Study Protocol

Study Title: Phase I clinical trial evaluating the safety and feasibility of bone marrow aspiration from ribs during thoracic surgery

Study Acronym: COBM
Phase of Development: Phase 1 clinical trial
Protocol Number: 1
Protocol Version and Date: V2.0 – 26th of October 2023
EudraCT Registry Number: N/A
ClinicTrials.gov Registry Number: NCT05251805
Investigational product or Medical Device: N/A
Sponsor: UZ Brussel
Coordinating/Principal Investigator: Domien Vanhonacker MD

PROTOCOL SIGNATURE PAGE

Protocol Version and date: 2.0 - 26th of October 2023

Protocol Title: Phase I clinical trial evaluating the safety and feasibility of bone marrow aspiration from ribs during thoracic surgery

Sponsor: UZ Brussel

Principal Investigator: Domien Vanhonacker MD

I agree:

- to assume responsibility for the proper conduct of this study
- to conduct the study in compliance with this protocol and any future amendments
- not to implement any deviations from or changes to the protocol without prior review and written approval from the Ethics Committee, except where necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the study (where permitted by all applicable regulatory requirements)
- that I am thoroughly familiar with the appropriate use of the investigational drug, as described in this protocol
- to ensure that all persons assisting me with the study are adequately informed about the investigational drug and their study-related duties and functions as described in the protocol
- that I am aware of and will comply with the current good clinical practice (GCP) guidelines and ethical principles outlined in the Declaration of Helsinki
- to conduct the study in accordance with all applicable laws and regulations

Printed name Signature Date

Domien Vanhonacker

Table of Contents

1 Trial Registration/Protocol Summary

Information	
EudraCT number: Date of registration:	N/A
ClinicalTrials.gov:	**NCT05251805**
Official Title:	**Phase I clinical trial evaluating the safety and feasibility of bone marrow aspiration from ribs during thoracic surgery**
Study Phase/Type:	Phase I clinical trial
Condition:	Lung cancer
Objectives:	Evaluate the safety and feasibility of bone marrow aspiration during thoracic surgery
Investigational Product or Medical Device:	None
Interventions:	1. Pre-operative blood sample collection 2. Costal bone marrow aspiration 3. Lung tumor and adjacent tissue sampling
Endpoints:	1. Safety: the occurrence of complications 2. Feasibility: Obtain a minimum of $2x10^6$ Lin^- $CD34^+$ Hematopoietic Stem and Progenitor Cells (HSPCs) to allow single cell RNA sequencing (10^6), bone marrow cultivation *in vitro* ($5x10^5$ cells per culture) and transplantation in immunodeficient mice (10^5 cells per injection).
Study population:	Lung cancer patients undergoing thoracic surgery
Number of patients:	10
Overview of study design:	Phase I feasibility trial
Statistical Considerations:	None
Sponsor:	UZ Brussel
Inclusion Criteria:	Confirmed or suspected lung cancer patients undergoing thoracic surgery
Exclusion Criteria:	1. ASA ≥ 3 2. Bleeding disorders
Target Date of first enrolment:	01/10/2022
Target sample size:	10

2 Protocol Version History

Version No.	Release Date	Summary of Changes
V1	**20 April 2022**	**-**
V1.1	28 June 2022	Upon request of the EC: made some minor changes concerning the reporting of SAE's and added an explanation on the multidisciplinary aspects of the research project.

3 Sponsor/Coordinating Investigator Information

Sponsor: UZ Brussel
Principal Investigator: Domien Vanhonacker MD
Co-investigators: Prof. Dr. Cleo Govyaerts, Prof. Dr. Dirk Smets and Yanina Jansen MD, PhD
Additional co-researchers: PhD students Thomas Benoot and Evelyn Calderon Espinosa
Statistician: N/A
Laboratory: Laboratory for Molecular and Cellular Therapy (LMCT), VUB
Pharmacy: N/A
Study Coordinator: N/A
Study sites: UZ Brussel and LMCT, campus Jette, VUB

4 List of Abbreviations

ICI: immune checkpoint inhibitors
NSCL: non-small cell lung cancer
EU: European Union
LuCE: Lung Cancer Europe
PD-L1: programmed death-ligand 1
TME: tumor micro-environment
BMN: bone marrow niche
HSPCs: hematopoietic stem and progenitor cells
LMCT: Laboratory for Molecular and Cellular Therapy
PBS: phosphate buffered saline

5 Introduction

5.1 Overview of Disease Pathogenesis

Non-small cell lung cancer (NSCLC) remains the leading cause of cancer-related deaths worldwide, with a staggering 2.21 million new cases in 2020 alone[1]. For patients with stage I and stage II disease, surgical resection is the primary modality of treatment. However, only 20-30% of lung cancer patients are suitable for curative treatment (surgery versus stereotactic radiotherapy) at the time of diagnosis. Moreover, following surgery, the risk of recurrence during the first 4-year ranges from 6% to 10% per person per year but decreases thereafter to 2%[2]. Current progress in the field of **anti-tumor immunotherapy** is based on the capacity of effector T cells to recognize and kill cancer cells specifically. As progressing tumors often master skills to restrict T-cell mediated killing via upregulation of immune checkpoint molecules, immune checkpoint inhibitors (ICIs) were designed to ameliorate antitumor immunity. Today, the latter represents a first-line treatment option for advanced NSCLC patients with curative potential[3]. Unfortunately, up to **75% of patients currently do not respond** to ICIs, suggesting that lung tumor cells master more skills to mislead effector T cells than upregulating immune checkpoint molecules alone, highlighting the ongoing medical need to develop additional/alternative (immune)therapeutics that enhance the overall survival rate of ICI-treated NSCLC patients.

5.2 Epidemiology

Lung cancer is the fourth most diagnosed cancer, affecting more than 312.000 people every year in the European Union (EU[4]). Cigarette smoking is the major cause of lung cancer, with around 80-90% of all lung cancer attributable to tobacco. In men, lung cancer is the second most diagnosed cancer. In women, lung cancer is the third most frequent cancer. However, this gender gap is closing. Lung cancer survival is poor, although new treatments lead to a slightly better prognosis. The overall 5-year survival is around 13% since diagnosis.

5.3 Current Treatments

1. Stage I disease: surgery or stereotactic radiotherapy in patient unfit for surgery
2. Stage II disease: surgery followed by adjuvant chemotherapy
3. Stage III disease: chemoradiotherapy
4. Stage IV disease: chemotherapy, targeted therapy, and immunotherapy. Immunotherapy is used as a first-line treatment in NSCLC patients without driver mutations whose tumors express PD-L1 (programmed death ligand-1) at levels of 50% or greater.

5.4 Study Rationale and Purpose

In general, antitumor immunotherapy tends to spark the patients' own tumor-specific effector T cells to multiply, recognize and kill tumor cells. Yet, a typical characteristic of immunotherapy-resistant (lung) tumors' deceptive gambit, is their molding of an effector T-cell hostile **tumor microenvironment** (**TME**). The latter comprises cellular, molecular, vascular, and physical **barriers** for effector T cells which hamper effective tumor cell killing. With regard to the cellular barriers, we and others have appointed a leading role to the immunosuppressive myeloid cells[5,6]. These myeloid cells represent a heterogeneous group of subsets with embryonic origin (macrophages) and/or hematopoietic origin (dendritic cells, macrophages, granulocytes, mast cells, and platelets), suggesting that the **bone marrow niche** (**BMN**) is involved in the creation of an immunosuppressive T-cell hostile TME. Moreover, this niche has been shown to aid in pre-metastatic niche formation while it has also been proposed as a supportive **hideout** for disseminated cancer cells, as reported by the detection of $Epcam^{+}$ $CD45^{-}$ disseminated cancer cells in 43,5% of sternal aspirated BM samples from 62 NSCLC patients[7]. Nevertheless, as BM aspiration is a painful process and therefore, unlike blood drawing not routinely performed, it remains a tremendously **understudied niche in solid cancers.**

Next to the fundamental information that BMN investigation would deliver to ameliorate our understanding of NSCLC progression and resistance to (immuno)therapy, bone marrow represents an extremely valuable biomaterial for preclinical onco-immunology research. More specifically, the Lin- CD34+ hematopoietic stem (and progenitor) cells or **HSPCs** have been used to **generate humanized mice** by transplanting total body irradiated mice with human bone marrow[8–10]. Antitumor immunotherapy (and research thereof) relies on histocompatible cell-to-cell interactions between the immune system, tumor cells and their TME. Hence, humanized patient-derived xenograft models in which the immune system and tumor are derived from the same patient, a.k.a. 'murinized patients', represent the ultimate model to study human tumor immunology and evaluate innovative immunotherapies.

To study the BMN and autologous tumor-bone marrow interactions, we want to collect **blood, bone marrow and lung tissue of 10 lung cancer patients.**

1) This pilot trial is designed to **evaluate the feasibility and safety of obtaining bone marrow upon costal aspiration** during surgery. The rationale for using rib bone marrow aspiration is the easily accessible localization due to trocar placement (in minimally invasive surgery) or during thoracotomy. Previous trials demonstrated the feasibility of micrometastasis detection in rib marrow aspiration[11–13]. This procedure was safe without any documented adverse events for patients. We hypothesize that we can use this technique to obtain sufficient bone marrow-derived HSPCs (which represent only 0,1% of the total bone marrow fraction) for *ex vivo* single-cell evaluation[14], optimization of a 3D BMN platform[15] and preclinical ex/*in vivo* assessment in patient-derived organoids[16,17] and murine xenograft models[18]. The use of rib marrow eliminates the need for extra incision as incisions used for trocar placement or thoracotomy will be used. We

hypothesize that the patient will not experience extra discomfort from this technique since every patient undergoing thoracic surgery is treated according to the ERAS protocols with a major emphasis on pain control.

2) If the feasibility and safety of bone marrow aspiration are confirmed by this pilot trial, we plan to conduct a large-scale study in which we will collect and bank blood, bone marrow and tumor per NSCLC patient. With this unique autologous biological material collection, we aspire to conduct innovative research into the current resistance mechanisms of NSCLC to immunotherapy via at least two ongoing research projects:

a) **X-talk project** with the hypothesis: 'The bone marrow niche of NSCLC patients withholds invaluable information about tumor-installed resistance mechanisms to immunotherapy[15]. By deciphering this information, we aspire to serve the diagnostic and therapeutic field.'
b) **RADar project** with the aim to answer the following research questions: 1) can we efficiently deliver RADars specifically to human lung tumor cells? and 2) will RADar delivery result in tumor cells' sensitivity to and killing by patient-specific human T cells? To be able to answer these questions in a relevant way, we opt for two meticulous human(ized) immunological models namely:

- an ***in vitro*** **Predixoid platform** in which NSCLC organoids are grown as TME-on-a-chip[19,20].
- '**murinized patients'**, in which patient-derived NSCLC cells or organoids are engrafted intrathoracic into immunodeficient mice to generate orthotopic lung cancer patient xenografts[8,9]. Next, we will optimize the generation of humanized mice, transplanted with NSCLC patient-derived HSPCs and subsequent autologous and orthotopic NSCLC tumor engraftment to generate 'murinized patients.

Hence, by exploiting patient-derived material, we aspire to perform **more clinically relevant fundamental and translation research**, especially as the use of both platforms will allow us to take **inter-patient-heterogeneity** into account (unlike immortalized lung tumor cell lines).

The PI of this feasibility trial, Domien Vanhonacker (MD), is an anesthesiologist. He has written the study protocol in close cooperation with Yanina Jansen (MD, PhD) and Cleo Goyvaerts (PhD). In conjunction with Dirk Smets (MD) he will include the patients in this trial, attend to the patient during and after the surgical procedure and perform the bone marrow aspiration.

5.5 Rationale for Study Design

This trial is a feasibility trial evaluating the possibility to obtain bone marrow-derived HSPCs by bone marrow aspiration from ribs.

5.6 Rationale for Dose and Regimen/Schedule Selection

Not applicable

6 Study Schematic and Schedule of Activities

6.1 Study Schematic

1. Patient screening and informed consent
2. Collection of samples:
    a. Blood sample collection during pre-operative blood collection
    b. Bone marrow aspiration during thoracic surgery
    c. Lung tumor and adjacent tissue true cut on removed lung tissue
3. Observation of adverse events during the first 30 days following surgery
4. Processing of obtained tissue at LMCT

6.2 Primary Objective

The safety and feasibility of rib bone marrow aspiration during thoracic surgery

6.3 Secondary Objectives

None

6.4 Endpoints

1. Safety: the occurrence of complications
2. Feasibility*:* Obtain a minimum of $2x10^6$ Lin- CD34+ HSPCs to allow single cell sequencing (10^6), bone marrow cultivation ($5x10^5$ cells per culture) and transplantation in immunodeficient mice (10^5 cells per injection).

7 Investigational Plan

7.1 Overall Study Design

1) This trial is a phase I feasibility trial. It is designed to evaluate the feasibility and safety of obtaining bone marrow upon costal aspiration during surgery.
2) If feasibility and safety of rib bone marrow aspiration are confirmed in this pilot trial, a large-scale study will be started to optimize and conduct fundamental and translational research via the X-talk project and RADar project (see details under 5.4, page 9).

7.2 Study Duration for Subjects

7.2.1 Screening

All patients undergoing thoracic surgery (by minimally invasive technique or by thoracotomy) will be screened for their eligibility by the principal investigator.

7.2.2 Treatment Period

a. Blood sample collection during pre-operative blood collection
b. Bone marrow aspiration during thoracic surgery – no additional incisions will be made
c. Lung tumor collection – only tumor not used for diagnostic purpose

7.2.3 Unscheduled Visit(s)

All patients will be hospitalized the day before surgery and between 5-7 days following surgery. This is standard practice for all patients undergoing thoracic surgery in our department. No unscheduled visits will be necessary

7.2.4 Early Study Termination

The study will be closed prematurely if 2 patients experience a grade 3 adverse event directly related to the bone marrow aspiration

7.2.5 End of Study

The study will end once 10 study patients have been included.We forsee that the end will be scheduled for 31st of December 2025.

8 Selection of Subjects

8.1 Selection of Study Population

All patients undergoing thoracic surgery for a confirmed or suspected lung cancer.

8.2 Inclusion Criteria

Patient undergoing thoracic surgery for a confirmed or suspected lung cancer. All patients eligible for major surgery, as defined by a thoracic procedure, are considered eligible for participation in this trial.

8.3 Exclusion Criteria

1. Uncertainty of pre-operative diagnosis, exception is per-op frozen section analysis confirming malignity
2. ASA ≥ 3
3. Bleeding disorders
4. Contraception/Pregnancy Avoidance

The aspiration of bone marrow will not impact contraception/pregnancy avoidance.

9 Screening

9.1 Screening and Enrollment

All patients undergoing thoracic surgery will be screened pre-operative by the principal investigator. When eligible, patients will be asked for their participation and included after signing the informed consent.

10 Interventions/Treatment

10.1 Intervention

a. Blood sample collection during pre-operative blood collection
   A total of 12 ml will be collected
b. Bone marrow aspiration during thoracic surgery
   Following the surgical intervention, bone marrow aspiration will be performed through the incisions made for the thoracic procedure. The ribs lying directly above and below the trocar incision will be exposed. A bone marrow needle will be advanced into the periost until the needle tip reaches the bone marrow. After removing the stylet a 2ml syringe will be attached to the aspiration needle and 0.5ml to 2ml of bone marrow will be aspirated before removing the needle. This procedure will be repeated twice for every trocar site, for a maximum of 3 trocar sites, or until 5ml of bone marrow aspirate is obtained. This part of the trial is experimental. Literature on rib bone marrow aspiration is scares but good results are obtained. The procedures does not seem to be correlated with major AE[11,12,21].
c. Lung tumor and adjacent healthy tissue collection – only tumor not used for diagnostic purposes.
   All surgical samples will be analyzed by our anatomopathological department. Viable and fresh material, obtained via true cute biopsy on the excised tissue and not used for the diagnostic purposes, will be collected by the LMCT.

All samples (blood, bone marrow, and fresh tumor tissue), will be transferred to the LMCT for immediate processing of viable cells (within 2 hours after isolation) and subsequent cultivation and/or cryopreservation for later usage.

Randomization and Stratification

No randomization or stratification will be performed. All patients will be included in a consecutive manner.

10.2 Prohibited Medication

All anti-coagulants should be interrupted before the procedure as is standard practice for thoracic surgery.

10.3 Known Undesirable Effects of costal rib aspiration

- Pain
- Bleeding
- Infection

In a survey of members of the British Society of Haematology reported in 2003, there were 26 adverse events, including one fatality, out of an estimated total of 55,000 bone marrow biopsy procedures (0.05 percent) [22]. A follow-up survey of this group, reported in 2006 involving 20,323 procedures, resulted in the reporting of 15 adverse events (0.07 percent) [13]. Previous research evaluating the feasibility to detect disseminated tumor cells in bone marrow aspirate from the ribs couldn't identify any adverse events related to the bone marrow aspiration.

11 Study Assessments and Procedures

11.1 Study Assessments

11.1.1 Screening

All patients undergoing thoracic surgery for lung cancer will be screened by the principal investigator for inclusion in this trail.

11.1.2 Baseline

Baseline screening consists of the pre-operative assessment for all patients undergoing thoracic surgery: blood tests (including coagulation testing), CT or PET CT, as well as consulting an anesthesiologist. No additional tests are required for this trial.

11.1.3 Treatment Period

a. Blood sample collection during pre-operative blood collection
   A total of 12ml (3 EDTA tubes) will be collected
b. Bone marrow aspiration during thoracic surgery
   Following the surgical intervention, a bone marrow aspiration will be performed through the incisions made for the thoracic procedure. The ribs lying directly above and below the trocar incision will be exposed. A bone marrow needle will be advanced into the periost until the needle tip reaches the bone marrow. After removing the stylet, a 2ml syringe will be attached to the aspiration needle and 0.5ml to 2ml bone marrow will be aspirated before removing the needle. This procedure will be repeated twice for every trocar site, for a maximum of 3 trocar sites, or until 5ml of bone marrow aspirate is obtained.
c. Lung tumor collection – only tumor not used for diagnostic purposes.
   All surgical samples will be analyzed by our anatomopathological department. Viable and fresh material, obtained via true cute biopsy on the excised tissue and not used for the diagnostic purposes, will be collected by the LMCT.

11.1.4 Follow-Up

All patients are hospitalized one day prior to surgery. After surgery, patients will stay in the post-anesthesia care unit (PACU) for one night. Following surgery, the patients will stay in the hospital for 5-7 days. All patients will be seen by their surgeon at the consultation 2 weeks following surgery. We don't expect a prolonged hospital stay or additional hospital visits will be necessary for the patients partaking in the trial.

11.1.5 End of Study

Adverse events will be followed up to four weeks postoperatively.

11.1.6 Re-screening

No

11.1.7 Early Termination

The trial will be ended prematurely if grade 3 (or higher) adverse events occur in 2 patients

11.1.8 Unscheduled Visits

Not expected

11.2 Assessment Types

11.2.1 Efficacy Assessment

In accordance with a recent single-cell analysis study on patient-derived bone marrow[14], we will process blood and bone marrow specimens from patients within two hours of collection. The bone marrow mononuclear cells (BM-MNCs) will subsequently be isolated by density centrifugation using LSM Lymphocyte Separation Medium (#50494X, MPbio). In short, the bone marrow will be diluted twofold using phosphate-buffered saline (PBS), layered on top of 1 volume LSM Lymphocyte Separation Medium in a 50-ml Falcon tube, and centrifuged at 2300 rpm for 25 minutes at room temperature without brake. Next, the MNC layer will be isolated and washed with PBS after lysis of red blood cells with RBC lysing buffer. To count and purify the Lin- CD34+ HSPCs per patient using our BD FACSAria™ III Cell Sorter, BM-MNCs will be stained with monoclonal antibodies for 30 minutes on ice: anti-Lineage antibody, anti-CD34 antibody, anti-CD45 antibody, and anti-Epcam antibody to divide each bone marrow sample over HSPCs (Lin/CD45/Epcam- CD34+), immune cells (Lin/CD34/Epcam- CD45+), disseminated cancer cells (CD45/CD34- Epcam+) and the rest fraction.

The endpoint will be the determination of viable HSPC numbers per sample as well as detection of disseminated Epcam+ NSCLC cells. For future experiments we aim at a minimum of **2x10E6 Lin- CD34+ HSPCs** to allow single-cell sequencing (10^6), bone marrow cultivation ($5x10^5$ cells per culture), and transplantation in immunodeficient mice (10^5 cells per injection).

11.2.2 Safety and Tolerability Assessments

- NRS pain score: The NRS pain score will be used to evaluate postoperative pain. Bone marrow aspiration will be performed at a maximum of 3 trocar sites. Thoracic procedures are known as painful procedures mandating multimodal pain treatment. Bone marrow aspiration is not expected to add to the pain experience.
- Bleeding: in all patients undergoing a thoracic procedure a thoracic drainage system is used and all patients will stay at the recovery ward following surgery. Since the bone marrow aspiration is done under visual control we don't expect any significant postoperative bleeding.
- Infection: the bone marrow aspiration is done under sterile conditions, using the incisions made to introduce the trocars. Follow-up is provided 14 days following surgery.

11.2.3 Laboratory Evaluation

Hematology: a preoperative coagulation screening will be performed for all patients; this is the standard procedure for all patients undergoing thoracic surgery.

12 Safety Monitoring and Reporting

12.1 Adverse Events

12.1.1 Definitions and Reporting

All Adverse events will be graded according to the Clavien Dindo classification system:

Grade	
1	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions. Acceptable therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics and electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside.
2	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions, antibiotics and total parenteral nutrition are also included.
3	Requiring surgical, endoscopic or radiological intervention
3a	Intervention under regional/local anesthesia
3b	Intervention under general anesthesia
4	Life-threatening complication requiring intensive care/intensive care unit management
4a	Single organ dysfunction
4b	Multi-organ dysfunction
5	Patient demise

Clavien Dindo classification system

12.1.2 Reporting Period

All adverse events related to the bone marrow aspiration will be evaluated and classified by their treating physician. Adverse events occurring during the first postoperative month (30 days) will be registered.

Serious Adverse Events

12.1.3 Definitions

All adverse events of grade 3 or higher (according to the Clavien Dindo classification) will be reported as serious adverse events.

12.1.4 Immediate Reporting

In the occurrence of adverse events of grade 3 or higher related to the trial procedure, the ethical committee will be contacted. In the occurrence of 2 adverse events of grade 3 or higher the trial will be prematurely discontinued.

12.2 Suspected Unexpected Serious Adverse Events (SUSAR)

12.2.1 Definitions

All adverse events of grade 3 or higher (according to the Clavien Dindo classification) that might be related to a trial related procedure will be reported as a serious adverse event

12.2.2 Reporting

In the occurrence of adverse events of grade 3 or higher related to the trial procedure, the ethical committee will be contacted. In the occurrence of 2 adverse events of grade 3 or higher the trial will be prematurely discontinued and the ethical committee will be notified.

12.3 Procedures for Handling Special Situations

12.3.1 Pregnancy

Pregnancy is no contra-indication for participating in the trial, however due to the context of the underlying disease we don't expect to include any pregnant patients.

12.3.2 Overdose Management: not applicable

12.4 Annual Safety Report

A yearly safety report will be submitted to the ethical committee up to 1 year following recruitment of the final study patient

13 Data Collection and Management

13.1 Monitoring

13.1.1 Composition of data monitoring committee

Given the low number of included patients, the low risk of serious adverse events, and the single-center set-up, no external data monitoring committee is foreseen.
During patient recruitment and up to 3 months following the inclusion of the last study patient, the principal investigator and the co-investigators will review and confirm all data on a 3-monthly basis during a teleconference. All data (outcome, duration of hospital stay, complications) will be gathered on red-cap. No statistical analyses will be performed.

13.1.2 Interim analysis

After the inclusion of the first 5 patients, an interim analysis will be performed to confirm whether the size of the blood sample is adequate, how much bone marrow is extracted and whether this is adequate to do the lab analysis.

13.2 Data Collection

Redcap will be used to collect clinical data and translational data.

13.3 Database Management and Quality Control

Database management and quality control will be performed by the principal investigator and co-investigators. All study-related documents will be collected and kept in a closed location.

13.4 Statistical Considerations and Data Analysis

No statistical analysis will be done in this feasibility trail

14 Ethical Considerations

14.1 Ethical conduct of the study

14.1.1 Declaration of Helsinki

The Investigator shall be responsible for ensuring that the clinical study is performed in accordance with the protocol, the ethical principles that have their origin in the Declaration of Helsinki (as amended by the 59th WMA General Assembly, Seoul, October 2008, to be found at www.wma.net) as well as with the ICH Note for GCP (ICH, Topic E6, 1995) and applicable regulatory requirements. These documents state that the informed consent of subjects is an essential precondition for participation in the clinical study.

14.1.2 Ethics Committee

Prior to commencement of the study, the study protocol will be submitted together with its associated documents (informed consent, IB, insurance policy and insurance conditions, and all written information regarding this study to be provided to the subject or the subject's legal guardian) to the relevant IRB/EC for their favorable opinion/approval. The favorable opinion/approval of the IRB/EC will be filed in the Investigator Site File and a copy in the Study Master File by the Sponsor.
The study will only commence following provision of a written favorable opinion/approval. The date of the meeting, constitution of the committee, and voting members present at the meeting will be documented. Written evidence that clearly identifies the study, protocol version, and consent documents reviewed is required. Where possible, copies of the minutes of this meeting will be obtained.

14.2 Informed Consent

Each subject will sign an informed consent for participation in the clinical part of this study.
The written informed consent of the subject must be obtained before any study related activities are carried out. It must be signed and personally dated by the subject and by the Investigator/person designated by the Investigator to conduct the informed consent discussion.
The subject will be given sufficient time to read the information and the opportunity to ask questions and to request additional information and clarification about the study. The subject information sheet will be revised whenever important new information becomes available that may be relevant to the consent of subjects. Subjects with legal incapacity or limited legal capacity should be excluded from participation in the clinical study.
When informed consent is obtained, this will be documented in the CRF by the Investigator. The signed and dated declaration of informed consent will remain at the Investigator's 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 forms should be provided to the subject prior to participation.

14.3 Patient and Study Data Protection

A unique subject number will be assigned to a subject at inclusion, immediately after informed consent has been obtained. This number serves as the subject's identifier in the trial as well as in the clinical trial database. The subject's data collected in the trial will be stored under this number. Only the Investigator will be able to link the subject's trial data to the subject via an identification list kept at the site.

The subject's original medical data reviewed at the site during source data verification by the monitor, audits, and authority inspections, will be kept strictly confidential.
Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing subject data. Subjects will be informed accordingly and will be requested to give their consent on data handling procedures in accordance with national regulations.

14.4 Subject Identification

All patients undergoing thoracic surgery for lung cancer will be screened by the principal investigator for inclusion in this trial.

15 Finance and Insurance

For all subjects enrolled in the study, appropriate insurance coverage is provided in line with legal requirements and GCP guidance. Details can be asked for at the investigational site (certificates and conditions in the Investigator Site File). Insurance coverage shall be provided by a Global Insurance Provider. Since no additional visits are mandatory for this trial, no financial compensation is provided for the patients taking part in the trial.

16 Reporting and Dissemination

Valuable data will be disseminated via national (e.g. ORC, BACR, *etc.*) and international conferences (e.g. CIMT, SITC, IASLC, ESMO, *etc.*). Moreover, the obtained results of the trial will be summarized for publication in a peer-reviewed journal with impact factor > 5. Finally, the co-investigator Prof. Dr. Cleo Goyvaerts will disseminate valuable results in layman's' terms as she gives seminars on a monthly basis to explain the concepts of cancer and immunotherapy to a broad audience.

17 Conflict of Interest Statement

Domien Vanhonacker MD has received financial compensations for talks on advanced hemodynamic management and proctoring sessions from Edwards Lifesciences.
Yanina Jansen MD, PhD: travel, accommodation, and expenses— Bristol-Myers Squibb, MSD, Pfizer

18 Tables and Figures

Not applicable

19 References

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