CLINICAL STUDY PROTOCOL

Effects of genomic profiles on thromboembolic risk in patients with locally advanced or metastatic non-small-cell lung cancer

Protocol number (PRIN): Prot. 20223ZYAJL

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Synopsis

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Full title	**Effects of genomic profiles on thromboembolic risk in patients with locally advanced or metastatic non-small-cell lung cancer**
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Co-Investigators	Andrea Ardizzoni- University of Bologna Francesco Grossi- University of Varese
Study Design	Multicenter, prospective observational study
Number of Centers	10-15 Oncologic Centers, in Italy
Purpose and rationale	The purpose of the study is to assess the thromboembolic potential in patients with oncogene-addicted and wild type NSCLC. Recent evidence has suggested that certain gene mutations and molecular aberrations expressed by tumor cells may influence the thrombotic risk in NSCLC patients.
Primary objective	The primary aim of this project is to evaluate the association between oncogene mutations and levels of plasma parameters of activated coagulation cascade as the plasma levels of TF, thrombin generation, IL 6, vWF, ADAMTS-13 activity PAI-1 and soluble P-selectin in both, oncogene-addicted and wild type NSCLC patients.
Secondary Objectives	-to assess the association between levels of plasma TF, thrombin generation, IL 6, vWF, ADAMTS-13 activity PAI-1 and soluble P-selectin and cancer stage and oncogene profile in NSCLC patients;

	- to assess the association between levels of plasma TF, thrombin generation, IL 6, vWF, ADAMTS-13 activity PAI-1 and soluble P-selectin and VTE in NSCLC patients; - to assess the association between oncogene mutations and VTE in NSCLC patients; - to assess the value of combined strategies including clinical patients' features, levels of plasma TF, thrombin generation, IL 6, vWF, ADAMTS-13 activity PAI-1 and soluble P-selectin and oncogene mutations to assess the risk of VTE in patients with NSCLC.
Population	A total of 500 NSCLC patients with diagnosis (cytologically or histologically confirmed) of locally advanced or metastatic disease will be enrolled in the study with a ratio 1:1 for oncogene addicted or wild type group. The oncogene-addicted group (Group A): patients with at least one oncogene mutation (ie. patients expressing EGFR mutations, KRAS mutation, ALK or ROS1 rearrangements); the wild type group (Group B): patients without oncogene mutations, categorized in 2 subgroups according to expression of PD1/PD-L1 mutation or not.
Inclusion criteria	• Patients aged 18 years or older, • Cytological or histological confirmation of NSCLC, • Locally advanced or metastatic disease (Stage III-IV), • Patients starting a new anticancer treatment for locally advanced/metastatic disease (first or further line of treatment), • Testing for oncogenic (EGFR, KRAS, ALK, ROS1 and PD-1/PD-L1) profile performed, • Written informed consent
Exclusion criteria	• Patients received surgery or radiotherapy for lung cancer within the past 3 months before recruitment or chemotherapy within the past 1 months before recruitment, • Patients with a history of VTE after cancer diagnosis or evidence of VTE events at enrollment • Continuative use of anticoagulant drugs for any indication (atrial fibrillation or previous VTE) • ECOG performance profile 3 or 4 • Life expectancy of less than 3 months
Blood sampling	Levels of plasma TF, thrombin generation, IL6, vWF, ADAMTS-13 activity PAI-1 and soluble P-selectin in patients with NSCLC before the starting of anticancer therapy, at 3 months and at 6 months or in case of clinical suspicion of symptomatic VTE event or documented incidental proximal deep vein thrombosis or pulmonary embolism will be collected. Each collected blood sample will be collected and stocked at each participating center and subsequently all collected blood samples will be sent for the laboratory analysis..
Primary outcome	Levels of plasma TF, thrombin generation, IL6, vWF, ADAMTS-13 activity, PAI-1and soluble P-selectin in patients with NSCLC before the starting of the new line of anticancer therapy and after 3 and 6 months of anticancer treatment, across different patterns and association of oncogene mutations.

Secondary outcomes	The further endpoints of the study will be to evaluate: -Levels of plasma TF, thrombin generation, IL6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin across different cancer stages and oncogene profile; -The potential correlation between VTE events with Levels of plasma TF, thrombin generation, IL6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin; -VTE risk across different patterns of oncogene mutations in NSCLC patients; -Role of combined strategies including clinical patients' features, Levels of plasma TF, thrombin generation, IL6, vWF, ADAMTS-13 activity, PAI-1and soluble P-selectin and oncogene mutations to assess the risk of VTE in patients with NSCLC; - Role of anticancer treatments in modifying the rate of clinically overt VTE events in patients with oncogene addicted or wild type pattern of NSCLC, -Analysis of survival based on oncogenic profile and Levels of plasma TF, thrombin generation, IL6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin.
Criteria for diagnosis of venous thromboembolism	During follow up visits, occurrence, and clinical features of objectively confirmed VTE events will be recorded such as presence of symptoms and sign of proximal deep vein thrombosis (DVT) or pulmonary embolism (PE), location of VTE. The time from the diagnosis of lung cancer and the occurrence of VTE will be also recorded. VTE event will be defined as objectively confirmed VTE episode, which include proximal deep-vein thrombosis of the lower limbs (symptomatic or incidental), symptomatic deep-vein thrombosis of the upper limbs, and pulmonary embolism (symptomatic, incidental, or fatal) occurring during the 6 months follow up period. The criteria for proximal DVT diagnosis will be the presence of a new filling defect in the iliac, femoral or popliteal veins, in the vena cava veins or in the axillary or subclavian veins. The criteria for PE diagnosis will be the presence of a new filling defect in the pulmonary arteries that will be classified as central, lobar, segmental, or sub-segmental, according to the location of emboli. Incidental DVT or PE will be thromboembolic events detected on imaging tests performed for reasons other than clinical suspicion of VTE (i.e. imaging performed for cancer re-staging). Incidental PE will be considered with a filling defect involving a segmental or more proximal pulmonary artery. An independent adjudication committee of vascular and radiological experts, with the scope to confirm or rule out diagnosis and classify cause of the deaths as VTE related or not VTE related.
Duration of enrollment	24 months
Follow up	Patients will be followed up prospectively for 6 months or until death, VTE event, loss to follow-up or voluntary consent withdrawal. Visits at enrollment and 3 and 6 months will be scheduled. Additional visits will be provided in case of clinical suspicion of symptomatic VTE event or in case of documented incidental proximal lower limb deep vein thrombosis or pulmonary embolism.

Data analysis	Characteristics of patients were described by mean, SD, median, first and third quartiles for continuous variables and by frequencies and percentages for categorical variables. Differences in patient characteristics between the two groups will be analyzed with descriptive statistics. The study outcomes will be reported according to pre-categorized group. The association between specific oncogene mutations and the occurrence of VTE events will be also analyzed. A competing risk analysis will be performed to determine the cumulative incidence of VTE with death considered a competing event. Cumulative incidences will be presented as proportion with 95% confidence interval. Gray's test will be used to identify statistically significant differences between different groups. Overall survival rate will be analyzed by the Kaplan–Meier survival curve, and the differences among groups were assessed by the log-rank test. Time–to-outcome event will be analyzed using a Cox proportional hazards model that included predefined group. An event-free survival analysis will be also performed. Event-free survival will be defined as the absence of VTE and death. All statistical tests will be two-sided and P value <0.05 was considered statistically significant.
Expected results	Our study will evaluate the effects of EGFR, KRAS mutations and ALK/ROS 1 and PD-1/PD-L1 rearrangements on the expression of TF and thrombin generation or the interaction between inflammation and endothelial or platelet and cancer cells, in patients with NSCLC. The study will also evaluate the potential correlation between VTE events and the expression of oncogene mutations in patients with NSCLC. The results of this study could generate the hypothesis of including the genetic profile as variable for a risk-stratification tools and decision-making algorithms in NSCLC patients.

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1. Introduction

Patients with cancer are at 4- to 7-fold higher risk for venous thromboembolism (VTE) than patients without cancer. The risk for cancer-associated VTE depends on cancer type and is generally higher in patients with advanced disease. The occurrence of VTE is associated with increased morbidity and mortality in the short- and long-term course. According to global cancer statistics 2020, lung cancer is the second most prevalent sex-unrelated cancer, with an estimated 2.3 million new cases (11.4%). Pharmacological anticancer therapies significantly increase the risk of VTE. Patients with VTE have an approximately 50% higher risk of mortality than those without VTE. Patients with Non-Small Cell Lung Cancer (NSCLC) present a risk of VTE about two-fold higher in comparison with those affected by other lung cancers. The assessment of genomic profile in patients with new diagnosis of NSCLC can indicate specific treatments, which is associated with high response rates and prolonged progression-free survival. Recent evidence has suggested that certain gene mutations and molecular aberrations may impact thrombotic development in various tumor types. Epidermal growth factor (EGFR) or kirsten rat sarcoma (KRAS) mutations and anaplastic lymphoma kinase (ALK) and C-ros oncogene 1 receptor tyrosine kinase (ROS1) gene rearrangements are the most common oncogene mutations in oncogene-addicted NSCLC. These mutations are considered mutually exclusive in most patients. The programmed cell death 1-ligand 1 (PD-L1) protein expression is usually examined to target the use of immune checkpoint inhibitors in patients with Wild Type NSCLC.

The specific contribution of the genomic profile to thrombotic risk in patients with locally advanced or metastatic NSCLC is still to be completely defined. The KRAS and EGFR mutations are expressed in 20-30% and 15-17% of patients, respectively whereas ALK or ROS 1 rearrangement occurs in 5-7% of patients with NSCLC. An about four-fold increase in risk of venous thrombotic events have been reported in patients with the ALK rearrangement in comparison to those without. Compared to patients with ROS1 and ALK rearrangements, patients with EGFR and KRAS mutations seemed to have a lower risk of thrombotic events. Patients with Wild Type NSCLC expressing PD-L1, especially with a percentage of expression above 50%, are responsive to immunomodulating therapies. A recent study reports a PE rate of 24% in patients with wild Type NSCLC expressing PD-L1. The relative risk of PE was 1.7 (95%CI 1.1-2.2) in patients with positive PD-L1 in comparison with patients without PD-L1 expression. Mechanisms of hypercoagulable state in NSCLC. Hypercoagulable state and VTE events are major contributors to cancer-associated mortality and morbidity. The identification of predictive biomarkers may be of help to identify patients who may benefit from prophylactic anticoagulant treatment, not only in terms of preventing thrombotic events but also to improve survival.

The pathophysiologic pathways that can contribute to the hypercoagulable state in cancer patients are represented by the activation of coagulation cascade, the inflammatory response to cancer cell spreading, the activation of endothelial cells and the activation of platelet aggregation.

2. Aim of the study

The main aim of this project is to assess the thromboembolic potential in patients with both oncogene-addicted and wild type NSCLC in terms of both biochemical markers and clinical events.

Biochemical thromboembolic potential will be measured by the assessment of levels of plasma TF, TG, IL 6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin. Biochemical parameters will be measured at patient enrollment and after 3 months or in case of clinically overt VTE events. Clinical thromboembolic and bleeding risk will be measured by the assessment of newly diagnosed VTE or major bleeding during study period.

The primary aim of this project is to evaluate the association between oncogene mutations and levels of plasma parameters of activated coagulation cascade as the plasma levels of TF, thrombin generation, IL 6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin in both, oncogene-addicted and wild type NSCLC patients.

The secondary objectives of the project are:
- to assess the association between levels of plasma TF, TG, IL 6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin in NSCLC patients and specific oncogene profile;
- to assess the association between levels of plasma TF, TG, IL 6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin in NSCLC patients and cancer stage;
- to assess the association between levels of plasma TF, TG, IL 6, vWF, ADAMTS-13 activity and the platelet aggregation tests and VTE, either clinically overt or incidentally detected;
- to assess the association between genomic profile and VTE, either clinically overt or incidentally detected;
- to assess the value of combined strategies including clinical patients' features, levels of plasma TF, TG, IL 6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin and genomic profile to assess the risk of VTE in patients with NSCLC.
- To assess the role of anticancer treatments in modifying the levels of plasma TF, TG, IL 6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin in patients with oncogene-addicted or wild type NSCLC.
- To assess the role of anticancer treatments in modifying the rate of VTE, either clinically overt or incidentally detected, in patients with oncogene-addicted or wild type NSCLC.

-Analysis of survival based on oncogenic profile and Levels of plasma TF, thrombin generation, IL6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin.

3. Experimental design

3.1 Design of the study

The project is a multicenter, prospective study, including consecutive patients with locally advanced or metastatic NSCLC (stage III or IV).

The project will be run in collaboration among centers with specific expertise in clinical research concerning the prevention and treatment of VTE in patients with cancer, a highly specialized coagulation laboratory for hemostatic assessments in patients with cancer, an Italian framework of oncologists dedicated to the care of patients with lung cancer, with specific expertise in clinical research.

3.2 Study population

Consecutive patients with diagnosis (cytologically or histologically confirmed) of locally advanced or metastatic NSCLC will be enrolled in the study. For the purpose of this study, after testing genomic profile, patients will be stratified as having oncogene-addicted NSCLC (Group A) (if cancer cells are expressing at least one mutation) or wild type NSCLC (Group B) (if cancer cells are not expressing mutations).
Patients with oncogene-addicted NSCLC will be categorized in 4 groups according to type of genetic mutation:
Group 1: patients with NSCLC expressing EGFR mutation
Group 2: patients with NSCLC expressing KRAS mutation
Group 3: patients with NSCLC expressing ALK or ROS1 rearrangements
Group 4: patients with NSCLC expressing mutations other than EGFR or KRAS mutations or ALK and ROS1 rearrangements (ie. BRAF, MET, RET, HER2).

Patients with wild type NSCLC, will be categorized in 2 sub-group according to expression of PD-1/PD-L mutation or not.

3.3 Inclusion criteria

Consecutive patients with:

- Patients aged 18 years or older
- Cytological or histological confirmation of NSCLC (advanced/metastatic disease)
- Locally advanced or metastatic disease
- Patients starting a new anticancer treatment for locally advanced/metastatic disease (first or further line of treatment)
- Testing for genomic status performed
- Written informed consent

3.4 Exclusion criteria

Patients are ineligible for the study in case of:

- Patients received surgery or radiotherapy for lung cancer within the past 3 months before recruitment or chemotherapy within the past 1 month before recruitment
- Patients with a history of VTE after cancer diagnosis or evidence of VTE at enrollment
- Continuative use of anticoagulant drugs for any indication (atrial fibrillation or previous VTE)
- ECOG performance status 3 or 4
- Life expectancy of less than 3 months

4. Study outcomes

4.1 Primary outcomes

Levels of plasma TF, TG, IL6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin in patients with NSCLC before the starting of new line of anticancer therapy and after 3 and 6 months of anticancer treatment, across different patterns and association of oncogene mutations.

4.2 Secondary outcomes

The following outcomes of the study will be to evaluate:
- levels of plasma TF, TG, IL6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin across cancer stages and oncogene profiles;
- the correlation between VTE events with levels of plasma TF, TG, IL6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin;
- VTE risk across different patterns of oncogene mutations in NSCLC patients;
- role of combined strategies including clinical patients' features, Levels of plasma TF, TG, IL6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin and oncogene mutations to assess the risk of VTE in patients with NSCLC;
- role of anticancer treatments in modifying the rate of VTE events, either clinically overt or incidentally detected, in patients with oncogene-addicted or wild type pattern of NSCLC.
- analysis of survival based on oncogene status and Levels of plasma TF, TG, IL6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin

5. Laboratory measurements

Plasma samples for the assessment of thromboembolic potential will be locally stored and centrally collected for central assessment in highly specialized coagulation laboratories.

Assessment of TG, IL-6, VWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin will be performed according to validated methodology. Values of these markers will be analyzed as continuous values and according to accepted cut-offs.

Specifically, the blood will be taken using a 21-gauge needle in trisodium citrate (3.2%, 0.109M, 1/10 v/v) tubes. Three tubes will be sufficient to assess all the plasma parameters.

Platelet poor plasma (PPP) will be obtained after centrifuging blood at 2000g for 20 at 4°C. Plasma will be immediately centrifuged at-20°C until they will be centralized and assayed. Plasma parameters of activated coagulation cascade, i.e. the plasma levels of TF, IL 6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin will be assayed on PPP by the ELISA method using commercially available kits.

Thrombin generation assay will be carried out on PPP using calibrated automated thrombin generation method (CAT). All materials needed for the test including the calibrator (640nM human thrombin), PPP-Reagent LOW (final concentration 1.0pM TF and a mixture of phospholipids at 84 microM), the fluorogenic substrate and fluorogenic buffer (FluCa-kit) will be supplied by Thrombinoscope (Stago, Italy). The CAT method will be carried out according to the instructions of the manufacturer. Analyses will be performed in triplicates 96-well round bottom microplates. The samples will be first pre-incubated for 10 minutes in an incubator and analysis performed at 37°C in a Microplate Fluorescence Reader equipped with 390/460 filter where the fluorescence intensity is monitored for 90 minutes. The fluorescence intensity (measured in relative fluorescence units (RFU) is proportional to the amount thrombin generated in the assay. Results will be delivered in a

thrombogram where the parameters lag time, endogenous thrombin potential (ETP), peak, and time to peak (ttpeak/second) will be presented.

An immunostaining score system will be performed to evaluate the thromboembolic potential associated with TF plasma level. Anti-TF-positive cell percentage was scored as 0, negative, <5%; 1, sporadic, 5–25%; 2, focal, 25–50%; and 3, diffuse, >50%. Anti-TF-positive staining intensity was scored as 0, negative; 1, weak; 2, moderate; and 3, strong. Both positive cell percentage and cell staining intensity will be determined in a double-blinded manner. The total score will be then defined as: staining index = positive cell percentage score × staining intensity score. A staining index ≤4 will be considered as normal expression, and a staining index >4 will be considered as high expression.

As far as the oncogene mutations is concerned, the oncogene assessment is currently required for scheduling the treatment of NSCLC. To ensure the patient timely initiation of proper treatment, these assessments will be locally performed according to validated and established methodology. EGFR, ROS1 and KRAS mutation will be locally tested by validated and internationally accepted methods with amplification refractory mutation system polymerase chain reaction; similarly, ALK rearrangement will be diagnosed by validated and internationally accepted methods as fluorescence in situ hybridization (FISH) or other. The expression of PD-L1 will be assessed by an immunohistochemical testing, according to validated methodology.

6. Study procedures

6.1 Subject information and informed consent

Before being admitted to the study, the subject must consent to participate after being fully informed about the nature of the observational study. A copy of the signed informed consent document must be given to the subject. The original signed consent document will be retained by the investigator.

6.2 Visits schedule and follow-up

All included patients will be subjected to blood sampling for levels of plasma TF, TG, IL6, vWF, ADAMTS-13 activity, PAI-1 and soluble P-selectin at predefined intervals: at the enrollment, before starting a new line of anticancer treatment (T0), and at 3 months (T1), and at 6 months (T2). Each blood sample will be collected and stocked at each participating center and subsequently all collected blood samples will be sent for the laboratory analysis.
Follow up visits at enrollment, 3, 6 months will be scheduled. Additional visits will be provided in case of clinical suspicion of symptomatic VTE event or in case of documented incidental proximal lower limb deep vein thrombosis or pulmonary embolism.

Data will be prospectively collected by electronical database with coded anonymous identification numbers from the patients' electronic medical records and from the patients themselves. The following variables will be collected for each patient: age, gender, smoking history (never vs. former/current), Eastern Cooperative Oncology Group (ECOG) performance status (PS), tumor histology (adenocarcinoma vs. non-adenocarcinoma), tumor stage (localized stage vs. advanced

stage), anticancer treatment (TKIs vs. other treatment without TKIs vs. immunotherapy vs untreated) and VTE history.

According to the International Association for the Study of Lung Cancer (version 7) TNM staging of lung cancer, all patients will be enrolled if they have an advanced stage of cancer disease (stage III B or IV).

All patients will be instructed about the symptoms and signs of VTE at recruitment and will be required to report to the doctors should these symptoms occur. VTE will be not actively screened. Once VTE symptoms appeared, objective imaging methods will be used to either confirm or rule out the diagnosis of VTE. DVT will be objectively confirmed by venous compressive ultrasonography of the lower or upper limbs or computed tomography venous angiogram. PE will be confirmed by computed tomography pulmonary angiography or radionuclide pulmonary ventilation/perfusion imaging. All VTE events will be reviewed and adjudicated by a centrally adjudication committee.

6.3 Observations and measurements

During follow up visits, occurrence and clinical features of VTE events will be recorded such as presence of symptoms and sign of proximal deep vein thrombosis (DVT) or pulmonary embolism (PE), location of VTE. The time from the diagnosis of lung cancer and the occurrence of VTE will be also recorded.
VTE event will be defined as objectively confirmed VTE episode, which include proximal deep-vein thrombosis of the lower limbs (symptomatic or incidental), symptomatic deep-vein thrombosis of the upper limbs, and pulmonary embolism (symptomatic, incidental, or fatal) occurring during the 6 month follow up period.
The criteria for proximal DVT diagnosis will be the presence of a filling defect in the iliac, femoral or popliteal veins, in the vena cava veins or in the axillary or subclavian veins.
The criteria for PE diagnosis will be the presence of a filling defect in the pulmonary arteries that will be classified as central, lobar, segmental, or sub-segmental, according to the location of emboli.
Incidental DVT or PE will be thromboembolic events detected on imaging tests performed for reasons other than clinical suspicion of VTE (i.e. imaging performed for cancer re-staging). Incidental PE will be considered with a filling defect involving a segmental or more proximal pulmonary artery.
In this study an independent adjudication committee of vascular and radiological experts, unaware of adjudication of VTE event at enrolling center, will review all VTE events with the scope to confirmed or ruled out diagnosis and classify cause of the deaths as VTE related or not VTE related.

7. Data quality control

The investigator will be provided with an access to an electronic investigator site file (eISF) at the start of the trial. The investigator will archive all trial data and relevant correspondence in the ISF. The ISF, all source data and all documents will be kept filed according to the requirements of the ICH-GCP guidelines not only during the study period but also after its termination. It is responsibility of the investigator to ensure that the subject-identification sheets and the originally signed informed consent forms are properly stored for at least 15 years beyond the end of the clinical trial.

Monitoring procedures will be followed to comply with Good Clinical Practice (GCP) guidelines. Three different types of monitoring activities will be performed by the appointed CRO: on site, remote and central monitoring.
Each center will be visited at regular intervals by a CRO Site Monitor to ensure compliance with the study protocol, GCP and legal aspects. This will include on-site checking of the electronic case report forms (eCRF) for completeness and clarity, cross- checking with source documents, and clarification of administrative matters.

The Site Monitor will ensure that the investigator will maintain a list of sub-investigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties (personnel log). During the on-site monitoring visits, the Site Monitor will review the entries into the eCRF for completeness and correctness and verify the entries on the basis of the source documents. The presence of correct informed consents will be checked for every subject.
The investigator must allow the Site Monitor to look at all relevant documents and must provide support at all times to the Site Monitor. Moreover, remote monitoring by means of phone calls between investigators and Site Monitors will be done.
The risk for the occurrence of quality and safety issues will be regularly and centrally monitored through the use of a “Risk Based Monitoring & Management” (RBM) platform. For each of following six risk categories: 1) site management quality, 2) data quality, 3) data timeliness, 4) source documents verification, 5) milestone delay, 6) subject safety, study specific risk indicators and related scores will be defined and closely and centrally monitored during the entire study period. This will allow prompt and targeted remedial actions.

8. Analysis

Continuous variables will be summarized using mean, SD, median, first and third quartiles, minimum, maximum, number of non-missing observations and number of missing observations. The 95% CIs will also be provided for selected categorical and continuous variables.
Categorical variables will be summarized as number of observations and percentages (%) of the observations in each category. Percentages do not include the missing category and are calculated over the number of subjects with available (non-missing) data.
The study outcomes will be reported according to pre-categorized group. Comparisons between groups will be analyzed with descriptive statistics. The two-tailed unpaired Student’s t-test will be used for comparisons of continuous variables and chi-square for categorical variables.
Data will be analyzed with the D’Agostino-Pearson normality test. Data not normally distributed will be analyzed with the Mann Whitney test.
Multiple comparisons will be performed with One-way ANOVA, followed by Dunn's or by Holm-Sidak's post-test where appropriate. Correlations between parameters will be assessed with the Spearman’s or Pearson's test, where appropriate. A p value <0.05 will be considered statistically

significant.
Raw incidence rates of study outcome events will be calculated at each time point in patients with oncogene addicted vs wild type NSCLC. The association between specific oncogene mutations and the occurrence of VTE events will be also analyzed.
Additional analyses and comparisons between patients with oncogene addicted vs wild type NSCLC will be performed using time to event analyses. After verification of the proportionality of the hazards, survival function and empirical cumulative hazards functions will be estimated via Kaplan–Meier estimator for various groups of patients; the differences between survival functions will be tested using the Log-rank statistic (or Mantel–Haenszel test). In this analysis, patients will be censored at the time of an outcome event, death, or if they were lost to follow-up. The relationship between the survival function and the set of explanatory variables will be explored with Cox proportional hazard models. Furthermore, we will estimate additional models to investigate for any possible effect of predictor variables. A competing risk analysis will be performed to determine the relationship between the survival function and the set of explanatory variables with death considered a competing event. Cumulative incidences will be presented as proportion with 95% confidence interval. Gray's test will be used to identify statistically significant differences between different groups. Overall survival rates will be analyzed by the Kaplan–Meier survival curve, and the differences among groups will be assessed by the log-rank test. These results will be reported as a hazard ratio with a 95% confidence interval. A 2-sided P value <0.05 will be considered significant.
An event-free survival analysis will be also performed. Event-free survival will be defined as the absence of VTE and death.
The analysis will be conducted using SPSS Win Package 25.0 and software R, version 3.0.3 (copyright (C) 2013 The R Foundation for Statistical Computing)

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