Ministero della Salute

DIREZIONE GENERALE DELLA RICERCA E DELL'INNOVAZIONE IN SANITA'
Ufficio 3

Convenzione tra il Ministero della salute, il destinatario istituzionale **Fondazione Policlinico San Matteo** e, per conoscenza, il PI della ricerca **MELONI FEDERICA**, per la regolamentazione dello svolgimento dei progetti di ricerca finalizzata, relativi al bando della ricerca finalizzata 2021, esercizi finanziari 2020-2021, afferenti alla tipologia progettuale - **Ordinari della ricerca finalizzata (RF)** - "**Theory enhancing**".

Convenzione progetto RF-2021-12374476

Premesso che

ai sensi di quanto disposto dall'art. 12 e dall'art. 12 bis del decreto legislativo 30 dicembre 1992 n.502, come modificato e integrato dal decreto legislativo n. 229/1999, concernenti il finanziamento da parte del Ministero della salute dei progetti di ricerca presentati dai destinatari istituzionali, individuati dalla normativa stessa, si rende necessario - ai fini dello svolgimento dei progetti di ricerca finalizzata, relativi agli anni finanziari 2018-2019 e approvati dal Comitato Tecnico Sanitario – sezione c), nella riunione del 5 giugno (progetti SG), nelle riunioni del 22 e 30 settembre 2020 (progetti RF-CO-GR), disciplinare i conseguenti rapporti di collaborazione e finanziari;

l'art.7 del decreto ministeriale 8 aprile 2015, recante il riordino degli uffici di livello dirigenziale non generale del Ministero della salute, ha individuato gli uffici in cui si articola la Direzione generale della ricerca e dell'innovazione in sanità, individuando, fra le altre, le specifiche competenze assegnate all'ufficio 3 della stessa;

con il Decreto Direttoriale dell'1 marzo 2022, registrato dall'Ufficio Centrale di Bilancio in data 4 marzo 2022, al n. 247, con il quale il Dott. Gaetano Guglielmi è stato autorizzato, tra l'altro, all'esercizio del potere di spesa sul capitolo di spesa 3398, piano gestionale 3 piani gestionali per i residui passivi perenti, limitatamente agli importi destinati agli IRCCS;

con Decreto del Ministro dell'economia e delle finanze 31 dicembre 2021, recante "*Ripartizione in capitoli delle unità di voto parlamentari relative al bilancio di previsione dello Stato per l'anno finanziario 2022 e per il triennio 2022–2024*";

con Decreto 12 novembre 2021 del Ministro della salute di concerto con il Ministro dell'istruzione dell'università e della ricerca, registrato all'Ufficio di controllo di bilancio con visto numero 592 del 22 novembre 2021, e alla Corte dei Conti in data 13 dicembre 2021 con numero 3018, è stato approvato il bando della ricerca finalizzata 2021, per gli anni finanziari 2020-2021, in cui confluiscono le somme disponibili di euro 50.000.000,00 (cinquantamilioni/00) riferite all'anno finanziario 2020 ed euro 50.000.000,00

(cinquantamilioni/00) riferite all'anno finanziario 2021, per complessivi euro 100.000.000,00 (centomilioni/00) ripartiti tra le diverse tipologie progettuali menzionate nel richiamato bando;

in data 27 dicembre 2021 è stato pubblicato sul portale del Ministero della salute il bando della ricerca finalizzata 2021, relativo agli anni finanziari 2020-2021;

nella riunione del 28 ottobre 2022 il Comitato tecnico sanitario sezione c), preso atto della regolarità del processo di valutazione, ha approvato la graduatoria finale distinta per ciascuna delle sezioni del Bando e delle tipologie progettuali, nonché il finanziamento da destinare ai progetti collocatisi in posizione utile;

con il Decreto direttoriale in data 28 ottobre 2022 - registrato dall'Ufficio centrale di bilancio in data 18 novembre 2022 al numero 1057 - con il quale sono state approvate le graduatorie relative alle previste sezioni e tipologie progettuali del Bando per la Ricerca Finalizzata anno 2021, relativo agli anni finanziari 2020-2021, con indicazione, per ciascun progetto collocatosi in posizione utile ai fini del finanziamento, della somma a carico di questo Dicastero per la realizzazione del progetto medesimo;

nel suddetto provvedimento è menzionato il progetto **RF-2021-12374476** denominato "***Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis***" - Destinatario istituzionale - **Fondazione Policlinico San Matteo** – utilmente collocato nella specifica graduatoria "**Ordinari della ricerca finalizzata (RF)**") ed al quale è stato attribuito un finanziamento complessivo di **€450.000,00** (***quattrocentocinquantamila/00***).

TANTO PREMESSO SI STIPULA E CONVIENE QUANTO SEGUE
TRA

Il Ministero della salute
rappresentato dal dott. Gaetano Guglielmi – Direttore dell'Ufficio 3 della Direzione generale della ricerca e dell'innovazione in sanità;

E
Il Destinatario istituzionale
Fondazione Policlinico San Matteo
(nel prosieguo denominato Destinatario istituzionale)
rappresentato da - **Dott. Stefano Manfredi**

Articolo 1

1. Le premesse sono parte integrante della presente convenzione.

Articolo 2

1. La presente convenzione regola l'affidamento da parte del Ministero della salute – Direzione generale della ricerca e dell'innovazione in sanità - al Destinatario istituzionale del progetto di ricerca finalizzata, **RF-2021-12374476** dal titolo *"**Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis**".*
2. Il principal investigator è individuato nella persona dott./dott.ssa **MELONI FEDERICA,** codice fiscale **MLNFRC61P70G388B.**

Articolo 3

1. Il finanziamento è di **€450.000,00** (***quattrocentocinquantamila/00***) a valere sui fondi del *capitolo 3398/1 ed i seguenti perenti 3398/83-84-87*

Articolo 4

1. Il Destinatario istituzionale ed il principal investigator svolgono congiuntamente il progetto di ricerca secondo quanto riportato nel piano esecutivo e finanziario presentato in ottemperanza a quanto previsto dal Bando per la ricerca finalizzata 2021 relativo agli anni finanziari 2020-2021 di cui in premessa ed allegato alla presente convenzione di cui è parte integrante.

Articolo 5

1. La presente convenzione ha la durata di tre anni prorogabile eventualmente di ulteriori 12 mesi come previsto dal successivo articolo 11.
2. L'attività di ricerca, da svolgersi nell'arco temporale della vigenza della convenzione, deve avere inizio improrogabilmente entro e non oltre il 30 aprile 2023, comunicando la data effettiva con nota sottoscritta digitalmente dal proprio rappresentante legale e dal principal investigator della ricerca almeno 20 giorni prima dell'inizio effettivo.
3. Il Destinatario istituzionale entro e non oltre 30 giorni dall'invio della presente convenzione da parte del Ministero della Salute per la sottoscrizione provvede alla restituzione della convenzione firmata dal legale rappresentate e controfirmata dal principal investigator, tramite il sistema di monitoraggio del WFR, accompagnato dalla comunicazione del codice CUP del progetto e i codici CUP e codici fiscali delle singole Unità operative;
4. La presente convenzione, vincolante all'atto della sottoscrizione per il destinatario istituzionale ed il principal investigator, diventa efficace per il Ministero a seguito della registrazione da parte dell'organo di controllo.
5. Il Destinatario istituzionale, entro e non oltre 20 giorni precedenti la scadenza del termine di cui al comma 2 del presente articolo, pena la decadenza dal finanziamento, è tenuto a trasmettere - con nota sottoscritta digitalmente dal proprio rappresentante legale e dal principal investigator della ricerca - la seguente documentazione, soggetta a verifica da parte del Ministero al fine di autorizzare l'avvio del progetto:
    a) il parere positivo del comitato etico e/o l'autorizzazione di cui all'articolo 31 del decreto legislativo n.26 del 4 marzo 2014, ove previsti;
    b) le dichiarazioni indicanti le unità operative coinvolte nel progetto nonché l'accettazione degli Enti che svolgono funzioni di unità operativa e dei relativi responsabili di unità operativa dell'accettazione dei termini della presente convenzione;
    c) la dichiarazione con la quale il destinatario istituzionale attesta che il principal investigator svolgerà la propria attività, relativamente al progetto in questione, esclusivamente presso la struttura del S.S.N. all'uopo individuata dal destinatario istituzionale medesimo. La dichiarazione di cui al presente comma dovrà essere controfimata dal Princiapl Investigator interessato;
    d) il certificato AIRE (Anagrafe degli italiani residenti all'estero) nel caso di collaborazione con ricercatori italiani residenti e operanti all'estero;
    e) La traduzione in lingua italiana della proposta progettuale senza apportare alcuna modifica alla versione in inglese allegata alla presente convenzione;
6. Il monitoraggio e la verifica del raggiungimento degli obiettivi del progetto di ricerca di cui alla presente convenzione sono affidati alla Direzione generale della ricerca e dell'innovazione in Sanità, Ufficio 3.
7. La scheda del piano finanziario è vincolante relativamente al solo totale del finanziamento assegnato e al riparto iniziale tra unità operative, mentre ha valore meramente indicativo per quanto riguarda la ripartizione tra voci di costo e le motivazioni a giustificazione di tali costi. Il Destinatario istituzionale si impegna a rispettare le quote percentuali previste dal bando per le varie voci di costo che saranno calcolate, a consuntivo, sulle spese rendicontate, al netto di eventuali economie riscontrate sul finanziamento assegnato e sulle sole spese eleggibili, dopo verifica da parte del Ministero della salute.
8. Le parti convengono che le comunicazioni relative al progetto di cui trattasi siano effettuate attraverso il sistema di monitoraggio delle ricerche denominato Workflow della ricerca a disposizione dei

Destinatari istituzionali. Il Ministero si riserva di attivare, qualora reso disponibile, il sistema di rendicontazione on-line sulla piattaforma del Workflow della ricerca, e lo stesso sarà vincolante dalla data di comunicazione della relativa disponibilità.

9. Il Destinatario istituzionale attraverso il proprio rappresentate legale, nonché il principal investigator devono firmare digitalmente tutti gli atti inerenti alla ricerca.

Articolo 6

1. La prima rata del finanziamento è pari a **€225.000,00** (***duecentoventicinquemila/00***) e la procedura per il pagamento della stessa è avviata solo a seguito dell'accertamento da parte del Ministero degli avvenuti adempimenti di cui al comma 2 e 4 dell'articolo 5 della presente. La predetta rata è imputata sull'esercizio finanziario 2022.
2. La seconda rata del finanziamento è pari ad **€135.000,00** (***centotrentacinquemila/00***) ed è erogata dopo la trasmissione da parte del Destinatario istituzionale della relazione intermedia di cui al successivo art. 7 e solo a seguito della valutazione positiva della stessa da parte del Ministero. La predetta rata è imputata sull'esercizio finanziario 2025.
3. La terza rata, a saldo del finanziamento, è pari ad **€90.000,00** (***novantamila/00***). Essa è corrisposta una volta accertata la sussistenza dei requisiti di cui al successivo articolo 9 e solo a seguito della valutazione positiva della relazione finale da parte del Ministero. La predetta rata è imputata sull'esercizio finanziario 2027.
4. A garanzia della coerenza con l'inizio dell'attività dichiarata, il Destinatario istituzionale si impegna ad anticipare le risorse economiche necessarie, nell'eventualità in cui le somme da corrispondersi da parte del Ministero siano in regime di perenzione**.**
5. Laddove non vengano rispettati i termini di cui alla presente convenzione, che non consentano la tempestiva erogazione dei fondi, il Destinatario istituzionale esonera il Ministero da eventuali ritardi nell'erogazione delle somme spettanti.

Articolo 7

1. Allo scadere dei 18 mesi dall'inizio dell'attività della ricerca e comunque non oltre i trenta (30) giorni da tale termine, il Destinatario istituzionale trasmette al Ministero la relazione intermedia sullo stato d'attuazione della ricerca - sottoscritta digitalmente dal legale rappresentante del Destinatario istituzionale e dal principal investigator - contenente la descrizione delle attività svolte dalle singole unità operative da cui risulti il regolare svolgimento del progetto secondo quanto riportato nel piano esecutivo . Tale relazione deve essere accompagnata da un documento di sintesi, a cura del principal investigator, che illustri, nella globalità, lo stato di avanzamento dei lavori, inclusa la descrizione delle attività realizzate da eventuali Enti co-finanziatori.
2. Il Ministero ha facoltà, previa comunicazione preventiva al destinatario istituzionale, di attivare le procedure per la sospensione del finanziamento ed il recupero delle somme erogate, comprensive degli eventuali interessi legali maturati, qualora il Destinatario istituzionale non adempia a quanto previsto entro i termini di cui al comma 1 del presente articolo.
3. il Ministero, previa comunicazione preventiva al Destinatario istituzionale, ha facoltà di non erogare la seconda rata di finanziamento, subordinandola all'eventuale esito positivo del giudizio in ordine alla relazione finale, qualora la relazione intermedia, all'esito dell'istruttoria, non sia considerata idonea a dimostrare che siano stati pienamente raggiunti gli obiettivi medio termine o emerga che essa sia stata condotta non in piena conformità con quanto previsto nel piano esecutivo approvato. In tal caso il Ministero potrà procedere alla erogazione della seconda rata contestualmente al saldo. Laddove non vengano rispettati i termini di cui alla presente convenzione, che non consentano la tempestiva erogazione dei fondi, il Destinatario istituzionale esonera il Ministero da eventuali ritardi nell'erogazione delle somme spettanti.
4. Il Ministero, previa comunicazione preventiva al Destinatario istituzionale, può sottoporre alle valutazioni al Comitato tecnico sanitario sez. c), un dossier, qualora la relazione intermedia, all'esito della istruttoria ministeriale, non consenta di esprimere un compiuto motivato parere. La decisione

del suddetto Comitato è vincolante per il Destinatario istituzionale ai fini del prosieguo della convenzione.

Articolo 8

1. A partire dal 6° mese successivo all'avvio del progetto e fino a 12 mesi prima della scadenza del progetto, il Destinatario istituzionale, con nota firmata dal proprio rappresentate legale e dal principal investigator - può apportare modifiche al piano esecutivo, coerenti con gli obiettivi progettuali, o alla distribuzione di fondi tra le unità operative, solo se approvato dal Ministero con espresso e formale atto preventivo di assenso e purché non comportino un aumento del finanziamento a carico del Ministero. Non è consentito oltre tale periodo avanzare richieste di modifica. In caso di una eventuale necessità di una ulteriore modifica progettuale è possibile presentare tale modifica solo dopo 6 mesi dall'approvazione da parte del Ministero dell'ultima modifica progettuale.
2. La distribuzione delle somme tra le diverse voci di costo, nell'ambito di ogni singola unità operativa, è consentita sotto la responsabilità del Destinatario Istituzionale che ha presentato il progetto e che dovrà verificare il rispetto delle percentuali previste dal bando.
3. Qualsiasi proposta emendativa deve essere adeguatamente motivata dal principal investigator per documentare che quanto richiesto risulti indispensabile per assicurare il raggiungimento degli obiettivi a suo tempo prefissati.
4. Solo dopo l'approvazione del Ministero, il Destinatario istituzionale potrà procedere a dare attuazione alle modifiche di cui al comma 1 del presente articolo. In caso di eventuali inadempimenti al presente articolo il Ministero ha facoltà di procedere sia alla risoluzione della convenzione, dandone comunicazione al Destinatario istituzionale, sia alla sospensione del finanziamento nonché al recupero dell'importo erogato.

Articolo 9

1. Fatta salva l'eventuale concessione di proroga della durata delle attività progettuali, al termine di trentasei mesi - e comunque non oltre trenta (30) giorni dopo la data fissata per il termine della ricerca – ai fini dell'erogazione del saldo, il Destinatario istituzionale, con nota firmata digitalmente dal rappresentante legale, trasmette contestualmente al Ministero la seguente documentazione, redatta dal principal investigator e recante la firma digitale dello stesso:
   a) la relazione finale della ricerca contenente quanto posto in essere da eventuali Enti co-finanziatori, documenti, per ciascuna unità operativa, la coerenza delle attività svolte con il programma esecutivo approvato e gli obiettivi raggiunti;
   b) copia dei lavori pubblicati su riviste impattate a seguito dello svolgimento della ricerca di cui all'articolo 1 della presente;
   c) la rendicontazione delle spese sostenute con i fondi ministeriali, utilizzando se disponibile il sistema di rendicontazione on-line del WFR;
   d) indicazioni della URL del repository pubblico dove sono resi disponibili i dati grezzi progettuali e quelli utilizzati per le pubblicazioni scientifiche correlate.
2. Tutta la soprarichiamata documentazione deve essere redatta utilizzando esclusivamente la modulistica reperibile sul sistema Workflow della ricerca.
3. La documentazione di supporto deve essere a disposizione del Ministero presso il Destinatario istituzionale, che deve provvedere alla relativa custodia.
4. Il Ministero provvede ad applicare una decurtazione pari al 10% della rata del saldo, qualora la documentazione di cui alle lettere a) b) c) d) del comma 1 del presente articolo sia trasmessa al Ministero in un periodo compreso tra il trentunesimo ed il sessantesimo giorno dalla data di conclusione del progetto.
5. Il Ministero provvede ad applicare una decurtazione pari al 20% della rata del saldo, qualora la documentazione di cui alle lettere a) b) c) d) del comma 1 del presente articolo sia trasmessa al Ministero in un periodo compreso tra il sessantunesimo ed il novantesimo giorno dalla data di conclusione del progetto.

6. Il Ministero, previa comunicazione preventiva al Destinatario istituzionale, attiva le procedure per la sospensione del finanziamento e la conseguente economia della rata finale nonché per il recupero delle somme già erogate, comprensive degli interessi legali maturati, qualora la documentazione di cui alle lettere a) b) c) d) del comma 1 del presente articolo non sia trasmessa al Ministero entro il novantesimo giorno dalla data di conclusione del progetto.
7. Il Ministero si riserva la facoltà di chiedere informazioni ed eventuale documentazione integrativa al Destinatario istituzionale, che deve fornire riscontro entro e non oltre i successivi 15 giorni, qualora:
   - la relazione finale non sia considerata idonea a dimostrare il regolare svolgimento della ricerca, in conformità di quanto previsto nel piano esecutivo e nel piano finanziario approvati;
   - la rendicontazione risulti incompleta o incongruente sia sui dati contabili sia sulle descrizioni.
8. Il Ministero provvederà ad emettere la valutazione finale sulla base di quanto acquisito agli atti, in caso di mancato o esaustivo riscontro da parte del Destinatario istituzionale delle richieste di cui al precedente comma 7.
9. Il Ministero comunica al Destinatario istituzionale il parere negativo in ordine alla relazione finale e conseguentemente in ordine alla erogazione del saldo ed ha facoltà di chiedere la restituzione delle somme già erogate comprensive degli interessi legali maturati, in caso di mancato riscontro oppure laddove dalla istruttoria della documentazione integrativa emerga che sono stati disattesi gli obiettivi di cui al piano esecutivo.
10. Il Ministero, previa comunicazione preventiva al Destinatario istituzionale, può sottoporre al Comitato tecnico sanitario sez. c). un dossier, qualora la relazione finale all'esito della istruttoria ministeriale, non consenta di esprimere un compiuto motivato parere. La decisione del suddetto Comitato è vincolante per il Destinatario istituzionale ai fini del prosieguo della convenzione.
11. Il Ministero, previa comunicazione preventiva al Destinatario istituzionale, attiva le procedure per la sospensione del finanziamento e la conseguente messa in economia delle rate residue, nonché per il recupero delle somme già erogate, comprensive degli interessi legali maturati, in caso di mancato rispetto da parte del PI dell'orario di lavoro contrattuale, ovvero di quello minimo stabilito tramite convenzione con altro Ente, durante il periodo di svolgimento della ricerca. A tal fine, il Ministero si riserva la facoltà di richiedere al Destinatario Istituzionale i tabulati relativi alla durata dell'orario di lavoro giornaliero svolto dal PI, rilevato con sistema di misurazione oggettiva; il Destinatario istituzionale dovrà fornire riscontro entro e non oltre 15 giorni dalla richiesta.

Articolo 10

1. Il Ministero della salute – Direzione generale della ricerca e dell'innovazione in sanità, in via autonoma o sentito il Comitato Tecnico Sanitario, ha facoltà di chiedere chiarimenti e può disporre verifiche in ogni momento e anche durante lo svolgimento della ricerca.

Articolo 11

1. Il termine della ricerca può essere prorogato dal Ministero per un periodo massimo di mesi 12 dalla data di scadenza, solo a seguito di formale, motivata e documentata istanza firmata digitalmente dal legale rappresentante del Destinatario istituzionale e del principal investigator. A detto periodo possono essere applicate eventuali deroghe a seguito di provvedimenti della Direzione generale della ricerca e dell'innovazione in sanità per eventi emergenziali.
2. La richiesta di cui al comma 1 può essere avanzata dopo la presentazione della relazione intermedia di cui all'articolo 7 e fino a 12 mesi precedenti il termine del progetto con formale e motivata istanza da parte del Destinatario istituzionale e del principal investigator che avrà efficacia solo dopo l'approvazione da parte del Ministero.

Articolo 12

1. La proprietà degli studi, dei prodotti e delle metodologie sviluppati nell'ambito del progetto è regolamentata dalla normativa vigente in materia, salvo particolari accordi stipulati tra le parti firmatarie del presente atto, ferma restando la possibilità dei soggetti istituzionali del Servizio Sanitario Nazionale di fruirne, previa richiesta alle parti firmatarie.

2. Nel caso in cui il contraente intenda trasferire ad altri soggetti qualsiasi diritto, anche parziale, relativo alla ricerca in questione, ai risultati della stessa o ad eventuali brevetti derivati deve darne preventiva comunicazione al Ministero.
3. Qualsiasi documento prodotto, ivi comprese le pubblicazioni scientifiche inerenti al progetto di ricerca oggetto della presente convenzione – per i quali deve essere assicurato l'accesso non oneroso al Dicastero - deve contenere l'indicazione del finanziamento ministeriale e del codice del progetto finanziato.
4. Il Ministero non riconosce l'eleggibilità dei costi delle pubblicazioni sui propri fondi qualora in dette pubblicazioni non si faccia espressa menzione del finanziamento ministeriale e del codice progetto.
5. Il Ministero provvede ad una decurtazione pari al 10% dell'intero finanziamento, nel caso in cui il Destinatario istituzionale al termine delle attività progettuali non inoltri documentazione relativa a quella indicata alla lettera b) del comma 1 dell'articolo 9.
6. Il Ministero provvede ad una decurtazione pari al 10% dell'intero finanziamento, nel caso in cui il Destinatario istituzionale al termine delle attività progettuali non inoltri documentazione relativa a quella indicata alla lettera d) del comma 1 dell'articolo 9.
7. Il Ministero provvede ad una decurtazione pari al 5% dell'intero finanziamento, nel caso in cui il Destinatario istituzionale al termine delle attività progettuali inoltri documentazione relativa a quella indicata alla lettera b) del comma 1 dell'articolo 9 priva della menzione del Ministero della salute e del codice progetto.
8. Il Ministero provvede ad una decurtazione pari al 5% della rata del saldo, nel caso in cui il Destinatario istituzionale al termine delle attività progettuali inoltri documentazione relativa a quella indicata alla lettera b) del comma 1 dell'articolo 9 dalla quale risulti che solo alcune pubblicazioni prodotte recano la menzione del Ministero quale istituzione finanziatrice e del codice progetto.
9. Le parti convengono che il Ministero della salute possa dare direttamente diffusione, anche attraverso il proprio sito web, dell'estratto della proposta progettuale e dei risultati della ricerca sia in forma completa che sintetica e delle pubblicazioni scientifiche da essa derivate.

Articolo 13

1. I beni e gli strumenti necessari per l'esecuzione del presente progetto di ricerca possono essere posti a carico dei fondi ministeriali qualora acquisiti a mezzo leasing, noleggio ovvero in comodato d'uso, per un periodo pari alla durata del progetto.
2. È fatto divieto di utilizzare i fondi del Ministero della salute per l'acquisto diretto di apparecchiature e materiale inventariabile.
3. Relativamente ai progetti RF-CO-GR, per il pagamento di quote parte stipendiali è riconosciuto un contributo fisso al limite di euro 40.000,00 l'anno per *"full time equivalent"*, nei limiti del 50% del finanziamento complessivo del progetto ovverosia della quota totale rendicontata a carico del Ministero della salute e riconosciuta eleggibile.
4. Relativamente ai progetti SG, per il pagamento della borsa di studio del ricercatore proponente, è riconosciuto un contributo fisso al limite di euro 90.000,00 per l'intera durata del progetto.

Articolo 14

1. Le parti contraenti prendono atto che il finanziamento del presente progetto di ricerca afferisce alla gestione dei fondi per il finanziamento delle attività di ricerca o sperimentazione, "Ricerca Scientifica" *capitolo 3398/1 ed i seguenti perenti 3398/83,84 e 87, ad esclusione degli importi destinati agli IRCCS,* di pertinenza del centro di responsabilità Direzione generale della ricerca e dell'innovazione in sanità, dello stato di previsione del Ministero della salute, in relazione a quanto disposto dal decreto legislativo n. 502/1992 e successive modifiche ed integrazioni**.**

Articolo 15

1. Le parti si impegnano all'osservanza, per quanto di rispettiva competenza, delle disposizioni inerenti alla tracciabilità dei flussi finanziari di cui all'art.3 della legge 13 agosto 2010 n.136, e successive modifiche ed integrazioni.

Letto, confermato e sottoscritto con firma digitale, ai sensi dell'art. 21 del decreto legislativo 7 marzo 2005, n.82.

Roma, *(data della sottoscrizione come quella dell'ultima firma digitale apposta)*

per il Ministero della salute
Dott. Gaetano Guglielmi
Direttore dell'Ufficio 3
Direzione generale della ricerca e dell'innovazione in sanità

per il Destinatario istituzionale
Fondazione Policlinico San Matteo
Dott. Stefano Manfredi
Codice fiscale **MNFSFN62D12D150X**

Il presente atto è sottoscritto per presa visione
il principal investigator - **MELONI FEDERICA**
Codice fiscale **MLNFRC61P70G388B**

CAVALLINI

SAN MATTEO
RF-2021-12374476

Ministero della Salute Direzione generale della ricerca e dell'innovazione in sanità **BANDO RICERCA FINALIZZATA 2021** **esercizio finanziario anni 2020-2021 - Progetto Completo**	**Project Title:** Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis **Project duration (months):** 36
Project Code: RF-2021-12374476	**Principal Investigator:** MELONI FEDERICA
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

MDC primary: Cardiologia-Pneumologia

MDC secondary: Trapiantologia

Project Classification IRG: Cardiovascular and Respiratory Sciences

Project Classification SS: Lung Injury, Repair, and Remodeling - LIRR

Project Keyword 1: Pulmonary fibrosis and interstitial lung diseases: Includes granulomatous diseases (such as sarcoidosis), idiopathic pulmonary fibrosis, interstitial pneumonias, autoimmune lung diseases, and lymphangioleiomyomatosis. This would also include involvement of mesenchymal stem cells, epithelium dysfunction, and epithelial-mesenchymal transition.

Project Keyword 2: treatment

Project Keyword 3: liposomes

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

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

Patent number: n°102021000022253

Type patent owner: Public Inst. Company **Patent owner:** Policlinico San Matteo / Universita di Torino

Operative Units

	INSTITUTION	Department/Division/Laboratory	Role in the project
1	Fondazione Policlinico San Matteo	UOS transplant center UOCRheumatology	PI and CO-PI
2	University of Messina	Department of Pharmacology	collaborator
3	IRCCS Mario Negri	Department biochemical research and molecular pharmacology	collaborator and in charge for animal experiment

Operative Unit not SSN: OU2

Investigators, Institution and Role in the Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	di paola rosanna	2 - University of Messina	collaborator	10/08/1975
2	BIGINI PAOLO	3 - IRCCS Mario Negri	Collaborator	07/01/1970
3	Codullo Veronica	1 - Fondazione Policlinico San Matteo	CO-PI	21/08/1980
4	Fusco Roberta	2 - University of Messina	collaborator	21/01/1991

Co-PI: Codullo Veronica

Person in charge for the animal experiment: BIGINI PAOLO

Retired personnel: None within three years of project start

Overall Summary

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

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Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

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The study aims to develop an innovative nano-platform for the treatment of lung fibrogenic disorders (LFD). The approach involves local delivery by inhalation of drug-loaded liposomes, coated with hyaluronic acid (HA) to directly target CD44+ pathogenic cells. We aim to expand and develop the formulation "XHALIP¿ (patent pending) by the following steps: 1) Characterization of safety and bioavailability in healthy and lung fibrogenic disorders (LFD) mice; 2) Evaluation of the pharmacokinetics and uptake by human LFD fibroblasts and macrophages and on healthy/LFD mice; 3) testing of anti-fibrotic/-inflammatory activities of the most promising XHALIP on mouse LFD models and translational studies on lung cells/tissues from LFD patients. This research will allow XHALIP to scale up to human testing. The multidisciplinary consortium with clinical, chemical, pharmacological biological expertise, and the preliminary basis of available stable formulations, guarantee the achievement of the aims.

Background / State of Art

In inflammatory LFD chronic allo- or auto specific immune insults lead to mesenchymal cell (MC) proliferation and ExtraCellular Matrix (ECM) deposition involving lung interstitial spaces and small airways. Bronchiolitis obliterans syndrome (BOS), a lung or allogeneic bone marrow transplant complication, and interstitial fibrosis in collagen vascular diseases (CTD-ILD) represent the most severe disorders and, albeit rare, they bear a bad prognosis and significant mortality. Few therapeutic options are available: immune modulators (conventional or small molecules such Janus Kinase Inhibitors: JKI) and the oral antifibrotic tyrosine kinase inhibitor (TKI) Nintedanib, which shows a slight improvement of clinical progression but is burdened by significant toxicity. Among the different options to improve its therapeutic index, a liposomal formulation linking HA represents an interesting and innovative strategy to improve targeted lung delivery of TKI or JKI. The tissue amount of HA, an ECM component synthesized by MC, is related to inflammation, influences MC migration and immune cell activation. HA plays a key role in fibrosis by interacting with its receptor, CD44. As recently demonstrated, primary MC cell lines isolated from Broncho-alveolar lavage (BAL) of patients (pts) with CTD-ILD and BOS highly express CD44, while normal, non-inflamed epithelium does not. A nanoparticle dependent targeting to CD44 thus could be a promising approach to maximize delivery of TKI and other anti

It's available a Systematic Review on this topic? No

Hyphotesis and Specific AIMS

Hyphotesis and Significance

To satisfy the unmet need in the field of LFD, and from the evidence of a high CD44 expression by primary MCs, in the last years novel therapeutic approaches for LFD have been designed on the basis of the following considerations of nanocarriers: 1)Local delivery allows to reach high drug concentration in the lung avoiding systemic toxicity; 2)they permit to deliver to lung cells hydrophobic/toxic drugs otherwise difficult to administer as free drugs by inhalation; 3) they can be functionalized to induce an active uptake and targeting of pathogenic cells, sparing broncho-alveolar epithelium. A first evidence of the validity of this approach has been gained both in vitro on different lung cell populations (MC, epithelial cells, macrophages and other immune cells) and in vivo (mouse models of bleomycin induced pulmonary fibrosis (BM) and of heterotopic tracheal transplantation (HTT-M)). When anti CD44 coated imatinib loaded gold nanoparticles were administered intratracheally (see as reference Publication list from PI and Co-PI).
The exploitation of this approach has been hampered by the potential toxicity of gold after chronic intratracheal administration due to its poor biodegradability. To overpass this limitation, a liposomal formulation named XHALIP was therefore designed and fully characterized. We here provide limited data on the in vitro and in vivo activity of XHALIP since details are still confidential during patent evaluation. XHALIP was designed jointly by Turin University (Department of Drug Science Technology Prof Silvia Arpicco¿s Lab) and IRCCS San Matteo Foundation (PI lab) which presented a patent file on

August 2021 (n° 102021000022253).
XHALIP are functionalized on the surface by HA in order to specifically target CD44+ cells and loaded in their internal aqueous core with either a TKI (including imatinib and nintedanib) or a JKI (ruxolitinib). When tested on MC and immune cells XHALIP showed a high stability in hydrophilic solutions or plasma and a significantly higher uptake by CD44+ than by CD44- cells. XHALIP were also able to significantly: inhibit MC proliferation and viability, decrease in vitro collagen production by MC, and inhibit molecular drug targets, and were lacking intrinsic pro-inflammatory activity. Finally, encouraging preliminary results were obtained in the BM by Unit2. Unit 1 and 2 are already performing preliminary toxicity studies with XHALIP, Unit 3 has been recently involved in the research group thanks to its expertise in the field of pharmacology, pharmacokinetics and nanomedicine. Our consortium includes: clinical competences from Unit 1 to obtain human cells and tissues for in vitro tests with the Ethics already set up; a longstanding experience in animal models mirroring human diseases (HTT-M and BM) from Unit 2 (UniMe) and finally of pharmacokinetics and tissue and cell tracking systems (Unit 3 IRFMN). Unit 1 will provide XHALIP, under the supervision of UniTo that agrees in the presentation of present project.Since the last months, in collaboration with Unit 1, all groups have been working on the issue of pulmonary delivery of NPs in both healthy and LFD mice. In particular, we were able to select the intranasal and inhalatory way of NP administration to obtain the better lung/filter organs ratio. Very interestingly, intranasally administered fluorescent NPs were able to cross the lung barrier and to rapidly segregate in lung immune cells, mainly macrophages .Moreover, in the last years UO3 optimized a combined visualization of both fluorescent NPs and of the drug conjugated to them by a serial sectioning of different organ tissues (tumors, brains, lungs) alternating MALDI mass spectrometry imaging (MALDI-MSI) and and fluorescent microscopy acquisitions. This has an extreme biological relevance since it will enable us to monitor different time-points both the kinetics of NP penetration and the processes of drug release and its potential degradation or clearance.

Preliminary Data

Over the last years Units 1 and 2 have been performing preliminary toxicity studies with XHALIP, whereas Unit 3 has been recently involved in the research group thanks to its expertise in the field of pharmacology, pharmacokinetics and nanomedicine. A series of preliminary results have been recently provided by our team. Below we have summarized the most significant unpublished preliminary results (PR, see attached supporting picture):
PR 1: Based on previous experimental data from our group, a biocompatible formulation of XHALIP was designed according to the following process: drug-loaded liposomes were prepared by hydrating a lipid film composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, cholesterol and L-¿-phosphatidylglycerol (70:30:3 molar ratio) in citrate buffer pH 4.5. The obtained suspension was then extruded and gel filtration with HEPES buffer pH 7.4 was used to change the external buffer. To further encapsulate the drug into liposomes the transmembrane pH gradient method was used. XHALIP decorated liposomes were prepared as described (S. Arpicco, 2013). In these conditions, drug encapsulation efficiency value is about 85%, liposome size around 200 nm and zeta potential -30 mV. After 4-week storage at 4°C the formulations still conserve at least 90%-95% of the initial drug content and over this period no appreciable size and zeta potential change, precipitation or aggregation are observed.
PR2: Imatinib-loaded XHALIP were tested in vitro on primary MC from patients with CTD-ILD or BOS, lung-derived CD44+/- epithelial cell lines, and macrophages. Uptake of HA-conjugated liposomes by CD44+ cells was significantly higher than that of uncoated vehicles (Fig1 PR2). CD44-negative epithelial cells showed a significantly lower liposome uptake, while alveolar macrophages were actively internalizing this formulation. In vitro cell treatment caused a significant inhibition of cell proliferation (fig2 PR2), a significant inhibition of collagen production and of the expression of phospho-c-abl (Fig3 PR2).
PR 3: Intranasal XHALIP administration leads to a quick and massive penetration in lung parenchyma.
PR 4: Integrated spectroscopy and imaging enabled us to follow the fate of both nanoparticles and associated drugs in mouse sections over time.

PR5: Research lab of Unit1 has recently established thin cut lung slice cultures. (Fig1 UPR5)
PR6: preliminary experiments in the mouse model of bleomycin-induced pulmonary fibrosis have been conducted by Unit 3 with positive encouraging results. Specific data are not available since confidential due to pending patent extension.

Picture to support preliminary data
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Specific Aim 1

Our project will be carried out through three, tightly related, aims.
AIM 1: XHALIP characterization and bio-nano interaction
As previously explained, the project makes use of nanocarriers already extensively characterized in vitro in terms of colloidal stability, loading capacity and drug release. The different XHALIP encapsulating different agents (Imatinib, Nintedanib and Ruxolitinib) have been already assessed and will be made available for the study by Unit 1 with the support and supervision of Prof Arpicco¿s laboratory at UniTo. Two different nanoformulations will be employed: drug-unloaded fluorescent liposomes (F-LIP) for distribution studies and drug loaded liposomes (XHALIP) for safety/toxicology studies.
F-LIP will be firstly delivered by three administrations to a small group of healthy animals: aereosolization, intratracheal and intranasal route to choose the one which is associated to the highest and even lung distribution. The best method will be used for further experiments. Further experiments will be performed on healthy animals and in a small subgroup of BM animals (to assess whether in pathologic conditions we assist to a different permeability and distribution).
The safety of these liposomal formulations will be tested after an acute and a chronic treatment (following Acute Toxicity EOCD 425 and Chronic toxicity EOCD 452 protocols) at Unit 2 and 3. To discriminate between a potential toxicity due to the nanocarriers and the drug incorporated, another group of mice receiving the empty liposome and three groups receiving the three drugs respectively will be enrolled. To determine the impact of the different formulations observational, haemato-chemical and histopathological analyses will be carried out.
In addition to the toxicity analyses, we will evaluate the potential risk due to bio-accumulation of both liposomes and drugs in the main off-target organs (liver, spleen and kidneys) combining histology (liposomes will be labeled with a dye detectable by confocal laser excitation) and mass spectrometry (the three drugs will be HPLC/MS/MS). Moreover, by the overlapping of serial micrometric sections alternatively processed for the fluorescence detection (by virtual slider Olyvia-Olympus scanning) and the Maldi-Imaging (Hamamatsu Instruments) we will be able to follow the fate of carrier and cargo in different organs and at different time-points.
Finally we will also explore in vitro different-fluorescent XHALIP preparations on a model of alveolo-capillary barrier (Unit1) in order to analyze the ability to interact with mucous/surfactant layers and to cross the alveolo-capillary interface.

Specific Aim 2

Organ and cell lung Targeting
The second aim of this project is the evaluation of the lung lung penetration, the release and the potential accumulation in macrophages of the formulations selected by the safety studies. The in vivo study will be carried both in healthy and LFD mice at Unit 3 and 2 respectively. Firstly, an acute (single) treatment will be observed at different time-points (from 15 mins to 24 hours) to determine the kinetics of penetration of XHALIP in lungs, the ability to interact with target cells and the organ distribution of both liposomes and incorporated molecules. These analyses will be carried out by the procedures described in AIM 1. Both histology and pharmacokinetic analyses will be performed. Subsequently, in lungs collected from the animals used for safety studies, we will evaluate the amount of drug(s) and the interaction with target cells (for this study the animals will be sacrificed 1 hour after the last administration). This phase will be pivotal to select the best formulation able

to improve the lung delivery of drugs and minimizing the side-effects in off-target organs.
In order to get an adequate statistical robustness, according to the 3R principles, the sample-size of each experimental group will be calculated (with the aim of avoiding any unjustified and unethical use of an excessive number of animals). In order to make our study more translational, a comparison between the behavior of primary MC, lung epithelial and lung endothelial cells, macrophages, lymphocytes and neutrophils deriving from either mice or LFD patients will be carried out and comparing the uptake ability, the subcellular localization, interaction with molecular drug targets, and drug interference in response to pro-inflammatory or pro-fibrotic stimuli. Finally, Unit 1 will perform an analysis of F-LIP uptake and XHALIP activity on specific lung cells on thin cut lung slice (TCLS) cultures obtained from normal mouse and human lungs and from lungs of patients with LFD (explanted in case of lung Transplantation or Re-transplantation). These studies will be performed with confocal microscopy and electron microscopy + histology + immune histochemical examination of TCLS cultures. The results that will emerge from this part of the project will be crucial for defining the best treatment schedule and most relevant cellular targets in animal models of diseases (AIM 3).

Specific Aim 3

Efficacy assessment in lung fibrosis models

To this aim two well-characterized animal LFD models(the Bleomycin lung fibrosis Model and the Heterotopic trachea transplantation model respectively) will be used.The two highest performing XHALIP formulations (selected in 1st and 2nd phase of the project as for lung biodistribution and safety profile) will be administered to animals following specific administration time points (every 5 days to sacrifice starting from day 10 after intratracheal Bleomycin administration or from day 10 after heterotopic tracheal transplantation). The HTTM will require local administration of a liposome suspension into a subcutaneous pouch, while in BM model the best administration way among aereosolization, intratracheal and intranasal selected in AIM1 will be adopted. These time points have been selected in order to reflect at best human treatment needs (diagnosis of both these disorders is usually made at a relatively late time points when the fibrogenic process has already started) and considering the drug releasing profile of XHALIP. Liposome dosage will be selected according to results obtained in AIM1 and 2. As a control, the same dose of the selected free drug will be administered by parenteral/subcutaneous route. A vehicle-treated group will be included as control. In each model, the efficacy of the therapy will be evaluated by assessing a number of endpoints: mortality, histological damage by HE and Masson staining for architectural damage and fibrosis, Collagen determination, myeloperoxidase and Malondialdehyde staining and immunohystochemistry for TGF beta, lymphocyte, neutrophil and macrophage infiltration as well as Western blot analysis for several factors including, among others, TRAF-6, NF-kb, IKKb, mmp 9, mmp2, p-JNK, p-p38 and specific drug molecular targets analysis.
In addition, the expression of several cytokines and mediators involved in the development of the inflammatory /fibrogenic response and endothelial mesenchymal transition (EMT) will be evaluated by RT-qPCR. The evaluation of these expression patterns at time 0 and at end of treatment with different XHALIP formulation will help in defining the best treatment schedules.

From this project we expect to both provide further indications on the potential translation of XHALIP and give an important boost to the development of innovative devices in the field of materials science and nanomedicine. If successful, the results that will emerge from this study may pave the way towards a future clinical translation.

Experimental Design Aim 1

XHALIP will be produced for the study by Unit 1 with the support and supervision of Prof Arpicco's laboratory at UniTo. Two

different nanoformulations will be employed: drug-unloaded fluorescent liposomes (F-HALIP) for distribution studies and drug-loaded liposomes (XHALIP) for safety/toxicology studies (some XHALIP will also be produced as fluorescent with Rhodamine linked to the outer layer). F-HALIP will be firstly delivered to a small group of healthy animals to compare 3 different administration routes, namely aerosolization, intratracheal, and intranasal instillation with the aim of choosing the formulation with the highest and more uniform intrapulmonary distribution. The most efficient method will then be used for further experiments that will be performed in a small subgroup of BM animals to assess whether pathologic conditions may lead to different permeability and distribution. To discriminate the potential toxicity of empty nanocarriers, mice receiving F-HALIP will be then compared with 3 groups each receiving a XHALIP formulation loaded with one of the three drugs. To determine the impact of the different formulations, biochemical and histopathological analyses will be performed after the sacrifice of mice. We will measure and discriminate the biodistribution of both liposomes and drugs after synchronous administration of F-HALIP and XHALIP in all organs by an innovative approach combining serial micrometric sections alternatively processed for fluorescence detection (F-HALIP labeled with a dye detectable by confocal laser excitation) and Matrix Assisted Laser Desorption/Ionization (MALDI) imaging, where the spatial distribution of the chemical compounds incorporated into the liposomes will be detected by mass spectrometry. Moreover, a quantitative measurement of drug accumulation will be carried out by HPLC/MS/MS. This approach will allow us to follow the fate of carrier and cargo in different organs at different time-points. Finally Unit 1 will explore in vitro different F-HALIP preparations on a model of alveolo-capillary barrier in order to analyze their ability to interact with mucous/surfactant layers and to cross the alveolo-capillary interface.

Experimental Design Aim 2

The in vivo study will be performed both in healthy and LFD mice at Units 3 and 2 respectively. Firstly, an acute (single) treatment will be followed at different time-points from 15 minutes to 24 hours to determine the kinetics of penetration of XHALIP in lungs, their ability to interact with target cells and the organ distribution of both liposomes and incorporated molecules. Both histology and pharmacokinetic analyses will be performed. Subsequently, from lungs collected from the animals used for safety studies (see experimental design aim 1), we will evaluate the amount of drug(s) and the interaction with target cells (for this study the animals will be sacrificed 1 hour after the last administration). This phase will be pivotal to select the formulation providing the most efficient lung delivery of drugs and lowest side-effects in off-target organs. In order to make our study more translational, a comparison between the behavior of primary MC, lung epithelial and endothelial cells, macrophages, lymphocytes and neutrophils deriving from both mice and LFD patients will be performed to compare uptake ability, subcellular localization, interaction with molecular drug targets, and drug interference in response to pro-inflammatory or pro-fibrotic stimuli. Finally, Unit 1 will perform an analysis of F-HALIP uptake and XHALIP activity on specific lung cells on thin-cut lung slice (TCLS) cultures obtained from normal mouse and human lungs and from lungs of patients with LFD (explanted in case of lung Transplantation or Re-transplantation). These studies will be performed with confocal microscopy, electron microscopy, conventional histology and immunohistochemistry of TCLS cultures. The results that will emerge from this part of the project will be crucial for defining the best treatment schedule and most relevant cellular targets to be translated in animal models of diseases (AIM 3).

Experimental Design Aim 3

Animal models will be prepared as follows

Animals: CD1 mice (6-12 weeks old) for BM model and C57BL/6 and BALB/c mice (6¿12 weeks old) for HTT model, will be housed in a controlled environment and will be provided with standard rodent chow and water. Procedures involving animals and their care will be conducted in conformity with the Italian regulations on protection of animals used for experimental and other scienti¿c purpose (D.M. 116192) as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986).

BM: Bleomycin administration will be performed as previously described (Di Paola et al., 2016). Bleomycin sulphate (0.1 IU per mouse) is delivered by an intratracheal administration (Cross et al., 2017). Experimental groups: 1) Bleomycin: Mice receiving bleomycin administration and treated daily with F-HALIP; 2) Control group: mice receiving intratracheal instillation of saline (0.9% w/v) instead of bleomycin, and treated daily with F-HALIP; 3) Treatment group: Mice receiving bleomycin and treated with XHALIP every 5 days to sacrifice starting from day 10 after intratracheal bleomycin administration.
HTT: Tracheas from C57BL/6 mice will be implanted into Balb/c mice (allogeneic) or C57BL/6 mice (syngeneic) as previously described (Ming Dong et al., 2015). Experimental groups: 1) Control group: animals subjected to orthotopic tracheal transplantation and treated with F-HALIP; 2) Treatment group: animals subjected to orthotopic tracheal transplantation and treated with XHALIP every 5 days to sacrifice starting from day 10 after heterotopic tracheal transplantation; 3) Sham-operated mice not subjected to orthotopic tracheal transplantation but treated with F-HALIP.
XHALIP will be administered to animals following a specific time schedule (every 5 days to sacrifice starting on day 10 after intratracheal bleomycin administration or heterotopic tracheal transplantation). HTTM will require local administration of a liposome suspension into a subcutaneous pouch, while in BM model the best administration route between aereosolization, intratracheal and intranasal instillation selected in AIM1 will be adopted. This schedule has been designed in order to reproduce at best human treatment requirements, since diagnosis of both disorders is usually made at a relatively late stage when the fibrogenic process is already established, and taking into consideration the profile of drug release from XHALIP. Liposome dosage will be decided according to the results obtained in AIM1 and 2. As a control, the same dose of the selected free drug will be administered by parenteral/subcutaneous route. A
In each model, the efficacy of the therapy will be evaluated by assessing the following endpoints: mortality, histological damage by HE, Masson staining for architectural damage and fibrosis quantification, immunohistochemical stains for collagen, myeloperoxidase, TGF beta, lymphocyte subset characterization, neutrophils and macrophages count, as well as Western blot analysis for several factors including, among others, TRAF-6, NF-kb, IKKb, mmp 9, mmp2, p-JNK, p-p38 and specific drug molecular targets (Impellizzeri et al., 2015),(Conte et al., 2014),(Di Paola et al., 2016). In addition, the expression of mRNA levels of several cytokines and mediators involved in the development of the inflammatory/fibrogenic response and endothelial mesenchymal transition (EMT) will be evaluated by RT-qPCR. The evaluation of these expression patterns at time 0 and at the end of treatment with different XHALIP formulations will help in defining the best treatment schedules.

Metodologies and statistical analyses:

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

In vitro experiments:
F-HALIP uptake in lungs will be evaluated both by a qualitative (confocal microscopy) and by a quantitative method (flow cytometry). All other variables (cell viability and cell proliferation, endothelial-mesenchymal-transition) will be evaluated by quantitative/semi-quantitative methods as flow cytometry, MTT test, expression studies and WB analysis. In order to avoid bias replicate experiments and adequate negative and positive controls (untreated cells, cells treated with free drugs -not loaded in liposome- HA-uncoated drug-loaded liposomes) will always be included in the experiments. We will also perform replicated experiments on the in vitro model of alveolo-capillary unit and on precision cut lung slices of fresh explanted fibrotic and BOS lungs. Histological and immunohistochemcal evaluation of PCLS with blinded lectures will be provided in order to avoid interpretation bias.
Animal studies:
In order to get an adequate statistical robustness, according to the 3R principles, the sample size of each experimental group will be calculated to avoid any unjustified and unethical use of an excessive number of animals.

1. Longitudinal Biodistribution: In vivo ad ex vivo imaging to track the fate of fluorescent liposome composing the XHALIP in mice will be carried out measuring tissue fluorescence levels by Lumina-X5 IVIS Imager system (PerkinElmer) as previously described (Violatto MB. Et al., 2019.). To maximize the signal/background ratio, liposomes will be functionalized with a dye falling in the infra-red that is the best condition to minimize the tissue autofluorescence.
2. PK Studies: To detect the presence of the therapeutic agents in plasm, lungs and off target tissues drug levels will be measured by HPLC/MS in plasma and different tissues harvested at sacrifice, in order to determine pharmacokinetic profiles (e.g. the half-time of elimination in plasma) and, mainly, the liver-to-plasma ratios as previously described (Ongaro A. et al., 2022). Positive standars (the calibration curve of the drug alone) and inner controls (measurements in mice not receiving the drugs) will be used to minimize the experimental bias.
3. Combined Confocal-Maldi imaging: this approach will be carried out to determine the interaction between carriers and drugs in tissues. The evaluation of spatial distribution of both liposomes and therapeutic agents forming the XHALIP will be achieved by serial sectioning of lung and off-target tissues and alternate evaluation of the fluorescence with confocal microscopy and the spectroscopy of analytes (drugs) by Maldi Imaging, as previously reported (Giordano S Sci Rep. 2016). The calibration curve associated with the spatial spectroscopy will be drawn using internal standards, whereas untreated animals will be used as inner control in order to avoid confounding results due to the tissue background signals.
Animal models pitfalls and Solutions
To monitor any adverse effects not foreseen following the administration of the substances under examination, the state of health of the animals will be kept under observation by the researchers and by the staff of the animal facility for the duration of the study.
The risks for the operator are linked to the use of laboratory animals (possibility of being bitten or scratched by animals or injuring oneself with sharp objects such as needles). All staff are trained in the use of appropriate personal protective equipment and suitable hoods (eg hoods for the elimination of litter); in addition, the staff is trained in the use of specific operating procedures to minimize the risk of injury.

Methods of data collection (Indicate the data that will be collected, the tools used)
All experimental data will be collected by the three Units according to their coordinating role in the specific WPs. Raw data obtained in each single experiments (conducted in any of the participant Units) will be collected in excel files under the responsability of the WP coordinator, and will be available for review for the whole length of the project.

Statistic plan (calculation of statistical data)
Since clinical variables are not included in this study, statistical analysis will be performed straightforward on experimental replicates including appropriate negative and positive controls.

Statistical analysis (describe the main statistical analysis)
When normally distributed variables will be analysed, groups will be compared by means of T-Student test or one-way ANOVA, and group means will be then compared by Dunnett's test using a statistical package (GraphPad Prism 4.0). A p value of <0,05 will be considered as statistically significant. If variables will not be normally distributed non parametric tests will be applied.

Timing of analysis data (indicate duration of study: duration of enrollment, of therapy, follow-up etc)
Planning of the study waypoints is detailed below.
We do not envisage difficulties in patient recruitment for the ex vivo experiments (BAL lung cell isolation and cultures, precision cut lung slices cultures) since these experiments are already performed routinely in Unit 1 lab on samples obtained from patients referring to UNIT1 for diagnosis and treatment of lung conditions acconding to protocols approved by EC.

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Analysis of in vitro and pharmacokinetic/biodistribution experiments will be crucial for the calculation of animal model sample size and planning of dose and time of liposome administration to mice.
Animal experiments will be planned according to the below reported schedule (see also GANTT Chart); statistical analysis will be performed at the completion of experiment sets.

WP 1 liposome preparation (1-30M)
task 1.1 preparation of fluorescent HA-coated and uncoated LIPOSOMES (1-24M); task 1.2 preparation of Imatinib-loaded HA-coated and uncoated Liposomes (7-30M); task 1.3 preparation of nintedanib-loaded HA-coated and uncoated liposomes (10-30M); task 1.4 preparation of ruxolitinib-loaded HA-coated and uncoated Liposomes (10-30M).
WP 2 in vitro assessment of uptake and activity of different XHALIP preparations (1-12M)
task 1.1 uptake by MC, epithelial cells, endothelial cells, macrophages and other inflammatory cells (3-12M); task 2.2 distribution across alveolo-capillary barrier in vitro (3-12); task 2.3 assessment of in vitro activity on different cell types (proliferation, collagen production, migration, viability, cytokine release): (3-12M); task 2.4 assessment of distribution in PCLS cultures (3-30M)
WP 3 pharmacokinetics studies in Animals (3-29M)
task 3.1 Combined Maldi-Confocal Imaging optimization for the tracking of XHALIP in lungs (3-29M); task 3,2 Biodistribution, nanosafety assessment in animals (10-23M); task 3.3 Pharmacokinetics (17-29M)
WP4 Animal Toxicology (8-31M)
task 4.1 acute toxicity studies (8-21M); task4.2 chronic toxicity studies (8-30M); Task 4.3 analysis of cell toxicity (20-31M)
WP5: Activity in animal models (8-30M)
Task 5.1 animal model of fibrosis (8-30M); Task 5.2 animal model of BOS (8-30M).
WP6 coordination and dissemination Task 6.1 coordination (1-36M); Task 6.2 dissemination (10-36M)

Expected outcomes

We have previously demonstrated that XHALIP nanoformulation is a reliable and innovative device to maximize the stability and lung macrophage uptake of TK inhibitors drugs. From this project we expect both to provide further indications on the potential translation of XHALIP (Toxicity, PK and Biodistribution, Ativity in animal models BM and HTT) and to give an important boost to the development of innovative devices in the field of materials science and nanomedicine. If successful, the results that will emerge from this study may pave the way towards future clinical translation.

Risk analysis, possible problems and solutions

Aim 1 Protocols of liposome preparation have been fully standatdized. For this reason we do not envisage any potential risk
Aim:2.1 Potential risk: Toxicity or bio-accumulation in off-target organs of XHALIPs. Solution: Modify the posology (doses, frequency, route of administration).
Aim:2.2 Potential risk: Reduced release of drug at the late symptomatic phase. Solution: Modify the temporal window by increasing the doses or the frequency of treatment along the clinical progression.
Aim:2.3 Potential risk: Low efficacy due to accelerated clearance of XHALIP or limited penetration in target cells Solution: Modify the liposome surfaces to accelerate the penetration in lungs and in target cells
Aim 3.Potential risk : we do not envisage problems in the assessment of XHALIP efficacy thanks to satisfactory preliminary data in BM; technical problems related to HTT can be forecast, due to the need of XHALIP instillation in the subcutaneous pouch In this case the use of Alzet pump will be considered to guarantee adequate drug delivery.

	Project Title: Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis **Project duration (months):** 36
Project Code: RF-2021-12374476	**Principal Investigator:** MELONI FEDERICA
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Significance and Innovation

Significance: Dissimilarly to other projects of preclinical nanomedicine where the goal is to bypass filter organs to increase the accumulation of nanoparticles into the target, we are exploiting the inhalatory way in order to maximize drug load in lungs. Although this proposal focuses on inflammatory-fibrotic lung disorders, in case of positive results this approach could be easily extended to other lung disorders, such as pulmonary hypertension or lung cancer. In this latter case the evidence that XHALIP are efficiently internalized in macrophages will be relevant in the view of modulation of tumor-specific immune responses (often inhibited by tumours).-
Innovation: None of the liposomes registered for inhalation so far (amikacin- or amphotericin B-loaded vehicles) are targeted or addressed to fibrogenic disorders. The development of a targeted TKI formulation adequate for inhalatory administration would represent an absolute innovative step in the field of nano-based therapy.

Description of the complementary and sinergy research team

Our consortium includes: -clinical expertise (Unit1) in diagnosis and management of LFD,crucial to obtain cells and tissues for in vitro tests (available approval EC); -longstanding experience in mice models which mirror human chronic airway rejection (HTT-M) and pulmonary fibrosis (BM) from Unit 2 (UniMe), and -extended experience of pharmacokinetics and tissue and cell tracking systems (Unit 3 IRFMN). Unit 1 will provide XHALIP, under the supervision of UniTo that agrees in the presentation of this project. The present project will involve also some young researchers at the three Units. The coordinator of the project, Prof. Federica Meloni is a respiratory and lung transplant physician with research experience who already acted as PI of an euronanomed research consortium, the PI of UNIT 2 Rosanna di Paola, as a pharmacologist, has developed and studied previously the 2 animal models and performed animal toxicology studies also in collaboration with Unit 1. The PI of the UNIT 3, Dr Paolo Bigini, has a relevant experience in pharmacology and nanomedicine and his group is specialized in nanotracking and pharmacokinetics. He is collaborating with Unit 1 in the last year for the development of a nanotechnological approach to chronic lung diseases . The close relationship between researcher coming from different scientific fields is strongly required to get to a real impact in terms of technological exploitation of nanomedicine products and dissemination of the results.

Training and tutorial activities

The complementary background of the project members will allow the organization of multidisciplinary training sessions to share ideas, knowledge, and skills bridging different expertise from chemistry to medicine. A workshop on nanotechnology and clinical translation will be organized Unit 1. Attendance will be open to University students,hospital researchers and general public. A series of seminars on preclinical imaging and nanokinetics will be held at the Unit 3. In this context, the speakers will consider the chemical problems related to both diagnostics and therapy. A selected number of students and researchers will have the possibility to visit the mouse clinic of Unit 3 and the Radiology department ofUnit 1 to compare in vivo imaging instruments used for mice and patients (e.g. MicroCT, MRI, EcoScan). Finally, symposia and conferences on preclinical/clinical lung disorders will be hosted both at the IRCCS Foundation Policlinico San Matteo and at Unit 2.

Bibliography

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Di Paola R, Impellizzeri D, Fusco R, et al. Ultramicronized palmitoylethanolamide (PEA-um((R))) in the treatment of idiopathic pulmonary fibrosis. Pharmacol Res 2016; 111:405-412

Di Paola R, Cordaro M, Crupi R, et al. Protective effects of ultramicronized palmitoylethanolamide (PEA-um) in myocardial ischaemia and reperfusion injury in VIVO. Shock 2016;46:202-13.

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Giordano S, Morosi L, Veglianese P, et al. 3D Mass spectrometry imaging reveals a very heterogeneous drug distribution in tumors. Sci Rep 2016;6:37027

Impellizzeri D, Talero E, Siracusa R, et al. Protective effect of polyphenols in an inflammatory process associated with experimental pulmonary fibrosis in mice. Br J Nutr 2015;114:853-65.

Ongaro A, Violatto MB, Casarin E, et al. The mode of dexamethasone decoration influences avidin-nucleic-acid-nano-assembly organ biodistribution and in vivo drug persistence. Nanomedicine 2022;40:102497

Violatto MB, Casarin E, Talamini L, et al. Dexamethasone conjugation to biodegradable avidin-nucleic-acid-nano-assemblies promotes selective liver targeting and improves therapeutic efficacy in an autoimmune hepatitis murine model. ACS Nano 2019;13:4410-4423

Timeline / Deliverables / Payable Milestones

Deliverables

D 1.1 preparation of stable F-HALIP and XHALIP with the 3 drugs [M3¿>30].

D 1.2: Release and sharing of protocols for nanosafety and PK studies of XHALIPs in healthy and diseased mice, and standardization of procedures for the generation of models [M9].

D 1.2: Mid-term report with main results achieved in toxicity screening of XHALIPs in cells and mice [M18]

D 2.1: Release and sharing of protocols of combined in vivo and ex vivo analyses in mice treated with XHALIP [M18].

D 2.2: Final report with the results on the biodistribution and pharmacokinetics of different XHALIP preparations in cells and in healthy and diseased mice [M 36].

D2.3 fFnal report on the toxicity studies of different XHALIP preparations both in vitro and in vivo

D 3.1 Identification of the treatment schedule providing the best disease control according to histo-molecular studies [M 36].

D 3.2 Final report describing the main results of therapy achieved in the chronic treatment of BM and HTT [M 31].

Milestones 18 month

M18 1 conclusive evidence on XHALIP and F-HALIP in vitro uptake and activity on different cell types, including their ability to cross alveolo-capillary barrier and in vitro uptake

Milestones 36 month

M36 1 conclusive evidence on acute and chronic toxicity of different XHALIP preparations
M36 2 conclusive evidence on biodistribution and phamacokinetic studies
M36 3 conclusive evidence on the activity of different XHALIP preparations in animal models (BM and HTT)
M36 4 conclusive evidence on specific cell uptake and activity on PCLS cultures

Gantt chart

gantt finalizz2022 meloni pi.pptx

Equipment and resources available

Facilities Available

The group of Dr Bigini (IRCCS-Mario Negri Institute) has access to a fully equipped laboratory with modern mass spectrometers.and instruments available for performing HPLC-MS/MS at low and high resolution: API 3000 and API 5500 triple quadrupoles, and a nano-HPLC LTQ-Orbitrap-XL FT/MS and MALDI-Imager-Shimatsu. In addition, a mouse clinic equipped with modern instruments for in vivo imaging in rodents (Magnetic Resonance Imaging, Fluorescence Molecular Tomography, MicroCT, Eco-Scan, Two-Photon Confocal Microscopy) and instruments for imaging of cells and tissues at cellular and subcellular resolution (Olympus Fluoview microscope, Atomic Force Microscope, transmission electron microscope and Super-Resolution Microscope) are available and currently in use. Dr. Meloni¿s Laboratory has an established qualification in nanomedicine research including study of cell uptake and biological activity of new nanocarriers. The lab has also acquired experience in the synthesis of XHALIP according to protocols established and shared with University Of Turin (Prof Arpicco¿s laboratory). Lab is provided with equipment for biochemistry and molecular biology, cell cultures, cellular and molecular biology, confocal laser scanning microscope (Olympus) and vibratome (Compresstome® VF 210-0Z Vibrating Microtome). Finally Unit2 has an animal facility + all equipment for histochemistry and immunohistochemistry as well as WB or expression studies.

Subcontract

In this project subcontract is not required. Prof Arpicco from the University of Turin agreed in providing full technical assistance to Unit 1 for the synthesis of XHALIP according to the already established protocol that Unit 1 and University of Turin developed over the last three years.

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

The present project has a concrete translational spillover. XHALIPare a very promising formulation and, according to preliminary data, their further development by means of this research, will provide a strong base for the scale up to human application. Our liposomes will be the first targeted drug-loaded liposomes to be applied for the local treatment ofLFD which, whilst classified as rare diseases, are still associated toa high rate of morbidity and mortality. In addition, there is a strong rational basis for the application of XHALIP in the treatment of other pulmonary disorders, where the use of a TKI: imatinib has been halted by its systemic side effects. Finally, as a consequence of this research, other possible targeted liposomal formulations specifically designed for the inhalatory application could be developed for human application.

Ministero della Salute Direzione generale della ricerca e dell'innovazione in sanità **BANDO RICERCA FINALIZZATA 2021** **esercizio finanziario anni 2020-2021 - Progetto Completo**	**Project Title:** Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis **Project duration (months):** 36
Project Code: RF-2021-12374476	**Principal Investigator:** MELONI FEDERICA
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Principal Investigator (PI) Profile

MELONI FEDERICA

Birth date: 30/09/1961

Institution: Fondazione Policlinico San Matteo
Department/Unit: UOS transplant center
UOCRheumatology
Position Title: Principal investigator

Education and training

Institution and Location	Degree	Year(s)	Field of study
University of Pavia	Specialization in Oncology	1993	thesis on small cell lung cancer
University of Turin	PhD in pharmacology and Toxicology	1995	immunotoxicology of the lung
University of Pavia	Degree in medicine and Surgery	1986	degree thesis was held in the hemathology deptm .

Personal Statement:

F.M has experience as project cocordinators and , thanks to an already established collaboration with both Unit 2 and 3, PI and Co PI (who possess a solid clinical background and provide skills and samples from ex vivo studies) will be able to:

- Coordinate the project

- Provide already set up- stable XHALIP preparations to the consortium

- Perform all ex vivo tests on Human samples (in vitro test on primary cell cultures, on models of alveolar -capillary barrier, and on thin cut lung tissue slices) from patients with CTD-ILD or BOS

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
IRCCS San Matteo	Respiratory Disease Unit	Diagnostic and Research Laboratory of Respiratory Diseases (SMEL 836)	Chief	2010	2022
IRCCS San Matteo	general direction	UOS transplant center	chief	2021	2022
University of Pavia	Respiratory Diseases	IRCCS Policlinico San Matteo In convention	Associate Professor	2001	2022
University of Pavia	Respiratory Diseases	Policlinico San Matteo (in convention from 1/11/1997)	Researcher	1992	2001

Project Title:
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Project duration (months): 36

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Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Official H index: 33.0 (autocertificated)

Scopus Author Id:7005166124 **ORCID ID:**0000-0003-4014-0617 **RESEARCH ID:**K-5828-2016

Other awards and honors

From September 2018 to September 2021 secretary of Assembly 8 (Thoracis Surgery and Transplantation) of the European Respiratory Society, Chair Elect since 2021
Member of the ERS Long range planning committee of the Thoracic Surgery and Lung Transplantation assembly
Since 2018 she gained the National Scientific Qualification as Full professor in the Discipline of Respiratory Disease

Other CV informations

During her career she developed a scientific interest on the immunological mechanisms of respiratory diseases, PHD was completed at the University of Bern focusing on the biological activity of IL8 in lung fibrosis. Later, she was involved in the following fields: the pathogenesis of interstitial lung fibrosis associated to collagen vascular diseases (CTD-ILD) and in the last 20 yrs, immune and fibrogenic mechanisms of chronic lung rejection. Since 2014 she started developing a local nano-based treatment of these diseases. Present project represent the continuation of a 2017 euro-nanomed ARROW NANO project (PI F.M), that brought to the development of XHALIP. From the clinical point of view F.M is now responsible of the follow up of Lung recipients and , together with Co-PI of CTD-ILD.

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

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
miRNAs Potentially Involved in Post Lung Transplant-Obliterative Bronchiolitis: The Role of miR-21-5p	Article	688	10	2021	10.3390/cells10030688	33804639	6.6	0	L
Neutrophil Extracellular Traps Induce the Epithelial-Mesenchymal Transition: Implications in Post-COVID-19 Fibrosis.	Article	663303	12	2021	10.3389/fimmu.2021.663303	34194429	7.561	5	L
Liposomes Loaded with Everolimus and Coated with Hyaluronic Acid: A Promising Approach for Lung Fibrosis.	Article	7743	22	2021	10.3390/ijms22147743	34299359	5.924	0	L
Loading Imatinib inside targeted nanoparticles to prevent Bronchiolitis Obliterans Syndrome.	Article	20726	10	2020	10.1038/s41598-020-77828-y	33244143	4.38	1	L
Calcineurin Inhibitor-Based Immunosuppression and COVID-19: Results from a Multidisciplinary Cohort of Patients in Northern Italy. Microorganisms.	Article	977	8	2020	10.3390/microorganisms8070977	32629788	4.128	28	L
Broncho-alveolar inflammation in COVID-19 patients: a correlation with clinical outcome.	Article	301	20	2020	10.1186/s12890-020-01343-z	33198751	2.99	27	L
Peripheral CD19+CD24highCD38high B-regulatory cells in lung transplant recipients.	Article	101245	57	2019	10.1016/j.trim.2019.101245	31526864	1.624	1	L

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
Hyaluronic Acid-Decorated Liposomes as Innovative Targeted Delivery System for Lung Fibrotic Cells.	Article	3291	24	2019	10.3390/molecules24183291	31509965	3.286	7	L
Analysis of long term CD4+CD25highCD127- T-reg cells kinetics in peripheral blood of lung transplant recipients.	Article	102	17	2017	10.1186/s12890-017-0446-y	28720146	1.0	7	L
Bioengineered gold nanoparticles targeted to mesenchymal cells from patients with bronchiolitis obliterans syndrome does not rise the inflammatory response and can be safely inhaled by rodents.	Article	534-545	11	2017	10.1080/17435390.2017.1317862	28415888	5.996	5	L
Identification of miRNAs Potentially Involved in Bronchiolitis Obliterans Syndrome: A Computational Study.	Article	e0161771	11	2016	10.1371/journal.pone.0161771	27564214	2.806	4	L
Cova E, Colombo M, Inghilleri S, Morosini M, Miserere S, Peñaranda-Avila J, Santini B, Piloni D, Magni S, Gramatica F, Prosperi D, Meloni F. Antibody-engineered nanoparticles selectively inhibit mesenchymal cells isolated from patients with chronic lung allograft dysfunction. Nanomedicine (Lond). 2015 Jan;10(1):9-23. doi: 10.2217/nnm.13.208. Epub 2014 Feb 21. PMID: 24559038. Citazioni 16	Article	9-23	10	2015	10.2217/nnm.13.208	24559038	4.756	37	L
Systemic inflammatory response and downmodulation of peripheral CD25+Foxp3+ T-regulatory cells in patients undergoing radiofrequency thermal ablation for lung cancer.	Article	477-86	70	2009	10.1016/j.humimm.2009.03.012	19332094	1.0	36	L
Correlation of rhinovirus load in the respiratory tract and clinical symptoms in hospitalized immunocompetent and immunocompromised patients.	Article	1498-1507	81	2009	10.1002/jmv.21548	19551831	2.37	82	L
Bronchoalveolar lavage fluid proteome in bronchiolitis obliterans syndrome: possible role for surfactant protein A in disease onset.	Article	1135-43	26	2007	10.1016/j.healun.2007.08.009	18022079	3.087	11	F
Peripheral CD4(+)CD25(+) TREG cell counts and the response to extracorporeal photopheresis in lung transplant recipients.	Article	213-7	39	2006	10.1016/j.transproceed.2006.10.227	17275508	0.962	46	F
Cytokine profile of bronchoalveolar lavage in systemic sclerosis with interstitial lung disease: comparison with usual interstitial pneumonia.	Article	892-4	63	2004	10.1136/ard.2003.014019	15194596	3.916	9	F
Bronchoalveolar lavage cytokine profile in a cohort of lung transplant recipients: a predictive role of interleukin-12 with respect to onset of bronchiolitis obliterans syndrome. 2004 Sep;23(9):1053-60. doi: 10.1016/j.healun.2003.08.019. PMID:	Article	1053-60	23	2004	10.1016/j.healun.2003.08.019	15454171	2.813	21	F

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
BAL cytokine profile in different interstitial lung diseases: a focus on systemic sclerosis.	Article	111-8	21	2004		15281432	2.82	46	F
Regulatory CD4+CD25+ T cells in the peripheral blood of lung transplant recipients: correlation with transplant outcome.	Article	762-6	77	2004	10.1097/01.tp.0000116565.86752.6b	15021844	3.568	100	F

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

** Autocertificated

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

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
Post-transplant lymphoproliferative disorders and Epstein-Barr virus DNAemia in a cohort of lung transplant recipients.	Article	421	8	2011	10.1186/1743-422X-8-421	21892950	5.402	15	L
Surfactant apoprotein A modulates interleukin-8 and monocyte chemotactic peptide-1 production.	Article	1128-35	19	2002	10.1183/09031936.02.00211102	12108868	8.0	9	F
Indoleamine 2,3-dioxygenase in lung allograft tolerance.	Article	1185-92	28	2009	10.1016/j.healun.2009.07.023	19783182	2.091	12	F
Chemokine redundancy in BOS pathogenesis. A possible role also for the CC chemokines: MIP3-beta, MIP3-alpha, MDC and their specific receptors.	Article	275-80	18	2008	10.1016/j.trim.2007.08.004	18047937	0.892	23	F
Elevated IL-8 and MCP-1 in the bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis.	Article	655-9	149	1994	10.1164/ajrccm.149.3.8118632	8118632	4.705	107	O
The interferon landscape along the respiratory tract impacts the severity of COVID-19.	Article	4953	184	2021	10.1016/j.cell.2021.08.016	34492226	41.582	6	O
Hyaluronic Acid-Decorated Chitosan Nanoparticles for CD44-Targeted Delivery of Everolimus.	Article	2310	19	2018	10.3390/ijms19082310	30087241	4.183	27	O
Cyclosporine in anti-Jo1-positive patients with corticosteroid-refractory interstitial lung disease.	Article	484-92	40	2013	10.3899/jrheum.121026	23418387	2.932	32	O
Foxp3 expressing CD4+ CD25+ and CD8+CD28- T regulatory cells in the peripheral blood of patients with lung cancer and pleural mesothelioma.	Article	1-12	67	2005	10.1016/j.humimm.2005.11.005	16698419	2.605	81	O

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
Human CD4+CD25+ regulatory T cells selectively express tyrosine hydroxylase and contain endogenous catecholamines subserving an autocrine/paracrine inhibitory functional loop.	Article	632-42	109	2006	10.1182/blood-2006-01-028423	16985181	10.896	154	O

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

** Autocertificated

Project Title:
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Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

CO-PI Profile

Codullo Veronica

Birth date: 21/08/1980

Institution: Fondazione Policlinico San Matteo
Department/Unit: UOS transplant center
UOCRheumatology
Position Title: CO-PI

Education and training

Institution and Location	Degree	Year(s)	Field of study
University of Pavia	Specialization in Rheumatology	4	Connective tissue disorders and their pulmonary complications
University of Pavia	PhD	3	Internal Medicine with a special focus on systemic autoimmune diseases
University of Pavia	MD	6	Medicine

Personal Statement:

Clinical expertise in the management of pulmonary fibrosis with an inflammatory background, patient enrolment, in vitro and in vivo data interpretation

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
University of Pavia	Rheumatology Unit	Pavia, Italy	Resident in Rheumatology	2007	2011
University of Glasgow	Department of Immunity, Infection and Inflammation	Glasgow, Scotland	Clinical Research Fellow	2009	2009
University of Amsterdam, Academic Medical Centre	Rheumatology and Immunology Unit	Amsterdam, Netherlands	Clinical Research Fellow	2009	2010
IRCCS Policlinico San Matteo	Rheumatology	Pavia, Italy	Rheumatologist (consultant)	2011	2018
Hopital Cochin	Rheumatology	Paris, France	Medical Assistant	2018	2019
IRCCS Policlinico San Matteo	Rheumatology Unit	Pavia, Italy	Medical officer (rheumatology)	2019	2022

Official H index: 25.0 (autocertificated)

Scopus Author Id:17134491200 **ORCID ID:**0000-0003-2557-8514 **RESEARCH ID:**AAC-7384-2022

Other awards and honors

Since 2017 National Qualification of Associate Professor in Rheumatology (ASN)

Project Title:
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Other CV informations

Dr Codullo is an expert in pulmonary complications of systemic autoimmune diseases, with a focus on connective tissue diseases. Since 2017 she is in charge of the Scleroderma Unit at IRCCS Policlinico San Matteo Foundation, a dedicated outpatient clinic for patients with Systemic Sclerosis (Ssc). She has a lab backgroud which she has consolidated in numerous experiences abroad (University of Glasgow, Amsterdam and Paris) and she has ongoing collaborations with European experts in the field (Prof Y Allanore, Paris, France, Prof J Distelr, Erlangen, Germany)

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

Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
Role of placental inflammatory mediators and growth factors in patients with rheumatic diseases with a focus on systemic sclerosis	Article	3307	60	2021	10.1093/rheumatology/keaa782	33313931	7.6	1	F
Undiagnosed connective tissue diseases: High prevalence in pulmonary arterial hypertension patients	Article	e4827	95	2016	10.3899/jrheum.180594	27684814	2.13	11	F
Scleroderma Renal Crisis: Still a Lot To Do	editorial	3	46	2019	10.3899/jrheum.180594	30600234	3.37	1	F
Serologic Profile and Mortality Rates of Scleroderma Renal Crisis in Italy	Article	1464	36	2009	10.3899/jrheum.080806	19487262	4.5	28	F
An investigation of the inflammatory cytokine and chemokine network in systemic sclerosis Annals Rheumatic Diseases	Article	1115	70	2011	10.1136/ard.2010.137349	21285114	8.7	44	F
Stress doppler echocardiography in systemic sclerosis: Evidence for a role in the prediction of pulmonary hypertension Arthritis Rheum	Article	2403-2411	65	2013	10.1002/art.38043	23754201	6.4	43	F
Serum prealbumin is an independent predictor of mortality in systemic sclerosis outpatients Rheumatology	Article	315	55	2016	10.1093/rheumatology/kev322	26359329	4.8	30	F
Imatinib-loaded gold nanoparticles inhibit proliferation of fibroblasts and macrophages from systemic sclerosis patients and ameliorate experimental bleomycin-induced lung fibrosis. Journal of Controlled Release	Article	198	310	2019	10.1016/j.jconrel.2019.08.015	31430501	7.6	12	F

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

Ministero della Salute Direzione generale della ricerca e dell'innovazione in sanità **BANDO RICERCA FINALIZZATA 2021** **esercizio finanziario anni 2020-2021 - Progetto Completo**	**Project Title:** Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis **Project duration (months):** 36
Project Code: RF-2021-12374476	**Principal Investigator:** MELONI FEDERICA
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

** Autocertificated

	Project Title: Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis **Project duration (months):** 36
Project Code: RF-2021-12374476	**Principal Investigator:** MELONI FEDERICA
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Biographical Sketch Contributors. N. 2

di paola rosanna

Birth date: 10/08/1975

Institution: University of Messina
Department/Unit: Department of Pharmacology
Position Title: collaborator

Education and training

Institution and Location	Degree	Year(s)	Field of study
University of Messina	specialization in Clinical Pathology	2013	Ruolo dello Stress Ossidativo nella patogenesi dell¿ischemia riperfusione miocardica
University of Perugia	PhD in Biotecnologie Farmacologiche e Farmacologia Clinica	2011	New Pharmacological target for the treatment of inflammatory process associated with myocardial ischemia reperfusion injury
University of Messina	PhD	2005	Role ofGlucocorticoid-induced TNFR Related Gene (GITR) in the regulation of Inflammatory response 2during acute and chronic inflammation
Universityof Messina	Biological sciences	1998	Prevalence of anti Chlamydial antibodies in patients with Myocardial Infarction

Personal Statement:

Prof Di Paola will keep on with XHALIP development planning all toxicology and all animal model of LFD. Prof Di Paola, PhD in Experimental Medicine, PhD in Biotechnology Pharmacology and Clinical Pharmacology and Specialized in Clinical Pathology, is now Full Professor of Biochemistry at the Department of Veterinary Sciences of the University of Messina, Italy. Author of over 335 publications in renowned journals and 11,692 citations. His areas of scientific interest are autoimmunity, acute and chronic inflammation and modulation of the signal pathway. She has various skills in analytical methodologies including experimental animal model, histology, immunohistochemistry and molecular biology techniques for the study of protein expression.

Positions and honors

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Positions

Institution	Division / Research group	Location	Position	From year	To year
University of Messina	Department: scienze chimiche biologiche farmaceutiche e ambientali dell¿Università degli studi di messina.	Sezione farmacologia	Full professor	2022	2022
Università di Messina	di scienze chimiche biologiche farmaceutiche e ambientali dell¿Università degli studi di messina.	Sezione di Farmacologia	Associate Professor	2019	2021
University of Messina	Dipartimento di scienze chimiche biologiche farmaceutiche e ambientali	Sezione di Farmacologia	Ricercatore a tempo determinto cat B	2015	2018

Official H index: 56.0 (autocertificated)

Scopus Author Id:57218288397 **ORCID ID:**0000-0001-6725-8581 **RESEARCH ID:**K-5175-2016

Other awards and honors

Dr. Di Paola won in 2009 the "Young Researcher" for the scientific production for the macro-area drug Biomedical conferred by the University of Messina. Additionally, she is member of several scientific society including: the Italian Society of Pharmacology (SIF),
the Italian Biochemistry Society (SIB), the American Society Pharmacology and experimental Therapeutics (ASPET)

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Biographical Sketch Contributors. N. 3

BIGINI PAOLO

Birth date: 07/01/1970

Institution: IRCCS Mario Negri
Department/Unit: Department biochemical research and molecular pharmacology
Position Title: Collaborator

Education and training

Institution and Location	Degree	Year(s)	Field of study
Open University UK	PhD in Biotechnology	2003	apoptosis in cellular and animal models of motor neuron degeneration
Mario Negri Milan	post graduate specialization in Pharmacology	2000	Biotechnology Field: cell apoptosis
University of Pisa	Biology	1995	cell biology

Personal Statement:

Dr Bigini will be involved in the supervision of all animal tests and will be designing and supervising biodistribution and pharmacokinetic studies in animals.
His experience in the field of pharmacology and pharmacokinetics is large and longstanding, this will guarantee the completion of Aim 1 and 2
From 2004 to 2014 he was mainly involved, first as post-doc and then as permanent researcher, in studies focused on stem cell therapy and preclinical imaging. Since 2014 he is the leader group of the Nanobiology Unit at the Mario Negri. During his activity he published and revised more than 150 manuscripts and coordinated Italian and European projects. He belongs to preclinical Ethical Committees

Positions and honors

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Positions

Institution	Division / Research group	Location	Position	From year	To year
Bicocca University of Milano	Technology Converging to Biomolecular Science	Milano	Doctoral School Panel Member	2020	2022
Mario Negri Institute of Pharmacological Science	Department of Molecular Biochemistry and Pharmacology/Unit of Nanobiology	Milano	Ethical Committee for preclinical studies Member	2017	2022
Mario Negri Institute of Pharmacological Science	Department of Molecular Biochemistry and Pharmacology/ Unit of Nanobiology	Milano	Unit Head	2014	2022
Mario Negri Institute of Pharmacological Science	Department of Molecular Biochemistry and Pharmacology/ Laboratory of Biochemistry and Protein Chemistry	Milano	Researcher - Preclinical Imaging Specialist	2009	2013
Mario Negri Institute of Pharmacological Science	Department of Molecular Biochemistry and Pharmacology/ Laboratory of Receptor Pharmacology	Milano	Post-Doc	2004	2008

Official H index: 24.0 (autocertificated)

Scopus Author Id:55911359200 **ORCID ID:**0000-0002-0239-9532 **RESEARCH ID:**AAB-2901-2020

Other awards and honors

No award to be reported

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Biographical Sketch Contributors. N. 5

Fusco Roberta

Birth date: 21/01/1991

Institution: University of Messina
Department/Unit: Department of Pharmacology
Position Title: collaborator

Education and training

Institution and Location	Degree	Year(s)	Field of study
University of Messina	PhD	2018	Applied Biology and Experimental Medicine
University of Messina	Chimica e Tecnologia Farmaceutiche	2014	preclinical studies of newdrugs

Personal Statement:

Dr. Roberta Fusco, Researcher in Biochemistry at the University of Messina, completed her PhD in Applied Biology and Experimental Medicine at the University of Messina and the Yale University School of Medicine and postdoctoral studies at the University of Messina. She has worked as a researcher for preclinical pharmacology studies and biochemical activities (primary cultures in vitro and experimental models in vivo). You have published more than 80 articles in renowned journals on biochemistry and oxidative stress.
Her role in this project will be to collaborate in the generation and analysis of all models of disease

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
University of Messina	Department " Scienze Chimiche Farmaceutiche Biologiche ed Ambientali"	sezione farmacologia	RTDA BIO/10 researcher	2021	2022

Official H index: 27.0 (autocertificated)

Scopus Author Id:57192308076 **ORCID ID:**0000-0003-0223-1403 **RESEARCH ID:**AAC-1745-2019

Other awards and honors

Dr. Fusco won in 2018 the SIF Research Scholarship for Short Periods Abroad for a study stay at Yale University, School of Medicine, Department of Pharmacology, USA. Additionally, she won the travel grant for the participation in the 45th FEBS Congress for the Italian Society of Biochemistry and Molecular Biology (SIB). she is member of several scientific society including: the Italian Society of Pharmacology (SIF), SIB, the American Society Pharmacology and experimental Therapeutics (ASPET)

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Expertise Research Collaborators

Selected peer-reviewed publications of the Research Group / Collaborators

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
Codullo Veronica	Systemic sclerosis: Recent insight in clinical management	Article	293	87	2020	10.1016/j.jbspin.2019.09.015	31568838	2.45	17	O
Codullo Veronica	Elevated ACKR2 expression is a common feature of inflammatory arthropathies	Article	1607	56	2019	10.1093/rheumatology/kex176	28486662	5.6	6	O
Codullo Veronica	Telemedicine in rheumatology: a reliable approach beyond the pandemic	Article	366	60	2021	10.1093/rheumatology/keaa554	32893293	7.5	12	O
Codullo Veronica	Comparison of efficacy of first- versus second-line adalimumab in patients with rheumatoid arthritis: Experience of the Italian biologics registries	Article	660	4	2016		28516879	2.9	5	F
Fusco Roberta	Focus on the Role of NLRP3 Inflammasome in Diseases. Int J Mol Sci. 2020 Jun 13;21(12):4223. doi: 10.3390/ijms21124223. PMID: 32545788; PMCID: PMC7352196.	Review	4223	21	2020	10.3390/ijms21124223	32545788	4.556	53	F
Fusco Roberta	Modulation of NLRP3 Inflammasome through Formyl Peptide Receptor 1 (Fpr-1) Pathway as a New Therapeutic Target in Bronchiolitis Obliterans Syndrome.	Article	E2144	21	2020	10.3390/ijms21062144	32244997	5.923	19	O
Fusco Roberta	Inhibition of inflammasome activation improves lung acute injury induced by carrageenan in a mouse model of pleurisy.	Article	3497-3511	31	2017	10.1096/fj.201601349R	28461340.d	5.191	30	F

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	IF	Cit.**	P.*
Fusco Roberta	Involvement of TLR4 and PPAR-¿ Receptors in Host Response and NLRP3 Inflammasome Activation, Against Pulmonary Infection With Pseudomonas Aeruginosa.	Article	221-7	51	2019	10.1097/SHK.0000000000001137	29547450	2.33	33	O
di paola rosanna	Effect of PD98059, a selective MAPK3/MAPK1 inhibitor, on acute lung injury in mice.	Article	937-50	22	2009	10.1177/0394632009022200409	20074457	3.0	24	F
di paola rosanna	Adrenomedullin in inflammatory process associated with experimental pulmonary fibrosis	Article	41	12	2011	10.1186/1465-9921-12-41	21477302	3.36	23	F
di paola rosanna	Adelmidrol: A New Promising Antioxidant and Anti-Inflammatory Therapeutic Tool in Pulmonary Fibrosis.	Article	601	9	2020	10.3390/antiox9070601		6.312	15	L
di paola rosanna	Ultramicronized palmitoylethanolamide (PEA-um(®)) in the treatment of idiopathic pulmonary fibrosis.	Article	405-12	111	2016	10.1016/j.phrs.2016.07.010	27402190	7.658	29	F
BIGINI PAOLO	Blood protein coating of gold nanoparticles as potential tool for organ targeting	Article	3455 - 3466	35	2014	10.1016/j.biomaterials.2013.12.100	24461938	8.6	65	L
BIGINI PAOLO	Organ distribution and bone tropism of cellulose nanocrystals in living mice	Article	2862 - 2871	16	2015	10.1021/acs.biomac.5b00805	26226200	5.6	13	C
BIGINI PAOLO	Influence of Size and Shape on the Anatomical Distribution of Endotoxin-Free Gold Nanoparticles	Article	5519 - 5529	11	2017	10.1021/acsnano.7b00497	28558193	13.7	82	L
BIGINI PAOLO	Repeated administration of the food additive E171 to mice results in accumulation in intestine and liver and promotes an inflammatory status	Article	1087 - 1101	13	2019	10.1080/17435390.2019.1640910	31271314	4.9	8	C

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

** Autocertificated

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Total proposed budget (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1a Staff Salary	75.459,00	75.459,00	not permitted	0,00
1b Researchers' Contracts	195.000,00	0,00	195.000,00	43,33
2 Equipment (Leasing - Rent)	0,00	0,00	0,00	0,00
3a Supplies	145.690,00	0,00	145.690,00	32,38
3b Model Costs	38.210,00	0,00	38.210,00	8,49
3c Subcontracts *	0,00	0,00	0,00	0,00
3d Patient Costs	0,00	0,00	0,00	0,00
4 IT Services and Data Bases	0,00	0,00	0,00	0,00
5 Publication Costs	8.000,00	0,00	8.000,00	1,78
6 Convegni	4.500,00	0,00	4.500,00	1,00
7 Travels	5.100,00	0,00	5.100,00	1,13
8 Overheads *	25.000,00	0,00	25.000,00	5,56
9 Coordination Costs	28.500,00	0,00	28.500,00	6,33
Total	525.459,00	75.459,00	450.000,00	100,00

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

Report the Co-Funding Contributor:

salaries of Meloni (2pm/yeart), Codullo (2pm/year), Bigini (2pm/year) and Di Paola (1 pm/year) are included. Salaries are provided by IRCCS San Matteo, IRCCS Mario Negri and University of Messina, respectively.

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Budget Justification

1a Staff Salary	Support NOT required from MOH Unit1: Meloni (2pm/year), Codullo (2pm/year) salaries are provided by Fondazione S. Matteo; As For Unit 2Di Paola (1pm/year) salaries are provided by University of Messina. Bigini (2pm/year) salary provided byIRCCS M Negri.
1b Researchers' Contracts	as for Unit 1 (San Matteo IRCCS) : Salary support is required for biologist (40.000€/year) to manage the workload of in vitro experiments, according to Law 205/2017; as for Unit 3 (IRCCS M NEGRI): Research Contract Full Time Annalisa Morelli (post Doc)
2 Equipment (Leasing - Rent)	none
3a Supplies	sodium HA, Phospholipids, cholesterol,fluoresceinated agents, TKIs, antibodies, cell culture- RT PCR- WB-reagents, materials for analytics, vital dyes, surgical instuments, anaesthetics,eluition columns for HPLC,-MS
3b Model Costs	Animals (C 57 Bl/6J mice),
3c Subcontracts	none
3d Patient Costs	none
4 IT Services and Data Bases	none
5 Publication Costs	publication of research results on peer reviewed J. open access
6 Convegni	participation to international meetings (dissemination at EULAR, ERS, ISHLT, ATS, Nano meetings)
7 Travels	travels to consortium meetings
8 Overheads	Institutional indirect cost and general expenses, within 10% of MoH funding
9 Coordination Costs	Organization of 2 research meetings: 1st - Startup meeting; 2nd - end of the project (800€); logistic for samples shipments (200€ each), patent cost (9000€)

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Proposed total budget UO1 Institution: Fondazione Policlinico San Matteo (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1a Staff Salary	26.100,00	26.100,00	not permitted	0,00
1b Researchers' Contracts	120.000,00	0,00	120.000,00	51,61
2 Equipment (Leasing - Rent)	0,00	0,00	0,00	0,00
3a Supplies	63.700,00	0,00	63.700,00	27,40
3b Model Costs	0,00	0,00	0,00	0,00
3c Subcontracts	0,00	0,00	0,00	0,00
3d Patient Costs	0,00	0,00	0,00	0,00
4 IT Services and Data Bases	0,00	0,00	0,00	0,00
5 Publication Costs	5.000,00	0,00	5.000,00	2,15
6 Convegni	3.000,00	0,00	3.000,00	1,29
7 Travels	2.300,00	0,00	2.300,00	0,99
8 Overheads	10.000,00	0,00	10.000,00	4,30
9 Coordination Costs	28.500,00	0,00	28.500,00	12,26
Total	258.600,00	26.100,00	232.500,00	100,00

Report the Co-Funding Contributor:

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Budget Justification

1a Staff Salary	Meloni (2pm/year), Codullo (2pm/year) salaries are provided by Fondazione San Matteo. Support is not required from the MoH.
1b Researchers' Contracts	Salary support is required for biologist (40.000€/year) to manage the workload of in vitro experiments, according to Law 205/2017
2 Equipment (Leasing - Rent)	none
3a Supplies	Sodium hyaluronate Phospholipids cholesterol chemicals for conjugate synthesis, flouresceinated compounds, antibodies, imatinib, nintedanib, cell cultures media and reagents, agarose, WB reagents, RT-PCR reagents.
3b Model Costs	not for this Unit
3c Subcontracts	none
3d Patient Costs	none
4 IT Services and Data Bases	none
5 Publication Costs	open access publication of research results
6 Convegni	registration fees and travel/accomodation for the dissemination of research results at international meetings in the field of pulmonary diseases or Rheumatology (ERS ATS ISHLT EULAR)
7 Travels	travel to meetings of the consortium
8 Overheads	Institutional indirect cost and general expenses, less than 10% of MoH fundiing
9 Coordination Costs	Organization of 2 research meetings: 1st - Startup meeting; 2nd - end of the project (800€); logistic for samples shipments (200€ each), patent cost (9000€)

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Proposed total budget UO2 Institution: University of Messina (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1a Staff Salary	25.869,00	25.869,00	not permitted	0,00
1b Researchers' Contracts	0,00	0,00	0,00	0,00
2 Equipment (Leasing - Rent)	0,00	0,00	0,00	0,00
3a Supplies	37.500,00	0,00	37.500,00	55,56
3b Model Costs	30.000,00	0,00	30.000,00	44,44
3c Subcontracts	0,00	0,00	0,00	0,00
3d Patient Costs	0,00	0,00	0,00	0,00
4 IT Services and Data Bases	0,00	0,00	0,00	0,00
5 Publication Costs	0,00	0,00	0,00	0,00
6 Convegni	0,00	0,00	0,00	0,00
7 Travels	0,00	0,00	0,00	0,00
8 Overheads	0,00	0,00	0,00	0,00
9 Coordination Costs	not permitted	not permitted	not permitted	0,00
Total	93.369,00	25.869,00	67.500,00	100,00

Report the Co-Funding Contributor:

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Budget Justification	
1a Staff Salary	Di Paola (1pm/year) salaries are provided by University of Messina. Support is not required from the MoH.
1b Researchers' Contracts	none
2 Equipment (Leasing - Rent)	none
3a Supplies	antibodies, reagents for RT-PCR, reagents for Immuno-histochemistry
3b Model Costs	direct and indirect cost for animals: C57 mice for toxicity and animal modeS: Bleomycin-induced fibrosis and HTT model.
3c Subcontracts	none
3d Patient Costs	none
4 IT Services and Data Bases	none
5 Publication Costs	none
6 Convegni	none
7 Travels	none
8 Overheads	none
9 Coordination Costs	none

Ministero della Salute Direzione generale della ricerca e dell'innovazione in sanità **BANDO RICERCA FINALIZZATA 2021** **esercizio finanziario anni 2020-2021 - Progetto Completo**	**Project Title:** Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis **Project duration (months):** 36
Project Code: RF-2021-12374476	**Principal Investigator:** MELONI FEDERICA
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Proposed total budget UO3 Institution: IRCCS Mario Negri (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1a Staff Salary	23.490,00	23.490,00	not permitted	0,00
1b Researchers' Contracts	75.000,00	0,00	75.000,00	50,00
2 Equipment (Leasing - Rent)	0,00	0,00	0,00	0,00
3a Supplies	44.490,00	0,00	44.490,00	29,66
3b Model Costs	8.210,00	0,00	8.210,00	5,47
3c Subcontracts	0,00	0,00	0,00	0,00
3d Patient Costs	0,00	0,00	0,00	0,00
4 IT Services and Data Bases	0,00	0,00	0,00	0,00
5 Publication Costs	3.000,00	0,00	3.000,00	2,00
6 Convegni	1.500,00	0,00	1.500,00	1,00
7 Travels	2.800,00	0,00	2.800,00	1,87
8 Overheads	15.000,00	0,00	15.000,00	10,00
9 Coordination Costs	not permitted	not permitted	not permitted	0,00
Total	173.490,00	23.490,00	150.000,00	100,00

Report the Co-Funding Contributor:

Project Title:
Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis

Project duration (months): 36

Project Code: RF-2021-12374476

Principal Investigator: MELONI FEDERICA

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

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Budget Justification

1a Staff Salary	Bigini (2pm/year) salary provided by Istituto di Ricerche Farmacologiche Mario Negri IRCCS. Support is not required from the MoH.
1b Researchers' Contracts	Research Contract Full Time Annalisa Morelli (Post-Doc)
2 Equipment (Leasing - Rent)	none
3a Supplies	Antibodies, Reagents, materials for analytics, vital dyes, surgical instuments for in vivo studies, anaesthetics, eluition columns for HPLC-MS
3b Model Costs	Animals (C 57 Bl/6J mice)
3c Subcontracts	none
3d Patient Costs	none
4 IT Services and Data Bases	none
5 Publication Costs	Costs related to fees for publication of project results on peer-reviewed journals
6 Convegni	Institutional indirect cost and general expenses, within 10% of MoH funding
7 Travels	Travel, living and accommodation costs related to participation to thematic conferences for project dissemination
8 Overheads	Institutional indirect cost and general expenses, within 10% of MoH funding
9 Coordination Costs	none

Ministero della Salute Direzione generale della ricerca e dell'innovazione in sanità **BANDO RICERCA FINALIZZATA 2021** **esercizio finanziario anni 2020-2021 - Progetto Completo**	**Project Title:** Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis **Project duration (months):** 36
Project Code: RF-2021-12374476	**Principal Investigator:** MELONI FEDERICA
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Principal Investigator Data

Cognome: MELONI
Nome: FEDERICA
Codice fiscale: MLNFRC61P70G388B
Documento: Passaporto, Numero: YA8963743
Data di nascita: 30/09/1961
Luogo di nascita: pavia
Provincia di nascita: PV
Indirizzo lavorativo: piazzale Golgi 19
Città: Pavia
CAP: 27100
Provincia: PV
Email: federica61.meloni@outlook.it
Altra email: f.meloni@smatteo.pv.it
Telefono: +393479254674
Altro telefono: 3479254674
Fax: 0382502719
Qualifica: Universitario covenzionato
Struttura: uos transplant center
Istituzione: fondaz irccs policlinico san matteo
Datore/ente di lavoro? Si
Datore/ente di lavoro SSN? No
Nome datore/ente di lavoro non SSN: Università di Pavia
Nome istituzione SSN: Fondazione irccs policlinico san matteo
Tipo contratto: Distaccato presso IRCCS/Ente SSN a seguito Convenzione Università/Altro Ente Ricerca

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

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

Ministero della Salute Direzione generale della ricerca e dell'innovazione in sanità **BANDO RICERCA FINALIZZATA 2021** **esercizio finanziario anni 2020-2021 - Progetto Completo**	**Project Title:** Development of innovative inhalatory targeted liposomal formulations for the treatment of inflammatory driven pulmonary fibrosis **Project duration (months):** 36
Project Code: RF-2021-12374476	**Principal Investigator:** MELONI FEDERICA
Research Type: d1) Theory-enhancing: sviluppare studi rilevanti per la sanità, il benessere animale e la sicurezza alimentare	**Applicant Institution:** Fondazione Policlinico San Matteo

Project Type: Ordinary/Progetti ordinari di Ricerca Finalizzata

Project validation result

Message: Success

WorkPackages	Unit 1 IRCCS San Matteo	Unit 2 IRCCS M. Negri	Unit 3 Univ of Messina		First Year												Second Year												Third Year											
					1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
WP 1 liposome preparation	C																																							
task 1.1 preparation of fluorescent HA coated and Un-coated LIPOSOMES	X																																							
task 1.2 preparation of Imatinib loaded HA coated and UN-coated Liposomes	X																																							
task 1.3 preparation of nintedanib cloaded HA coated and un-coated liposomes	X																																							
task 1.4 preparation of ruxolitinib loaded HA coated and Un-coated Liposomes	X																																							
WP 2 in vitro assessment of uptake and activity of different XHALIP preparations	C	P																																						
task 1.1 Uptake by MC, Epithelial cells, Endothelial cells macrophages and other inflammatory cells	X																																							
task 2.2 distribution across alveolo-capillary barrier in vitro	X																																							
task 2.3 in vitro activity on different cell types (prioliferation , colagen production, migration,viability, cytokine release)	X																																							
task 2.4 distruibution in PCLS cultures	X	X																																						
WP 3 pharmacokinetics studies in Animals	P	C																																						
task 3.1 Combined Maldi-Confocal Imaging optimization for the tracking of XHALIP in lungs																																								
task 3,2 Biodistribution, nanosafety assessment in animals																																								
task 3.3 Pharmacokinetics																																								
WP4 Animal Toxicology	P	P	C																																					
task 4.1 acute toxicity studies			x																																					
task4.2 chronic toxicity studies			x																																					
Task 4.3 analysis of cell toxicity	x	x	x																																						
WP5: Activity in animal models	P	P	C																																					
Task 5.1 animal model of fibrosis	X	X	X																																					
Task 4.5 animal model of BOS	X	X	X																																					
WP6 coordination and dissemination	C	P	P																																					
Task 6.1 coordination	X																																							
Task 6.2 dissemination	X	X	X																																					

C: coordinator / **P** participant

PR2

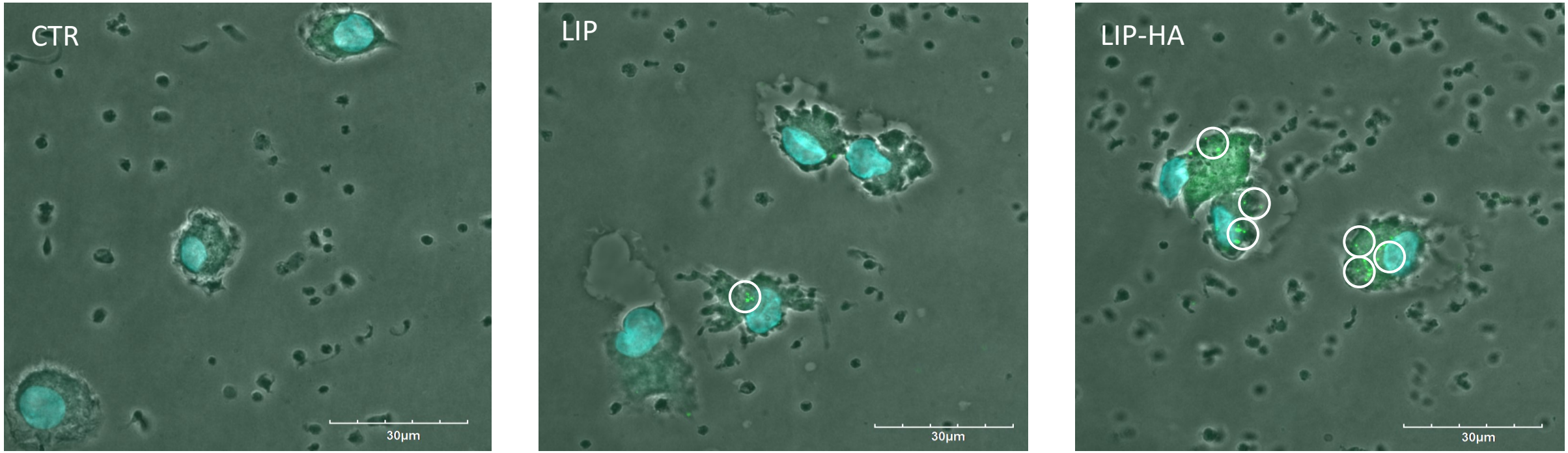


Fig. 1: Uptake of liposomes coated with HA (LIP-HA) and uncoated (LIP) by MC isolated from ILD-CTD

PR2

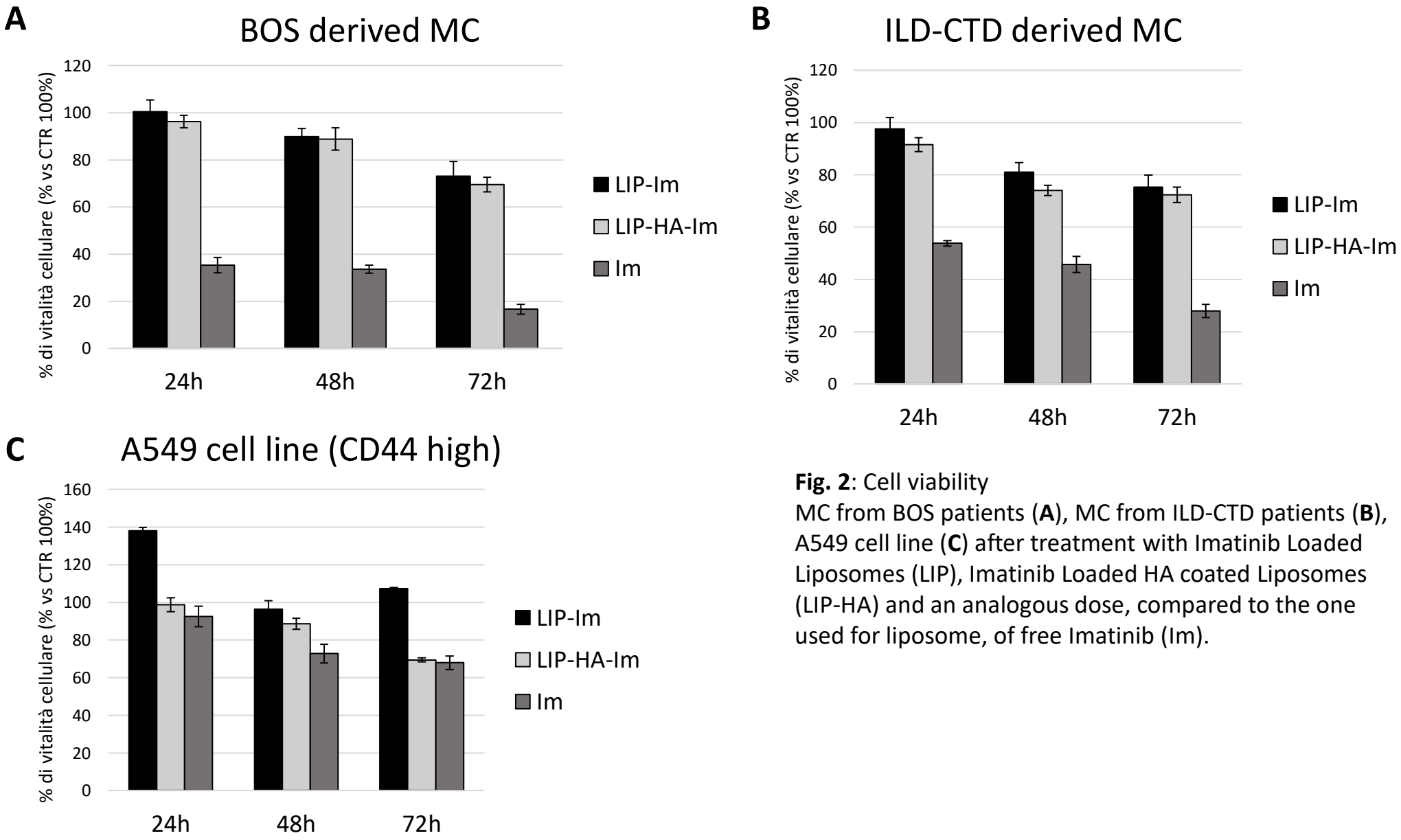


Fig. 2: Cell viability
MC from BOS patients (**A**), MC from ILD-CTD patients (**B**), A549 cell line (**C**) after treatment with Imatinib Loaded Liposomes (LIP), Imatinib Loaded HA coated Liposomes (LIP-HA) and an analogous dose, compared to the one used for liposome, of free Imatinib (Im).

PR2

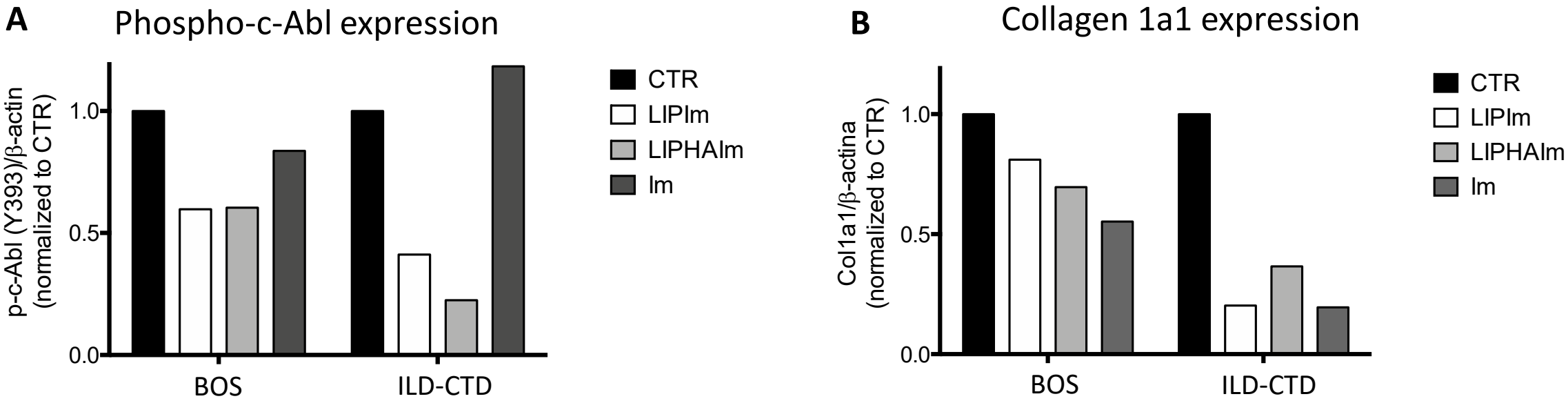
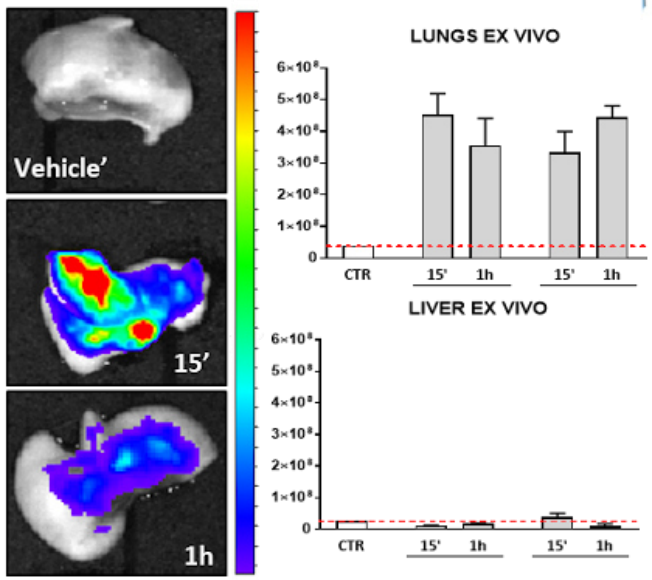
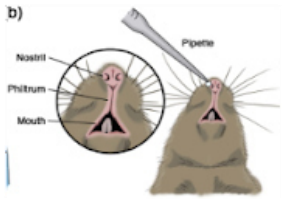


Fig. 3: (**A**) Expression of Phospho-c-Abl by cells treated with Imatinib Loadesd Liposomes coated with HA and uncoated. Free drug (at the same concentration of liposome) is used as control. (**B**) Collagen 1a1 expression in cells treated under the same conditions as in Fig. 3A.

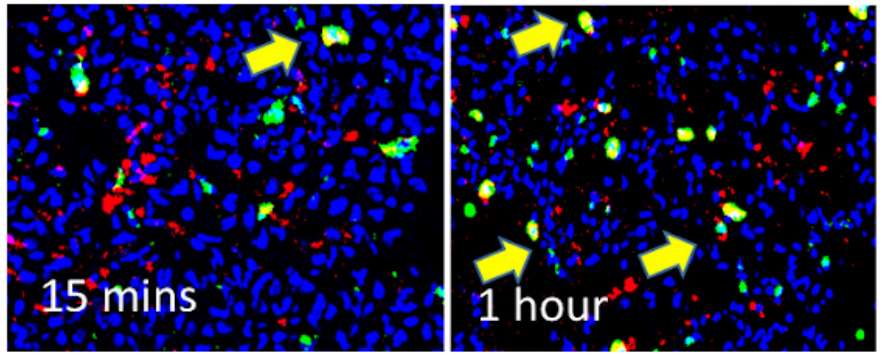
NP localization: *ex vivo* optical imaging

PR3





NP-Macrophages co-localization: confocal microscopy



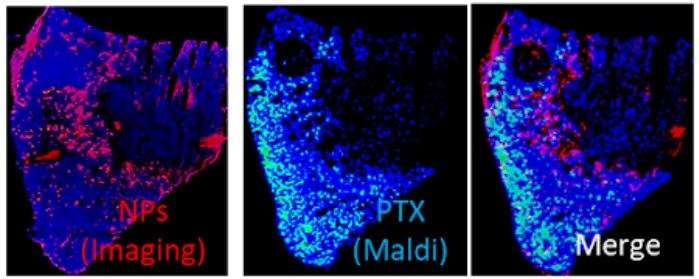
Nuclei — CD68-Macrophage Marker

FluoNPs — Merge NPs & CD68

After a single intranasal instillation, an intense and protracted fluorescence signal was detected in lungs in both experimental groups up until 1 h. No fluorescent signal was found in other organs. The selective localization of NP (red signal in figures on the right panels) inside macrophages (green signal) can be seen by the merged color in yellow (see arrows). In particular this colocalization increases from 15 minutes to 1 hour after intranasal administration.

NP-DRUG Maldi-Imaging and optical scanner

PR4



In this study paclitaxel loaded fluo NP were administered in tumor bearing mice. In red (left panel) the distribution of NPs in the section counterstained with the nuclear marker Hoechst 33258 is detectable 4 hours after treatment is mainly detectable in the central area. In the central panel the intensity of signal associated with PTX and measured by spatial spectrometry (MALDI-IMAGING) shows a stronger concentration of the drug in periphery. In the merge (right panel) it is possible to see that the PTX has been released and that is dissociated with NPs.

PR5