Observational Study Protocol

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Title of the study	**I³LUNG: Integrative science, Intelligent data platform for Individualized LUNG cancer care with Immunotherapy**
Background and Rationale	**BACKGROUND** Lung cancer is the leading cause of cancer-related mortality worldwide. The global incidence in 2020 was an estimated 2.2 million cases with an estimated 1.8 million deaths. A 70% increase in the number of new cases of lung malignancy is expected over the next 2 decades (1). There are two major families of lung cancers: small cell lung cancer (SCLC), which accounts for approximately 15-20% of total cases, and non-small cell lung cancer (NSCLC), which accounts for approximately 80-85% (2). 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 (3). 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 cell biology have led to the identification of specific genetic alterations, known as "driver" alterations, at the basis of tumorigenesis, including mutations of EGFR, KRAS and BRAF and rearrangements of ALK, ROS1, RET genes. The use of "target therapies" is now the treatment choice in this subgroup of patients (4-11). 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 destruction by the immune system. By blocking these inhibitory pathways, which physiologically control the immune response, ICIs reactivate and sustain the immune system response against tumors cells (12). Cytotoxic T-lymphocyte-associated-4 (CTLA-4) and programmed cell death protein 1 (PD-1) are receptors expressed on T cells that, by interacting with CD80/CD86 (13) and programmed cell death protein 1 (PD-L1) or PD-L2 ligands, respectively (14), can promote and foster immune evasion by tumor cells. PD-L1 evaluation on tumor remains the only used and approved predictor of response to immunotherapy (IO) treatment. Although the probability that a patient will benefit from IO treatment is higher in high PD-L1 expression, the role of PD-L1 remains unclear as a predictive role (15-20). As a general guideline, the lack of alterations in target genes and PD-L1 expression > 50% allow to candidate a patient for first-line treatment, single-agent IO or its combination with chemotherapy based on platinum regimen (CHT). In patients with PD-L1 expression 1-49%, IO alone showed modest activity, while the CHT-IO therapy demonstrated to significantly prolong patient survival compared to CHT alone, in both squamous and non-squamous NSCLC (10). To date, FDA and EMA approved for use in advanced NSCLC patients of nivolumab and pembrolizumab (antibodies against PD-1), and atezolizumab and durvalumab (antibodies against PD-L1). Due to the better efficacy and safety profile compared with chemotherapy, pembrolizumab, nivolumab, and atezolizumab were approved as monotherapies, pembrolizumab as first and second line treatment, whereas nivolumab and atezolizumab only in second line (15-19). Durvalumab is

instead indicated as maintenance therapy in patients with locally advanced NSCLC undergoing concomitant chemo-radiotherapy treatment (20).

Approximately 16% of NSCLC patients are still alive at 5 years compared to 5% with cytotoxic CHT. However, about 50% of patients do not obtain a real long-term benefit from this treatment modality. (21) Furthermore, in subjects carrying driver alterations, no significant advantage of single-agent IO over CHT has been reported (15-20). Lastly, there is a subgroup of 13-26% patients for whom IO treatment may even be detrimental, inducing a hyper-progressive disease (HPD) (22-26). Beyond PD-L1, several other biomarkers have been identified and used to profile patient prediction, including Tumor Mutational Burden (TMB) (27), tumor microenvironment (TME) (28), microRNA (miRNA) (29), immune gene signatures (30), gut microbiome (31), radiomics (32), or baseline clinical features or their combination in different scores (33,34).

Radiomics regards the quantitative analysis of digital imaging, informs future precision medicine, and supports objective decisions for cancer diagnosis and treatment. MAASTRO clinic has successfully experienced and validated fifty-six 3D-radiomic features. Recently, radiomics has demonstrated correlation between CT images and clinicopathologic features in NSCLC-IO patients (35). Finally, radiomics have been lately applied to PET/CT, in which context metabolic tumor volume (MTV) and total lesion glycolysis (TLG) in NSCLC demonstrated correlation with response to IO (36). There are several ongoing EU projects that use radiomics for NSCLC, but none of them is IO-specific and integrates multi- OMICs.

In the last decade, an impressive amount of knowledge has been achieved regarding the many other putative predictive factors, but no one has been applied in clinical practice. This is mainly due to unstructured and spared data among the different institutions, generating less consistent and therefore, less predictive datasets. Today, the medical community is claiming for an overall integration of clinical data and molecular biomarkers, in order to generate predictive information to address therapeutic administration in cancer patients, specifically in the immunotherapy era. In fact, it is unlikely that a single biomarker will profile prediction or prognosis with a high accuracy considering the role played by the immune system, and the complexity of the TME.

Artificial Intelligence (AI) frameworks and Machine Learning (ML) processes synthesize and correlate information from multiple sources and develop learning methods from it. These are a powerful, innovative, and potentially highly efficient instrument to construct decision-making (DM) tools providing individualized prediction of response to treatment and finally cope with the overmentioned complexity (37).

Therefore, the I³LUNG project aims to achieve the highest performance in personalized medicine through AI/ML modelled on multimodal patients' data, together with implementing an AI/ML model in a real-life setting. A set of patient-centered ML tools designed and validated for the project, which make use of the novel virtual patient AVATAR entity for predicting progression and outcome. To maximize its impact, the use of Trustworthy Explainable AI (XAI) methodology will integrate the AI's inherent performances with the input of Human Intuition (HI) to construct a responsible AI application able to fully implement truly individualized treatment decisions in NSCLC interpretable and trustworthy for clinicians. (37) The final objective is the establishment of a European (potentially worldwide) Data Sharing and Elaboration Platform (DSEP), presented in Figure 1. The DSEP will provide guiding tools for patients, providing information to generate awareness on treatments. Lastly, it gives access to researchers and the general scientific community to the most up-to-date data sources on NSCLC.

Within the I3LUNG project, an ad-hoc Individual Patient Decision Aid System (IPDAS) for NSCLC patients will be developed. Patient decision aids are tools that might be used by patients either before or within a consultation with physicians. Patient decision aids explicitly represent the decision to be made and provide patients with user-friendly information about each treatment option by focusing on harms and benefits. They allow patients to clarify what matters to them the most. These decisional support systems have been demonstrated to be effective in empowering patients, improving their knowledge, promoting their active participation in clinical decision-making about treatments, and improving overall patient satisfaction with care while decreasing decisional conflict and decisional regret (38-41) (see Psychological Study Annex I).

Finally, within the I^{3}LUNG project it will be assessed whether using the IPDAS during the clinical consultation would foster the quality of the shared decision-making as well as the quality of the doctor-patient communication. Alongside the evaluation of the impact of the IPDAS, it will be also evaluated whether the inclusion of the AI/ML predictive models in clinical practice will be added value in supporting oncologists' clinical decision-making and decreasing cognitive fatigue and decisional conflict (see Psychological Study Annex II).

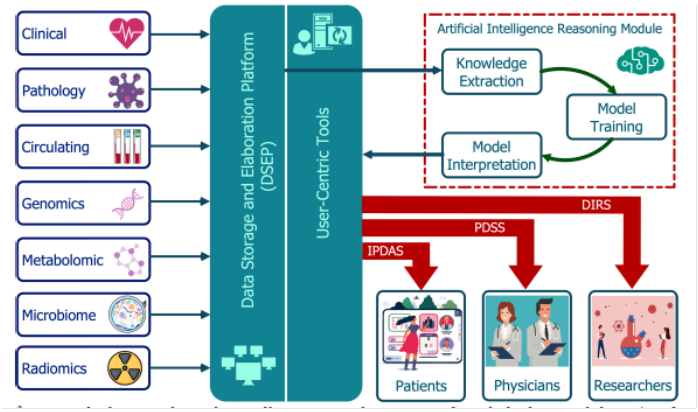

Figure 1. I^{3}LUNG platform, tools, and overall structure. The AVATAR based platform and the AI/ML based tools.

I^{3}LUNG will foresee two study phases: Retrospective and prospective.

I^{3}LUNG adopts a two-pronged approach to develop a medical device through the creation and validation of retrospective and prospective AI-based models to predict immunotherapy efficacy for NSCLC patients using the integration of multisource data (real word and multi-omics data) through a retrospective – setting up a transnational platform of available data from 2000 patients – and a prospective – multi-omics prospective data collection in 200 NSCLS patients – study phase.

The retrospective part of the I^{3}LUNG project includes the analysis of a multicentric retrospective cohort of more than 2,000 patients. This cohort will be used to perform a preliminary knowledge extraction phase and to build a retrospective predictive model for IO (R-Model), that will be used in the prospective study phase to create a first version of the Physician Decision Support System (PDSS) tool, an AI-based tool to provide an easy and ready-to-use access to predictive models, increasing care appropriateness, reducing the negative impacts of prolonged and toxic treatments on wellbeing and

	healthcare costs. Also, CT and PET scans will be collected and a first radiomic signature will be created to feed the R-Model. **The prospective part** of the project includes the collection and the analysis of multi-OMICs data from a multicentric prospective cohort of about 200 patients. This cohort will be used to validate the results obtained from the retrospective model through the creation of a new model (P-Model), which will be used to create the final Physician Decision Support System (PDSS) tool.
Objectives and clinical relevance	**Overall Primary Objective** • Prediction of response to immune checkpoint inhibitors in NSCLC by using clinical, biological, metabolomics, genomics, pathomics and radiomics data. **Secondary Objectives** • Prediction of Progression-free survival (PFS) in response to immune checkpoint inhibitors in NSCLC by using clinical, biological, metabolomics, genomics, circulomics, pathomics, microbiome and radiomics data. • Prediction of Objective Response Rate (ORR) in response to immune checkpoint inhibitors in NSCLC by using clinical, biological, metabolomics, genomics, circulomics, pathomics, microbiome and radiomics data. • Prediction of Overall survival (OS) in response to immune checkpoint inhibitors in NSCLC by using clinical, biological, metabolomics, genomics, circulomics, pathomics, microbiome and radiomics data. **Exploratory Objectives** • Added value of PET/CT into the prediction model for the Primary and Secondary Objectives. • Prediction of adverse events to immune checkpoint inhibitors in NSCLC by using clinical, biological, genomics, pathomics and radiomics data. Evaluate the addition of sarcopenia analysis into the prediction model to the response of immune checkpoint inhibitors in NSCLC **Specific Objectives:** **Retrospective Study Phase** The primary objective of the retrospective clinical study within the I3LUNG project is to create a model that will be used in the prospective phase to compare the predictive accuracy of ML methods (created using multi-OMICs analyses) with current validated biomarkers (e.g., PD-L1 or available scores). All available jeopardized data of 2000 aNSCLC patients treated at any point with any IO-based therapy will be collected in the first 18 months of the I3LUNG project, including all the data and radiomics detailed in the "Scientific Clinical Study Design" section. This model will be used to create a first version of the Physician Decision Support System (PDSS) tool. **Prospective Study Phase** The primary objective of the prospective phases to compare the predictive accuracy of ML methods (created using multi-OMICs analyses) to current validated biomarkers (e.g., PD-L1). In this prospective part of the study, multi-OMICs data (pathology, genomics, transcriptomics, metabolomics, circulating immune profiling, microRNA signature classifier, liquid biopsy, microbiome) of 200 patients recruited by 5 cancer centers from the Consortium (INT, GHD, VHIO,

MH and SZMC) will be collected and then integrated in the platform and used to feed the AI models learned on retrospective data, previously collected in the retrospective study phase.

Other objectives of the prospective phase in particular will include:

1. Integrate both retrospective and prospective data to improve and refine the predictive capabilities of the AI models compared to existing models;
2. Integrate radiomics signature originating from both Computed Tomography (CT) and Positron Emission Tomography–CT (PET-CT) images of the prospective cohort to refine and validate the retrospective radiomics signature;
3. Measure, within the prospective cohort new sensor-based QoL model and behavior data (e.g.: cough, physical and social activities, and mood states/emotions) that complements standard QoL measures (Patient Reported Outcome Measures or PROMs and Patient Reported Experience Measures or PREMs); the ad-hoc developed sensor- based monitoring system of patients' QoL and standardized PROMs and PREMs (ii) if co-shared decision-making impact QoL at least +10% compared to published prospective study data (Figure 1)
4. Use AI/ML to create new models in order to increase cognitive abilities of patients and physicians in shaping trust, usability, acceptability and attitude towards AI/ML;
5. Provide guidelines for an efficient collection of data to support diagnosis and select treatment in NSCLC will be developed thanks to the validated AI and Explainable AI (XAI) techniques;
6. Put in place preliminary tasks for the CE marking certification and future exploitation of the "medical device developed tools".

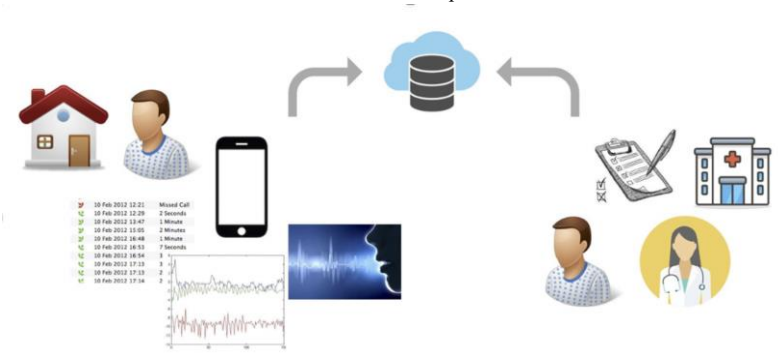

Figure 1. QoL sensor-based and transparent monitoring using app and cloud-collection on patients' information.

Clinical relevance

Currently, no clear data support the use of any biomarker, beyond PD-L1, to predict the patients benefit from IO and clinicians are often left alone to make decisions. The I³LUNG study aims to combine different types of markers (clinical, radiologic, genetic, molecular and immunological) through Artificial Intelligence (AI) frameworks and Machine Learning (ML) techniques. This will permit to fit it in a model that will be tested and integrated in the prospective phase of the study. In the final scenario, a pragmatic tool will be provided to clinicians in order to help predicting the benefit of IO, choosing the best candidate to immunotherapy and finally guide the therapeutic choice (IO alone vs IO-CHT combinations vs IO-IO combinations).

Study Endpoints	**Primary endpoint** • The predictive model will be developed through artificial intelligence and machine learning analysis of the integrated clinical, biological, genomics, metabolomics, circulomics, pathomics, microbiome and radiomics data. **Secondary endpoints** • Using the predictive model index as classificatory biomarker in Progression-free survival (PFS), defined as the time interval from the date of the first dose of the study treatment until the first date at which disease progression is objectively documented, or death due to any cause, whichever occurs first and calculated through censored Kaplan-Meier analysis. • Prediction of Objective response rate (ORR) classification through the predictive model and compared to the assessed by the investigator and defined as the proportion of the participants with confirmed complete response (CR) or partial response (PR) according to Response Evaluation Criteria in Solid tumors (RECIST) v1.1, after initiation of immune checkpoint inhibitors treatment. • Using the predictive model index as classificatory biomarker in Overall survival (OS) calculated from diagnosis to death or last follow-up through censored Kaplan-Meier analysis. **Exploratory endpoints** • If available PET/CT will be added to the prediction model and the increment in the prediction performance will be analyzed for the primary and secondary endpoints and the adverse events categories. • Machine learning algorithm will be used to predict adverse events categories in response to immune checkpoint inhibitors treatment through the integration of the clinical, biological, genomic, pathomics and radiomics. Sarcopenia will be calculated through radiomics by measuring the fat and muscular mass on L1 slice of the chest CT scan and added to the prediction model for the Primary and Secondary Objectives.
Study population	RETROSPECTIVE PHASE: The study cohort consists of aNSCLC patients treated with IO. Data from an estimated 2000 patients treated with IO-based therapy (any treatment line) in the period 2012-2021 will be collected from all the clinical partners (INT, GHD, VHIO, MH, SZMC and UOC). Inclusion criteria will be checked and informed consent for the study and for the re-use of prior collected medical data will be obtained before enrolment. If not feasible, i.e. patients not alive, the approval to Privacy Guarantee will be obtained. **Inclusion criteria** retrospective phase: 1. Age ≥ 18 years 2. Presence of an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2; 3.Histologically confirmed diagnosis of stage III-IV Non-Small-Cell Lung Cancer patients receiving also maintenance therapy with durvalumab or other immune/combination are allowed; 4. Patients who received any line of IO-based therapy (immunotherapy monotherapy or immunotherapy in combination with any other treatments); 5. Patients with any CNS mets (stable or not) are allowed.

6. Patients with driver alteration (e.g., EGFR, BRAF, KRAS mutations or ALK, ROS1) who underwent an immunotherapy line will be included.

Exclusion criteria retrospective phase:

1. Patients without minimal treatment information data (e.g. start and stop date of immunotherapy and type of immunotherapy agent or protocol name)

PROSPECTIVE PHASE:

In the prospective phase, the study cohort consists of aNSCLC patients candidate for first-line IO-based therapy with available surgical samples (enough to perform OMICs). Baseline data of an estimated 200 patients from 5 clinical centers (INT, GHD, VHIO, MH and SZMC) will be collected including complete clinical, multi- OMICs analysis, imaging of CT and PET scan at baseline IO, behavioral, health economic, QoL measurements with based-sensor techniques and standard QoL. Data treated with IO-based therapy (any treatment line) will be collected. Inclusion criteria will be checked and informed consent for the study and for the re-use of prior collected medical data will be obtained before enrolment.

Inclusion criteria for prospective phase:

1. Age >/= 18 years.
2. Evidence of a personally signed and dated ICF indicating that the patient has been informed of and understands all pertinent aspects of the study before enrolment.
3. Eastern Cooperative Oncology Group (ECOG) performance status " 0-2.
4. Histologically confirmed diagnosis of stage IIIB/C-IV Non-Small-Cell Lung Cancer
5. No activating EGFR mutations or ALK/ROS1 rearrangements
6. No prior treatment for advanced disease
7. Availability of at least 1 (one) FFPE block for -omics data generation (for cohort 200 pts)
8. Clinical indication for frontline treatment with immunotherapy (alone or in combination), as first line of treatment for metastatic disease or consolidation treatment for locally advanced disease
9. Patients with untreated or unstable CNS brain metastases are allowed

Exclusion criteria for prospective phase:

1. Prior systemic treatment for NSCLC for advanced disease
2. Absence of sufficient amount of tumor tissue for -omics data generation for OMIC cohort (200 pts).
3. Unavailability or inability to comply with the requested study procedures, including compilation of QoL questionnaires and behavioral data collection (mobile app).
4. Unable to participate in the psychological study

<table>
<tr><td></td><td>Data for –omics analysis from patients considered screening-failure and, therefore, not eligible for the prospective phase of the protocol, will also be collected to feed the AI models using their real-world data generated during IO-based treatments. There is no limit for sample size in this phase and are not counted within the 200 patients who will be eligible for the multi-OMICs analysis.</td></tr>
<tr><td>Methodology and study design</td><td>Scientific Clinical Study Design

I³LUNG will conduct an observational retrospective/prospective multicenter international project on advanced Non-Small-Cell Lung Cancer patients treated with IO. Its main objective is to study, train and validate the predictive accuracy of ML methods created by using real world data and multi-OMICs analyses, compared to the current standard of care biomarkers (e.g., the PD-L1).

The prospective study part of the project will start at month 3 and will continue up to 36 months, NSCLC patients candidates (n=200) to first-line IO-based therapy with available surgical samples (enough to perform -omics) will be enrolled.

In particular, will be collected the following data for Retrospective Phase (when available) and for Prospective phase:

<table>
<tr><th>Baseline patients' data</th><th>Retrospective phase Jeopardized</th><th>Prospective phase Homogenized</th></tr>
<tr><td>Demographic characteristics (age, gender, height, body weight, ethnicity, family history of cancer, Smoking History, asbestos exposure, alcohol consumption, drug use history).</td><td>✓</td><td>✓</td></tr>
<tr><td>Medical history (personal medical history of cancer and other severe chronic diseases, allergies and concomitant medications)</td><td>✓</td><td>✓</td></tr>
<tr><td>Health-economic details (site of treatment administration, lost workdays, etc.)</td><td>If available</td><td>✓</td></tr>
<tr><td>Lifestyle factors (diet and use of supplements, physical activity, daily heart rate, sleep patterns, QoL)</td><td></td><td>✓</td></tr>
<tr><td>Clinical findings:
- Performance Status (ECOG), Vital signs (blood pressure, oxygen saturation, (BP, SpO2), Temp, breaths/min, pulse).
- Main symptoms leading to diagnosis, main symptoms at initiation of immunotherapy. Presence of main disease-related symptoms (shortness of breath, cough, pain and fatigue).</td><td>✓</td><td>✓</td></tr>
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	- Body weight/height/BMI/BSA and weight loss prior to diagnosis and prior to initiation of IO (kg/months). - Major findings at clinical examination at diagnosis.		
	Laboratory findings: Hb, WBC, Neutrophils, Lymphocytes, NLR (neutrophil/lymphocyte ratio) at IO initiation and post-treatment change in NLR, Platelets, Albumin, LDH, Ferritin, Creatinine, Creatinine clearance, Na, ALP, Glucose, CRP.	✓	✓
	Tumor characteristics: Histology, Grade, Ki 67, mutational status (e.g. EGFR, BRAF KRAS, ALK, ROS1) and PD-L1 expression, TNM stage, Sites/organs of metastases. Time from initial diagnosis to first metastatic disease.	✓	✓
	Treatment Characteristics: - previous treatments, previous platinum use and response, - 1st line treatment combination (IO vs CT+IO or other IO combinations). - Regimen duration, number of cycles, details of maintenance treatment. Best response to 1st line. Duration of response, PFS, OS. - Toxicity details (type of toxicity, grade). Use of supportive medication (growth factors, anti-emetics).	✓	✓
	Radiomics	✓ (CT +PET, the later when available)	✓ (CT + PET scan)
	Immunohistochemistry data (e.g., CD3, CD4, CD8, CD20, CD11c, CD68, PD-1, PD-L1 and TIGIT)		✓
	Citomic: Immune phenotype and single cell analysis of circulating populations (i.e. PBMCs, PMNs)		✓
	Cytokines and MicroRNA signature (MSC test)		✓
	Cell free DNA and ctDNA sequencing		✓

Genomics analysis: tumor mutational burden (TMB), somatic copy number alterations, whole genome sequencing, immune infiltration transcriptomic profile		✓
Metabolomics and Microbiome		✓

The clinical/synthetic data will be collected within I3LUNG web-based platform. To each patient will be assigned a code. The CRF form will be filled in from each center with a dedicated account and all these workflows will be addressed using the Data Management Plan.

In particular, for the prospective phase the following multi-OMICs data will be collected for all 200 patients with available tissue and blood. For details see Table 2.

Omics type	**Collection**	**Analyses**
Tissue-based **(Tasks 3.2, 3.3, 3.4)**	Formalin-Fixed Paraffin Embedded (FFPE) tissue blocks from NSCLC patients who underwent surgery or large biopsy will be collected. Every 5 collected samples, FFPE blocks will be shipped to GHD. GHD will then extract samples for immune profiling/TME and will prepare 4 total cores, 2 for genomic profiling and 2 for metabolomics.	· TME and Tissue Immune Profiling - Reference tissue spots with known expression of the target proteins will be added to each microenvironment Tissue Microarrays (ME-TMA) · Genomic profiling (DNA and RNA extraction) and transcriptomics profiling. Metabolomics, metabolite extraction for targeted and untargeted metabolomics analysis

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Blood-based **(Task 3.5)** (NB: PNMs isolation and Single Cell analysis and will be performed only in INT, because PNMs lability will not permit freeze-thaw).	30 ml of WB samples will be collected from each patient only at baseline therapy. 1x2 ml WB tube will be used for DNA extraction 1x2 ml K2EDTA for cytokines assay 2x10 and 1x6 ml K2EDTA tubes for PBMC, PNMs, miRNA Signature Classifier (MSC) and cell-free DNA (cfDNA)	· Circulating Immune profiling PBMCs by flow cytometry staining. Assessment of membrane or intracellular markers (T-cells, T-regulatory cells, natural killer cells, monocytes, neutrophils, myeloid derived suppressor cells, B-cells and T-cell exhaustion, immune suppression, activation, proliferation, cytotoxicity, polarizations) · Single cell analysis – T-cell receptor RNA sequencing · Cytokines plasma analysis · MSC test - panel of lung cancer related miRNAs will be measured in plasma samples · cfDNA and ctDNA sequencing.
Microbiota analysis **(Task 3.6)**	Baseline IO stool samples of all prospective patients enrolled will be collected and shipped to INT each 10 samples for microbiome analysis.	· NGS libraries sequencing · Metagenomic DNA and RNA extraction Organism-specific gene hits will be assigned to the Kyoto Encyclopaedia of Genes and Genomes (KEGG) Orthology (KO) DB, metagenomes for each sample will be reconstructed into metabolic pathways
Advanced Imaging **(Task 3.7)**	CT scans in both in retrospective and prospective study phase and PET-CT in prospective phase scans are taken to perform the radiomic signature. The images will be anonymized and uploaded in a dedicated platform in order to be centralized to BGU.	· Tumour and lymph nodes/metastasis manual segmentation, and interactive segmentation, using algorithms of varying complexities, already evaluated. · Extraction of four classes of image features describing tumour features: i) intensity, ii) shape, iii) texture and iv) Fourier or wavelet features (also MTV and TLG for PET-CT). Radiomic signatures: unsupervised clustering to reveal clusters of patients with similar radiomic expression patterns

QoL Monitoring

Within the prospective part of the study, among all the prospective enroller centers (INT, GHD, MH, VHIO and SZMC) we will evaluate whether making decisions using our prediction system would lead to an improvement in patient's QoL using (i) the ad-hoc developed sensor- based monitoring system of patients' QoL and standardized PROMs and PREMs (ii) if co-shared decision-making impact QoL at least +10% compared to published prospective study data (Figure 6);

App Mobile

Sensor-based monitoring system of QoL This task foresees the development of a mobile application for the transparent monitoring of the patient's quality of life. It will include: an automatic cough detector system, an assessment of patient's mood state and emotional functioning, a human activities and gait recognition system and an evaluation of smartphone usage to monitor changes of social interactions and habits. Smartphone-based features collected daily will be correlated with clinical outcomes (e.g., monitoring toxicity and clinical benefit) collected at specific time points during the prospective clinical study in order to evaluate their capability to predict temporal variations of QoL, physical, social, and emotional functioning. Data collection within preliminary controlled studies are foreseen in this task to validate the app accuracy.

Using self-reported QoL measures to identify trajectories over time

Patients' lifestyle factors, daily habits, and quality of life will be collected at baseline and at baseline IO at 3, 6, 9 and 12 months and when progressive disease occurs through validated and self-reported measures. Physical activity, metabolic equivalent of task, and QOL will be assessed with the WHO Global Physical Activity Questionnaire. Moreover, patients will be administered with reliable, valid, and standardized cancer QoL measures such as EORTC QLQ-C30 , EQ-5D and lung cancer-specific QoL measures such as QLQ-LC13.

Image analysis methodology

The analytical process for the medical images will include:

- Data acquisition
- Image segmentation
- Analysis and features extraction

Data acquisition

Digital Imaging and Communications in Medicine (DICOM) medical images will be exported from the PACS system pseudonymized and uploaded to the central cloud server for Radiomics analysis.

Image segmentation

The pseudonymized DICOM images will be process through automatic segmentation by artificial intelligence using deep learning algorithms to recognize the lung field and automatically detect and segment the lung tumor lesions.

An independent qualified radiologist will centrally review the results and performance of the tumor segmentation algorithm.

Image features extraction

The study will extract and analyze a list of radiomics features according to the Image Biomarker Standardization Initiative (IBSI) recommendations. Features extracted from the segmented lesions will characterize different properties of the images, including:

i) pixel intensity,
ii) lesion shape,
iii) lesion texture,
iv) Fourier or wavelet-based features.

The parameters set obtained by RFE will be used to create radiomic signature following three steps:

i) identification of cluster and possible expression patterns;
ii) evaluation of relationship between these patterns and tumor/patient's;
iii) generation of radiomic signature;

To remove redundancy within the radiomic information, one or two best-performing radiomic feature from each of the four-feature groups will be selected and combined into a multivariate Cox proportional hazards regression model for prediction of survival. For the training phase, feature stability will be assessed in inter-observer settings.

The radiomic signature will be developed in the first 1000 CT and PET-CT images and will be then validated in the ~1000 remaining patients. The generated and validated radiomic signature will be integrate to the R-model generated to improve its prediction and make the R-model.

Radiomic Signature

To assess the value of radiomic features to capture phenotypic differences of tumors, the features obtained by the RFE will be subjected to unsupervised clustering to reveal possible clusters of patients with similar radiomic expression patterns. For PET-CT metabolic tumor volume (MTV), total lesion glycolysis (TLG) and radiomics features such as volume and heterogeneity will be added to the analysis of PET-CT images.

The main clusters of patients will be related with other collected parameters, looking for association with tumor stage, histology etc. Finally, the generated radiomics signature will be validated and then integrated in the PDSS tool to improve prediction.

Artificial intelligence methodology

I³LUNG will develop a Data Sharing and Elaboration Platform (DSEP) that gathers and manages virtual entities, namely patient AVATARS, corresponding to real patients, and will provide a set of tools that integrate physician expertise and the knowledge extracted from the patient avatars data to create individualized predictions for NSCLC patients treated with IO. As depicted in Figure 2, data is gathered from the DB and a context is generated for each patient, which, in its turn, is fed to the ML models to make decisions.

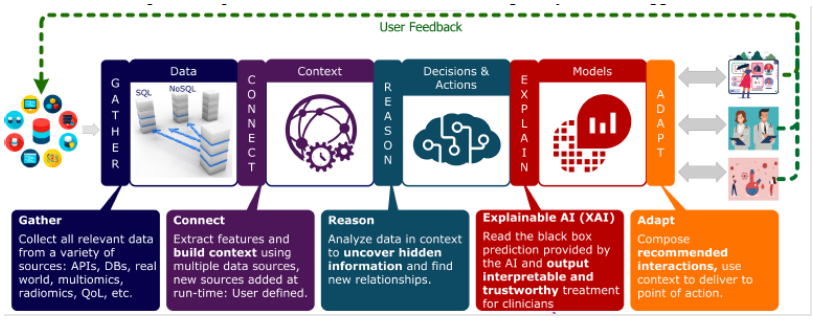
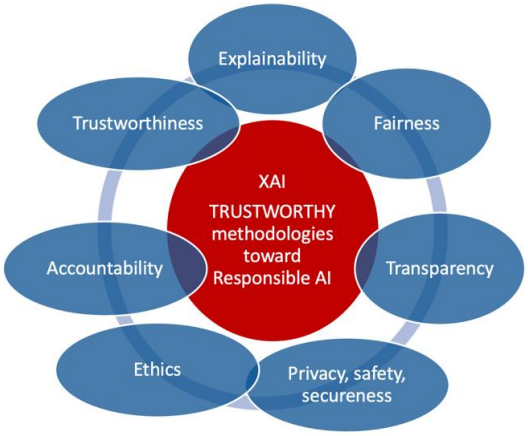


Figure 2. A summary of the ML pipeline we will adopt in the I³LUNG project.

	Patient data collected through the I³LUNG platform will be utilized to prototype, train, and host validated ML predictive algorithms to help with prognosis and IO response predictions. A careful ML data preparation for each of the data types (real world, radiomics) is essential to build both independent and merged models. ML informed features will be derived from the raw data types. **Explainability of the AI Models** The aims of the Explainable AI (XAI) are to produce more explainable models while maintaining a high level of learning performance (e.g., prediction accuracy) and to enable humans to understand, appropriately trust, and effectively manage the emerging generation of AI partners. In I³LUNG, a suite of XAI techniques will be used to make ML results interpretable and available to both clinicians and patients, comparing results with HI, so to support the clinical decision for the best IO-treatments for NSCLC, and the sharing of decisions with aware patients. XAI methodologies will be adopted and applied to the developed AI algorithms, such as the use of text, visual and local explanations, explanations by examples, and feature and input relevance (37). Moreover, simplifications to the model will be applied; for example, the creation of new models, based on the trained one, where part of the complexity is neglected without decreasing in the accuracy.  **Figure 3.** XAI methodology approaches.
Sample size and statistical analysis and AI analysis plan	For the retrospective phase, we have planned to enroll patients based on a first check availability of already treated patients with IO-based therapy among centres. The primary objective of the study is to compare the predictive accuracy of ML methods (created using multi-OMICs analyses) with current validated biomarkers (e.g., PD-L1 or available scores, see Table 2). Considering the number of 200 prospective cases, 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. Stratification Factors: Country (Italy or others), Gender (male vs females), ECOG PS (0-1 vs 2), Histology

	(squamous vs non-squamous), Stage (III vs IV), PD-L1 status, and treatment administered (Chemo-Immuno vs immuno-alone vs others IO combinations) will be considered. **AI analysis plan** After Ethical approval in each Cancer Center, the third part of the study will start at month 3 and will continue up to 36 months. These periods will allow sufficient time for prospective enrolment and follow-up of patients for at least for 18 months. At month 30, an intermediate version of the P-Model will be released after the collection of the first batch (~100) of patients including multi-OMICs data. At months 54, after the predictive models are build using all the data from 200 patients, the final P-Model will allow to verify if: (i) new predictive system using multi-OMICs results perform better compared to current ones (e.g., PD-L1 or other state of the art biomarkers), (ii) if the model using -omics data perform better compared to the R model, (iii) if there is any specific -omics analysis which perform better compare to other -omics, which would give more value to the model (iv) if the P-Model perform better compared to current biomarker, and compare to the R-model when applied to specific subgroups of patients (e.g., non-squamous vs squamous patients). We will consider this phase successful if the new P-models outperforms current biomarkers prediction at least for 10% in terms of correct prediction of either 2-year PFS and 2-year OS, measured with Harrel's C statistic.
Ethical considerations and Informed Consent	The study is conducted in accordance with the Declaration of Helsinki Ethical Principles and Good Clinical Practices and was approved at each site by an independent local ethics committee. During the clinical visit, after receiving adequate study information, the patient may sign an informed consent form, in accordance with the provisions of the local ethical regulations. regulations. The consent of the patient must be signed and personally dated by the patient and by the investigator/person designated to conduct the informed consent discussion. The signed and dated declaration of informed consent will remain at the researcher site and must be safely archived by the investigator so that the forms can be retrieved at any time for monitoring, auditing and inspection purposes. A copy of the signed and dated information and consent should be provided to the patient prior to participation. In the retrospective part of the study, in case it is not possible to ask and obtain for the informed consent (due to death or inability to reach the patients), to process the patient data and samples, for ethical-administrative reasons, the study falls within the scope and purposes specified by the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation), in its article 89.1. All clinical centers will be involved in the retrospective phase, while in the prospective phase only 5 out 6 centers will participate (INT, GHD, VHIO, MH and SZMC). The study will involve exporting of CT and PET images from both retrospective and prospective studies from EU cancer centers and US to Israel, while clinical data will be exported from all the EU cancer centers and Israel to US for AI analyses. Biological samples (tissue, blood and stool samples) will be collected from SZMC (Israel) and will be imported in EU countries (Italy, Germany, Spain) for pathology, genomic, circulating and microbiome analyses.
References	1. Globocan (2018) Available from http://globocan.iarc.fr/Default.aspx (Accessed on 25-Nov-2020).

2. Howlader N, et al. SEER Cancer Statistics Review, 1975−2012. National Cancer Institute, 2015 [resource on the Internet]. Available from: http://seer.cancer.gov/csr/1975_2012/http://seer.cancer.gov/csr/1975_2012/.

3. Sulpher JA, et al. Factors influencing a specific pathologic diagnosis of non-small-cell lung carcinoma. Clin Lung Cancer. 2013 May;14(3):238-44.

4. Siegel RL,et al. Cancer Statistics, 2017. CA Cancer J Clin 2017; 67:7−30.

5. Lynch TJ, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350: 2129-39.

6. Rosell R, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009; 361: 958-67.

7. Fukuoka M, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 2011; 29: 2866-74.

8. Maemondo M, et al. Gefitinib or chemotherapy for non-smallcell lung cancer with mutated EGFR. N Engl J Med 2010; 362: 2380-88.

9. Mitsudomi T, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010; 11: 121-128.

10. Rosell R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive nonsmall- cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012; 13: 239-46.

11. Sequist LV, Yang et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 2013; 31: 3327-34.

12. Wu YL, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. Lancet Oncol 2014; 15: 213-22.

13. Zhou C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011; 12: 735-42.

14. Soria JC, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non–Small-Cell Lung Cancer. N Engl J Med 2018; 378:113-25

15. Mok TS, et al. Osimertinib or platinum–pemetrexed in EGFR T790M–positive lung cancer. N Engl J Med 2017;376:629-40.

16. Sullivan I, Planchard D. ALK inhibitors in non-small cell lung cancer: the latest evidence and developments. Ther Adv Med Oncol 2016;8:32-47.

17. Karachaliou N, , et al. Anaplastic lymphoma kinase inhibitors in phase I and phase II clinical trials for non-small cell lung cancer. Expert Opin Investig Drugs 2017;26:713-22.

18. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–64.

19. Hathcock KS, et al. Identification of an alternative CTLA-4 ligand costimulatory for T cell activation. Science 1993; 262:905–7.

20. Thompson RH, et al. Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A 2004;101:17174–9.

21. Borghaei H, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med. 2015; 373: 1627–39.

22. Brahmer J, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. New Engl J Med 2015

23. Herbst RS, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016; 387: 1540–50.

24. Fehrenbacher L, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet. 2016; 387: 1837–46.

25. Reck M, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2016;375:1823–33.

26. Antonia SJ, et al. Durvalumab after Chemoradiotherapy in Stage III Non–Small-Cell Lung Cancer. N Engl J Med 2017; 377:1919-29.

27. Gandhi L, et al. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med 2018;378:2078-92.

28. Paz-Ares L, et al. Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial. Lancet 2019;394:1929-39.

29. Brahmer JR, et al: five-yer follow-up from the CA209-003 study of nivolumab in previously treated advanced non small cell lung cancer: clinical characteristics of long-term survivors. Presented at 2017 AACR Annual meeting Washington, DC, USA, 1-5 April 2017.

30. Adashek JJ, et al. Hyperprogression and Immune Checkpoint Inhibitors: Hype or Progress? Oncologist 2020; 25(2):94-98. doi: 10.1634/theoncologist.2019-0636.

31. Champiat Set al. Hyperprogressive disease: recognizing a novel pattern to improve patient management. Nat Rev Clin Oncol. 2018;15(12):748-762

32. Lo Russo G, et al. Antibody-Fc/FcR Interaction on Macrophages as a Mechanism for Hyperprogressive Disease in Non-small Cell Lung Cancer Subsequent to PD-1/PD-L1 Blockade Clin Cancer Res 2019; 25(3):989-999.

33. Ferrara R, et al. Hyperprogressive Disease in Patients With Advanced Non-Small Cell Lung Cancer Treated With PD-1/PD-L1 Inhibitors or With Single-Agent Chemotherapy JAMA Oncol 2018;4:1543–52.

34. Champiat S, et al. Hyperprogressive Disease Is a New Pattern of Progression in Cancer Patients Treated by Anti-PD-1/PD-L1. Clin Cancer Res 2017;23:1920–8.

35. Sun, Z. et al. Radiomics study for predicting the expression of PD-L1 in non-small cell lung cancer based on CT images and clinicopathologic features. J. X-Ray Sci. Technol. J Xray Sci Technol. 2020;28(3):449-459.

36. Polverari, G. et al. 18F-FDG Pet Parameters and Radiomics Features Analysis in Advanced Nsclc Treated with Immunotherapy as Predictors of Therapy Response and Survival. Cancers. 2020;12(5):1163.

37. Miller, T. Explanation in artificial intelligence: Insights from the social sciences. Artif. Intell. 2019;267:1–38.

36. Conforti, F. et al. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta- analysis. Lancet Oncol. 2018;19:737–746.

37. Triberti, S., Durosini, I. & Pravettoni, G. A "Third Wheel" Effect in Health Decision Making Involving Artificial Entities: A Psychological Perspective. Front. Public Health 8, (2020)

38. Stacey D, Légaré F, Lewis K, Barry MJ, Bennett CL, Eden KB, et al. Decision aids for people facing health treatment or screening decisions. Cochrane Database Syst Rev. 2017;4:CD001431

39. Søndergaard, S. R., Madsen, P. H., Hilberg, O., Jensen, K. M., Olling, K., & Steffensen, K. D. (2019). A prospective cohort study of shared decision making in lung cancer diagnostics: impact of using a patient decision aid. Patient Education and Counseling, 102(11), 1961-1968.

40. Hoffman, A. S., Hempstead, A. P., Housten, A. J., Richards, V. F., Lowenstein, L. M., Leal, V. B., & Volk, R. J. (2018). Using a patient decision aid video to assess current and former smokers' values about the harms and benefits of lung cancer screening with low-dose computed tomography. MDM policy & practice, 3(1), 2381468318769886.

41. Shay LA, Lafata JE. Where is the evidence? A systematic review of shared decision making and patient outcomes. Med Decis Mak. 2014;35:114–31.