n. 1 posto aggiuntivo del corso di perfezionamento in Nanoscienze sarà asseganto in relazione a temi di ricerca inerenti la convenzione tra SNS e CHIESI Farmaceutici S.p.A.

L'attività di ricerca del perfezionando si svolgerà sia presso la Scuola ovvero presso strutture di volta in volta individuate dagli organi del Corso e in coerenza con il programma del Corso stesso e delle regole della Scuola, sia presso i laboratori e sedi di ricerca di Chiesi o di soggetti con i quali Chiesi stesso intrattiene rapporti di collaborazione. Il periodo di formazione e ricerca del perfezionando presso la Scuola si svolgerà in 24 mesi (eventuali prolungamenti dovranno essere concordati dai responsabili dell'Accordo per Scuola e Chiesi).
Questo particolare percorso di ricerca rappresenta un'occasione unica per affrontare l'attività di perfezionamento con un orizzonte allargato partendo dalla realtà accademica con uno spiccato orientamento verso il mondo della ricerca nell'industria farmaceutica.

PRETERM RABBIT PRECISION CUT LUNG SLICES AS A TOOL FOR DRUG DISCOVERY

Principal Investigator (PI): **Francesco CARDARELLI**
Institution hosting the project: **SCUOLA NORMALE SUPERIORE, PISA, ITALY and CHIESI FARMACEUTICI S.p.A., PARMA, ITALY**

Bronchopulmonary dysplasia (BPD) is a disease that effects the lung in extremely preterm neonates. BPD etiology and pathophysiological characteristics are very heterogenous, however, prematurity is the determinant factor. Different BPD animal models have been developed: preterm large animals (lambs and baboons) and rodents born at term and exposed to hyperoxia (rats and mice). The preterm rabbit exposed to hyperoxia has been recently described as an alternative BPD model that shares, as the large BPD models, the same lung development as humans, i.e. birth at term in the alveolar phase of lung development, but it is still a small model with large litters that can be born preterm, unlike rodents. These features allow to closely model lung prematurity, the most important BPD determinant in humans. This model has been transferred to the Chiesi's Research Center in 2018 and it is now being employed to validate new drug targets and test new compounds for the treatment and prevention of BPD.
Chiesi recently decided to identify an ex vivo tool that recapitulates lung development in vitro to allow preliminary drug screening and dose-finding before moving into the in vivo model.
Precision cut lung slices (PCLS) harvested from adult animals have been described to study in vitro different pathophysiological lung processes. More recently, few studies that investigate neo alveolarization in newborn mouse lung slices have been published. Chiesi decided to start to set up this tool also starting from preterm rabbits' lungs. PCLS are obtained from preterm rabbits delivered in the saccular stage of lung development at 28 days gestational age (term pregnancy 31 days, alveolar stage of lung development). Slices are then incubated in either "normoxia" (oxygen 5% to mimic in utero oxygen concentration) or hyperoxia (oxygen 70% to mimic the hyperoxic insult at which preterm babies are often exposed after birth to allow gas exchange in premature lungs). Preliminary data show that lung slices in normoxia continue the alveolarization process for few days in culture. If exposed to hyperoxia, the neo-alveolarization process is blunted and a hyper-proliferative response leads to septal thickening.
As next steps for the PhD student, these PCLS need to be fully investigated by performing the following activities:
-identify from published literature key proteins involved in alveologenesis and validate antibodies on

rabbit PCLS to perform immunofluorescence.
-use RNA scope as an alternative if no suitable antibodies are identified (very few antibodies specific for rabbit antigens are available on the market; commercial antibodies for other species can be used if good cross-reactivity and homology with the rabbit protein is present. If this approach fails, in situ RNA hybridization is the other option to be explored)
-live imaging of alveolarization and immunofluorescence on fixed sections, employing the tools identified above.
-consolidate cell markers and fluorescent tracers to "live" track the different cell subsets of the rabbit lungs (epithelial/mesenchymal/immune cells) to be used for two-photon microscopy.
-regular use of fluorescence lifetime imaging microscopy (FLIM) to quantify the enzyme-bound and enzyme-free fractions of coenzyme NADH, a major intrinsic biomarker of cellular metabolic state, and simultaneously probe the concentration of reactive oxygen species (ROS) in different experimental conditions.
Once the PCLS are characterized, they will be used to perform target validation and study how selected drugs can re-stimulate a correct neo-alveolarization process and tissue metabolic state in preterm lungs exposed to hyperoxia.