness ratio (ICER) as follows:

$$ICER = \frac{\left(Costs_{\text{rHuIFN}-\gamma} - Costs_{\text{comparator}}\right)}{\left(Effectiveness_{\text{rHuIFN}-\gamma} - Effectiveness_{\text{comparator}}\right)}$$

Where 'comparator' corresponds to the placebo. Effectiveness will be assessed in terms of QALYs. QALYs are constructed by weighting each period of time, typically one year, by a health-related quality of life index (called a utility score) ranging from 0, that represents "being dead", to 1, that corresponds to a state of "perfect health". The utility scores will be determined by asking the patients to fill in the EQ-5D-3L health-related quality of life questionnaire.

The results of the cost-effectiveness analysis (i.e. the ICER) will be expressed in terms of costs per QALY gained. See paragraph 6.2.8. for statistical analysis.

The secondary outcomes to determine the **suitability and acceptability** of rHu-IFNγ **from the patients' and relatives' perspectives** are:

<u>Suitability</u>

- Changes in health-related quality of life (HRQoL) from one (M1) to three months (M3) after randomization measured with the Short Form **(SF)-36 scale** validated in French, Greek, and Spanish
- Changes in anxiety and depression from M1 to M3 measured with the **HADS scale** validated in French, Greek, and Spanish
- Changes in subjective well-being from M1 to M3 measured with the **Satisfaction With Life Scale (SWLS)** validated in French, Greek, and Spanish

At M1 and M3, these questionnaires will be filled in by the patient (patient's perspective) and

by one relative (relative's perspective). The relatives could be the legal representative who have signed the consent for the inclusion of their relative, or another relative; the aim being that it should be the person closest to the patient emotionally, and that it is the same person who answers the questionnaires at M1 and M3, if they consent to. If the patient is discharged from hospital before M1, the questionnaires will be given to the patients at discharge, or sent by post, to be returned to the clinical team, and the patient and his/her relative will be contacted by phone at M1 and M3, to ensure the good completion of the questionnaires. If his/her condition does not allow him/her to answer the questionnaires, only the questionnaire of the relative will be collected (from the relative's perspective).

Acceptability

Adaptation of the patients to their health state and its evolution from M1 to M3 using differential item functioning and **response shift analyses** for HRQoL, anxiety and depression.

2.3. Objective and endpoints for ancillary studies

We hypothesized that if rHu-IFNγ will reduce the risk of HAP, thus potentially reducing of hospitalization and sequalae. Although this treatment should be highly accepted by patients and relatives assessing its suitability and acceptability from a quantitative and a qualitative perspective are important information to assess before making recommendations of clinical use.

This ancillary study thus aims to to provide additional evidence on the suitability and the acceptability (in terms of HRQoL and mental health) of rHu-IFNγ from the patients' and relatives' perspectives

To test the suitability and the acceptability of rHu-IFNγ, we will scrutinize and provide more insight, from a qualitative perspective in psychology, into the effects of the treatment on the patients' and relatives' adaptation (response shift between M1 and M3) to their health state and its changes. Using qualitative methods will enable to explore the ways in which patients and relatives respond to their circumstances in the contexts of their lives, and contextualize the ways in which they understand and define their experience following ICU stay. Allowing participants time to freely explain cognitions and experiences will provide a more thorough understanding on how patients and relatives approach their experience.

At Day 90, the qualitative study will be proposed to 20 consecutive patients from one center (Nantes Hospital) and 20 relatives (1 per patient), who will thus be distributed in a balanced way between each group. The relatives may be the legal representative who has signed the consent for the inclusion of the patient, or another relative; the aim being that it should be the person closest to the patient emotionally, and that it is the same person who has answered the questionnaires for the quantitative study at M1 and M3, provided they consent to do so.

Semi-structured interviews will be conducted by a researcher in psychology with patients and relatives (dyads) to gain more insight into the understanding and the interpretation of quantitative data, as recommended by literature on human sciences[17]. An interview guide will be developed on the basis of the literature on the psychological consequences of the immune intervention for patients. The interviews will be recorded with the participant's consent to allow

[17] Bioy A., Castillo M-C., Koenig M. (2021). Les méthodes qualitatives en psychologie clinique et psychopathologie. Paris:Dunod.

full transcription while respecting their anonymity and confidentiality. This interviews are highly specialized and thus can only be performed in one center of the study.

Qualitative data will be analyzed using a lexical analysis to describe what patients have told, together with a content analysis (thematic categorical classification, Bardin, 2003; Blanchet & Gotman, 2007) to highlight the topics of the corpus and interpret data[18]. More specifically, the interview guide will include 5 areas: 1/ Current life story and changes caused by the intervention in terms of HRQoL and mental health, 2/ Management of emotions such as distress and well-being, 3/ Management of fundamental cognitive schemas such as beliefs and goals, 4/ Behavioral management and social (inter)actions, 5/ Reassessment of self and HRQoL (i.e. response shift).

The discourses of the patients and their relatives will be analyzed in the following manner to answer the three following questions: “What are the topics addressed by the patients and relatives?”, “What is being said?", and “How is this verbalized?". The transcribed text of the interviews will be divided into segments allowing for categorical classification according to the topics that were addressed by patients and relatives. Numerical analysis of the lexicon will enable to identify what was being said, and numerical analysis of linguistics will explore how things were expressed.

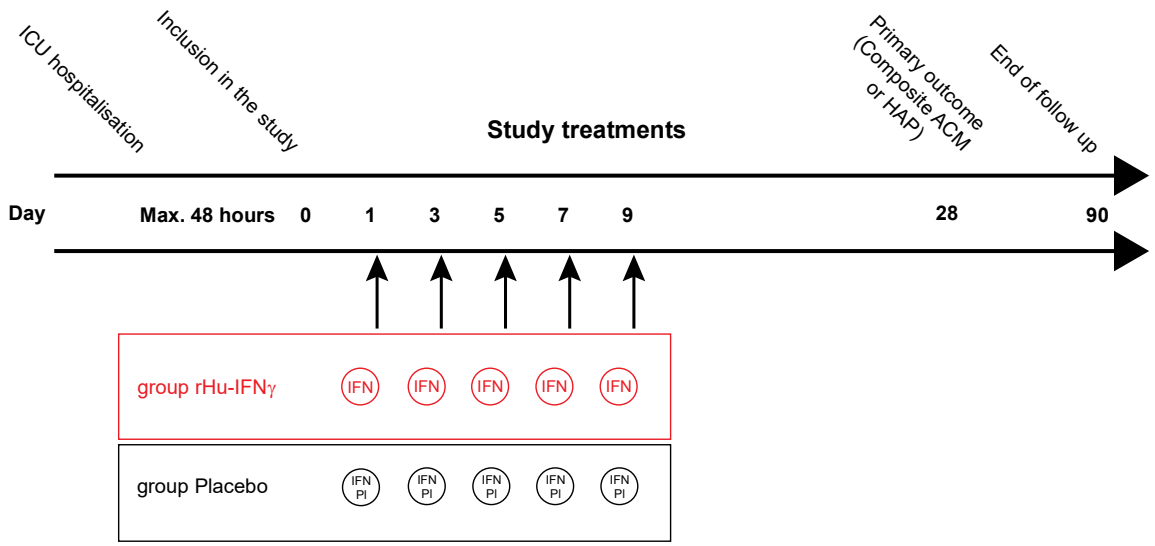
Briefly, the division of the discourses will be based on the proximities between the words used. Using open-source softwares “Iramuteq” and/or “Tropes” speech will be divided into units based on frequency which will then be classified into categories according to thematic groupings. The topics will be identified according to the study of co-occurrences and recurrences in discourse. Using open-source software “Sonal” a lexicometric analysis will enable to extract meaning from the structure and organization of discourse. The linguistic discourse analysis will assign words in lexical categories and subcategories to perform a content analysis, a cognitive-emotional discursive analysis, and diagnose the structure of the speech and the intention of the respondent (e.g. style (argumentative, narrative...), detection of doubts, removal of ambiguities, words occurrence).

We will use a mixed method design combining this qualitative approach with the quantitative approaches used for response shift analyses by drawing a parallel between them in order to provide narrative contexts to numerical data, providing stories behind the numbers and the comparsion of the HRQoL and mental health between the two study groups.

This mixed method approach will provide more insight into the suitability and acceptability of rHu-IFNγ from the patients’ and relatives’ perspectives, in terms of changes in HRQoL and in anxiety and depressive disorders after ICU stay. It will expand the knowledge on the patients’ and relative’s journey during this recovery period which remains understudied. Combing qualitative and quantitative approaches will allow painting a more complete picture of the recovery process which is a complex phenomenon encompassing many dimensions of physical, emotional, economic, and social health, and having different meanings to different individuals.

[18] Blanchet, A., &Gotman, A. (2007). L’enquête et ses méthodes: L’entretien. Paris: Armand Colin, et Bardin, L. (2003). L’analyse de contenu. Paris: PUF.

3. STUDY TREATMENTS



The patient will receive the following Investigational Medicinal Products (IMPs), depending on his/her randomization arm:

- **Arm 1 (Recombinant Interferon gamma 1b):**
  - **Recombinant Interferon gamma 1b** (IMUKIN®, from Clinigen®): 5 subcutaneous injections (100 µg/0,5ml) from day 1 to day 9 (i.e. 1 injection every 48h)
- **Arm 2 (Placebo):**
  - Recombinant Interferon gamma 1b placebo (IFN Pl): 5 subcutaneous injections (0,5ml) from day 1 to day 9, i.e. 1 injection every 48h

3.1. Description and mode of administration

3.1.1. IMP1: IMUKIN® (recombinant interferon gamma-1b – rHu-IFNγ)

Molecule name: IMUKIN® (recombinant interferon gamma-1b – rHu-IFNγ)

Qualitative and quantitative Product composition:

Each vial (0.5 ml) contains 2 x 10^6 IU (0.1 mg) recombinant human interferongamma-1b. Interferon gamma-1b is produced in an E. coli expression system.
List of excipients:
D-Mannitol
Disodium succinate hexahydrate
Polysorbate 20
Succinic acid
Water for injections

Manufacturer:

CLINIGEN HEALTHCARE B.V.
SCHIPHOL BOULEVARD 359
WTC SCHIPHOL AIRPORT, D TOWER 11TH FLOOR
1118BJ SCHIPHOL
NETHERLANDS

The drug has marketing authorization for the reduction of the frequency of serious infections in patients with chronic granulomatous disease or severe, malignant osteopetrosis.

Pharmaceutical form and packaging used:

IMUKIN® 2 x 10^6 IU (0.1 mg) is a clear, colourless solution for injection (subcutaneous use).
2 ml glass vials (Type I borosilicate glass) which are stoppered with grey butyl rubber stoppers with aluminium/polypropylene flip-off type caps.
Pack sizes: 1 vial in one folding box.

Storage and Handling

The vials need to be stored in a refrigerator (2-8°C). The vials must not be frozen.

Administration
IMUKIN® will only be used in the randomization arm 1:

- **Recombinant Interferon gamma 1b** (IMUKIN®, from Clinigen®): 100µg/0,5ml subcutaneous injections from day 1 to day 9 (5 injections, i.e. 1 injection every 48h,),

Dose adjustment
No dose adjustment is foreseen, but a drug discontinuation car occur in case of liver cytolysis (AST and/or ALT > 5N).

Reference documents
The reference document for this IMP is the current version of the Reference Safety Information provided by the sponsor.

3.1.2. IMP2: IMUKIN® Placebo

Molecule name: IMUKIN® placebo

Qualitative and quantitative product composition:

Clear and colourless solution of NaCl 0.9%.

Manufacturer:

PPRIGO – Rennes University Hospital
Pharmacy Department – Hôpital Sud
Pharmacotechnology Unit
16 Boulevard de Bulgarie
35200 Rennes
France

Pharmaceutical form and packaging used:

IMUKIN® placebo (NaCl 0.9%) is a clear, colourless solution for injection (subcutaneous use).

2 ml glass vials (Type I borosilicate glass) which are stoppered with grey butylrubber stoppers with aluminium/polypropylene flip-off type caps.

Storage and Handling
The vials need to be stored in a refrigerator (2-8°C). The vials must not be frozen.

Administration
IMUKIN® placebo is to be administered by subcutaneous injection, in randomization arm 2:

- Recombinant Interferon gamma 1b placebo (IFN PI): 0,5ml subcutaneous injections from day 1 to day 9 (5 injections, i.e. 1 injection every 48h).

Dose adjustment
No dose adjustment is foreseen, but a drug discontinuation car occur in case of liver cytolysis (AST and/or ALT > 5N).

Reference documents
The Reference Safety Information is in the current version of NaCl SmPC (section 4.8).

3.1.3. Other study treatments

Not applicable

3.2. Treatment compliance follow-up

For the compliance to the rHuIFN-γ and placebo treatments, all vials will be stored after use, for counting and auditing. All processing units (used or not) will be stored in the ICU and sent for destruction to the site pharmacy (when applicable) after use. The administrations will be recorded in the medical file of the patients, and these pieces of information will be collected in the eCRF.

A CRA of the sponsor will verify the returned units during monitoring visits, and the destruction may be carried out after each monitoring visit, after receipt of authorization for destruction from the sponsor.

3.3. Experimental drug circuit

3.3.1. General circuit

The sponsor will provide the clinical sites with the IMUKIN® and placebos vials:

- IMUKIN® vials will be purchased by the Sponsor
- IMUKIN® placebo vials will be manufactured by a subcontractor mandated by the sponsor, and provided to the French coordinating center (CHU Nantes).

The supplying will be adjusted according to the rhythm of the inclusions, of the study progress and of the expiry dates of IMPs.

All vials of IMUKIN® and placebo will be labelled, packed and stored under supervision of the pharmacist of the French coordinating center (CHU Nantes). IMPs will be delivered to each specific study site. Only the pharmacy of the French study's coordinating center will be aware of vials' composition (coding list).

At each study site, local investigators (surgeons, anesthetists), nurses and the patient will be blinded to the allocation group, and each site will be responsible of the storage, delivery and accounting of the treatments.

3.3.2. Experimental drug storage conditions

Description of dispensary storage

Upon receipt at each site, IMPs are kept in a cool dry place, protected from light, where the temperature stays between 2-8°C. As of reception by the pharmacy (or directly in the ICU, depending on local SOP) of the institution, the vials will be stored in a secured place whose temperature is monitored.

Description of department storage

The IMPs dispensing will be made starting from the medical prescriptions, by the pharmacy of the institution and according to local SOPs or by the local investigators' team of the institution according to the local SOPs.

Upon receipt in each department, IMPs are kept in a cool dry place, protected from light, where the temperature stays between 2-8°C. The vials will be stored in a secured place whose temperature is monitored.

Description of storage at patient's home

Not applicable

3.3.3. Unblinding procedure

Unblinding procedure can be requested by the sponsor :

- where knowledge of this information is necessary for the management of SUSAR and for the quality of the information transmitted to the DSMB, the competent authorities and Ethics Committees. An unblinding procedure is then carried out in agreement with the Safety Unit of the Sponsor;
- when drafting the Annual Safety Report if the analysis of the listing of the SAEs or AEs reveals a significant difference between the encoded randomization arms (up to the discretion of the Safety Unit of the Sponsor, and the DSMB).

Unblinding procedure only takes place under the conditions described in the protocol, and in compliance with the Sponsor's internal procedure (0062-PR-049_PROM-COORD-Blind lifting procedure). The Sponsor's Vigilance Unit is duly authorised to ask the sponsor's data manager to lift the blind according to this procedure : the Vigilance Unit analyses the SAEs for all the IMPs. It assesses causality and uses the reference information identified in the protocol to define the expected or unexpected character. Only in the event of SUSAR or a New Safety Information occurring in the patients, the data-manager lifts the blind for this event only, at the request of the vigilance officer.

Unblinding is also required at the end of the research by the biostatistician in charge of statistical analysis and by the Safety Unit of the Sponsor for the recoding of all SAEs in the Safety database of the Sponsor and for the drafting of the Final Safety Report.

Only data managers and clinical trials pharmacists at the University Hospital of Nantes can be informed of the treatments given to patients included (via the list or individual notifications sent by email).

3.4. Authorised and unauthorised treatments

3.4.1. Authorised treatments

All treatments usually used to prevent and treat hospital-acquired pneumonia in critically ill patients are authorized, notably (not exhaustive) Antimicrobial therapy (all molecules): antibiotics, antiviral therapy; selective oropharyngeal decontamination, selective digestive decontamination.

3.4.2. Unauthorised treatments

No treatment is forbidden in this protocol with the exception of open-labelled treatment with rHuIFN-γ during the first 28 days of the trial. The participation to another drug clinical trial is not authorized during the trial's participation, but the participation to a clinical trial with minimal risks and constraints (such as taking blood for example) is authorized.

3.4.3. Emergency treatments

Not applicable.

4.STUDY POPULATION

4.1. Description of the population

We will include 200 adult patients hospitalized in intensive care units, under mechanical ventilation in three European countries (see Statistic section for justification of the statistical power). The investigators working in Intensive Care Units will be responsible for the screening and the inclusion of critically ill patients. Patients under guardianship or trusteeship, pregnant women, minors won't be included in the trial.

The participation to another drug clinical trial is not authorized during the trial's participation, but the participation to a clinical trial with minimal risks and constraints (such as taking blood for example) is authorized.

In this study, inclusions will be realized in immediate vital emergency situation. Indeed, the treatment of hospital-acquired pneumonia is an emergency and it is recommended to start the treatment within the first hour after the diagnosis (Torres et al. European Guidelines for hospital-acquired pneumonia. Eur Respir. J 2017). Given the inclusion criteria (critically ill patients suffering acute pneumonia, or at risk of pneumonia due to critical illness), patient won't be able to express their consent before the inclusion in the study. Thus, an emergency consent procedure is needed and justified.

This procedure will allow some sites (depending on national regulatory approvals) to include the patients without having the patient's consent in a first time, nor the legal representative consent (but the consent will be sought as soon as possible).

Patients will be recruited in **3 European countries** during a 2-year period. Available data from these centers indicate that 10 to 20 patients fulfil the inclusion criteria monthly. A mean number of patients meeting non-inclusion criteria (including refusal to participate) of no more than 50% has been anticipated (worse scenario). The recruitment is competitive between the centers. The population recruitment will be stopped as soon as 200 patients are included in the study.

The criteria for site selection are:

- Potential recruitment (>10 patients/month with inclusion criteria)
- Previous experience of investigation in at less one randomized clinical trial in the last 5 years.
- Capacity to include patients 7 days a week.
- Capacity to manage the collection and the storage of the biological samples, ideally access to a dedicated facility.
- Capacity to guarantee the access to the study treatments 7 days a week to the investigators.

To ensure the feasibility of the study, we have taken the following decisions:

- Inclusion/non-inclusion criteria are consistent with routine care. A mean number of patients meeting non-inclusion criteria (including refusal to participate) of no more than 50% has been anticipated (worse scenario).
- Decisions about most aspects of patient care will be performed according to the expertise and routine clinical practice at each center. Little differences with standard practice set the stage for good adherence to the study protocol.

- A steering committee will insure the supervision of the trial. This committee will be composed of Pr Roquilly (HAP² coordinator), Pr Torres (National Coordinator for Spain and leader of HAP² WP4) and Dr Koulenti (Scientific Coordinator for Greece and leader of HAP² WP6), of Pr Sébille (leader of HAP² WP5 and methodologist for the 2 clinical trials of this European project, University Nantes) and of Dr Flet (Dept. of pharmacy, CHU Nantes). Regular meetings will be planned to evaluate the progress of the trial and adherence to the protocol.
- Dedicated clinical research associates on sites and/or study nurses will be made available at each center for follow-up and data registration.
- The 28-days and 90-days follow-up will be realized by the investigating center.
- The members of the steering committee, and the participating centers, are all experienced in the conduct of multi-center randomized clinical trials published in the field of hospital-acquired pneumonia: *JAMA (Torres et al. 2015, Roquilly et al. 2011)*, Lancet Infectious Diseases (Torres et al. 2018) *Lancet Resp Med (Roquilly et al. 2014), Am J RespirCrit Care Med (Roquilly et al. 2013) and Intensive Care Medicine (Roquilly 2017), Critical Care Med (Koulenti et al. 2009, national coordinator of the SAATELLITE trial and of the* COMBACTE network*)*.

4.2. Inclusion criteria

- **Adult** patients (18yr to 85yr).
- Hospitalized in intensive care unit for less than 48 hours**.**
- Receiving **invasive mechanical ventilation** at the time of inclusion.
- **One or more acute organ failure** at the time of inclusion **among**: neurological (Glasgow coma scale <13 before sedation), hemodynamic (norepinephrine, epinephrine, or any other vasopressor at a dose of ≥ 0.1 µg per kilogram of body weight per minute or ≥0.5 mg per hour for at least 6 hours), respiratory (PaO_2 / FiO_2< 200) and/or renal (creatininemia > 2 fold higher than the basal value and/or oliguria < 0.5 mL/kg/hour for at less 12 hours).
- **Informed consent** from a legal representative, or emergency procedure (when possible according to national regulation, see below). As is not possible to obtain the patient consent prior the inclusion (comatose patients), patient consent for the study continuation will be obtained as soon as deemed possible.
- Person insured under a health insurance scheme.

4.3. Non-inclusion criteria

These criteria define the characteristics meaning that the subject is not eligible for inclusion in the study:

- Pregnant women (serum or urine test), breastfeeding women
- Patient under legal protection (incl. under guardianship or trusteeship)
- **Hypersensitivity** to the active substance (interferon gamma-1b) or known hypersensitivity to related products, such as another interferon, or to any of the following excipients: Mannitol, Disodium succinate hexahydrate, Succinic acid, Polysorbate 20
- Severe **hepatic insufficiency** (Child Pugh score B or C)
- Liver cytolysis with hepatic enzymes (AST and/or ALT) > 5N

- **Severe chronic renal insufficiency** (MDRD Creatinine Clearance < 10 ml/min/1.73m^2)
- **Immunosuppression** (hematologic cancer, aplasia, chemotherapy/radiotherapy for cancer within 3 months prior to the inclusion, known infection Human immunodeficiency virus, concomitant use of any anti-graft rejection drug).
- **Coma after resuscitated cardiac arrest**
- **Cervical spinal cord injury**
- Participation to a drug interventional study within 1 month prior to the inclusion
- Hospital-acquired pneumonia before inclusion in the study during the current hospitalization.
- Sustained hyperlactatemia > 5 mmol/L.

5. STUDY DESIGN AND CONDUCT

5.1. Study schedule

Activities	V0 Inclusion visit within 6h before 1st injection	V1 Day 1	V2 Day 3	V3 Day 5	V4 Day 7	V5 Day 9	V6 Day 15	V7 Day 28	V8 Day 90
Inclusion and non-inclusion criteria verification + Patient and/or legal representative information, and consent (+ relative's consent for the questionnaires at M1 and M3)	X (legal representative) *and patient and relative as soon as deemed possible*								
Pregnancy test – urine or blood	X								
Randomisation	X								
Clinical examination	X	X	X	X	X	X	X	X	
IMP administration (IMUKIN® or placebo)		X	X	X	X	X			
Collection of the respiratory fluid and of blood (peripheral blood mononuclear cells)	X		X		X				
Liver Function Test (AST, ALT, bilirubin) + Blood count test	X		X		X		X		
Lipase test (or amylase if the lipase test cannot be carried out, according to the usual practice of the centres)	X				X		X		
Compliance		X	X	X	X	X			
Adverse events		X	X	X	X	X	X	X	X
Concomitant medications		X	X	X	X	X	X		
Patient notebook (+ extraction of hospital database if applicable) for consumption of pharmaceuticals, consultations...									X
Patient's and relative's perspective**: Health-related quality of life (SF-36), anxiety/depression (HADS), subjective well-being (SWLS) ; Patients perspective only: EQ-5D-3L	X *(EQ-5D-3L only)****							X	X
Interview with a researcher in psychology (20 patients and their relative in Nantes)									X

** These data will be measured at D28 and at D90 using quality of life questionnaires, sent by post (or given directly at the patient's discharge), or collected directly during the phone call.
These questionnaires will be filled by the patient (patient's perspective) and by one relative (relative's perspective), except for EQ-5D-3L, filled in by the patient only. If the patient is discharged from hospital, he will receive the questionnaires by post, to be returned to the clinical team, and he and his/her relative will be contacted by phone, to ensure the good completion of the questionnaires. If the patient is still hospitalized and his/her condition does not allow him to answer the questionnaires, only the questionnaire of the relative will be collected (from the relative's perspective). The phone call will ensure proper completion of the quality of life questionnaires (and the patient notebook for the patient). If the questionnaires haven't been completed and returned by post, the patient and the relative will answer to them during 10 to 15 minutes directly by phone.

*** At baseline, given that all patients will be unable to answer to a questionnaire, we will assume an equal level of quality of life for all. This level will be determined by a group of expert physicians before the inclusion in the trial. This score will be applied to all patients in the trial, at the baseline.

- **Screening (<48 hours after admission in intensive care unit).**

- **Inclusion/non-inclusion criteria:** all consecutive adult patients under mechanical ventilation will be assessed for eligibility. The clinicians will verify inclusion and exclusion criteria.

- **Informed Consent Form (ICF).** We anticipate that most patients will be unable to give consent before their inclusion (e.g. patients will be under invasive ventilatory support, sedated, or unconscious). In accordance with EU and national regulations, an emergency procedure for obtaining written consent from a legal representative will be submitted for approval to the relevant authorities and Ethics Committees. This procedure will allow some sites (depending on national regulatory approvals) to include the patients without having the patient's consent in a first time, nor the legal representative consent if he's not present (but the consent will be sought as soon as possible).

Apart from this emergency procedure, prior to the patient's inclusion in the trial, written consent from a legal representative will be obtained. The investigator informs the legal representative and answers all questions about the objective, the nature of the constraints, the foreseeable risks, the expected benefits of the research. He/she also specifies the patient's rights in the research and provides a copy of the information sheet and consent to the patient (or the legal representative). The legal representative will be invited to take time to reflect on the information provided and ask further questions. If the legal representative agrees to participate, the legal representative and the investigator record their full names, date and sign the consent form. A copy of this document is given to the legal representative and the original is kept by the investigator. The investigator will subsequently obtain patient consent to continue the research **as soon as the patient becomes able to consent** and clearly explain the study's aim and requirements to the patient, using the informed consent form, to be dated and signed.

Some patients may still be unable to give their consent before discharge from hospital, because of cognitive impairment either due to the initial pathology causing their hospitalization (for example: traumatic brain injury, stroke) or due to complications arising during their stay in ICU. If no legal representative is present to consent, best efforts will be made to obtain consent from a representative for the study. If the patient's representatives remain unreachable at the end of the study, patient's data will be analyzed. This procedure will be followed only in countries allowing such procedure, after ethics committee approval.

Information will be given in both **oral and written form** in the native language and non-medical terms so it can be fully understood. The trial will be described truthfully with regards to the purpose, nature, scope and possible consequences of the study. Patients and/or legal representative will be ensured that whatever their choice may be, there will be no consequences on the standard medical care received by the patient. It will be explained that agreement to participate must be made freely and willingly. Participants will be invited to take time to consider the information, to ask questions and to make further enquiries.

The trial information sheet will also contain detailed explanations about biological samples collected during the trials: type, quantity, destinations. Patients will also be required to consent to secondary use of their samples for further research after the end of the project (specific **biobank consent form**).

- The relative consent will also be obtained before asking them to complete the 1 month and 3 months questionnaires (SF-36, HADS, SWLS)

- In any case, the sponsor will comply with the regulations in force in each country regarding the collection of consent from persons unable to give consent

- For **women in child bearing age**, a urinary or a blood pregnancy test will be performed to rule out any ongoing pregnancy. As soon as patients are able to express their consent to continue the research, they will be asked to take effective contraception for up to 28 days after

the end of treatment.

- **Inclusion visit (within 48 hours after admission in intensive care unit).**
  The local investigator will perform a clinical examination of the patient.
  After the signature of the informed consent by the legal representative if available (emergency procedure for inclusion without patient or legal representative signed consent will be possible according national regulations), enrolled patients will be randomized by local investigators using a dedicated, password-protected, SSL-encrypted website (eCRF, Ennov Clinical) to allow immediate and concealed allocation. Each patient will be given a unique patient-number and a randomization number. Randomization sequence will be generated by blocks, and will be stratified according to cause of admission in ICU (sepsis or no), and according to the country (France, Spain or Greece). Patients will be randomized to:
    - **Arm 1: Recombinant Interferon gamma 1b** (IMUKIN®, from Clinigen®)
    - **Or arm 2: Placebo**.

  The investigator will provide the pharmacist, or the delegated ICU personnel (when applicable, depending on national SOP) with a prescription including the randomization number.
  EQ-5D-3L: given that all patients will be unable to answer to a questionnaire at the baseline, we will assume an equal level of quality of life for all. This level will be determined by a group of expert physicians.

Blinding

All vials of IMUKIN® and placebo will be labelled, and packed under supervision of the pharmacist of the French coordinating center (CHU Nantes). IMPs will be delivered to each specific study site. Only the pharmacy of the French study's coordinating center will be aware of vials' composition (coding list). At each study site, local investigators (surgeons, anesthetists), nurses and the patient will be blinded to the allocation group.

The computer program will assign kit numbers to the patient. Each study site will have sufficient IMPs to be allocated to include patients. This will ensure that the patient will receive only the treatment of the arm in which he was randomized. The IMUKIN® and placebo vials are manufactured, labelled and packaged to maintain the blind.

At each participating center, data will be collected and entered into the electronic web-based case report form (eCRF) by trial or clinical trained personal (clinical research associate), blinded to the allocation group, under the supervision of the trial site investigators.

Moreover, double blinding and the use of well-defined and pre-specified primary/secondary outcome measures will control for the risk of evaluation and reporting bias, respectively.

- **Recombinant Interferon gamma 1b or placebo administrations (day 1 to day 9).**

  The first injection of the study treatments (IMUKIN® or its placebo) is performed in the 6 hours following the randomization, followed by 4 injections of IMUKIN® or its placebo (1 injection every 48 hours +/- 2 hours until day 9).

  The pharmacist (or the investigator, depending on local SOP) will provide the allocated treatment (identified only by its identifying number) to the nurses of the intensive care unit. All members of sites pharmacy and intensive care unit including the doctor and the nurses will remain blinded to the allocated treatment group. The patient will receive the following Investigational Medicinal Products (IMPs), depending on his/her randomization arm:

Arm 1 (rHu-IFNγ):

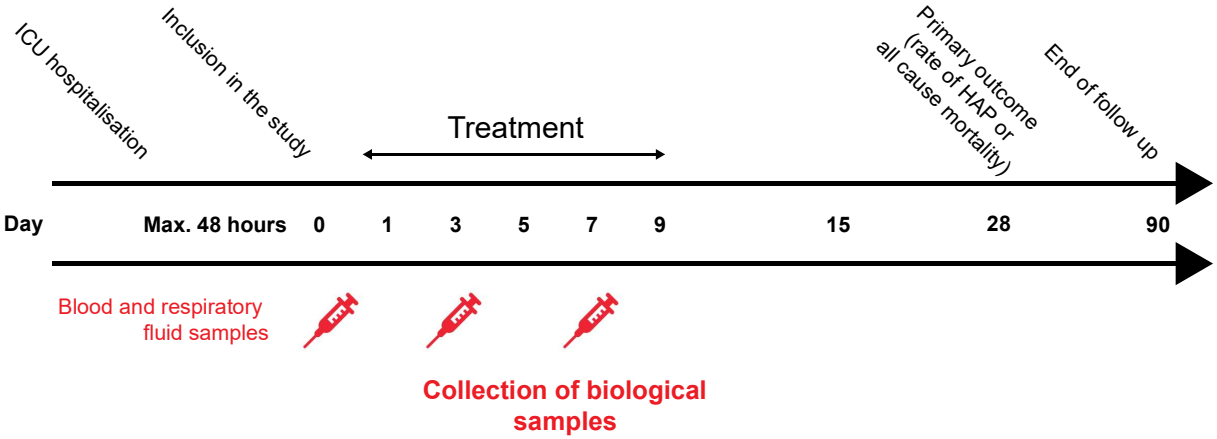
- **Recombinant Interferon gamma 1b** (IMUKIN®, from Clinigen®): 100 μg/0,5ml subcutaneous injections from day 1 to day 9 (5 injections, i.e. 1 injection of 100 μg every 48h, regimen adapted from SmPC),

Arm 2 (Placebo):

- **Recombinant Interferon gamma 1b placebo**: 5 subcutaneous injections from day 1 to day 9 (i.e. 1 injection of 0,5ml every 48h).

- **Collection of human samples**

  - Blood is collected at inclusion visit (between the randomization and the first administration of the study treatment), day 3 (before the 2nd injection), day 7 (before the 4th injection).
  - Tracheal aspirates are collected with Fibro mucus aspirators (provided to the sites by the sponsor) in patients with tracheal intubation at day inclusion (immediately before the first administration of the study treatment), day 3 (before the 2nd injection), day 7 (before the 4th injection). If the patient is extubated, no respiratory sample is to be collected.
  - In case of impossibility to collect the biological samples (blood and or respiratory fluid), the patients continue to receive the study treatment and is clinically followed according to the protocol.



- **Follow-up**

Standard of cares for the prevention of hospital-acquired pneumonia will comply with the international guidelines[19].

In each group, patients will be assessed:

- During ICU hospitalization:

- Daily clinical evaluation for the diagnosis of hospital-acquired pneumonia; follow-up of any AE/SAR/SAE;
- Liver function tests and blood count tests at Visit 0, Visit 2 (day 3), Visit 4 (day 7), Visit 6 (day 15)
- Lipase test (or amylase if the lipase test cannot be carried out, according to the

[19]Kallil et al. Clin Infect Dis 2016 (American guidelines); Torres et al. Eur Respir J 2017 (European guidelines); Leone et al. ACCPM 2018 (French guidelines)

usual practice of the centres) at Visit 0 (inclusion), Visit 4 (day 7) and Visit 6 (day 15)
- Compliance and concomitant medication follow-up

- Day 28: collection of the primary outcome. If the patient is discharged before day 28, the patient notebook will be given to the patient at discharge, and the investigator team will contact the patient and the relative at D28 to collect the outcome and to ensure a good completion of the questionnaires (EQ-5D-3L for patient and Health-related quality of life (SF-36) questionnaire, anxiety/depression (HADS) questionnaire and subjective well-being (SWLS) questionnaire for both patient and relative), and to check the correct completion of the patient notebook. The questionnaires will be completed by the patient and by the relative from their own perspectives (except for EQ-5D-3L, from patient's perspective only). The method for completion of the questionnaires will be recorded in the eCRF (patient alone; assistance required (identification of the person who provided assistance, e.g. relative, formal caregiver…); completion by the clinical team during the phone contact).

- Day 90: the study nurse or a local investigator will call the patient and his/her relative or the patient's family doctor to find out their vital status. Data for utility scores is collected with the EQ-5D-3L, quality of life is assessed using the SF36 questionnaire. HADS and SWLS will be filled for anxiety and depression signs, and for subjective well-being respectively. The 4 questionnaires (EQ-5D-3L, SF-36, HADS and SWLS) will be completed by the patient, and the questionnaires SF-36, HADS and SWLS by the relative from their own perspectives, and the method of completing the questionnaires will be recorded in the eCRF (patient alone; assistance required (identification of the person who provided assistance, e.g. relative, formal caregiver…; completion by the clinical team during the phone contact). As for the patient notebook, the study nurse or the investigator will verify the correct completion during the phone call, and it will be asked to the patient to send the notebook and the questionnaires back to the team.

- Day 90 (An ancillary study) will concern 20 patients included in Nantes and their relative, if they consent to. Semi-structured interviews will be conducted by a researcher in psychology to gain more insight into the understanding and the interpretation of quantitative data, as recommended by literature on human sciences.

- **Differences with routine clinical practice**
  The differences with routine clinical practice include:
  - the administration of IMUKIN® or of the placebo,
  - the liver blood tests at inclusion, day 3, day 7, day 15
  - the lipase test (or amylase if the lipase test cannot be carried out, according to the usual practice of the centres) at Visit 0 (inclusion), Visit 4 (day 7) and Visit 6 (day 15)
  - the collection of blood samples (a maximum of 19 blood tubes by catheter or direct vessel puncture) and respiratory fluid samples will be collected by the ICU nurses for each patient during the study (3 visits). It will represent a maximum amount of 183 ml of collected blood per patient.

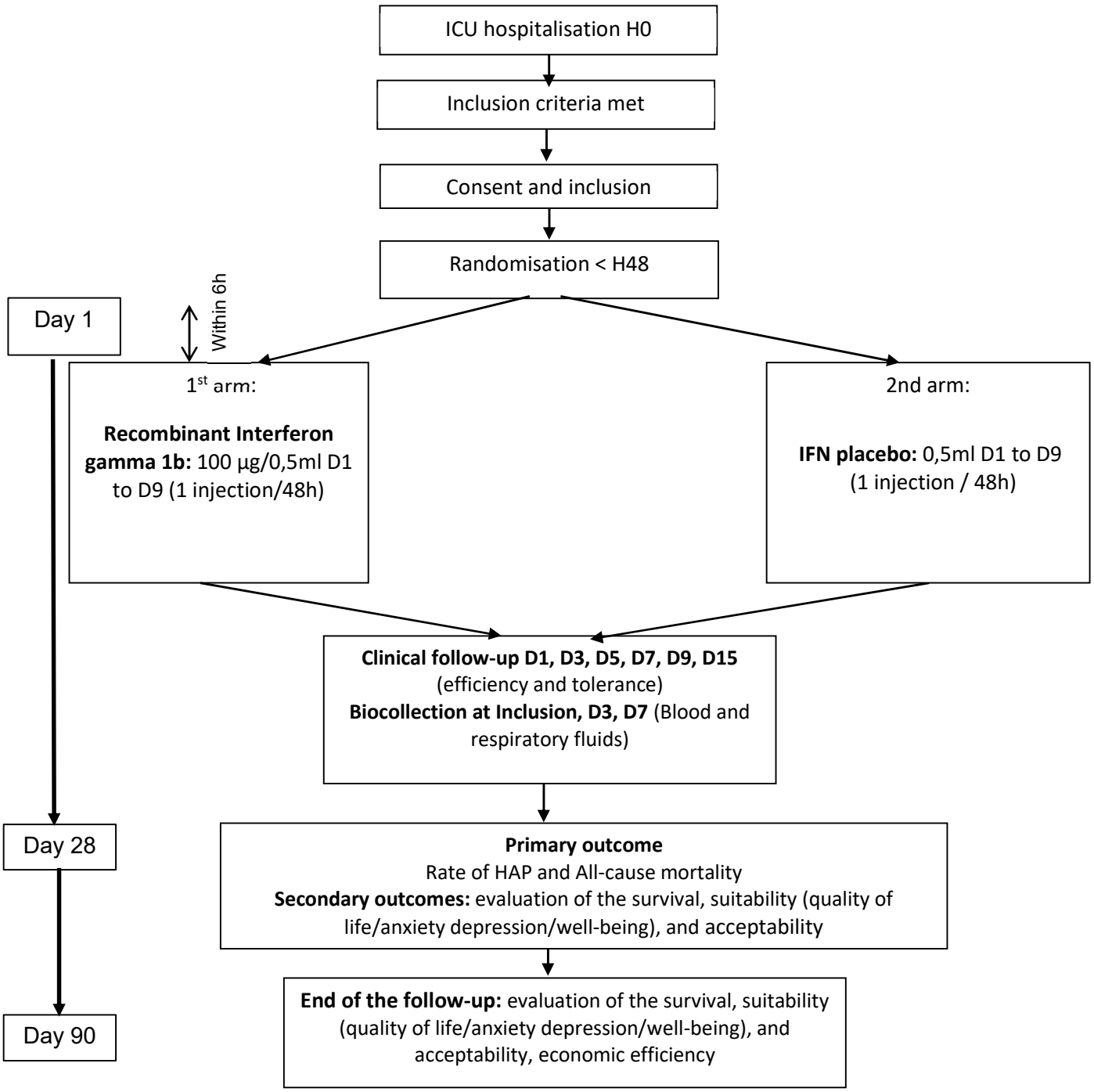
5.2. General study methodology

- Type of study: drug (**phase II**)
- **Multi-centre** international study
- **Placebo-Controlled, superiority study**
- **Randomised, stratified on the cause of hospitalization (sepsis or other) and country (France, Greece, Spain).**

- **Double-blind**
- **Parallel groups** study.

5.3. Study diagram

Recruitment duration: 24 months.
Follow-up duration / patient: 3 months
Duration of the entire trial: 27 months



5.4. Description and justification of the treatment plan

5.4.1. Cumulative Safety Information

IMUKIN® (recombinant interferon gamma-1b* – rHu-IFNγ*)
The drug has marketing authorization for the reduction of the frequency of serious infections in patients with chronic granulomatous disease or malignant osteopetrosis.

The real mechanism of action of interferon gamma-1b in such indications remained still unknown. Interferons are a family of functionally related proteins synthesized by eukaryotic cells in response to viruses and a variety of natural and synthetic stimuli. Findings related to superoxide anion production remain unequivocal. However, it is presumed that interferon gamma-1b increases macrophage cytotoxicity by enhancing the respiratory burst via generation of toxic oxygen metabolites capable of mediating the killing of intracellular micro-organisms. It increases HLA-DR expression on macrophages and augments Fc receptor expression, which results in increased antibody-dependent cell-mediated cytotoxicity.

In its MA indications, expected AE are detailed in 4.8 section of the SmPC. The most common adverse events are flu-like symptoms characterized by fever, headache, chills, myalgia or fatigue (with sometimes incomplete symptomatology). Hypersensitivity to the active substance (interferon gamma-1b) or to any of the excipients can't be excluded. Because Interferon gamma-1b is an exogenous protein, it may lead to the occurrence of antibodies during the course of treatment.

Regarding the study population, a Reference Safety Information (RSI) adapted to the study indication is provided by the sponsor. No SAR is expected and all serious adverse effects are considered as SUSARs.

Caution should be exercised when treating patients with known seizure disorders and/or compromised central nervous system function, cardiac disease, serious hepatic insufficiency and patients with severe renal insufficiency, because possible other adverse reactions, including those arising in special conditions.

Indeed, nausea, vomiting, abdominal pain seems to be common as well as depressive mood, reversible neutropenia and thrombocytopenia that can be severe and may be dose related have been observed. Liver enzyme increased that has been noted, especially in young children.

At high dosage or in case of overdose, reversible central nervous system adverse reactions including decreased mental status, gait disturbance and dizziness have been observed. Blood disorders including reversible neutropenia and thrombocytopenia as well as the onset of increased hepatic enzymes and of triglycerides have also been observed. Patients with pre-existing cardiac disease may experience an acute, self-limited exacerbation of their cardiac condition.

As pancreatitis (including fatal outcome) has also been reported as adverse effect (frequency not known), the sponsor will pay particular attention to lipase monitoring (or amylase if the lipase test cannot be carried out, according to the usual practice of the centres), considering diagnostic criteria of acute pancreatitis based on the fulfillment of two of the following ones: clinical (abdominal pain), laboratory (serum lipase or amylase > 3x upper limit of normal) and/or imaging criteria with characteristic findings of acute pancreatitis.

Interactions:

Interaction studies have only been performed in adults.

The interferon gamma 1-b does not reduce the efficacy of antibiotics or glucocorticoids; However, caution should be exercised when interferon gamma 1-b shall be associated with concomitant drugs, because It's also theoretically possible that hepatotoxic and/or nephrotoxic drugs might have effects on the clearance of interferon gamma 1-b or that interferon gamma 1-b potentially can prolong the half-lives of simultaneously administered drugs, which are metabolized by the cytochrome P-450 system.

So, concurrent use of drugs having neurotoxic (including effects on the central nervous system), haemotoxic, myelosuppressive or cardiotoxic effects may increase the toxicity of interferons in these systems.

The concomitant administration of heterologous serum protein preparations or immunological preparations (e.g. vaccines) may increase the immunogenicity of interferon gamma 1-gamma.

In conclusion

No SAR is expected and all serious adverse effects are considered as SUSARs Moreover, the sponsor will also pay particular attention to lipase monitoring (or amylase if the lipase test cannot be carried out, according to the usual practice of the centres), in order to prevent any risk of pancreatitis, considering diagnostic criteria of acute pancreatitis based on the fulfillment of two of the following ones: clinical (abdominal pain), laboratory (serum lipase or amylase > 3x upper limit of normal) and/or imaging criteria with characteristic findings of acute pancreatitis.

Placebo:

IMUKIN® placebo is a clear and colorless solution of NaCl 0.9%, to be administered by subcutaneous injection.

Regarding the composition, the secured process of preparation and the route of administration, only local AEs with pain, erythema, and irritation are expected. The amount of NaCl does not suggest systemic hydro electrolytic or blood pressure adverse effects, nor infection.

5.4.2. Cumulative Efficacy Information

Animals

In mice models of secondary pneumonia, treatment with IL-12 restores the production of IFN-γ by natural killer cells, increases the bacterial clearance and decrease mice weight loss.

Humans cells

In vitro treatment of PBMCs with rHuIL-12 restores the production of IFN-γ by natural killer cells collected in hospitalized patients. *In vitro* treatment of PBMCs with rHu-IFNγrestores the metabolic function of lymphocytes collected in hospitalized patients. Treatment of severe septic patients with rHu-IFNγ restores phagocytosis and antigen presentation by monocytes.

Humans patients

Case series of critically ill patients treated with rHu-IFNγ has confirmed the properties of immune-stimulation (intermediate outcomes). rHu-IFNγ was associated in clinical cure of hospital-acquire infections.

5.5. Samples management

Each center will prepare and freeze biological samples according to procedures imposed by the sponsor (centrifugation, aliquoting, freezing) and in accordance with the needs of the analytical laboratories. All the sites will use the reagents and consumables described in the SOP provided by the sponsor in order to limit preparation bias.

Samples will be stored in different boxes, depending on the nature of the samples.

A unique ID will be assigned to each aliquot. This ID will be reported on the label of each aliquot. At each visit, the samples will be recorded in the eCRF.

Each site will send every 3 months the prepared samples to the Biological Resources Centre of the Nantes University Hospital (CRB Nantes), whose follows the OCDE guidelines for biobanks and which is certified according to ISO 9001:2015 and to the French Afnor quality standard NF S96-900.

These samples will be transported in the presence of dry ice in order not to thaw the samples by a carrier authorized for transport classified UN3373. The list of sent samples will be exported from the eCRF.

At each reception, the CRB will carry out an inventory of each sample and check its labelling. PBMC samples will be stored in liquid nitrogen tanks (-196°C) and other types of samples will be stored in freezers at -80°C. The CRB's storage tanks and freezers are monitored and under automatic surveillance 24 hours a day to ensure the safety of the samples.
Finally, the samples will be sent to the various analytical laboratories twice: at mid-term and at the end of the studies. The transports will be carried out in dry ice by a carrier authorized for transports classified UN3373.

PREV-HAP Trial – Horizon2020 – RC20_0082

Day 1 | Day 3 | Day 7

Investigational sites

BLOOD

1 EDTA tube 3 ml (whole blood)

6 EDTA tubes (10ml) at D1, D3 and D7

5 2D barecode tubes (1ml with $10x10^6$ cells) for 5 initial EDTA PBMC (Nitrogen or -80°C for 3 months max)

A B C Biobank

4 2D barecode tubes (1ml) for 1 initial EDTA – Plasma (-80°C)

A B D Biobank

RESPIRATORY

1 Sterile cup / day D1, D3, and D7

3 2D barecode tubes (≥ 500µl) for 1 sterile cup - RESPIRATORY SECRETIONS

D E Biobank

Sample centralization and

*All samples centralized in **CRB Nantes** before dispatching in the Partners Labs*

Analytical labs

A

Radboudumc - Genetic, Epigenetic, Transcriptomic, Metabolomic

Total / patient: 1 EDTA tube 3ml (D1) ; 3 cryotubes PBMC (1/visit) on selected patients ; 3 cryotubes Plasma (1/visit)

B

Univ-Nantes - In vitro Functional Assays

Total / patient: 3 cryotubes PBMC (1/visit)

C

UZH - CyTOF

Total / patient: 6 cryotubes PBMC (2/visit)

D

Univ Nantes / Lyon Hosp Virome

Total / patient: 3 cryotubes plasma (1/visit) ; 3 resp. cryotubes (1/visit)

E

UMichigan - Microbiome

Total / patient: 3 resp. cryotubes (1/visit)

Samples collection details per patient

	Blood			Respiratory secretions
Inclusion – Day 1	✖		✖	✖
V2 – Day 3	✖			✖
V4 – Day 7	✖			✖
Samples collected:				
	EDTA tube 10ml		EDTA tube 3 ml	Sterile cup
Collected per visit	**6**		**1**	**1**
To prepare :				
Aliquots : 2D barcode microtubes	**4 x** 1ml  (1ml tubes)	**5 x** 1ml with $10x10^6$  (2ml tubes)	No aliquoting	**3 x <** 500µl  (0.5ml tubes)
Aliquot type	Plasma	PBMC	Whole blood	Respiratory secretions (transparent)
Sample requirements :				
Equipment & reagents needed for sample preparation	Centrifugation (1000xg, 10 min at RT) and aliquoting of plasma	Equipment: • safety cabinet II (sterile conditions) • centrifuge 1200xg • microscope & Malassez slide or equivalent Reagents: • Sterile Centrifuge Tube Falcon (50ml & 15 ml) • UNISEP tubes (provided by the Sponsor); • PBS 1x • hemolytic buffer • 4% Human albumin • DMSO	Freezing for DNA extraction	Aliquoting upon sterile conditions
Storage	-80°	Liquid nitrogen Or: -80°C for up to **3 months max**: →*Samples to be sent every 3 months to Nantes BRC for storage in liquid nitrogen*	-80°C	-80°C
Red flags	N/A	SOP provided by Sponsor - Personnel has to be trained	N/A	No centrifugation & and no additive

5.6. Identification of all data sources not included in the medical record

All the data sources will be included in the medical record of the patient including the quality of life questionnaires (EQ-5D-3L, Quality of life (SF-36), anxiety/depression (HADS), subjective well-being (SWLS)) at D28 and D90 and the patient's notebook, which will be then reported in the CRF.

5.7. Rules for discontinuing subject participation

5.7.1. Criteria in respect of early withdrawal of a subject from the study

Withdrawals from the study can only be effective after confirmation by the investigator and the sponsor. These withdrawals are always definitive.

These criteria should be clearly defined and validated by the study methodology expert.

In case of early withdrawal from the study, patients will be followed up until hospital discharge, according to routine clinical practice in each participating center.

Patients will be withdrawn **from the study** if:

- the patient, or his/her legal representative, withdraws consent,
- in case of legal criterion of non-inclusion not known at the time of inclusion due to the emergency inclusion procedure (patient under guardianship, curatorship, etc.)

Clinical data obtained before the consent withdrawal will be kept for the analyses. According to analysis populations, the patient will be excluded from the analyses or data will be imputed for the primary endpoint. These patients will not be replaced. In case of withdrawal after a SAR, patients will be followed up to the resolution or to the consolidation of this event.

A patient will be withdrawn from the **study treatment** for any of the following reasons (but are not limited to):

- Death
- Patient's request to withdraw his/her consent
- Patient becomes pregnant during the study period
- Liver Cytolysis (AST and/or ALT > 5N)
- Patient with suspicion and/or with identification of acute pancreatitis on the basis on the diagnostic criteria of acute pancreatitis including the fulfillment of two of the following ones: clinical (abdominal pain), laboratory (serum lipase > 3x upper limit of normal or amylase> 3x upper limit of normal if the lipase test cannot be carried out according to the usual practice of the centres) and/or imaging criteria with characteristic findings of acute pancreatitis
- Investigator's request to consider a change of therapy would be in the best interest of the patient
- Early termination of the study by the sponsor or a competent authority (safety reasons)

Patients should however remain in the trial for the purposes of follow-up and data analysis (with exception of patients who withdrew their consent).

5.7.2. Procedures in respect of early withdrawal of a subject from the study

For the data processing procedures in respect of subjects withdrawn early from the study, refer to the statistical section.

These patients will not be replaced.

In case of early withdrawal from the study treatment, patients should however remain in the trial for the purposes of follow-up and data analysis (with exception of patients who withdrew their consent). In case of withdrawal after a SAR, patients will be followed up to the resolution or to the consolidation of this event

In case of early withdrawal from the study, Clinical data obtained before the consent withdrawal will be kept for the analyses.

5.7.3. Criteria in respect of discontinuation of all or part of the study (excluding biostatistical considerations)

The end of the study is considered as the date of collection of all biomarker analyses.

This clinical trial will be followed by an independent Data and Safety Monitoring Board (DSMB); by a Steering Committee, as well as the entire consortium members of the HAP^2 project, in order to provide recommendations to the Sponsor regarding the safety of subjects, the conduct of the study and potential premature termination of the study.

An early, definitive or temporary discontinuation from part or all of the study can be done by Competent Authorities, Ethics committees, Sponsor or Data Safety Monitoring Board (DSMB)

In case of early discontinuation of the study on Sponsor's decision or DSMB, Ref-NCA, National Competent Authorities and Ethics Committees will be informed less than 15 days by mail.

In any case:

- A written confirmation of this early discontinuation of the study will be sent to Coordinator of this study and to each Principal Investigator of each center.
- All the patients included in the study will be informed and should realize the premature withdrawal visit.

The same applies to any investigator wanting to discontinue his/her participation to the study. The investigator must immediately inform the Sponsor in writing of this decision.

5.8. Patient medical care at the end of the study

No medical care related to the study will be continued after the end of the study.

The investigator will propose the best medical care to the patient, depending on his or her state of health at the end of the study

6. DATA MANAGEMENT AND STATISTICS

6.1. Data entry and data collection

6.1.1. Data entry, processing and circulation

Data collection for each person participating in the trial will be done with an electronic case report form (eCRF), created by the sponsor's data-management team, using a specialist software solution specifically designed for holding, auditing and checking trial data (Ennov Clinical Software).

Each person responsible for the filling of the eCRF (investigator, CRA...):

- will have to be identified in the table of delegations of responsibilities of each center (see investigator's file).
- Will have a "user" account with specific computer rights linked to his role (right to enter or modify a data, right to lock, monitor or sign a page of eCRF...)

Entering, viewing or modifying data will only be possible via the eCRF pages, on https://nantes-lrsy.hugo-online.fr/CSonline.

The data will be stored directly from the eCRF into the database hosted on a dedicated server, with controlled access (account/password) according to the user role. Any addition, modification or deletion of data will be recorded in a non-editable electronic file (the audit trail).

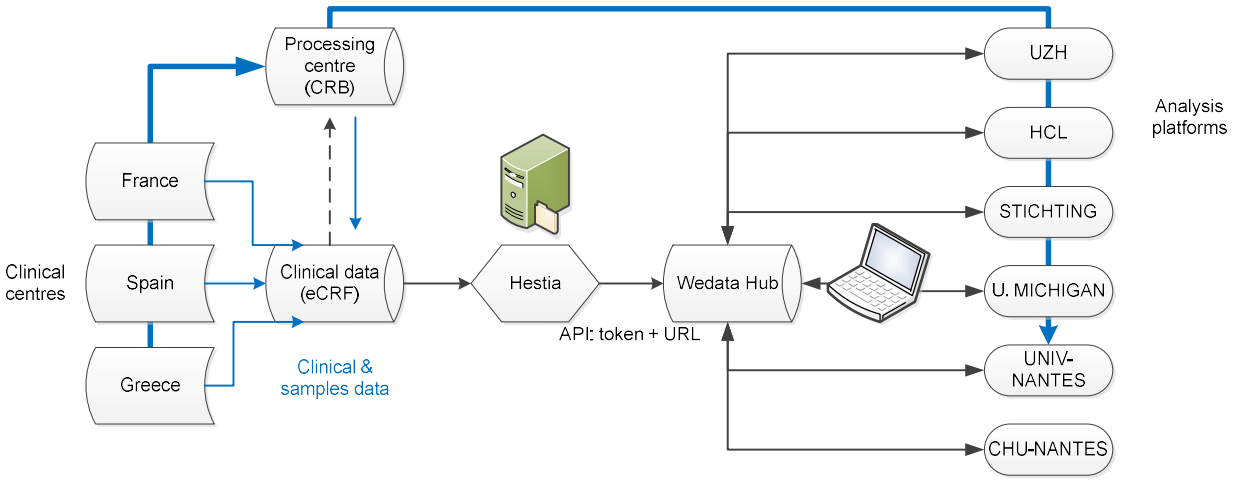
As for the health-economic analyse, extractions from the French hospitals database from the various participating centres will be sent by the investigation teams to the sponsor in a secure manner and stored on a secure server at the Nantes University Hospital accessible to the people responsible for the analysis.

6.1.2. Patient identification

The principal investigator and all co-investigators undertake to keep the identities of the persons who participate in the study confidential by assigning them a code (pseudonymisation). This code will be used for all the eCRF and all the attached documents (reports of imaging exams, biology, etc.). It will be the only information which will make it possible to make the connection with the patient retrospectively. The coding rule is the following: **month and year of birth, Inclusion number.**

6.1.3. Data Flow

The coded clinical data from the eCRF will be encrypted and automatically transferred to a different server of CHU Nantes, where it will be combined with the phenotypical, immunological, biomarker and multiomic's data generated in the context of HAP^2 WP3 for further analysis. A secure access to this second server will be created for the consortium's partner WeData (http://wedata.science/) for analysis, via a specific URL and token encrypted.



6.2. Statistics

6.2.1. Description of planned statistical methods, including planned intermediate analysis schedule

All analyses will be performed with the use of SAS software (version 9.4, NC, USA) before the breaking of the randomization code, according to International Conference on Harmonization-Good Clinical Practice guidelines.

Continuous variables will be presented as mean and standard deviations (as median and quartiles, otherwise). Categorical data will be presented as frequency and percentages.

All statistical analyses will take into account stratified randomization (cause of hospitalization (sepsis or not) and country (France, Greece, Spain)) as recommended in the CONSORT 2010 statement[20].

PRIMARY ANALYSIS

We will assess the efficiency of rHu-IFNγ for the prevention of hospital-acquired pneumonia with a Cox regression model (primary composite outcome: all-cause mortality at day 28 or the occurrence of hospital-acquired pneumonia within 28 days after randomization). Such an analysis combining the primary (occurrence of hospital-acquired pneumonia) and competing event (all-cause mortality) into a composite event has been recommended[21].

Crude and adjusted estimations on stratification factors will be given. The primary analysis will be adjusted on the stratification criteria and on center as a random effect.

SECONDARY ANALYSIS

To explore the risk of HAP in sub-populations (primary outcome), interaction terms between treatment arm and the following covariates will be tested in the Cox regression models (primary adjusted outcome):

[20] *CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials* BMJ *2010;340:c332*
[21] *Troendle JF et al.Stat Med.2018.*

- Randomization strata: Cause of hospitalization (sepsis or others), and country (France, Greece, Spain)
- Age (< or > 65 years)
- Severity upon ICU admission (Apache II 15-30, 30-45 or > 45)
- Time between the ICU admission and the first treatment injection (<24 hours; 24 to 36 hours, and 36-48 hours).
- Administration of glucocorticoid at the time of inclusion (yes or no)
- Analysis of the effect of treatment according to the COVID+ or COVID- status of the patients included in the study

All-cause mortality at day 90 will be analyzed with a Cox regression model, adjusted for stratification factors and on center as a random effect.

Categorical data (ventilator-associated tracheobronchitis at day 28, acute respiratory distress syndrome at day 28) will be analyzed with logistic regression model, adjusted for stratification factors and on center as a random effect.

Censored data (duration of antimicrobial therapy at day 28, duration of mechanical ventilation at day 90, duration of ICU hospitalization at day 90, duration of hospitalization at day 90) will be analyzed using Fine and Gray competing risks models to take into account the informative censoring and the competing risk due to death. The cumulative incidence functions (CIFs) of each competing event (death/end of antimicrobial therapy or death/extubation or death/end of hospitalization) will be estimated.

“Free-days” outcomes (antibiotic free days at day 28, mechanical ventilation free days at day 90, hospital free days at day 90) will be analyzed with a Mann-Whitney U.

Tolerance outcomes will be analyzed using logistic regression models.

PATIENT-REPORTED OUTCOMES DATA (SUITABILITY AND ACCEPTABILITY OF RHU-IFNγ)

Change in patient-reported outcomes data (quality of life, anxiety, depression) will be analyzed using longitudinal Rasch Measurement Theory (RMT)[22] models from the family of generalized random effects models. The RespOnse Shift ALgorithm at Item-level (ROSALI)[23] based on these models which has been showed to have good performance in a recently published simulation study[24] will be used. ROSALI will be developed and validated in WP5 using simulation studies to enable the use of RMT models as latent regression models to include covariates such as treatment, gender, and country. The development of ROSALI will allow investigating covariates’ effects on PRO (e.g. health-related quality of life) changes over time as well as on patients’ adaptation through response shift analyses (see WP5).

Patients' adaptation to their condition will also be investigated using regression analyses to test for the possibility of changes in the relationship between the patients' subjective well-being and their health-related quality of life[25]. Investigating this form of adaptation is important to assess the validity of one of the assumptions of the QALY (Quality-Adjusted Life-Years) measure that is commonly used to represent the effectiveness part of cost-effectiveness analyses (see the part describing the cost-effectiveness analysis). Various models will be estimated and compared to take into account factors such as unobserved individual heterogeneity for instance.

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22 Fischer GH, Molenaar IW, eds. Rasch Models: Foundations, Recent Developments, and Applications. New York: Springer-Verlag; 1995

23Guilleux A et al.. Qual Life Res.

[24] Comparison of structural equation modelling, item response theory and Rasch measurement theory-based methods for response shift detection at item level: A simulation study.Blanchin M, Guilleux A, Hardouin JB, Sébille V.Stat Methods Med Res. 2019 Oct 30:962280219884574. doi: 10.1177/0962280219884574.

[25] Tessier P, Blanchin M, Sébille V. Does the relationship between health-related quality of life and subjective well-being change over time? An exploratory study among breast cancer patients. Soc Sci Med. 2017 Feb;174:96-103. doi: 10.1016/j.socscimed.2016.12.021.

Semi-structured interviews will be conducted with patients and caregivers to gain more insight into the understanding and the interpretation of quantitative data [26]. An interview guide will be developed on the basis of the literature on the psychological consequences of the immune intervention for patients. Interviews will be audiotaped and transcribed verbatim by the interviewer with the patients' consent. Qualitative data will be analyzed using a lexical analysis to describe what patients have told, together with a content analysis (thematic categorial classification, Bardin, 2003 ; Blanchet &Gotman, 2007) to highlight the themes of the corpus and interpret data[27]. *See 2.3. Objective and endpoints for ancillary studies.*

6.2.2. Statistical justification of the number of inclusions

We will include 200 patients (100 patients receiving placebo, 100 patients receiving rHu-IFNγ). The rate of non survivors and/or hospital-acquired pneumonia in the placebo group is expected to reach 35%[28]. In this phase II clinical trial, the size of the effects with the studied treatment can not be estimated from current knowledge about the effect of these therapeutic strategies. We thus decided to rely on the recruitment capacity of the European centers allowing the inclusion of 100 patients / group over 24 months. This sample size will allow detecting a hazard ratio of 0.625 as compared to placebo with a 90% of statistical power and a double-sided type I error α at 5%.

6.2.3. Expected level of statistical significance

A two-sided P value of less than 0.05 will be considered for all analyses.

6.2.4. Statistical criteria for discontinuation of study

No interim analysis is planned for the efficiency.

6.2.5. Consideration method for missing, unused or invalid data

Lost to follow-up and missing data

There should be neither missing data nor lost to follow-up for the primary outcome which will be recorded in intensive care unit. Missing data will be described by treatment arm. According to the rate of missing data (over 5%) sensitivity analyses will be performed using multiple imputation methods.

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26 *Paillé, P., & Muchielli, A. (2005). L'analyse qualitative en sciences humaines et sociales. Paris: Armand Colin.*

27 *Blanchet, A., &Gotman, A. (2007). L'enquête et ses méthodes: L'entretien. Paris: Armand Colin, et Bardin, L. (2003). L'analyse de contenu. Paris: PUF.*

28Koulenti et al. Crit Care Med 2009 ; Asehnoune et al. Intensive Care Med 2017, Alvarez-Lerma et al. Crit Care Med 2018, and unpublished data from the Pneumocare study (1800 patients in 34 ICUs in France, 2018, numberclinical trial: NCT03348579)

Early withdrawals,
Withdrawals from the study can only be effective after confirmation by the investigator and the sponsor. These withdrawals are always definitive. In case of withdrawal from the study, patients will be followed up until hospital discharge, according to routine clinical practice in each participating center. Clinical data obtained before the consent withdrawal will be kept for the analyses. According to analysis populations, the patient will be excluded from the analyses or data will be imputed for the primary endpoint. These patients will not be replaced.
In case of withdrawal after a SAR, patients will be followed up to the resolution or to the consolidation of this event.

Non-compliance with the protocol
In case of non-compliance to the treatment regimen and/or to the collection of biological samples, the patients will be followed up to the end of the study, and the data will be kept for the analyze in intention to treat.

6.2.6. Management of changes made to the initial analytical strategy

An early, definitive or temporary discontinuation from part or all of the study can be done by Competent Authorities, Ethics committees, Sponsor or Data Safety Monitoring Board (DSMB)
In case of early discontinuation of the study on Sponsor's decision or DSMB, Ref-NCA, National Competent Authorities and Ethics Committees will be informed less than 15 days by mail.
In any case:

- A written confirmation of this early discontinuation of the study will be sent to Coordinator of this study and to each Principal Investigator of each center.
- All the patients included in the study will be informed and should realize the premature withdrawal visit.

The same applies to any investigator wanting to discontinue his/her participation to the study. The investigator must immediately inform the Sponsor in writing of this decision.

6.2.7. Choice of subjects to be included in analysis

Analyzes will be conducted, first, on data from the intention-to-treat (ITT) population, second, in the modified intention-to-treat (mITT) population as well as in the per-protocol population (PP).

- **Intention-to treat (ITT):** All randomized patients in the group in which they were randomised, regardless of the medical device/treatment received and breaches of the protocol. In case of missing data, the analysis of the ITT population will be performed by multiple imputation methods using demographic data (age, gender), stratification factors, IGS-II and cause of admission.
- **Modified intention to treat (mITT):** Randomized patients who have an assessable clinical outcome within the assessment window, fulfilling the major inclusion criteria, without major non-inclusion criteria, without consent withdrawal and who received at least one dose of treatmentare analyzed in the group in which they were randomised, regardless of the medical device/treatment received and other breaches of the protocol.

- **Per protocol (PP):** Randomized subjects who were treated in full compliance with the protocol (exclusion of the patients of the rHu-IFNγ group who have not received the complete drug regimen)

6.2.8. Economic evaluation

The cost-effectiveness analysis will be conducted from the perspective of the society with a three-month time horizon.

Assessment of costs

For all patients in the study the use of resources at the hospital and outside will be collected prospectively. Two modes of data retrieval will be combined: i) clinical research associates will record the consumption of resources in the hospital in combination with a database extraction of hospital information (outpatient consultations and procedures, hospitalizations) and ii) we will distribute diaries to patients to collect information about resources consumption after the initial hospitalization.

Patient pathways after discharge from initial hospitalization are defined as either follow-up within an after-care and rehabilitation structure, a return home, or a move to another care unit within the same hospital or one closer to the patient's home, cf. Figure 1.

We will retrieve within the hospital only resources related to pneumonia, for data retrieved outside the hospital we will be unable to differentiate what is due to pneumonia from other care so we will ask patients to record all care resources used over the period up to day 90. This will include, most notably, ICU length of stay, pharmaceuticals and consultations. We will also collect information about the time from caregivers (whether professional or informal) and about day out of work to value production losses.

To estimate costs, unit costs per each type of resource consumed will be estimated using accounting information, NHI official tariffs, and the hospitals' prices charged in different countries (France, Spain and Greece). The time of caregivers devoted to monitoring and assisting patients will be valued by applying a salary valorization equivalent to a home help job (proxy good method), and, we will calculate productivity losses using work stoppages.

Concerning the French patients, in case the return of patients' diaries on resource use would be deemed insufficient (less than 65% of the total) to carry out our study, we would use the database of the French Health Insurance in order to retrieve the consumption of ambulatory and hospital healthcare.

Resource use and resource unit costs will thus be collected and estimated for each country.

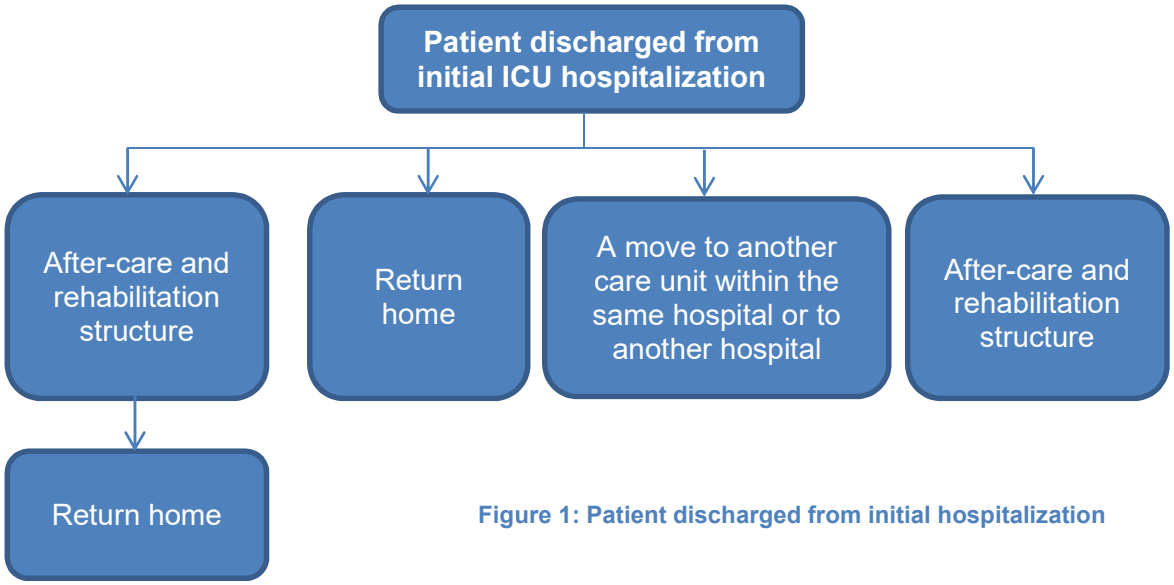


Figure 1: Patient discharged from initial hospitalization

Measures of effectiveness

The measure of effectiveness for the economic evaluation will be the number of QALYs. QALYs represent a measure of survival (life-years) weighted by health-related quality of life factors such that a weight of 0 represents death and a weight of 1 represents the best imaginable health state. An advantage of QALYs is that they allow to combine information about the length and the quality of life in a single index measure. In the study, QALYs will be estimated from answers to the EuroQol EQ-5D-3L quality of life questionnaire at the baseline, at day 28 and at three months after inclusion. Given that all patients will be unable to answer to a questionnaire at the baseline, we will assume an equal level of quality of life for all. This level will be determined by a group of expert physicians. If the patient is unable to complete the questionnaire at day 28 and at day 90, we will use the proxy version of the EQ-5D that will be completed by a physician.

To allow for the comparison between countries, we will use the European harmonized tariffs[29] to convert the EQ-5D answers into utility scores as was recently done in a multinational European cost-effectiveness analysis[30].

Results analysis

The cost-effectiveness analysis will be conducted on an intention to treat basis. Missing data about costs and QALYs will be imputed using multiple imputation methods.

Mean costs per type of resource used, mean total costs and mean QALYs per patient and their corresponding standard deviations will be presented. Differences in costs, in QALYs and the ICER will be estimated as well as the corresponding acceptability curve, i.e. the curve indicating the probability for an intervention to be cost-effective given the society's willingness to pay an additional unit of effectiveness (i.e. an additional QALY gained). We will also perform sensitivity analyses to assess the robustness of the results to the main assumptions of the analysis such as

[29] *Eur J Health Econ. 2003 Sep;4(3):222-31. A single European currency for EQ-5D health states. Results from a six-country study. Greiner W(1), Weijnen T,Nieuwenhuizen M, Oppe S, Badia X, Busschbach J, Buxton M, Dolan P, Kind P, Krabbe P, Ohinmaa A, Parkin D, Roset M, Sintonen H, Tsuchiya A, de Charro F*

[30] *J Antimicrob Chemother. 2018 Nov 1;73(11):3189-3198. doi: 10.1093/jac/dky309.*
Cost-effectiveness of internet-based training for primary care clinicians on antibiotic prescribing for acute respiratory tract infections in Europe.
Oppong R1, Smith RD2, Little P3, Verheij T4, Butler CC5, Goossens H6, Coenen S6,7, Jowett S1, Roberts TE1, Achana F8, Stuart B3, Coast J9

the methods to manage missing data, the methods to value units costs or the inclusion/exclusion of production losses for instance.

We will perform overall and country-specific cost-effectiveness analyses: incremental cost-effectiveness ratios (ICER) will be estimated for the whole sample (pooling together the data from the participating countries) and per country. Given the variety of approaches that may be followed to determine the cost-effectiveness ratios in multinational trials[31] [32], we will explore various approaches in sensibility analysis to assess the robustness of the results.

6.2.9. Randomisation

This is a **centralized randomization** performed directly on the eCRF with stratification on the cause of hospitalization (sepsis or not) and country.

The eCRF will be developed by CHU-Nantes, using a specialist software solution specifically designed for holding, auditing and checking trial data (Ennov Clinical Software - https://nantes-lrsy.hugo-online.fr/CSonline).

After the informed consent of patient's legal representative will be obtained, enrolled patients will be randomized by local investigators using this dedicated, password-protected, SSL-encrypted website to allow immediate and concealed allocation. Each patient will be given a unique patient-number and a randomization number. Randomization is performed in the first 48 hours following the admission in the intensive care unit.

[31] *Health Econ. 1998 Sep;7(6):481-93. Estimating country-specific cost-effectiveness from multinational clinical trials. Willke RJ1, Glick HA, Polsky D, Schulman K.*

[32] *Health Econ. 1998 Sep;7(6):481-93. Estimating country-specific cost-effectiveness from multinational clinical trials. Willke RJ1, Glick HA, Polsky D, Schulman K.*

7. PHARMACOVIGILANCE AND ADVERSE EVENT MANAGEMENT

7.1. Definitions

Pharmacovigilance	Science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem.
Adverse events (AE)	Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment An adverse event can therefore be any unfavourable and unintended sign (e.g. an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
Adverse reactions (AR)	A response to a medicinal product which is noxious and unintended. Response in this context means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility. An adverse reaction, in contrast to an adverse event, is characterised by the fact that a causal relationship between a medicinal product and an occurrence is suspected
Adverse reaction of an experimental medicinal product – Adverse Drug Reaction (ADR)	All untoward and unintended responses to an investigational medicinal product related to any dose administered.
Adverse reaction/event Intensity	Rated according to the CTCAE v.5.0 (excerpts in appendix XX) Any event not rated in the selected classification should be rated as follows: Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*. Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**. Grade 4 Life-threatening consequences; urgent intervention indicated. Grade 5 Death related to AE. *Activities of Daily Living (ADL)* **Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.* ***Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.*

Serious adverse events (SAE)	Any untoward medical occurrence or effect that: - results in death, - is life-threatening, - results in persistent or significant disability or incapacity, - requires hospitalisation or prolongation of existing hospitalisation, - is a congenital anomaly or birth defect. - is medically significant)
Unexpected adverse reactions	An adverse reaction, the nature or severity of which is not consistent with the applicable product information.
Suspected Unexpected Serious Adverse Reactions (SUSAR)	An untoward and unintended response to an investigational medicinal product, which is not listed is the applicable product information, and meets one of the serious criteria.
Emerging safety issue	Any new safety issue considered by the sponsor to require urgent attention by the competent authorities because of the potential major impact on the risk-benefit balance of the medicinal product and/or on patients' or public health and the potential need for prompt regulatory action and communication to patients and healthcare professionals.: ➢ Induce new evaluation of benefit/ risk ratio of the study or of the product object of the study, ➢ modify product utilization, the conduct of the study or documents related to the study ➢ Suspend or terminate the protocol under research or similar researches.
Causality	The Investigator must determine the relationship between the administration of IMP and the occurrence of an AE/SAE as Not Suspected or Suspected as defined below: *Not suspected:* A causal relationship of the adverse event to IMP administration is unlikely or remote, or other medications, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event. *Suspected:* There is a reasonable possibility that the administration of IMP caused the adverse event. 'Reasonable possibility' means there is evidence to suggest a causal relationship between the IMP and the adverse event. **Causality should be assessed and provided for every AE/SAE based on currently available information. Causality is to be reassessed and provided as additional information becomes available.**
Abuse	This corresponds to the persistent or sporadic, intentional excessive use of a medicinal product, which is accompanied by harmful physical or psychological effects.
Overdose	This refers to the administration of a quantity of a medicinal product given per administration or cumulatively, which is above the maximum recommended dose according to the authorized product information. Clinical judgement should always be applied.

	(Real overdose: due to a brut excessive amount / relative overdose: due to patient predisposal factors as renal insufficiency, hypo-albuminuria…)
Misuse	This refers to situations where the medicinal product is intentionally and inappropriately used not in accordance with the authorised product information.
Quality defect	Non conformity to the specifications described in the marketing authorisation file / CE marking / technical documentation or deviation against good manufacturing practices / good distribution/storage/labelling practices.
Medication Error	Medication errors are unintended failure (proved or potential) during the drug treatment process(from manufacturing to administration) that leads to, or has the potential to lead to, harm to the patient (risk or an adverse event for the patient).The risk of error or potential error concerns situations where the error did not happen, was intercepted but could have happen.

7.2. Safety evaluation parameters

7.2.1. Specific safety-related evaluation criteria

According to regulation, each AE/AR reported by the patient or identified by the investigator must be collected and reported to sponsor, as soon as he is aware, if it meets to seriousness criteria from inclusion of the subject, to the end of the participation. In addition, liver blood test will be carried out during the treatment period followed.

7.2.2. Methods and schedule envisaged to measure, compile and analyse safety evaluation parameters

Any AR/AE whether expected or unexpected, serious or not, must be real-time collected in the study eCRF.
Liver blood test will be followed at day 3, day 7 and day 15, and lipase test (or amylase if the lipase test cannot be carried out, according to the usual practice of the centres) at day 7 and Day 15.

7.3. Expected ARs

In this protocol, the expected Adverse Event and Reactions are associated with the drug under study (IMUKIN®) and its comparator (IMUKIN® Placebo), concomitant drugs (antibiotics, painkillers, bronchodilatators, hypnotics…), protocol and disease.

The IMUKIN® Reference Safety Information is provided by the sponsor.
For the IMUKIN® placebo, the Reference Safety Information is in the current version of NaCl SmPC (section 4.8). Even if the administration route is different in PREV-HAP study than in the MA indication, this is a common practice to inject NaCl subcutaneously, with much larger volumes in geriatrics and palliative care. There is no particular risk due to the very limited volume, the

infectious risk of any subcutaneous injection being managed by classic non-specific barrier measures

Expected ARs for IMP are:

IMUKIN®(recombinant interferon gamma-1b – rHu-IFNγ): No SAR is expected and all serious adverse effects are considered as SUSARs.

As pancreatitis (including fatal outcome) has also been reported as adverse effect (frequency not known), the sponsor will pay particular attention to lipase monitoring (or amylase if the lipase test cannot be carried out, according to the usual practice of the centres), considering diagnostic criteria of acute pancreatitis based on the fulfillment of two of the following ones: clinical (abdominal pain), laboratory (serum lipase or amylase > 3x upper limit of normal) and/or imaging criteria with characteristic findings of acute pancreatitis.

IMUKIN® placebo:

Regarding the composition, the secured process of preparation and the route of administration, only local AEs with pain, erythema, irritation are expected for the placebo ; the amount of NaCl does not suggest systemic hydro electrolytic or blood pressure adverse effects, nor infection.

The expected ARs for concomitant drugs are not different of those observed in standard care and are listed in current version of each product's SmPC (section 4.8).

Concerning the protocol, conventional medical examination/evaluation in the overall care of patients, the excess of risk identified protocol dependent should be un-frequent.
Indeed, current procedures will induce the most frequent expected disorders: all patients receiving mechanical ventilation and suspected of hospital-acquired pneumonia have an intravenous device for the realization of intravenous injections during several days, and daily biological monitoring is a standard of cares.

- Local complications of subcutaneous administration, inflammation, infections.
- Local complications of blood sampling (hematoma, moderate pain)
- Complication of ventilation and intensive care unit standard care (as MD for ventilation, urinary catheter…)
- Complication of prolonged hospitalization in ICU (nervous and musculoskeletal disorders…)

Concerning the disease, the most frequent expected AEs in patients hospitalized in ICU and requiring mechanical ventilations are (non exhaustive list):

- Death
- Hospital acquired infections (pneumonia, septicaemia, urinary tract infections, surgical site infection)
- Organ failure (Respiratory distress syndrome, Acute Kidney Injury, Liver insufficiency, Hemodynamic shock)
- Haemorrhage
- Gastric ulcer
- Venous Thrombosis, pulmonary embolism
- Stroke
- Neuromyopathy
- Bed sores

- Prolonged mechanical ventilation

7.4. Adverse Events of Special Interest

Regarding the specificity of the study, these Adverse Events/Reactions should be considered and reported as SAEs:

- all acute respiratory distress syndrome (ARDS) developed after the first IMP administration related or not to an IMP
- seizure related to Interferon-γ/NaCL

7.5. Adverse event management

7.5.1. AR/AEcollection

Any AR/AE (unless specify otherwise below), whether expected or unexpected, serious or not, must be real-time collected in the study eCRF.

7.5.2. SAR/SAE reporting

All SARs/SAEs initial and follow-up information (except those specified below), whether expected or unexpected, must be reported without delay, and at the latest within 24 hours to the sponsor from the day the investigator becoming aware of the event, using the eCRF (in case of unavailability, the SAE/SAR notification should be sent to the sponsor by e-mail to recherche-pv@chu-nantes.fr).

The investigator documents the event and the medical diagnosis as well as possible: the information on this SAE/SAR form and on the attached documents must be complete, precise, clear (no use of abbreviations...) and coded.

The Investigator will report the action taken with IMPs as a result of an AE or SAE, as applicable (e.g., discontinuation or reduction of IMP, as appropriate) and report if concomitant and/or additional treatments were given for the event.

The investigator must establish a causal link between the adverse events.

The occurrence of a new safety event should be reported to the sponsor.

Pregnancy:

If a woman becomes pregnant as part of the research or if her partner is participating in the research, the pregnancy must be notified immediately to the sponsor.
The investigator informs the sponsor using the Pregnancy Form.
The investigator should follow the patient until the term of pregnancy or its interruption and notify the sponsor of the outcome using the Pregnancy Follow-up Form.
In the case of paternal exposure, the investigator should obtain the consent of the parturient to collect the pregnancy information.

Special Situation:
Overdose, misuse, errors or potential errors, quality defects are also reported to the sponsor, even if there is no associated adverse event, using the Special Situation Report (SSR) form.

7.5.3. Exclusion from reporting/notification

Regarding the specificity of the study, some adverse events have not to be collected in safety section of the eCRF:
AEs related to the ICU management or related to the medical history (notably the normal course of cause of ICU admission) and which are those classically observed in this context will not be collected as part of this protocol, with the exception of those related to the medicinal products under study or its comparators and placebo, which will be properly collected and notified if necessary.

7.5.4. Reporting period

All SAE/SAR must be reported to the sponsor if it happens for a research participant:

- Since the consent signature date,
- During all the participant follow up period scheduled by the study up to the collection of the primary outcome (day 28)

After the end of the patient follow-up and without any time limit if the investigator becomes aware of a SADR possibly linked to one of IMP, including Placebo.

7.5.5. Data and Safety Monitoring Board (DSMB)

The sponsor is responsible for setting up a Data and Safety Monitoring Board (DSMB), and for providing all operating support. The DSMB is an independent advisory committee which will review the safety data and provide recommendations to the Sponsor regarding the safety of subjects, the conduct of the study and potential premature termination.
Its 5 members, well-versed in the field of clinical trials (pathology, safety, ethic and methodology), are not involved in the study. They are appointed for the period of the study and undertake to participate and to respect the data confidentiality. The members of the DSMB are selected collectively by the coordinator and the sponsor.
The DSMB will be the recipient of all suspected unexpected severe adverse reaction (SUSAR), the annual safety reports and may be consulted by the sponsor if a SUSAR or SADR involves a specific analytical problem or in the event of doubt on the risk benefit arising in the course of the study. Usually blinded data will be submitted, but in case of difficulty or of suspected unbalanced risk, unblinded data could be discussed and, if required, provided exclusively to the DSMB members by the statisticians.

A meeting will be planned at the beginning of the study, to present the protocol to the DSMB members, and to plan the next steps and meetings of the DSMB. There will be at least one annual meeting, and the frequency of other meetings will be determined during the first meeting

The list of members of the DSMB is attached in appendix 8.

The working group on Pneumonia of the **European Society of Intensive Care Medicine (ESICM)** and the "Comité Reanimation" from the **French Society of Anesthesiology and Reanimation (SFAR)** have reviewed the study protocol before the grant application, and they will receive regularly a summary of the safety reports, for informationyearly the safety reports and provide scientific advice.

7.5.6. Responsibilities of the sponsor

The sponsor is responsible for the ongoing assessment of the safety of the research, both in terms of the procedures performed and the treatments used.

In accordance with the applicable regulations, the sponsor will report any suspicion of SUSAR to the competent authorities within the regulatory timeframe (European and national's regulations).

The sponsor shall report relevant additional information regarding unexpected serious adverse reactions in a follow-up report to EMA and National Competent Authorities (ANSM, AEMPS, EOF).

7.6. Follow-up procedure and period for subjects following the onset of adverse events

7.6.1. Procedure to follow for the patient concerned by the SAE

All events/reaction must be followed up until recovery, consolidation or death (event closed).

Pregnancy occurring during the study should be followed up at least until birth or even until the child reaches adulthood.
Delayed adverse reactions must be reported to the sponsor (if known to the investigator) even after the end of the study.

8. ADMINISTRATIVE AND REGULATORY ASPECTS

8.1. Source data and document access rights

Each patient's medical data shall only be provided to the sponsor or any person duly authorised by the sponsor, and, where applicable, to authorised health authorities, in confidential conditions. The sponsor and the competent authorities may request direct access to medical records for the purposes of verification of the procedures and/or data in respect of the clinical trial, within the limits authorised by the legislation and regulations.
The data compiled during the trial may be processed electronically in compliance with GDPR requirements.

8.2. Trial monitoring

Monitoring will be carried out by the Sponsor in France, by the National Coordinating Center in Spain and a CRO in Greece. A Clinical Research Associate (CRA) shall visit each site (investigator and pharmacy) regularly to conduct quality control on the data reported in the case report forms. All the CRAs will work with the same procedures whatever the country. The Sponsor will provide the SOPs and the monitoring manual.

The protocol has been classified according to the estimated level of risk for the patient taking part in the study. It shall be monitored as follows:

Risk C: high foreseeable risk

The monitoring frequency and intensity is dependent on the risk associated with the study: 100% of data from 100% of the patients.
A monitoring plan, validated by the investigator, project manager and monitoring CRA defines the data to be monitored and the frequency of visits.

The on-site monitoring visits shall be organised after making arrangements with the investigator. The CRAs should be able to consult on each site:
- the enrolled patients' data compilation records,
- the patients' medical and nursing files,
- the investigator file.
- the treatment storage and dispensation place

The CRA will submit regular visit reports to the Sponsor’s Project Manager.

Each patient's medical data shall only be provided to the sponsor or any person duly authorised by the sponsor, and, where applicable, to authorised health authorities, in confidential conditions. The sponsor and the competent authorities may request direct access to medical records for the purposes of verification of the procedures and/or data in respect of the clinical trial, within the limits authorised by the legislation and regulations.
The data compiled during the trial may be processed electronically in compliance with data protection regulatory requirements of each country. Patient anonymity will be finally conserved.

8.3. Inspection / Audit

Within the scope of this study, an inspection or audit may be conducted. The sponsor and/or participating centres should be able to provide inspectors or auditors with access to the data.

8.4. Written informed consent/Emergency consent form collection

In the study's context, inclusions will be realized in immediate vital emergency situation. Indeed, the treatment of hospital-acquired pneumonia is an emergency and it is recommended to start the treatment within the first hour after the diagnosis (Torres et al. European Guidelines for hospital-acquired pneumonia. EurRespi J 2017). Given the inclusion criteria (critically ill patients suffering acute pneumonia, or at risk of pneumonia due to critical illness), patient won't be able to express their consent before the inclusion in the study. Thus, an emergency consent procedure is needed and justified.

Moreover, the exclusion of patients unable to provide informed consent before the inclusion of the HAP^2 trials would induce a major bias, jeopardizing the scientific quality of the project.

The procedure of acquisition of the consent of the legal representatives will be described as follows, and will comply the national regulations in force, after Ethics Committees approval:

In case of patients able to provide consent at the time of inclusion:

Patients will be informed in complete and faithful terms and in understandable language of the objectives and constraints of the study, the potential risks, the required observation and safety measures, and their right to refuse to participate in the study or to revoke their consent at any time. The investigator must also inform the subjects of the Ethics Committee opinion. All information appears in an information notice and consent form given to the patient. Written informed consent will be obtained by the investigator. These documents will be approved by the competent Ethics Committee. Two original copies will be co-signed by both the investigator and the patient. The second copy is to be kept in the patient's medical record.

In case of patients unable to provide consent at the time of inclusion:

1. **First, the authorisation will be given by the legal representative** (see description below 1.a**)** or failing that an emergency procedure will be applied, in countries where local regulations allow this procedure (see description below 1.b).
    a. The investigator agrees to provide the **legal representative** with clear and precise information about the protocol and request from him/her a written and signed consent form (information form and consent form appended). The **legal representative** will sign and date the consent form, after taking time to reflect on the matter. The investigator shall also sign and date the consent form.

    The investigator's original shall be placed in the investigator file. The consent form is signed in duplicate: the investigator keeps the original and gives the copy to the support person.

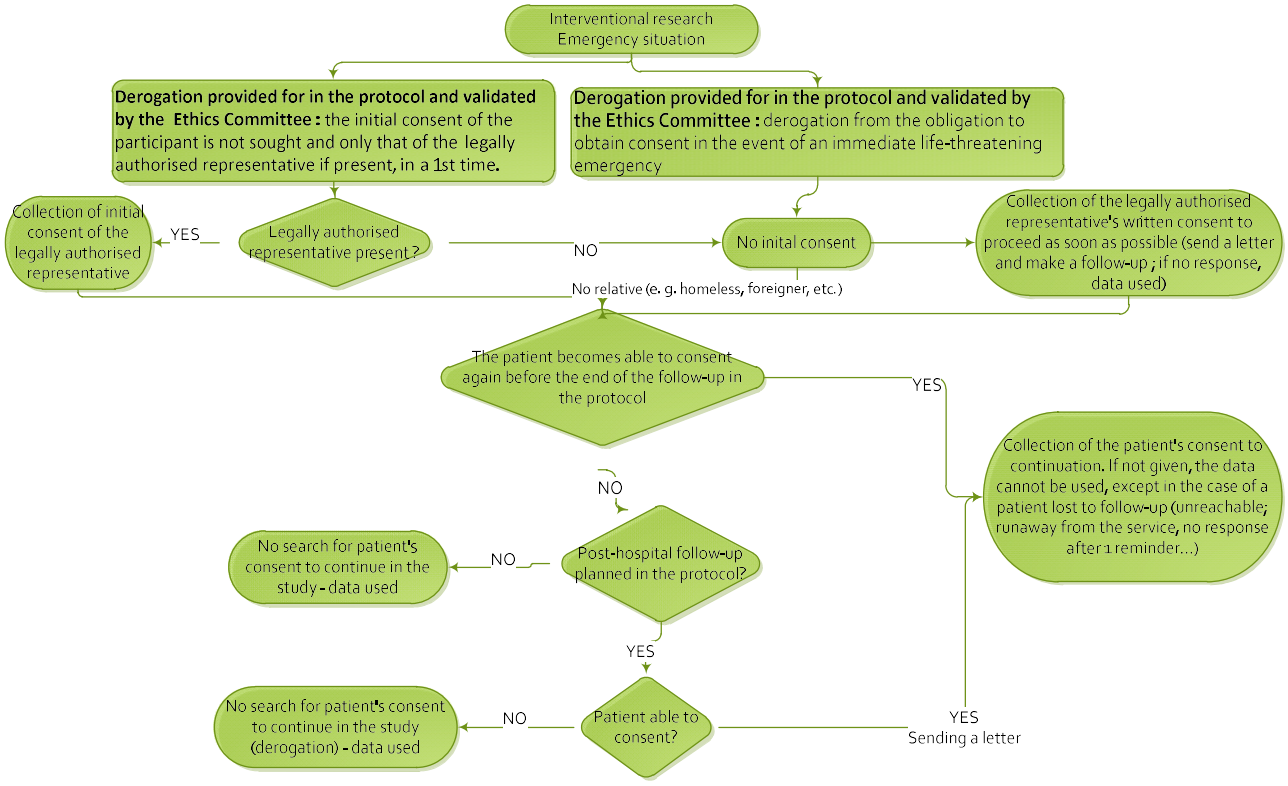
    b. Inclusions in the HAP^2 trials will be realized in situations of immediate vital emergency. Indeed, it is recommended to start the empiric antimicrobial therapy immediately after the collection of the respiratory fluids for patients with suspected hospital-acquired pneumonia (Torres et al. 2017). It will thus be possible to derogate to the consent collection obligation at the time of inclusion if the conditions for a fair information of the support person are not gathered. In this setting, the investigator will justify this procedure in the medical file of the patients,

and will seek the consent of the legal representative (if the patient is still unable to consent) to continue the study as soon as possible.

2. The **retrospective consent of the patient** will be collected by the investigator, or a physician who represents him. The participant will be informed as soon as deemed possible.

   Patient consent will be asked for the potential continuing of the research, and the utilization of his data, if he retrieves his ability to consent. The investigator agrees to provide the subject with clear and precise information about the protocol and request from him/her a written and signed consent form (information form and consent form appended) Patient will sign and date the consent form, after taking time to reflect on the matter. The investigator shall also sign and date the consent form. The investigator's original shall be placed in the investigator file. The consent form is signed in duplicate: the investigator keeps the original and gives the copy to the subject.

The emergency procedure is detailed below, and will be submitted to national regulatory approvals:



Patients can decide in the consent form if they want to be informed in case of incidental findings on samples analyzes or during the trials follow up.

In case of incidental findings, and if the patient has indicated that he/she wants to be informed, a specific consultation will be set up by the Medical Doctor in charge to propose adequate medical care, outside of the study.

8.5. Regulatory / ethics status

This clinical trial is conducted in 3 European countries and will comply with European Union clinical-trial and ethics legislation.

The sponsor is responsible for obtaining regulatory approvals of the clinical study before the initiation of the study:

- Competent Authorities: ANSM (Agence nationale de sécurité du médicament et des produits de santé) in France, AEMPS (Agencia Española de Medicamentos y Productos Sanitarios) in Spain and EOF in Greece ;
- National Ethics Committees ;
- Data protection regulation (GDPR + national regulations) ;

The sponsor will maintain close contact with regulatory authorities and independent ethics committees throughout the duration of the clinical trial and until the end of the study.

In any case, CHU Nantes, as sponsor, will ensure that all national and international regulatory and ethical requirements will have been met before initiating the clinical trial. The sponsor will rely on a coordinating structure in each country to independently submit to each NCA and EC, and on their expertise in the regulatory field specific to each country in terms of vigilance, data and personal protection, etc.

8.6. Amendments to the protocol

Requests for substantial modifications should be addressed by the sponsor for approval or notification to National competent authority and Ethics Committees concerned in compliance with the law and its implementing decrees.
The amended protocol should be a dated updated version.
The patient information and consent forms should be amended if required.

8.7. Study funding and insurance

This study is financed by the H2020 Programme of the European Union, under grant agreement number 847782.
The sponsor will take out an insurance policy covering the financial consequences of its civil liability in compliance with the regulations.

8.8. Publication rules

All trial sites including patients will be acknowledged, and all investigators at these sites will appear with their names under 'the HAP^2 investigators' in an Appendix to the final manuscript. The Steering Committee will grant authorship depending on personal involvement according to the Vancouver definitions.
If a trial site investigator is to gain authorship, the site has to include 10 patients or more. If the site includes 25 patients or more, two authorships will be granted.
The listing of authors will be as follows: A Roquilly (coordinator) will be responsible for the writing of the manuscript and the first author (and corresponding author), and the next authors (from the 2nd place in the list of authors) will be the other investigators according to the number of included patients per study site (for center with > 10 patients), and finally, D. Koulenti, A. Torres and K. Asehnoune will appear as the last three authors with equal participation to this work.

The study will be registered in clinicaltrials.gov database.

The sponsor will enter the study results in the European Union database as soon as the main publication from the research is released, in order to preserve intellectual property.

8.9. Outcome of biological samples

At the end of the study, biological samples resulting from sampling (blood and tracheal aspirates) shall be kept and the subject's written consent should be collected and the samples stored in one of Nantes University Hospital biocollections: biocollection section "IBIS - immunology " under the responsibility of Pr. Asehnoune. This biocollection and consent procedure have been registered under number DC-2012-1555 with the approval of CPP OUEST IV dated 30/06/2014. The patient's written consent (or legal representative consent, if applicable) will be collected by a specific consent form. **In case the patient has been included with Emergency consent form and no legal representative can be found, the biological samples will not be kept in this biocollection.**

8.10. Source data archiving

The investigator should archive all study data for at least 15 years after the end of the study.
At the end of the study, the investigator shall also receive a copy of the data for each patient in the investigator's centre sent by the sponsor.
Archiving procedure will be performed according to the relevant European / local regulations in place.

LIST OF APPENDICES

- **Appendix 1:** Investigator listing
- **Appendix 2:** Summary of protocol
- **Appendix 3:** ICF Emergency Procedure
- **Appendix 3bis :** Legal representative Information and consent form (ICF)
- **Appendix 4:** Patient ICF (study continuation)
- **Appendix 5:** Relative ICF (for Qol questionnaires)
- **Appendix 6:** Questionnaires (EQ5-D-3L, SWLS, SF36, HADS)
- **Appendix 7:** Patient notebook
- **Appendix 8:** Data and Safety Monitoring Board composition
- **Appendix 9:** Steering Committee composition
- **Appendix 10:** Adjudication Committee composition

APPENDIX 1: INVESTIGATOR LIST

Name	Department	Title	institution	Address	Telephone, fax and e-mail	Membership number
FRANCE						
ROQUILLY Antoine	Intensive care	Prof.	CHU Nantes (Hôtel Dieu)	Dept. of anesthesiology and critical care medicine / CHU Nantes (Hôtel Dieu) 1 place Alexis Ricordeau 44098 Nantes Cedex	Tel : +33.253482230 email :antoine.roquilly@chu-nantes.fr	10100176907
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Name	Area of medicine	Title	Name of institution	Name and address of affiliated department	Telephone, fax and e-mail	Membership number Order of Physicians
GREECE						
ARMAGANIDIS Apostolos	Intensive care	Prof.	Attikon University General Hospital	2nd Critical Care Department Attikon University General Hospital 1, Rimini street, Chaidari, Athens, Greece Postal Code: 12462	Tel: +302105832184 Fax: +302105326414 aarmag@med.uoa.gr	/
KOULOURAS Vasileios	Intensive care	Prof.	University General Hospital of Ioannina	Department of Intensive Care University General Hospital of Ioannina Av. Stavros Niarchos, Ioannina, Greece Postal Code: 45500	Tel.: mobile +30 6972840476 office +30 26510 99353 Fax +30 26510 99343 vpkoulouras@yahoo.gr	/
FILDISIS Georgios	Intensive care	Prof	Aghioi Anargyroi General Oncology Hospital	University ICU Aghioi Anargyroi General Oncology Hospital Noufaron & Timiou Stavrou Street, Kalyftaki, Kifisia, Athens, Greece Postal code: 14564	Tel: +302103501843 Fax: +302103501192 gfildis@nurs.uoa.gr	/
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Name	Area of medicine	Title	Name of institution	Name and address of affiliated department	Telephone, fax and e-mail	Membership number Order of Physicians
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Summary table of changes made to the study documents – Subtantial Amendment No 1

RC20_0082 – EudraCT : 2020-000620-18 - VHP1788 (VHP2020144)

Protocol Version 2.0 – 08/04/2021

Modification #	Page	Initial text	Modified or added text	Justification of the change
1	Page 17	**An adjudication committee**, composed of 1 investigator by country who will be blinded to the trial-group assignments, will review the medical charts of patients with respiratory tract infections of the 2 other participating countries, in order to review the diagnosis. Guidelines will be provided to the members by the sponsor. The primary endpoint concerning the occurrence of HAP will be based on the re-reading and review of each diagnosis by two adjudication committee members. In case of disagreement between the 2 adjudication committee members, the clinician's diagnostic will prevail.	**An adjudication committee**, composed of 1 investigator by country who will be blinded to the trial-group assignments, will review the medical charts of patients with respiratory tract infections of the 2 other participating countries, in order to review the diagnosis. Guidelines will be provided to the members by the sponsor. The primary endpoint concerning the occurrence of HAP will be based on the re-reading and review of each diagnosis by two adjudication committee members. In case of disagreement between the 2 adjudication committee members~~, the clinician's diagnostic will prevail,~~ a consultation meeting will be organised in the presence of an expert radiologist.	During the kick-off meeting of the Adjudication Committee, their members wishes to modify the process concerning the final decision in case of discrepancies between 2 members, and to add the expertise of an expert radiologist, who will help to decide the final conclusion.
2	Pages 19, 30, 34, 35, 37, 38, 42, 54, 55	Lipase	Lipase or amylase if the lipase test cannot be carried out, according to the usual practice of the centres	In some Greek sites, the lipase test is not so usual, so it has been decided to authorize the amylase test if the lipase test cannot be carried out, according to the usual practice of the centres

Protocol Summary Version 2.0 – 08/04/2021

Modification #	Page	Initial text	Modified or added text	Justification of the change
1	Page 2	**An adjudication committee**, composed of 1 investigator by country who will be blinded to the trial-group assignments, will review the medical charts of patients with respiratory tract infections of the 2 other participating countries, in order to review the diagnosis. Guidelines will be provided to the members by the sponsor. The primary endpoint concerning the occurrence of HAP will be based on the re-reading and review of each diagnosis by two adjudication committee members. In case of disagreement between the 2 adjudication committee members, the clinician's diagnostic will prevail.	**An adjudication committee**, composed of 1 investigator by country who will be blinded to the trial-group assignments, will review the medical charts of patients with respiratory tract infections of the 2 other participating countries, in order to review the diagnosis. Guidelines will be provided to the members by the sponsor. The primary endpoint concerning the occurrence of HAP will be based on the re-reading and review of each diagnosis by two adjudication committee members. In case of disagreement between the 2 adjudication committee members, ~~the clinician's diagnostic will prevail,~~ a consultation meeting will be organised in the presence of an expert radiologist.	During the kick-off meeting of the Adjudication Committee, their members wishes to modify the process concerning the final decision in case of discrepancies between 2 members, and to add the expertise of an expert radiologist, who will help to decide the final conclusion.
2	Page 3	• Rate of pancreatitis (Increase in Lipase) at D15.	• Rate of pancreatitis (Increase in Lipase or amylase if the lipase test cannot be carried out, according to the usual practice of the centres) at D15.	In some Greek sites, the lipase test is not so usual, so it has been decided to authorize the amylase test if the lipase test cannot be carried out, according to the usual practice of the centres

Investigator listing Version 2.0 – 08/04/2021

Modification #	Page	Initial text	Modified or added text	Justification of the change
1	Page 1	CINOTTI Raphael	~~CINOTTI Raphael~~LAKHAL Karim	- the PI of Nantes Laennec Hospital (France) has left the department, and is therefore replaced by a new PI
		SEGUIN Philippe	~~SEGUIN Philippe~~LAUNAY Yoann	- the PI of Rennes Hospital (France) wished to delegate his responsibilities to another doctor in his department
2	Page 3	Georgopoulos Dimitrios	VAPORIDI Aikaterini ~~Georgopoulos Dimitrios~~	- the PI of General University Hospital of Larissa (Greece) wished to delegate his responsibilities to another doctor in his department

Statistical Analyses Plan

Eudract: 2020-000620-18
Ref: RC20_0082

"Human recombinant interferon gamma-1b for the prevention of hospital-acquired pneumonia in critically ill patients: a double-blind, international, phase 2, randomized, placebo-controlled trial - the PREV-HAP study"

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Visit us:
hap2-project.com

6. DATA MANAGEMENT AND STATISTICS

6.1. Data entry and data collection

6.1.1. Data entry, processing and circulation

Data collection for each person participating in the trial will be done with an electronic case report form (eCRF), created by the sponsor's data-management team, using a specialist software solution specifically designed for holding, auditing and checking trial data (Ennov Clinical Software).

Each person responsible for the filling of the eCRF (investigator, CRA...):

- will have to be identified in the table of delegations of responsibilities of each center (see investigator's file).
- Will have a "user" account with specific computer rights linked to his role (right to enter or modify a data, right to lock, monitor or sign a page of eCRF...)

Entering, viewing or modifying data will only be possible via the eCRF pages, on https://nantes-lrsy.hugo-online.fr/CSonline.

The data will be stored directly from the eCRF into the database hosted on a dedicated server, with controlled access (account/password) according to the user role. Any addition, modification or deletion of data will be recorded in a non-editable electronic file (the audit trail).

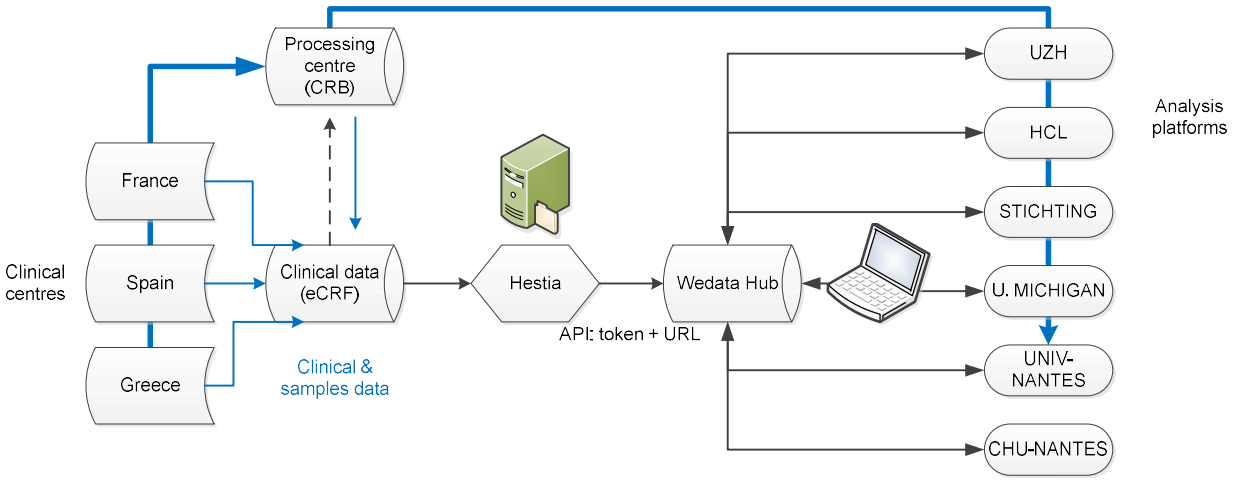
As for the health-economic analyse, extractions from the French hospitals database from the various participating centres will be sent by the investigation teams to the sponsor in a secure manner and stored on a secure server at the Nantes University Hospital accessible to the people responsible for the analysis.

6.1.2. Patient identification

The principal investigator and all co-investigators undertake to keep the identities of the persons who participate in the study confidential by assigning them a code (pseudonymisation). This code will be used for all the eCRF and all the attached documents (reports of imaging exams, biology, etc.). It will be the only information which will make it possible to make the connection with the patient retrospectively. The coding rule is the following: **month and year of birth, Inclusion number.**

6.1.3. Data Flow

The coded clinical data from the eCRF will be encrypted and automatically transferred to a different server of CHU Nantes, where it will be combined with the phenotypical, immunological, biomarker and multiomic's data generated in the context of HAP^2 WP3 for further analysis. A secure access to this second server will be created for the consortium's partner WeData (http://wedata.science/) for analysis, via a specific URL and token encrypted.



6.2. Statistics

6.2.1. Description of planned statistical methods, including planned intermediate analysis schedule

All analyses will be performed with the use of SAS software (version 9.4, NC, USA) before the breaking of the randomization code, according to International Conference on Harmonization-Good Clinical Practice guidelines.

Continuous variables will be presented as mean and standard deviations (as median and quartiles, otherwise). Categorical data will be presented as frequency and percentages.

All statistical analyses will take into account stratified randomization (cause of hospitalization (sepsis or not) and country (France, Greece, Spain)) as recommended in the CONSORT 2010 statement[20].

PRIMARY ANALYSIS

We will assess the efficiency of rHu-IFNγ for the prevention of hospital-acquired pneumonia with a Cox regression model (primary composite outcome: all-cause mortality at day 28 or the occurrence of hospital-acquired pneumonia within 28 days after randomization). Such an analysis combining the primary (occurrence of hospital-acquired pneumonia) and competing event (all-cause mortality) into a composite event has been recommended[21].

Crude and adjusted estimations on stratification factors will be given. The primary analysis will be adjusted on the stratification criteria and on center as a random effect.

SECONDARY ANALYSIS

To explore the risk of HAP in sub-populations (primary outcome), interaction terms between treatment arm and the following covariates will be tested in the Cox regression models (primary adjusted outcome):

[20] *CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials* BMJ *2010;340:c332*
[21] *Troendle JF et al.Stat Med.2018.*

- Randomization strata: Cause of hospitalization (sepsis or others), and country (France, Greece, Spain)
- Age (< or > 65 years)
- Severity upon ICU admission (Apache II 15-30, 30-45 or > 45)
- Time between the ICU admission and the first treatment injection (<24 hours; 24 to 36 hours, and 36-48 hours).
- Administration of glucocorticoid at the time of inclusion (yes/no)
- Analysis of the effect of treatment according to the COVID+ or COVID- status of the patients included in the study

All-cause mortality at day 90 will be analyzed with a Cox regression model, adjusted for stratification factors and on center as a random effect.

Categorical data (ventilator-associated tracheobronchitis at day 28, acute respiratory distress syndrome at day 28) will be analyzed with logistic regression model, adjusted for stratification factors and on center as a random effect.

Censored data (duration of antimicrobial therapy at day 28, duration of mechanical ventilation at day 90, duration of ICU hospitalization at day 90, duration of hospitalization at day 90) will be analyzed using Fine and Gray competing risks models to take into account the informative censoring and the competing risk due to death. The cumulative incidence functions (CIFs) of each competing event (death/end of antimicrobial therapy or death/extubation or death/end of hospitalization) will be estimated.

“Free-days” outcomes (antibiotic free days at day 28, mechanical ventilation free days at day 90, hospital free days at day 90) will be analyzed with a Mann-Whitney U.

Tolerance outcomes will be analyzed using logistic regression models.

PATIENT-REPORTED OUTCOMES DATA (SUITABILITY AND ACCEPTABILITY OF RHU-IFNγ)

Change in patient-reported outcomes data (quality of life, anxiety, depression) will be analyzed using longitudinal Rasch Measurement Theory (RMT)[22] models from the family of generalized random effects models. The RespOnse Shift ALgorithm at Item-level (ROSALI)[23] based on these models which has been showed to have good performance in a recently published simulation study[24] will be used. ROSALI will be developed and validated in WP5 using simulation studies to enable the use of RMT models as latent regression models to include covariates such as treatment, gender, and country. The development of ROSALI will allow investigating covariates’ effects on PRO (e.g. health-related quality of life) changes over time as well as on patients’ adaptation through response shift analyses (see WP5).

Patients' adaptation to their condition will also be investigated using regression analyses to test for the possibility of changes in the relationship between the patients' subjective well-being and their health-related quality of life[25]. Investigating this form of adaptation is important to assess the validity of one of the assumptions of the QALY (Quality-Adjusted Life-Years) measure that is commonly used to represent the effectiveness part of cost-effectiveness analyses (see the part describing the cost-effectiveness analysis). Various models will be estimated and compared to take into account factors such as unobserved individual heterogeneity for instance.

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We will include 200 patients (100 patients receiving placebo, 100 patients receiving rHu-IFNγ). The rate of non survivors and/or hospital-acquired pneumonia in the placebo group is expected to reach 35%[28]. In this phase II clinical trial, the size of the effects with the studied treatment can not be estimated from current knowledge about the effect of these therapeutic strategies. We thus decided to rely on the recruitment capacity of the European centers allowing the inclusion of 100 patients / group over 24 months. This sample size will allow detecting a hazard ratio of 0.625 as compared to placebo with a 90% of statistical power and a double-sided type I error α at 5%.

6.2.3. Expected level of statistical significance

A two-sided P value of less than 0.05 will be considered for all analyses.

6.2.4. Statistical criteria for discontinuation of study

No interim analysis is planned for the efficiency.

6.2.5. Consideration method for missing, unused or invalid data

Lost to follow-up and missing data

There should be neither missing data nor lost to follow-up for the primary outcome which will be recorded in intensive care unit. Missing data will be described by treatment arm. According to the rate of missing data (over 5%) sensitivity analyses will be performed using multiple imputation methods.

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Early withdrawals,
Withdrawals from the study can only be effective after confirmation by the investigator and the sponsor. These withdrawals are always definitive. In case of withdrawal from the study, patients will be followed up until hospital discharge, according to routine clinical practice in each participating center. Clinical data obtained before the consent withdrawal will be kept for the analyses. According to analysis populations, the patient will be excluded from the analyses or data will be imputed for the primary endpoint. These patients will not be replaced.
In case of withdrawal after a SAR, patients will be followed up to the resolution or to the consolidation of this event.

Non-compliance with the protocol
In case of non-compliance to the treatment regimen and/or to the collection of biological samples, the patients will be followed up to the end of the study, and the data will be kept for the analyze in intention to treat.

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In case of early discontinuation of the study on Sponsor's decision or DSMB, Ref-NCA, National Competent Authorities and Ethics Committees will be informed less than 15 days by mail.
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The same applies to any investigator wanting to discontinue his/her participation to the study. The investigator must immediately inform the Sponsor in writing of this decision.

6.2.7. Choice of subjects to be included in analysis

Analyzes will be conducted, first, on data from the intention-to-treat (ITT) population, second, in the modified intention-to-treat (mITT) population as well as in the per-protocol population (PP).

- **Intention-to treat (ITT):** All randomized patients in the group in which they were randomised, regardless of the medical device/treatment received and breaches of the protocol. In case of missing data, the analysis of the ITT population will be performed by multiple imputation methods using demographic data (age, gender), stratification factors, IGS-II and cause of admission.
- **Modified intention to treat (mITT):** Randomized patients who have an assessable clinical outcome within the assessment window, fulfilling the major inclusion criteria, without major non-inclusion criteria, without consent withdrawal and who received at least one dose of treatmentare analyzed in the group in which they were randomised, regardless of the medical device/treatment received and other breaches of the protocol.

- **Per protocol (PP):** Randomized subjects who were treated in full compliance with the protocol (exclusion of the patients of the rHu-IFNγ group who have not received the complete drug regimen)

6.2.8. Economic evaluation

The cost-effectiveness analysis will be conducted from the perspective of the society with a three-month time horizon.

Assessment of costs

For all patients in the study the use of resources at the hospital and outside will be collected prospectively. Two modes of data retrieval will be combined: i) clinical research associates will record the consumption of resources in the hospital in combination with a database extraction of hospital information (outpatient consultations and procedures, hospitalizations) and ii) we will distribute diaries to patients to collect information about resources consumption after the initial hospitalization.

Patient pathways after discharge from initial hospitalization are defined as either follow-up within an after-care and rehabilitation structure, a return home, or a move to another care unit within the same hospital or one closer to the patient's home, cf. Figure 1.

We will retrieve within the hospital only resources related to pneumonia, for data retrieved outside the hospital we will be unable to differentiate what is due to pneumonia from other care so we will ask patients to record all care resources used over the period up to day 90. This will include, most notably, ICU length of stay, pharmaceuticals and consultations. We will also collect information about the time from caregivers (whether professional or informal) and about day out of work to value production losses.

To estimate costs, unit costs per each type of resource consumed will be estimated using accounting information, NHI official tariffs, and the hospitals' prices charged in different countries (France, Spain and Greece). The time of caregivers devoted to monitoring and assisting patients will be valued by applying a salary valorization equivalent to a home help job (proxy good method), and, we will calculate productivity losses using work stoppages.

Concerning the French patients, in case the return of patients' diaries on resource use would be deemed insufficient (less than 65% of the total) to carry out our study, we would use the database of the French Health Insurance in order to retrieve the consumption of ambulatory and hospital healthcare.

Statistical Analysis Plan

Eudract: 2020-000620-18
Ref: RC20_0082

"Human recombinant interferon gamma-1b for the prevention of hospital-acquired pneumonia in critically ill patients: a double-blind, international, phase 2, randomized, placebo-controlled trial - the PREV-HAP study"

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Visit us:
hap2-project.com

6. DATA MANAGEMENT AND STATISTICS

6.1. Data entry and data collection

6.1.1. Data entry, processing and circulation

Data collection for each person participating in the trial will be done with an electronic case report form (eCRF), created by the sponsor's data-management team, using a specialist software solution specifically designed for holding, auditing and checking trial data (Ennov Clinical Software).

Each person responsible for the filling of the eCRF (investigator, CRA...):

- will have to be identified in the table of delegations of responsibilities of each center (see investigator's file).
- Will have a "user" account with specific computer rights linked to his role (right to enter or modify a data, right to lock, monitor or sign a page of eCRF...)

Entering, viewing or modifying data will only be possible via the eCRF pages, on https://nantes-lrsy.hugo-online.fr/CSonline.

The data will be stored directly from the eCRF into the database hosted on a dedicated server, with controlled access (account/password) according to the user role. Any addition, modification or deletion of data will be recorded in a non-editable electronic file (the audit trail).

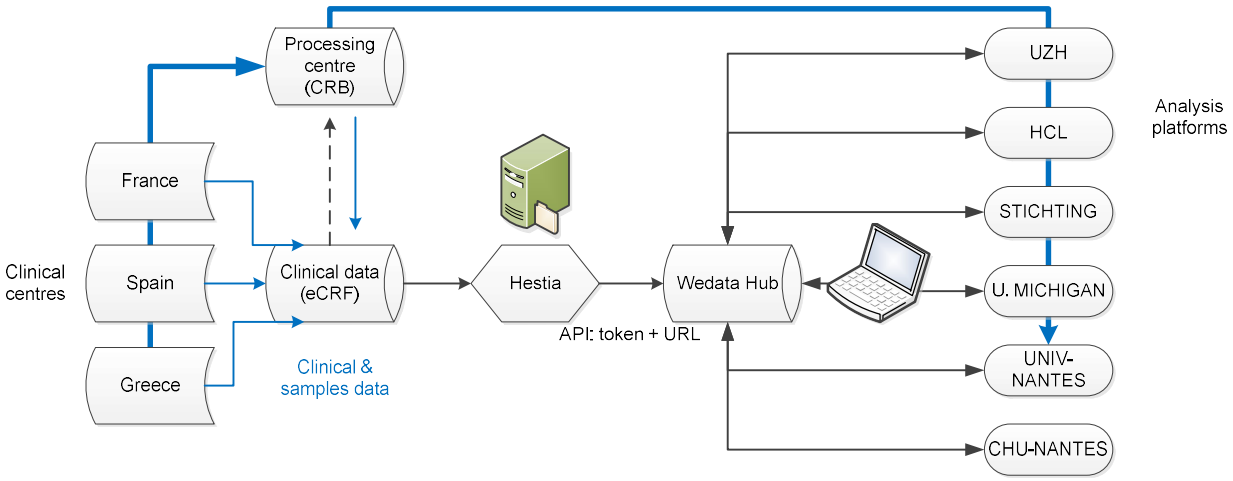
As for the health-economic analyse, extractions from the French hospitals database from the various participating centres will be sent by the investigation teams to the sponsor in a secure manner and stored on a secure server at the Nantes University Hospital accessible to the people responsible for the analysis.

6.1.2. Patient identification

The principal investigator and all co-investigators undertake to keep the identities of the persons who participate in the study confidential by assigning them a code (pseudonymisation). This code will be used for all the eCRF and all the attached documents (reports of imaging exams, biology, etc.). It will be the only information which will make it possible to make the connection with the patient retrospectively. The coding rule is the following: **month and year of birth, Inclusion number.**

6.1.3. Data Flow

The coded clinical data from the eCRF will be encrypted and automatically transferred to a different server of CHU Nantes, where it will be combined with the phenotypical, immunological, biomarker and multiomic's data generated in the context of HAP^2 WP3 for further analysis. A secure access to this second server will be created for the consortium's partner WeData (http://wedata.science/) for analysis, via a specific URL and token encrypted.



6.2. Statistics

6.2.1. Description of planned statistical methods, including planned intermediate analysis schedule

All analyses will be performed with the use of SAS software (version 9.4, NC, USA) before the breaking of the randomization code, according to International Conference on Harmonization-Good Clinical Practice guidelines.

Continuous variables will be presented as mean and standard deviations (as median and quartiles, otherwise). Categorical data will be presented as frequency and percentages.

All statistical analyses will take into account stratified randomization (cause of hospitalization (sepsis or not) and country (France, Greece, Spain)) as recommended in the CONSORT 2010 statement[20].

PRIMARY ANALYSIS

We will assess the efficiency of rHu-IFNγ for the prevention of hospital-acquired pneumonia with a Cox regression model (primary composite outcome: all-cause mortality at day 28 or the occurrence of hospital-acquired pneumonia within 28 days after randomization). Such an analysis combining the primary (occurrence of hospital-acquired pneumonia) and competing event (all-cause mortality) into a composite event has been recommended[21].

Crude and adjusted estimations on stratification factors will be given. The primary analysis will be adjusted on the stratification criteria and on center as a random effect.

SECONDARY ANALYSIS

To explore the risk of HAP in sub-populations (primary outcome), interaction terms between treatment arm and the following covariates will be tested in the Cox regression models (primary adjusted outcome):

[20] *CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials* BMJ *2010;340:c332*
[21] *Troendle JF et al.Stat Med.2018.*

- Randomization strata: Cause of hospitalization (sepsis or others), country was not tested since no patient was included in Spain and Greece.
- Age (< or > 65 years)
- Severity upon ICU admission (Apache II 15-30, 30-45 or > 45)
- Time between the ICU admission and the first treatment injection (<24 hours; 24 to 36 hours, and 36-48 hours).
- Administration of glucocorticoid at the time of inclusion (yes or no)
- Analysis of the effect of treatment according to the COVID+ or COVID- status of the patients included in the study

All-cause mortality at day 90 will be analyzed with a Cox regression model, adjusted for stratification factors and on center as a random effect.

Categorical data (ventilator-associated tracheobronchitis at day 28, acute respiratory distress syndrome at day 28) will be analyzed with logistic regression model, adjusted for stratification factors and on center as a random effect.

Censored data (duration of antimicrobial therapy at day 28, duration of mechanical ventilation at day 90, duration of ICU hospitalization at day 90, duration of hospitalization at day 90) will be analyzed using Fine and Gray competing risks models to take into account the informative censoring and the competing risk due to death. The cumulative incidence functions (CIFs) of each competing event (death/end of antimicrobial therapy or death/extubation or death/end of hospitalization) will be estimated.

“Free-days” outcomes (antibiotic free days at day 28, mechanical ventilation free days at day 90, hospital free days at day 90) will be analyzed with a Mann-Whitney U.

Tolerance outcomes will be analyzed using logistic regression models.

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Primary outcome analysis

The primary analysis was carried out in all randomized patients according to their assigned group (as-randomized population). No multiple imputations of primary outcome was performed because only one data was missing.