# Contrat doctoral – ED Galilée

**Titre du sujet :** ***Macrophages and Smooth Muscle Tone in Pulmonary Hypertension of Group 3 patients: Role of Omega-6/-3***


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- **Domaine de recherche :** Pharmacologie cardiovasculaire
- **Mots clés :** Hypertension pulmonaire, macrophage, artère pulmonaire, bronche, bioactive lipides.

**Background of the Project:**

Pulmonary hypertension (PH) is a severe disease with a half-life survival of 3-5 years. It is classified into five groups, with **Group 3 PH secondary to lung disease (COPD, emphysema, or fibrosis) being the most common and lethal form**. This pathology is frequently associated with inflammation and is characterized by elevated pulmonary arterial pressure, vascular remodelling, and impaired respiration. Therefore, it is crucial to enhance the relaxation of both **human pulmonary artery (HPA) and bronchial smooth muscle**. Bronchodilation will facilitate inhalation of drugs responsible for vasodilation and oxygenation. This was recently illustrated by our *in vitro* results, showing the (broncho-/vaso-) relaxations induced by treprostinil, a prostaglandin (PG)$I_2$ analogue (Norel et al., 2020, PMID 32962984), and *in vivo*, as this drug is a new inhaled treatment for Group 3 PH patients with interstitial lung disease (Waxman et al., 2021, PMID 33440084).

The **pathogenesis of PH is driven by inflammation**, endothelial dysfunction (deficient nitric oxide (NO), impaired $PGI_2$ pathways, excessive activation of endothelin (ET)-1 pathway), and abnormal proliferation of smooth muscle cells and fibroblasts. **Recent studies have highlighted the significant role of monocytes/macrophages (Mϕ**: alveolar, airway, and vascular) in these processes (Zhang et al., 2023, PMID 37153557), with Mϕ polarizing into pro-inflammatory M1 or pro-resolving M2 phenotypes in response to PH stimuli (Zuo et al., 2024, PMID 38555425). Tissue repair is also a new function associated with a switch in macrophage phenotype into mural cell (perivascular Mϕ PDGFR+, Amoedo-Leite et al., 2024, PMID 39196227). In contrast, in a murine PH model, this phenotype was responsible for increased muscularization (Sheick et al., 2018, PMID 29694892). **The presence and role of the perivascular Mϕ in Group 3 PH arteries will be explored in one part of this thesis project**.

Mϕ secrete various bioactive lipid mediators (LMs), such as omega-6 metabolites: $PGE_2$ and thromboxane $A_2$ (but no $PGI_2$), and the recently discovered specialized pro-resolving mediators (SPMs omega-3 metabolites), particularly maresin (MaR), resolvin (Rv)D2 and RvD5 (Werz et al., 2018, PMID PMID 29302056; Zhang et al., 1993, PMID 8427858; Serhan & Chiang, 2024, PMID PMID 36813255). **These LMs can influence vascular and respiratory functions through the HPA and bronchial muscular tones. This will be the second part of the project**, using an organ bath system to measure the modulation of muscular tone of HPA in the presence of the adjacent bronchus and reciprocally when SPM production is triggered.

**We aim to explore how Mϕ phenotypes and LM profiles impact PH progression. For the third part of the project**, we will use flow cytometry, immunofluorescence and lipidomic profiling (UPLC-MS/MS) to characterize the LM profiles of these Mϕ phenotypes. We will assess how specific Mϕ-derived SPMs could influence endothelial $PGI_2$ and NO productions. Furthermore, we will explore Mϕ-fibroblast and/or Mϕ-human pulmonary artery smooth muscle cells (hPASMC) interactions and their impact on HPA extracellular matrix and endogenous $PGI_2$/NO productions.

**Aim of the Project:**

We will attempt to demonstrate the beneficial role of the bronchial wall and certain Mϕ phenotypes in Group 3 PH patients, and whether they can restore (boost) pulmonary vasorelaxation and bronchodilation *in vitro* in tissue samples obtained from Group 3 PH patients. Targeting these interactions and the bioactive lipid mediators involved, could lead to novel PH therapeutic strategies.

**Methods:**

- Human tissues

Human lung samples (HPA, arterioles, bronchoalveolar lavage samples (BAL)) will be obtained, and coupled/adjacent arterial-bronchial rings will also be dissected. The experiments will be conducted using human pulmonary arterial (HPA) and human bronchial (HB) samples obtained at Bichat-Claude Bernard hospital (with informed consent of the patient and ethical advisory board "GHU Nord" agreement n°11-045). Samples will be obtained from patients with Group 3 PH or from controls. An array of complementary ex-vivo and in vitro pharmacological studies will be performed.

- *Ex-vivo* experiments:

Arterial preparations cut as rings will be set up in organ bath systems to investigate the muscular tone of the arterial samples with or without their adjacent bronchial segment. The modulation of physiological responses (contraction/relaxation) will be analyzed in the presence of SPM (Mar1, RvD2, and RvD5), which are produced in significant quantities by human macrophages. HPA contractions will be induced by $PGE_2$, a pro-inflammatory LM, or by norepinephrine; their relaxation will be induced by ATP or acetylcholine.

- *In-vitro* experiments:

Isolation of macrophages (from BAL or pulmonary sample homogenates. Primary cell culture (hPASMC, fibroblast, and pulmonary endothelial cells) derived from HPA will be performed.

Macrophage phenotype characterization (expression of the M1 markers CD80 and CD54 as well as the M2 markers CD206 and CD163 will be measured by flow cytometry. Mural macrophage phenotype mRNA (Pdgfrb, Myl9 Notch3).

Cell proliferation and migration will be analyzed with MTT assay and by *Incucyte® Scratch Wound Assay used for real-time measurements of both migration and invasion.*

Expression of different enzymes involved in vasodilation (e-NOS) and bioactive LM synthesis (mPGES1, PGIS, 5-LOX, 12-LOX, 15LOX-A, 15LOX-B, or COX-1/-2) will be measured by classical biochemistry techniques: Western blot, real-time PCR, immunofluorescence, or immunohistochemistry.

Measurements of endogenous LM (UPLC-MS/MS) mostly for SPMs.

NO production (using Griess assay), ROS generation (measured by DCF-DA assay), and ELISA for $PGE_2$, $PGI_2$, and endothelin levels quantifications.

The impact on extracellular matrix remodelling, particularly the deposition of fibronectin and collagen, will be analyzed by immunostaining or Western blot.

All these techniques are present at INSERM U1148 (Paris) or in collaboration with Pr. Oliver Werz's laboratory in Jena (Germany).

| Human arteries and bronchi:<br>effects and roles of bioactive lipids | Macrophages in pulmonary hypertension<br>Chen et al., 2020, PMID 32889242 |
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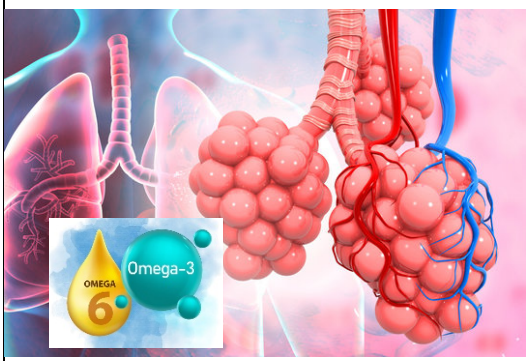
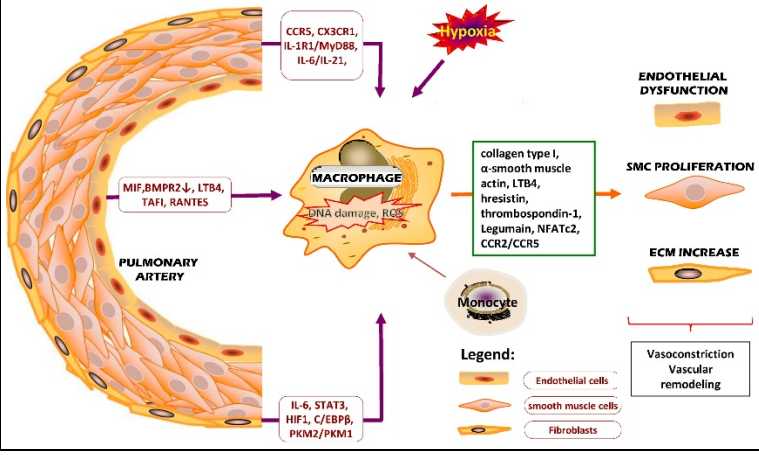
|  |  |