Observational Study Protocol

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Titolo dello studio	**APOLLO 11, CONSORTIUM OF LUNG CANCER PATIENTS TREATED WITH INNOVATIVE THERAPIES: INTEGRATION OF REAL WORLD DATA AND TRANSLATIONAL RESEARCH**

Rational	**BACKGROUND** Lung cancer is the leading cause of cancer-related mortality worldwide. The global incidence in 2018 was an estimated 2 million cases with 1.7 million deaths predicted (1). A 70% increase in the number of new cases of lung malignancy is expected over the next 2 decades (1). There are two major subtypes of lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), accounting for approximately 15-20% and 80-85% of total cases, respectively (2). Within NSCLC, 45-65% is represented by adenocarcinomas, 15-25% by squamous cell carcinomas, 5% by large cell neuroendocrine carcinomas, and the remainder by "not otherwise specified" carcinomas (3). About 70% of patients with NSCLC, at the time of diagnosis, have advanced disease not amenable to surgical resection while SCLC is always considered a systemic disease (4). For several decades, cytotoxic chemotherapy has been the only treatment able to prolong survival in advanced patients with both SCLC and NSCLC. In recent years, in NSCLC, progress in better understanding of cell biology has led to the identification of specific genetic alterations, known as "driver" alterations, at the basis of tumorigenesis, including mutations of EGFR, KRAS, BRAF and MET, and rearrangements of ALK, ROS1, RET genes. The use of "target therapies" is now the treatment choice in this subgroup of patients. About 15% of NSCLC harbour somatic mutations in EGFR. Tyrosine kinase inhibitors (TKIs) such as gefitinib, erlotinib, and afatinib resulted in significantly improved progression-free survival (PFS) compared with standard chemotherapy and were initially indicated as first-line therapy (6-13). More recently, osimertinib, a 3rd generation drug, has reported, compared with other TKIs, a significant advantage in terms of PFS and OS and has become the new first-line standard (14). In light of its superiority over chemotherapy in the AURA3 trial (15), osimertinib also represents the second-line standard in patients with T790M-mediated acquired resistance to first-line therapy with first- and second-generation EGFR-TKIs (approximately 60% of cases). The ALK gene is shown to be rearranged in 5% of NSCLC cases. The first agent approved for ALK-positive NSCLC was crizotinib. Following initial disease control almost all patients inevitably develop resistance within approximately 12 months with disease progression predominantly in the brain (16). Therefore, the development of next-generation ALK inhibitors has been encouraged. To date, ceritinib, alectinib, brigatinib and lorlatinib have received approval from the Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA), and the Agenzia Italiana del Farmaco (AIFA), for the treatment of ALK rearranged NSCLC demonstrating an enhanced ability to penetrate through the blood-brain barrier. Moreover, in ongoing clinical trials othe novel ALK-TKIs have provided promising preliminary data in terms of both clinical activity and safety (17). The advent of immunotherapy has further changed the therapeutic landscape in both NSCLC without "driver" alteration (NSCLC-WT) and SCLC. This is a treatment modality based on mobilization of the immune system in order to recognize and destroy tumor cells. Immune Checkpoint Inhibitors (ICIs), have been developed with the intent to act on those pathways of "self-tolerance" used by tumors to escape recognition and then destruction by the immune system. By blocking these inhibitory pathways, which physiologically control the immune response, they reactivate and sustain the immune system against tumor cells (18). In particular, cytotoxic T-lymphocyte-associated-4 (CTLA-4) programmed cell death protein 1 (PD-1) are receptors expressed on T cells that, by interacting with CD80/CD86 (19) and programmed cell death protein 1 (PD-L1) or PD-L2 ligands, respectively (20), are able to promote and foster immune evasion by tumor cells. Several ICIs, capable of blocking PD-1/PD-L1 and CTLA-4 inhibitory

Objectives and clinical relevance	**OBJECTIVES** **<u>Primary objectives:</u>** 1. To create a network of Italian Lung Cancer Centers (APOLLO 11 Consortium) through: a. The development of a National Real World distributed database assets for lung cancer patients (NSCLC and SCLC) treated with INNOVATIVE THERAPIES (immunotherapy, target therapy and next generation treatments). b. Building the consortium, harmonizing all the relevant ethical, legal, and data protection issues among centers. c. The development of a National "virtual multilevel biobank" in lung cancer patients through the creation of real local biobanks stored in each participating center. d. The set-up and harmonization of the operative procedures for collection, storage and shipment of all the biological specimens foreseen. 2. To identify a specific factor or combinations of factors (i.e. mutations, transcript abundances, protein levels and immunological markers) influencing response, progression/hyperprogression, treatment resistance, survivals in lung cancer patients (NSCLC and SCLC). **In particular in the first phase, we will focus our analysis in:** - Integrating retrospective and prospective multisource data collection of advanced NSCLC already treated or candidate to receive ICIs-based therapy to assist the creation of a predictive artificial intelligence (AI) model to improve response prediction, ultimately leading to better cancer patients survival and their Quality of Life. - Characterizing the immune circulating profile by single cell analysis, FACS analysis, and TCR sequencing in order to correlate these variables with clinical outcome of NSCLC patients receiving first line ICIs treatment feeding the predictive machine learning algorithms. - Collecting all the available ICIs baseline CT scans from retrospective and prospective aNSCLC patients treated with ICIs to create and validate a radiomic signature and use its value to refine the predictive algorithms. **<u>Further scientific objectives of APOLLO 11:</u>** 1. To evaluate the association of data on genomics, tumoural and immunological microenvironment with clinical and pathological features of lung cancer (NSCLC and SCLC) treated with innovative therapies (immunotherapy, target-therapies and next-generation therapies).

2. To identify how specific factors or combinations of factors (tumoral microenviromental and immunological markers) may influence response, progression, atypical response (hyperprogression or pseudoprogression) and treatment resistance in lung cancer (NSCLC and SCLC).
3. To evaluate the prognostic role of these factors or combinations of factors in lung cancer (NSCLC and SCLC).
4. To assess possible changes over time of specific factors or combinations of factors (gene, biomolecular and immunological markers) in relation to the clinical evolution of the disease.
5. To specifically assess the role of metabolism in influencing tumour biology, the biology of the anti-tumour immune response and the activity of cancer therapies.
6. To evaluate the possible impact of clinical characteristics, comorbidities and concomitant therapies on oncological outcomes
7. To develop prognostic/predictive models of response/resistance to innovative treatments analyzing "big data" through artificial intelligence methods such as machine learning and deep learning in order to tailor treatments for patients with lung cancer and guide decision-making processes.
8. To use XAI approach to study decision-making systems (such as traditional AI and ML methodologies), with the aim of making them more interpretable, transparent, explainable, and reliable for clinicians by unfolding the black box of the AI algorithms.
9. To compare mutational and gene expression profiles identified in tumour tissue with those identified in peripheral blood.
10. To identify factors or combinations of factors predictive of response, resistance, disease progression in blood, urine, faeces and/or any other available biological material in lung cancer (NSCLC and SCLC).
11. To define the immunological mechanisms underlying immunorelated toxicities or toxicities derived from target therapies and possible biomarkers associated with their development and clinical course.
12. To define the mechanisms of secondary resistance to specific treatments used in lung cancer e.g., in patients with EGFR, ALK, ROS1, RET, BRAF, HER2, KRAS, MET exon skipping 14 and other possible driver alterations and their relative driver TKIs.
13. To identify new potential therapeutic targets at the level of any biological material.
14. To identify the association between meta-genomic profiles on stool, patients' immune phenotype and cancer outcome.

CLINICAL RELEVANCE

Firstly, creating an Italian network of hospitals with expertise in the treatment of advanced LC and facilitating the integration of real-world data and biological samples

	from multiple centers, in line with the credo “union is strength”, will avoid unnecessary dispersion of data from single institutions that are often inconclusive, due to the limit sample number. This real-world data collection will also help to understand real-world outcomes, which frequently are not the same compared to results upcoming from clinical trials. With the advent of several innovative therapies (target, ICIs and next-generation therapies), there is an increasing attention towards mechanisms potentially involved in tumor escape in both NSCLC and SCLC. APOLLO 11 network aims to identify and combine different types of markers (clinical, radiologic, genetic, molecular and immunological) in different histologies (NSCLC and SCLC)capable of predicting response to therapy, toxicity, relapse after curative intervention or associated with primary and acquired resistance. Moreover, the identification of gene expression, biomolecular and immunological profiles may allow an increased personalization of lung cancer (NSCLC and SCLC) treatment. The aforementioned analyses/activities will be carried out on the collected data in the local databases. Moreover, the sample collected and stored in each local biobank will be shared and used to achieve the primary objectives. Moreover, biological samples will be accessible over time as soon as dedicated funds are available to address the specific objectives described above and future research in the light of the evolution of scientific evidence.

Study population	Patients affected by lung cancer will be enrolled according to the following *inclusion criteria:* 1. Provision of signed and dated written informed consent, when applicable, by the patient or legally acceptable representative prior to any mandatory study-specific procedures, sampling, and analyses; 2. Patients must be ≥18 years of age at the time of signing the informed consent; 3. Histologically or cytologically confirmed lung cancer (NSCLC or SCLC) at any stage (I-IV) candidate to receive or already treated with innovative therapies (ICIs, target therapies and next generation therapies). Patients must not enter the study if any of the following *exclusion criteria* are fulfilled: 1. Patients who are or will be taking other unapproved antineoplastic therapies concurrently are not eligible. 2. Patients who have not received an oncological systemic innovative therapy at any setting 3. Patients received only local treatments (e.g., only surgery, or only radiotherapy) 4. Patients who received only standard chemotherapy are excluded. 5. Patients who received treatment therapies before 2010 are excluded.

Methods and study design	This is a retrospective/prospective multicenter observational study that will enroll lung cancer patients (NSCLC and SCLC) treated with at least one innovative systemic treatment. **DATA and MATERIAL COLLECTION (Figure 1)** **Real world data collection on the local databases (REDcap)** Retrospective phase: all the involved centers will participate in the collection of clinical data that will be stored in the local database of each Center. This phase involves the collection of "real world" available *jeopardized* data (e.g., demographic, epidemiological, clinical, biochemical, biological, radiological including also data of treatments performed, survival, and toxicity outcomes) related to cases of lung cancer (NSCLC stage I - IV and SCLC-LD and ED) treated with innovative therapy (e.g., target therapy, immunotherapy and next generation therapies) since January 2010 at the moment of ethical committee approval if the patients have already started the innovative therapy. Prospective phase: each patient with new diagnosis of lung cancer (NSCLC stage I - IV and SCLC-LD and ED) candidate to receive an innovative therapy and able to sign the informed consent to the processing of personal data can be enrolled in the prospective phase. In this phase *homogenized* data will be collected from the date of the committee approval. The data will be entered into the local REDCAP database. **Imaging collection:** For both retrospectively and prospectively enrolled patients, CT, PET and MRI scans will be performed as per standard of care, locally stored and shared, with the same code assigned during enrollment, to achieve the described objectives as detailed in the data management plan. The collection of these images will be performed at each innovative treatment at first radiologic evaluation, best response and radiological progression. **Radiomics analysis:** the first analysis will start on ICIs baseline CT scans of aNSCLC-IO cohort. In the retrospective phase in around 1000 patients and prospectively in around 200 patients. The CT scans from the participating sites will be de-identified in accordance with GDPR standards and encrypted prior to transmission to the central server for Radiomics analysis. A dedicated radiologist will analyze CT images and 3D tumour targets volume delineation will be manually determined for each patient and image segmentation will be performed. The main clusters of patients will be related with other collected parameters and the generated radiomics signature will be validated and then integrated with AI models. **Biological samples collection for translational research** The study foresees the annotation of samples already available for standard of care (i.e. FFPE tumor tissue) for retrospective enrolled patients in dedicated CRF. In case of

previous other samples collection (e.g,. blood, feces etc) in this setting it is possible to record retrospectively these samples in the dedicated CRF.
The study foresees the collection, preservation and annotations of biological samples (peripheral blood, stool, urine, saliva as per protocol and, when available, tumor tissue collected as per standard of care) for prospective enrolled patients.
Each biological sample collected per protocol will be identified with the same code assigned to the patient when enrolled, with a label reporting also date of collection, and type of specimens. Moreover, these information and storage references will be entered into dedicated CRF.

Tissue collection
For both retrospective and prospective enrolled patients, cyto-histological tumor tissue collected for diagnostic purposes and during an innovative treatment per **standard of care**, will be annotated in the "virtual biobank" dedicated CRF. Two different types of storage are foreseen: 1) FFPE tissue sample 2) Frozen tissue, whenever possible.

Blood, urine, saliva, stool Samples Collection
For patients enrolled in the retrospective phase no blood specimens will be collected if the patients have already started the innovative therapy. A patient enrolled in the retrospective phase, could be also enrolled in the prospective phase, if he will start a subsequent innovative treatment. The same code patient will be assigned.

For patients enrolled in prospective phase, **blood, urine, saliva, stool** samples will be collected during the patient's appointments and therapies already scheduled on site at different timepoints for each innovative treatment (when more than one innovative therapy is foreseen for a single patient): 1) baseline, when treatment starts, 2) after the first cycle, 3) at the first radiological evaluation and 4) at evidence of progressive disease.

In particular, a total amount of 32 ml peripheral blood will be collected to obtain the following biological specimens for each timepoint.
Details for pre-analytical procedures are stated in detail in the **laboratory manual** of APOLLO 11 biobank.

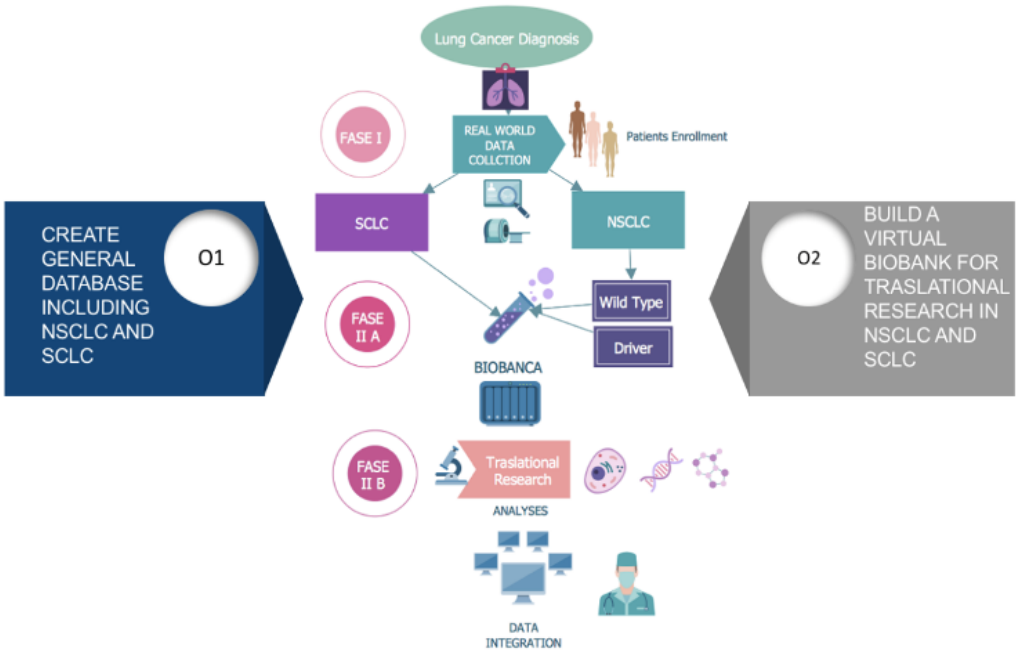

Figure 1. Master Protocol long-term scientific objectives of APOLLO 11.

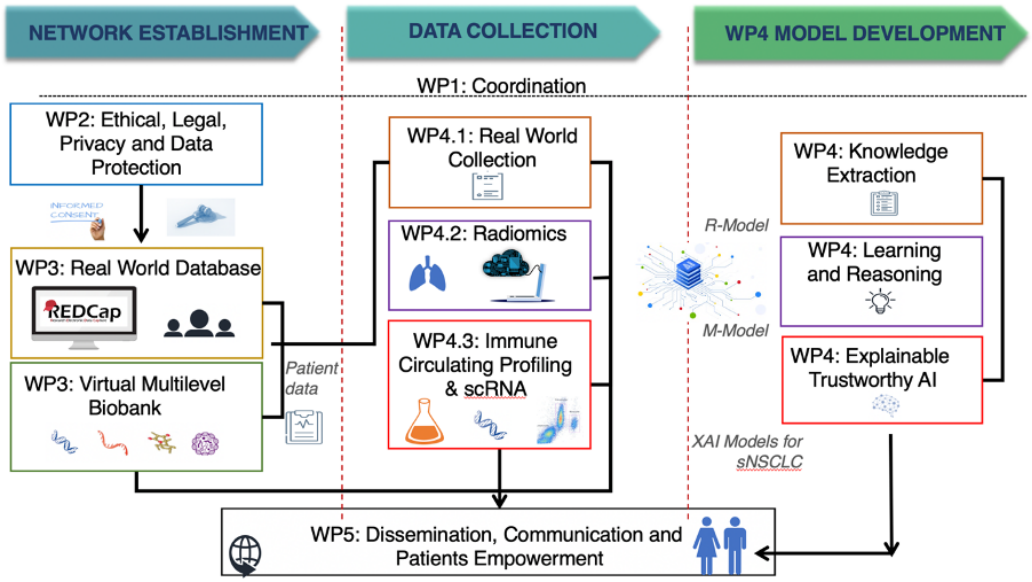

Figure 2. Project design of the first analysis of the APOLLO 11 project.

Sample size and statistical analysis	Since the aim of the project is to identify prognostic/predictive factors of response, progression or resistance to treatment, for which incidence and prevalence data are not available to date, and in light of the multicenter participation in the study, the total number of patients who can be enrolled cannot be defined at present. About 48 IRCCS, general and academic hospitals from different Italian Regions will be involved in this study.. The study cohort for the first analysis will consist of around 1000 retrospective and 200 prospective aNSCLC-IO cases. The primary objective of this part is to compare the predictive accuracy of ML/DL methods (created using multisource data) with current validated biomarkers (e.g., PD-L1 or available scores). The study will have at least 85% power to detect a 0.1 increase in predictive accuracy as measured by the area under the ROC curve (AUC), assuming an AUC for validated biomarker alone of at least 0.6, using a one-sided z-test at a significance level of 0,050. The data are assumed to be discrete (rating scale) responses.
Informed consent	During the visit, after adequate information, the patient may sign an informed consent form, in accordance with the provisions of the regulations set out in Legislative Decree of 01 March 2012 no. 85, OJ General Series no. 72 of 26-3-2012, for the prospective collection of clinical data and conservation of biological samples. As it is impossible to ask for consent to process data from patients entered retrospectively, for ethical-administrative reasons, the study falls within the scope and purposes specified by the Guarantor for the Protection of Personal Data (General authorization to process personal data for scientific research purposes and General authorization to process genetic data - 15 December 2016 - Official Gazette no. 303 of 29 December 2016).