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ABSTRACT

Introduction: Lung cancer (LC) is the leading cause of death from cancer. Early diagnosis of LC is of paramount importance in terms of prognosis. The health authorities of most countries do not accept screening programs based on low dose chest computed tomography (LDCT), especially in Europe, since they are flawed by a high rate of false positive results, leading to a large number of invasive diagnostic procedures. These authorities advocated further research, including companion biological tests that could enhance the effectiveness of LC screening. The present project aims to validate early diagnosis of LC by detection and characterization of circulating tumor cells (CTCs) in a peripheral blood sample taken from a prospective cohort of patients with a high-risk of lung cancer.

Methods and analysis: The AIR Project is a prospective, multicenter, double-blinded, cohort study (patients and investigators are blinded to the results of CTC search). The primary objective is to evaluate CTCs as a screening tool for LC in individuals at high-risk, i.e. smokers and ex-smokers (≥ 30 PY, quitted ≤ 15 years), aged ≥ 55 years, with chronic obstructive pulmonary disease (COPD). The volunteers will undergo yearly screening **ROUNDS** for 3 years plus a one year follow-up. Each round will include LDCT plus peripheral blood sampling for CTC detection. Assuming 5% prevalence of LC in the studied population and a 10% dropout rate, a total of at least 600 volunteers will be enrolled.

Ethics and dissemination: A consortium of 21 French university centers will run this study. The study sponsor is the University Hospital of Nice (Centre Hospitalier Universitaire de Nice). Ethics committee and ANSM (Ministry of Health) approvals were obtained in July 2015. The findings of the trial will be disseminated through peer-reviewed journals, and national and international conference presentations.

Trial registration number: NCT02500693

STRENGTHS AND LIMITATIONS OF THIS STUDY

This study focuses on an unmet need, *i.e.* identification of patients at high risk for lung cancer. Previous results from our group suggest that CTCs is an early event in LC and therefore could be used as a screening tool for LC. The main limitation is that lung cancer related mortality will not be assessed as the primary outcome. Therefore, the power of this study may not be adequate to show a clinical benefit

BACKGROUND AND RATIONALE

Lung cancer (LC) is a leading cause of death from cancer. In France, LC is the 1st and 2nd cause of cancer-related death in men and women, respectively, before prostate and colorectal cancers in men and after breast cancer in women [1]. In 2015 for the first time, the LC death rate in women overtook that of breast cancer in some European countries (UK & Poland) [2].

Despite recent progress in therapeutic strategies, overall survival marginally decreased in the past 30 years and almost all patients with a symptomatic LC die of their disease within 5 years of diagnosis [3-4]. The main reason is that surgery can be offered to less than 25% of patients since more than 75% of LC are diagnosed at advanced stages or are associated with comorbidities that contraindicate surgery [5-6].

Beside tobacco control, screening for early stage LC makes great sense. The National Lung Screening Trial (NLST), a methodologically rigorous trial conducted by the National Cancer Institute, provided evidence on the effect of low dose chest computed tomography (LDCT) screening on LC mortality [7]. This trial conducted in patients aged 55 to 74 years, current or former smokers (at least 30 pack-years) who quitted within fifteen years prior to recruitment, showed that LDCT screening decreased LC-specific mortality by at least 20% compared with chest X-ray screening. Several other LDCT screening trials are currently underway in Europe [8-9], but may not reach definite conclusions [10]

Implementation and generalization of LDCT screening generates a substantial number of patients seeking advice for lung nodules of an undetermined nature and is therefore under debate [10]. False positive tests (i.e. identified at least one nodule > 4 mm in size), occur in close to one third of LDCT screening examinations and less than 3% of the patients who underwent the 3 rounds ultimately had a confirmed LC [7,11-13]. In addition to generating anxiety and being costly these “incidental” nodules lead to multiple additional investigations that may per se be harmful to the patients. Even in North America, where LDCT screening is recommended, there is large debate on improving the LC screening efficiency by: (1) enhanced risk-based approaches, i.e. focusing on the appropriate high-risk patients, such as patients with chronic obstructive pulmonary disease (COPD) [14-22], (2) setting more conservative thresholds for a positive nodule (e.g., > 6 mm) instead of the current “minimal size” of 4 mm [23] or (3) development of novel biomarkers measured in different biological samples (e.g., blood, urine) that improve the ability to predict lung cancer risk [24]. Non-invasive biomarkers under investigation include circulating tumor cells (CTCs) [25-26], circulating specific non-protein-coding RNA [27-29] and methylation of circulating DNA [30]. Migration of CTCs into the blood stream is an early event of human carcinogenesis and tumors measuring around 300 microns can be associated with the presence of CTCs. Up to now, CTCs have been mainly analyzed in patients with an established diagnosis of cancer and used to monitor response to chemotherapy or too look for genomic alterations associated with a targeted therapy [25,31-32]. We recently showed that in high-risk patients, i.e. COPD patients, CTCs detected with the isolation by size of epithelial tumor cell (ISET) technique (RARECELLS®), were present up to 4 years before LC was identified on

LDCT [26]. The CTCs detected had a heterogeneous expression of epithelial and mesenchymal markers, which was similar to the corresponding lung tumor phenotype [33]. No CTCs were detected in control smoking and non-smoking healthy individuals. With these preliminary results we demonstrated for the first time, that in high-risk patients, CTCs can be detected very early in the course of lung cancer and therefore, could be used as a screening tool in high-risk patients or be a biomarker that helps focusing LDCT efforts on individuals who are at highest risk for LC. It also might help in distinguishing benign from malignant lesions in the 20% of patients who show lung nodules on LDCT in screening programs. To confirm these hypotheses, a national prospective cohort study will be run with high-risk patients.

STUDY DESIGN AND OBJECTIVES

The AIR Project is a prospective, non-randomized, multicenter, double-blinded, cohort study that will evaluate CTCs as a screening tool for LC in a population at high-risk. Patients and investigators will be blinded to the results of the CTC analysis. At inclusion in the trial patients will undergo LDCT. In subjects without prevalent cancer at baseline, 2 additional LDCT, one and two years after baseline will be offered for search of incidental cases. Search for CTC will be performed at baseline along with LDCT (± 1 months of LDCT) and then once a year for 2 years along with LDCT. The study sponsor is the University Hospital of Nice (Centre Hospitalier Universitaire de Nice). Twenty-one sites throughout France will participate in this study. The total duration is 4 years (inclusion period = 16 months; follow up = 1 to 4 years). ClinicalTrials.gov identifier NCT02500693.

The primary objective is to evaluate CTCs as a screening tool for LC in individuals at high-risk.

The secondary objectives are: i) to determine whether the presence of CTC may help to differentiate benign from malignant nodules in patients who show the presence of pulmonary nodules on LDCT, ii) to ask whether the presence of CTCs should prompt a special follow-up in a population at high-risk, iii) to study the miRNA signature from plasma and DNA methylation from circulating free DNA for prediction of LC in high-risk patients, iv) to study the relationship between the severity and distribution of emphysema and LC development, iv) to study the exposure of the cohort to professional carcinogens and v) to study the effect of screening on smoking behavior in smokers and to study the psychological impact of LDCT detection of lung nodules.

STUDY POPULATION

The study population will consist of at least 600 patients who will be enrolled in 21 centers in France. Inclusion and exclusion criteria are detailed in table 1.

The main inclusion criteria combine the NLST criteria [7], i.e. age 55 years, tobacco pack-years ≥ 30, if a previous smoker, quit within 15 yr, and the presence of COPD.

We chose to add the "COPD" criteria since we aim to focus on a population at high-risk for LC. In fact, accumulating data suggest that the presence of COPD or emphysema, not considered in the NLST trial [7], dramatically improves the selection process for LDCT cancer screening. For instance it was shown that after adjusting for sex, age and history of smoking, COPD is associated with a two-fold increase, and emphysema with a 3.5-fold increase in the LC risk [16,18,20, 34].

INCLUSION AND PROCEDURES

Patients who meet all the inclusion criteria and none of the exclusion criteria will be enrolled by a designated investigator from each center, after signing written informed consent. Patient inclusion will be performed online (secured internet protocol) using a dedicated web based platform; the patient inclusion number will be allocated online with the format: 00-00/ center number/ number of inclusion. No randomization is planned in this study. Patients and investigators are blinded to the results of CTC analysis.

The study flow diagram is displayed in figure 1. After the assessment for eligibility, enrolled patients will undergo a LDCT and a blood test for CTC search (CTCs detected with the ISET technique (RARECELLS®)) once a year for 3 consecutive years. Patients will be excluded from further screening when diagnosed with a lung cancer after a LDCT. All patients will be followed for at least one year after the last screening tour.

For low dose chest computed tomography, multidetectors scanners (≥16 detectors) will be used to ensure that the whole chest can be scanned in a single maximal breath hold. Unenhanced acquisitions (i.e. without contrast media) will use a low radiation exposure protocol consistent with lung cancer screening protocols with flexibility for making adjustments based on the patients' body mass index (BMI). The tube voltage will range from 100 to 140 kVp and the effective tube current time product will range from 20 to 60 mAs, according to the patient's BMI and on the CT apparatus. Each low dose CT will result in an average effective dose ≤ 1.5 mSv [35-36], for an average 70 kg adult. T corresponds to a dose length product (DLP) of about 75 mGy.cm.

During each round, 20 ml of peripheral blood on Streck tubes (Cell-Free DNA BCT® tubes, Streck, Omaha, USA) will be taken from each patient. These tubes will be sent at ambient temperature, to the coordinator center (Nice Biobank) within 48 hours. ISET will be carried out as previously described [26]. To compare the immediate delayed filtration, during the first round, the coordinator center (Nice) and the 4 other centers equipped with the ISET machines (Strasbourg, Toulouse, Nancy, Grenoble) will collect an additional 10 ml

on an EDTA tube, which will be processed for immediate ISET filtration. The ISET non-colored filters, stored at 4°C, will be shipped later to the coordinator center for further CTC analysis.

MANAGEMENT OF RADIOLOGICALLY SIGNIFICANT INCIDENTAL FINDINGS

As in the NLST trial [7], no formal recommendation will be made as to how a suspicious lesion should be managed in each center. For the management of a suspicious lesion all the centers involved in the present study will follow the strategies recommended by the French thoracic oncology groups [35] and the Fleischner society [37]. However, specific clinical presentations may fall outside of this general approach and require specific discussion in a multidisciplinary team meeting (MDT), which is the standard of care in all the centers involved in the present study. For patients for whom a suspicious lung nodule has been identified and in whom a final and definite diagnosis of cancer cannot be established because of premature death, a dedicated adjudication committee will review the entire medical chart and available documents to establish the probability of cancer.

OUTCOMES

The primary end point will be evaluated at the end of the follow-up period and will be represented by the rate of detection of CTCs in patients for whom lung cancer is detected during the study.

The secondary end points will include: (1) rate of detection of CTCs in the whole study population, (2) predictive value of CTC detection for the diagnosis of LC in patients identified as having a pulmonary nodule, (3) time span between detection of CTC and detection of lung cancer with LDCT and vice versa, (4) microRNA and DNA methylation profile in the study population, (5) smoking cessation rate and (6) pre/post-test changes in the Hospital Anxiety and Depression (HAD) scale.

COLLECTION OF DATA AND MONITORING

The usual demographic data will be recorded. The COPD phenotype will be recorded and thus will include measurement of airflow limitation by pulmonary function tests and assessment of COPD severity by the ABCD grading system (i.e. the ABCD grading system considers COPD symptoms along with the exacerbation frequency and severity, A is better, D is worse) [38]. Professional exposure to carcinogens will be evaluated with the RECAP questionnaire [39]. Lastly, the HAD scale will be measured during each protocol visit.

An electronic case report form (eCRF) has been created and is connected to a dedicated web-based biobank management module (BiobanK 06, Nice and Naeka, Grenoble France).

All chest-CT will be anonymized and stored in electronic (DICOM) format for further centralized analysis.

Quality control will be done by clinical research monitors appointed by the sponsor. The nature and frequency of monitoring will be based on the rate of inclusion. They will check for the accuracy and completeness of the Case Report Form entries, source documents and other trial-related records, they will verify that written informed consent was obtained and that in each center, the trial is in compliance with the currently approved protocol/amendment(s), with good clinical practice, and with the applicable regulatory requirement(s).

ADVERSE EVENTS

An adverse event (AE) is defined as any untoward medical occurrence occurring during the participation of a subject in the trial, independently of the relationship with the study related interventions and procedures. A serious adverse event (SAE) is defined as any AE that results in death, is life-threatening, requiring in patient hospitalization or prolongation of an existing hospitalization, results in persistent or significant disability/incapacity. Each adverse event (AE) must be judged by the investigator and the sponsor, for assessment of the severity of the causal link between AE and the procedure and the character expected or unexpected. A SUSAR (Suspected Unexpected Severe Adverse Reaction) is an adverse reaction that is both unexpected (not consistent with the study related interventions and procedures) and also meets the definition of a SAE. According to the law of 9 August 2004 of the Code of Public Health any occurrence of a SAE will immediately be reported to the sponsor. The sponsor will declare SAE likely to be related to biomedical research to the EC and to the French Agence Nationale du Medicament et des produits de Santé (ANSM) without delay and no later than 7 calendar days in case of death or life-threatening and without delay and at the latest within 15 days for the other SAE.

STATISTICAL ANALYSIS AND SAMPLE SIZE

Statistical analyses will be performed on the whole study population, those with lung cancer (LC positives) and those without (LC negatives), detected by means of LDCT or by any others means. Data from both groups will be summarized separately for all criteria, demographics and baseline characteristics by descriptive statistics. A flow diagram presenting the progress of both groups throughout the study (enrollment, intervention allocation, follow-up and techniques for definite LC diagnosis) in each case will be displayed. The sensitivity and specificity of the ISET technique (detection of CTCs) for the detection of LC will be determined, and presented with their 95% CI based on the Wilson score calculation method. Positive and negative predictive values (PPV, NPV) will be estimated assuming a 5% prevalence of LC in the studied population. The PPV and NPV of detection of CTCs for diagnosing LC in the subjects for whom clinically significant incidental findings will be revealed during the study course will be calculated, assuming a 25-35% prevalence of such findings in the studied population. In the LC positives group, descriptive

statistics describing the temporal relationship (first sign suggestive of LC) and positivity of CTC detection will be provided. Test assumptions will be verified before analyses. P-values below 0.05 will be considered to denote statistical significance. Based on the available literature and considering a 10% dropout rate, if we aim to study a total of 25-35 detected LC, including baseline and repeat screening round, we need to enroll a total of 600 patients.

ETHICAL AND REGULATORY CLEARANCES

Ethic Committee Approval, CPP Sud Méditerranée V and ANSM authorization were obtained on July 8 and 10, 2015. Liability Insurance: Hospital Mutual Insurance Company (SHAM n° 145.017). ClinicalTrial.gov N°: NCT02500693. ISET®-Rarecells® CE mark were obtained on May 12, 2013. A steering committee has been set up to monitor ethical aspects of the project.

DISCUSSION

The AIR Project is the first study to prospectively assess the value of CTC detection for lung cancer screening. We expect that in this high-risk population, CTC detection (via the ISET technology) will improve the detection rate of lung cancer, reduce the rate of false positive chest computed tomography, and act as a decision-making tool for patients with lung nodules of undetermined nature. Concurrent biomarker analysis (miRNA and DNA methylation) in blood samples may also offer the opportunity to validate a multi-modal signature predictive of lung cancer in a high-risk population.

The main limitation of this study is that lung cancer related mortality will not be assessed as the primary outcome. Therefore, the power of this study may not be adequate to show a clinical benefit. Another limitation may be the detection of CTCs from a cancer developing in another organ (such as the bladder) in this high-risk population. However, ancillary methods are under development to better characterize the origin of the detected CTCs [33].

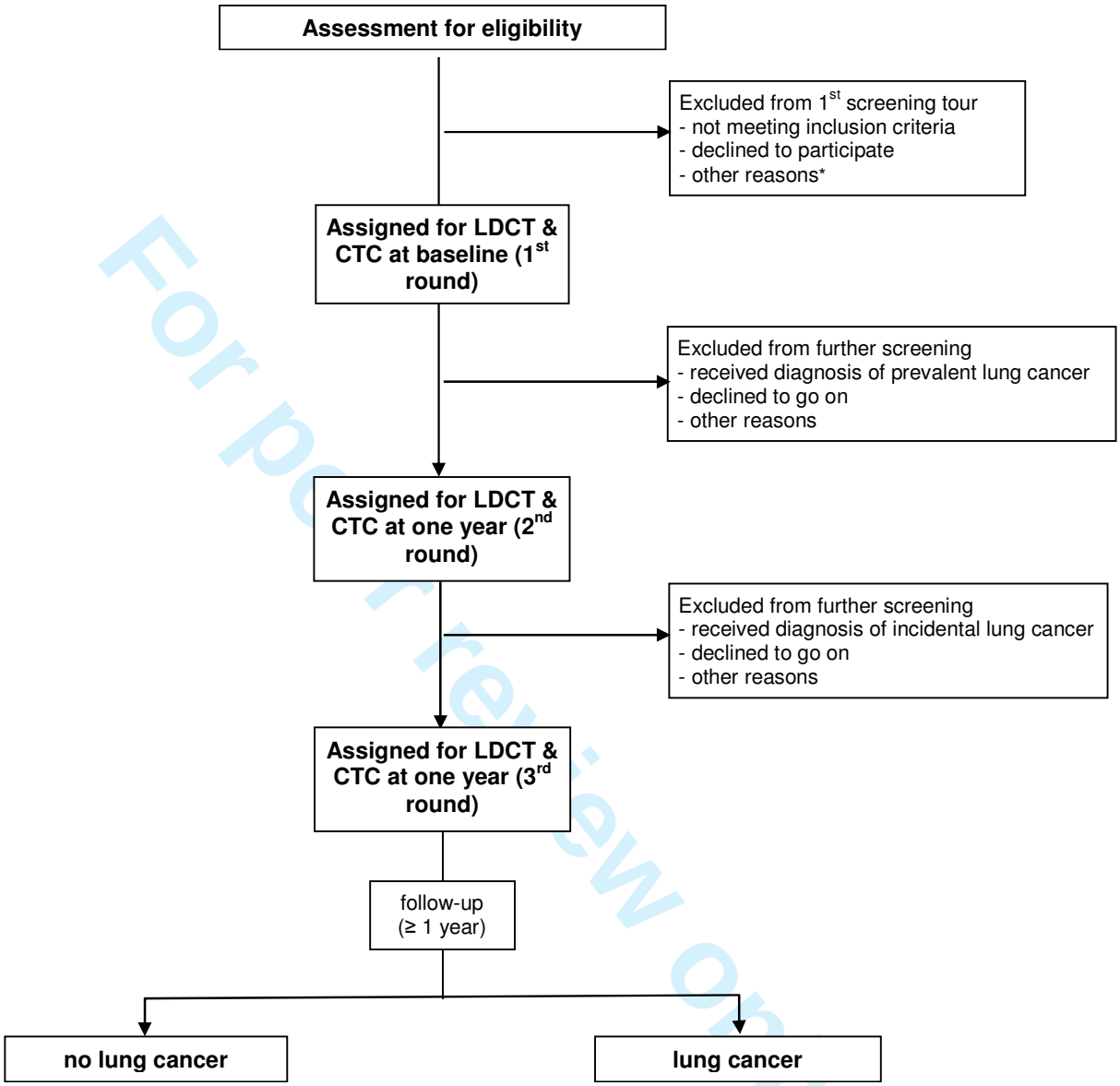
TABLES AND FIGURES

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. age ≥ 55 years 2. tobacco pack-years ≥ 30 3. if former smoker, quit within 15 years 4. presence of COPD[1] 5. affiliation to the French social security system	1. chest CT examination in the 12 months prior to eligibility assessment, except Chest CT made in the prior 6 weeks * 2. known pulmonary nodule or abnormality warranting radiological follow-up or further diagnostic work up 3. treatment for, or evidence of, any cancer other than skin basocellular carcinoma in the 5 previous years 4. acute respiratory tract infection treated with antibiotics in the previous 12 weeks 5. unexplained weight loss ≥ 10% in the previous 12 months 6. recent hemoptysis 7. history of lung volume reduction with coils, glue or valves 8. thoracic metallic implants or devices such as Harrington fixation rods 9. participation in another cancer screening trial 10. participation in a cancer prevention study, other than a smoking cessation study 11. vulnerable persons: adults under guardianship, adults under trusteeship or persons deprived of their liberty, patients under 18 years old , Medical and/or psychiatric problems
[1]compatible medical history AND fixed airflow limitation as defined by post-bronchodilator FEV1/FVC < 0.7 [38]	

Figure 1. Flow chart of the AIR Project.

LDCT, low-dose computed tomography; CTC, circulating tumor cells.



REFERENCES

1. INCA http://www.e-cancer.fr/Actualites-et-evenements/Actualites/Les-cancers-en-France-en-2016-l-essentiel-des-faits-et-chiffres
2. Malvezzi M, Bertuccio P, Rosso T, Rota M, Levi F, LaVecchia C, Negri E. European cancer mortality predictions for the year 2015: does lung cancer have the highest death rate in EU women? Ann Oncol 2015;26:779-86
3. Blanchon F, Grivaux M, Asselain B, Lebas FX, Orlando JP, Piquet J, Zureik M. 4-year mortality in patients with non-small-cell lungcancer: development and validation of a prognostic index. Lancet Oncol.2006;7:829-36.
4. Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2014, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site, April 2017.
5. Morgensztern D, Ng SH, Gao F, Govindan R. Trends in Stage Distribution for Patients with Non-small Cell Lung Cancer: a National CancerDatabase survey. J ThoracOncol.2010;5:29-33
6. Locher C, Debieuvre D, Coëtmeur D, Goupil F, Molinier O, Collon T, Dayen C, Le Treut J, Asselain B, Martin F, Blanchon F, Grivaux M. Major changes in lung cancer over the last ten years in France: the KBP-CPHG studies. Lung Cancer. 2013;81:32-8.
7. Aberle D, Adams A, Berg C, Black W, Clapp J, Fagerstrom, Gareen IF, Gatsonis C, Marcus PM, Sicks JD, National Lung Screening Trial Research Team. Reduced Lung-Cancer Mortality with Low-Dose Computed Tomographic Screening. N Engl J Med 2011;365:395–409.
8. Field JK, van Klaveren R, Pedersen JH, Pastorino U, Paci E, Becker N, Infante M, Oudkerk M, de Koning HJ; European Randomized Screening Trial Group. J Surg Oncol. 2013;108:280-6
9. Manser R, Lethaby A, Irving LB, Stone C, Byrnes G, Abramson MJ, Campbell D. Screening for lung cancer. Cochrane Database Syst Rev. 2013 Jun 21;6:CD001991
10. HAS http://www.has-sante.fr/portail/jcms/c_2001613/fr/pertinence-du-depistage-du-cancer-broncho-pulmonaire-en-france-point-de-situation-sur-les-donnees-disponibles-analyse-critique-des-etudes-controlees-randomisees
11. Bach PB, Mirkin JN, Oliver TK, Azzoli CG, Berry DA, Brawley OW, Byers T, Colditz GA, Gould MK, Jett JR, Sabichi AL, Smith-Bindman R, Wood DE, Qaseem A, Detterbeck FC. Benefits and Harms of CT Screening for Lung Cancer. A Systematic Review. JAMA 2012;307:2418-2429
12. Berg CD, Aberle DR, Wood DE. Lung Cancer Screening: Promise and Pitfalls. Am Soc Clin Oncol Educ Book. 2012:450-7
13. National Lung Screening Trial Research Team. Results of initial low-dose computed tomographic screening for lung cancer. N Engl J Med. 2013 ;368:1980-91
14. de Torres JP, Bastarrika G, Wisnivesky JP, Alcaide AB, Campo A, Seijo LM, Pueyo JC, Villanueva A, Lozano MD, Montes U, Montuenga L, Zulueta JJ. Assessing the relationship between lung cancer risk and emphysema detected on low-dose CT of the chest. Chest. 2007;132:1932-8
15. Li Y, Swensen SJ, Karabekmez LG, Marks RS, Stoddard SM, Jiang R, Worra JB, Zhang F, Midthun DE, de Andrade M, Song Y, Yang P. Effect of emphysema on lung cancer risk in smokers: a computed tomography-based assessment. Cancer Prev Res (Phila). 2011;4:43-50.
16. Young RP, Hopkins RJ. Diagnosing COPD and targeted lung cancer screening. Eur Respir J. 2012;40:1063-4.
17. Arenberg D. Searching for red shirts. Emphysema as a lung cancer screening criterion ? Am J Respir Crit Care Med. 2015;191:868-9.

18. de-Torres JP, Wilson DO, Sanchez-Salcedo P, Weissfeld JL, Berto J, Campo A, Alcaide AB, García-Granero M, Celli BR, Zulueta JJ. Lung Cancer in Patients with Chronic Obstructive Pulmonary Disease. Development and Validation of the COPD Lung Cancer Screening Score. Am J Respir Crit Care Med. 2015;191:285-91

19. Lowry KP, Gazelle GS, Gilmore ME, Johanson C, Munshi V, Choi SE, Tramontano AC, Kong CY, McMahon PM. Personalizing annual lung cancer screening for patients with chronic obstructive pulmonary disease: A decision analysis. Cancer. 2015 15;121:1556-62.

20. Sanchez-Salcedo P, Wilson DO, de-Torres JP, Weissfeld JL, Berto J, Campo A, Alcaide AB, Pueyo J, Bastarrika G, Seijo LM, Pajares MJ, Pio R, Montuenga LM, Zulueta JJ. Improving Selection Criteria for Lung Cancer Screening: the Potential Role of Emphysema. Am J Respir Crit Care Med. 2015;191:924-31

21. Katki HA, Kovalchik SA, Berg CD, Cheung LC, Chaturvedi A. Development and validation of risk models to select ever-smokers for CT lung cancer screening. JAMA 2016;315:2300-11

22. NCCN guidelines version 1.2017.Lung Cancer screening. https://www.nccn.org/professionals/physician_gls/pdf/lung_screening.pdf

23. Yip R, Henschke CI, Yankelevitz DF, Smith JP. CT Screening for Lung Cancer: Alternative Definitions of Positive Test Result Based on the National Lung Screening Trial and International Early Lung Cancer Action Program Databases. Radiology. 2014;273:591-6

24. Vachani A, Sequist LV, Spira A. AJRCCM: 100-Year Anniversary. The Shifting Landscape for Lung Cancer: Past, Present, and Future. Am J Respir Crit Care Med. 2017;195: 1150–1160

25. Ilie M, Hofman V, Long E, Bordone O, Selva E, Washetine K, Marquette CH, Hofman P. Current challenges for detection of circulating tumor cells and cell-free circulating nucleic acids, and their characterization in non-small cell lung carcinoma patients. What is the best blood substrate for personalized medicine? Ann Transl Med. 2014;2:107.

26. Ilie M (b), Hofman V, Long-Mira E, Selva E, Vignaud JM, Padovani B, Mouroux J, Marquette CH, Hofman P. "Sentinel'' Circulating Tumor Cells Allow Early Diagnosis of Lung Cancer in Patients with Chronic Obstructive Pulmonary Disease. PLoS One. 2014;9:e111597

27. Mazières J, Catherinne C, Delfour O, Gouin S, Rouquette I, Delisle MB, Prévot G, Escamilla R, Didier A, Persing DH, Bates M, Michot B. Alternative processing of the U2 small nuclear RNA produces a 19-22nt fragment with relevance for the detection of non-small cell lung cancer in human serum. PLoS One. 2013;8:e60134.

28. Montani F, Marzi MJ, Dezi F, Dama E, Carletti RM, Bonizzi G, Bertolotti R, Bellomi M, Rampinelli C, Maisonneuve P, Spaggiari L, Veronesi G, Nicassio F, Di Fiore PP, Bianchi F. miR-Test: A Blood Test for Lung Cancer Early Detection. J Natl Cancer Inst. 2015 ; 107:djv063.

29. Hofman P. Liquid biopsy for early detection of lung cancer. Curr Opin Oncol. 2017;29:73-78

30. Lu Y, Li S, Zhu S, Gong Y, Shi J, Xu L. Methylated DNA/RNA in Body Fluids as Biomarkers for Lung Cancer. Biol Proced Online. 2017 Mar 17;19:2. doi: 10.1186/s12575-017-0051-8.

31. Hofman V, Bonnetaud C, Ilie MI, Vielh P, Vignaud JM, Fléjou JF, Lantuejoul S, Piaton E, Mourad N, Butori C, Selva E, Poudenx M, Sibon S, Kelhef S, Vénissac N, Jais JP, Mouroux J, Molina TJ, Hofman P. Preoperative circulating tumor cell detection using the isolation by size of epithelial tumor cell method for patients with lung cancer is a new prognostic biomarker. Clin Cancer Res. 2011 ;17:827-35

32. Ilie M, Long E, Butori C, Hofman V, Coelle C, Mauro V, Zahaf K, Marquette CH, Mouroux J, Paterlini-Bréchot P, Hofman P. ALK-gene rearrangement: a comparative analysis on circulating tumour cells and tumour tissue from patients with lung adenocarcinoma. Ann Oncol. 2012;23:2907-13

33. Hofman VJ, Ilie M, Hofman PM. Detection and characterization of circulating tumor cells in lung cancer: Why and how? Cancer Cytopathol. 2016;124:380-7.

34. Wilson DO, Weissfeld JL, Balkan A, Schragin JG, Fuhrman CR, Fisher SN, Wilson J, Leader JK, Siegfried JM, Shapiro SD, Sciurba FC. Association of radiographic emphysema and airflow obstruction with lung cancer. Am J Respir Crit Care Med. 2008 ; 178:738-44

35. Couraud S, Cortot AB, Greillier L, Gounant V, Mennecier B, Girard N, Besse B, Brouchet L, Castelnau O, Frappé P, Ferretti GR, Guittet L, Khalil A, Lefebure P, Laurent F, Liebart S, Molinier O, Quoix E, Revel MP, Stach B, Souquet PJ, Thomas P, Trédaniel J, Lemarié E, Zalcman G, Barlési F, Milleron B; French lung cancer screening statement taskforce; groupe d'Oncologie de langue française. From randomized trials to the clinic: is it time to implement individual lung-cancer screening in clinical practice? A multidisciplinary statement from French experts on behalf of the French intergroup (IFCT) and the groupe d'Oncologie de langue française (GOLF). Ann Oncol 2013;24:586-97

36. Lederlin M, Revel MP, Khalil A, Ferretti G, Milleron B, Laurent F. Management strategy of pulmonary nodule in 2013. Diagn Interv Imaging. 2013;94:1081-94.

37. MacMahon H, Naidich DP, Goo JM, Lee KS, Leung AN, Mayo JR, Mehta AC, Ohno Y, Powell CA, Prokop M, Rubin GD, Schaefer-Prokop CM, Travis WD, Van Schil PE, Bankier AA. Guidelines for Management of Incidental Pulmonary Nodules Detected on CT Images: From the Fleischner Society 2017. Radiology. 2017 Feb 23:161659. doi: 0.1148/radiol.2017161659.

38. Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2017. Available from http://goldcopd.org.

39. Pélissier C, Dutertre V, Fournel P, Gendre I, Michel Vergnon J, Kalecinski J, Tinquaut F, Fontana L, Chauvin F. Design and validation of a self-administered questionnaire as an aid to detection of occupational exposure to lung carcinogens. Public Health. 2017;143:44-51.

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The AIR project: circulating tumor cells as a potential screening tool for lung cancer; a prospective multicenter cohort study

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The AIR project: circulating tumor cells as a potential screening tool for lung cancer; a prospective multicenter cohort study

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SL, CHM, PH, VH, MI, JB, BP and AM designed the study and protocol submission. SL and CHM and PH wrote the manuscript. DIB, CP, PC, JC and JM revised the manuscript.

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ABSTRACT

Introduction: Lung cancer (LC) is the leading cause of death from cancer. Early diagnosis of LC is of paramount importance in terms of prognosis. The health authorities of most countries do not accept screening programs based on low dose chest computed tomography (LDCT), especially in Europe, since they are flawed by a high rate of false positive results, leading to a large number of invasive diagnostic procedures. These authorities advocated further research, including companion biological tests that could enhance the effectiveness of LC screening. The present project aims to validate early diagnosis of LC by detection and characterization of circulating tumor cells (CTCs) in a peripheral blood sample taken from a prospective cohort of persons at high-risk of lung cancer.

Methods and analysis: The AIR Project is a prospective, multicenter, double-blinded, cohort study (study participants and investigators are blinded to the results of CTC search). The primary objective is to determine the operational values of CTCs for the early detection of LC in a cohort of asymptomatic participants at high risk for LC, i.e. smokers and ex-smokers (≥ 30 PY, quitted ≤ 15 years), aged ≥ 55 years, with chronic obstructive pulmonary disease (COPD). The volunteers will undergo yearly screening rounds for 3 years plus a one year follow-up. Each round will include LDCT plus peripheral blood sampling for CTC detection. Assuming 5% prevalence of LC in the studied population and a 10% dropout rate, a total of at least 600 volunteers will be enrolled.

Ethics and dissemination: A consortium of 21 French university centers will run this study. The study sponsor is the University Hospital of Nice (Centre Hospitalier Universitaire de Nice). Ethics committee and ANSM (Ministry of Health) approvals were obtained in July 2015. The findings of the trial will be disseminated through peer-reviewed journals, and national and international conference presentations.

Trial registration number: NCT02500693

STRENGTHS AND LIMITATIONS OF THIS STUDY

This study focuses on an unmet need, *i.e.* identification of persons at high risk for lung cancer. Previous results from our group suggest that CTCs is an early event in LC and therefore could be used as a screening tool for LC. The main limitation is that lung cancer related mortality will not be assessed as the primary outcome. Therefore, the power of this study may not be adequate to show a clinical benefit

BACKGROUND AND RATIONALE

Lung cancer (LC) is a leading cause of death from cancer. In France, LC is the 1st and 2nd cause of cancer-related death in men and women, respectively, before prostate and colorectal cancers in men and after breast cancer in women [1]. In 2015 for the first time, the LC death rate in women overtook that of breast cancer in some European countries (UK & Poland) [2].

Despite recent progress in therapeutic strategies, overall survival marginally decreased in the past 30 years and almost all patients with a symptomatic LC die of their disease within 5 years of diagnosis [3-4]. The main reason is that surgery can be offered to less than 25% of patients since more than 75% of LC are diagnosed at advanced stages or are associated with comorbidities that contraindicate surgery [5-6].

Beside tobacco control, screening for early stage LC makes great sense. The National Lung Screening Trial (NLST), a methodologically rigorous trial conducted by the National Cancer Institute, provided evidence on the effect of low dose chest computed tomography (LDCT) screening on LC mortality [7]. This trial conducted in patients aged 55 to 74 years, current or former smokers (at least 30 pack-years) who quitted within fifteen years prior to recruitment, showed that LDCT screening decreased LC-specific mortality by at least 20% compared with chest X-ray screening. Several other LDCT screening trials are currently underway in Europe [8-9], but may not reach definite conclusions [10]

Implementation and generalization of LDCT screening generates a substantial number of patients seeking advice for lung nodules of an undetermined nature and is therefore under debate [10]. False positive tests (i.e. identified at least one nodule ≥ 4 mm in size), occur in close to one third of LDCT screening examinations and less than 3% of the patients who underwent the 3 rounds ultimately had a confirmed LC [7,11-13]. In addition to generating anxiety and being costly these “incidental” nodules lead to multiple additional investigations that may per se be harmful to the patients. Even in North America, where LDCT screening is recommended, there is large debate on improving the LC screening efficiency by: (1) enhanced risk-based approaches, i.e. focusing on the appropriate high-risk patients, such as patients with chronic obstructive pulmonary disease (COPD) [14-22], (2) setting more conservative thresholds for a positive nodule (e.g., ≥ 6 mm) instead of the current “minimal size” of 4 mm [23] or (3) development of novel biomarkers measured in different biological samples (e.g., blood, urine) that improve the ability to predict lung cancer risk [24] or to distinguish benign from malignant screen-detected nodules. Non-invasive biomarkers under investigation include circulating tumor cells (CTCs) [25-26], circulating specific non-protein-coding RNA [27-29] and methylation of circulating DNA [30]. Migration of CTCs into the blood stream is an early event of human carcinogenesis and tumors measuring around 300 microns can be associated with the presence of CTCs. Up to now, CTCs have been mainly analyzed in patients with an established diagnosis of cancer and used to monitor response to chemotherapy or to look for genomic alterations associated with a targeted therapy [25,31-32]. We recently showed that in high-risk patients, i.e. COPD patients, CTCs detected with the isolation by size of epithelial tumor cell (ISET) technique

(RARECELLS®), were present up to 4 years before LC was identified on LDCT [26]. The CTCs detected had a heterogeneous expression of epithelial and mesenchymal markers, which was similar to the corresponding lung tumor phenotype [33]. No CTCs were detected in control smoking and non-smoking healthy individuals. With these preliminary results we demonstrated for the first time, that in high-risk patients, CTCs can be detected very early in the course of lung cancer and therefore, could be used as a screening tool in high-risk patients or be a biomarker that helps focusing LDCT efforts on individuals who are at highest risk for LC. It also might help in distinguishing benign from malignant lesions in the 20% of patients who show lung nodules on LDCT in screening programs. To confirm these hypotheses, a national prospective cohort study will be run with high-risk patients.

STUDY DESIGN AND OBJECTIVES

The AIR Project is a prospective, non-randomized, multicenter, double-blinded, cohort study that will evaluate CTCs as a screening tool for LC in a population at high-risk. Study participants and investigators will be blinded to the results of the CTC analysis. At inclusion in the trial study participants will undergo LDCT. In participants without prevalent cancer at baseline, 2 additional LDCT, one and two years after baseline will be offered for search of incidental cases. Search for CTC will be performed at baseline along with LDCT (± 1 months of LDCT) and then once a year for 2 years along with LDCT. The study sponsor is the University Hospital of Nice (Centre Hospitalier Universitaire de Nice). Twenty-one sites throughout France will participate in this study. The total duration is 4 years (inclusion period = 16 months; follow up = 1 to 4 years). ClinicalTrials.gov identifier NCT02500693.

The primary objective is to determine the sensitivity, specificity, PPV and NPV of CTCs for the early detection of LC in a cohort of asymptomatic participants at high risk for LC.

The secondary objectives are: i) to determine whether CTC and/or miRNA signature from plasma and/or DNA methylation from circulating free DNA can distinguish benign from malignant screen-detected pulmonary nodules in persons at high risk for LC, ii) to check whether the presence of CTCs should prompt a special follow-up in a population at high-risk of LC with negative LDCT screening, iii) to study the relationship between the severity and distribution of emphysema and LC development, iv) to study the exposure of the cohort to professional carcinogens and v) to study the effect of screening on smoking behavior in smokers and to study the psychological impact of LDCT detection of lung nodules.

STUDY POPULATION

The study population will consist of at least 600 patients who will be enrolled in 21 centers in France. Inclusion and exclusion criteria are detailed in table 1.

The main inclusion criteria combine the NLST criteria [7], i.e. age 55 years, tobacco pack-years ≥ 30, if a previous smoker, quit within 15 yr, and the presence of COPD.

We chose to add the "COPD" criteria since we aim to focus on a population at high-risk for LC. In fact, accumulating data suggest that the presence of COPD or emphysema, not considered in the NLST trial [7], dramatically improves the selection process for LDCT cancer screening. For instance it was shown that after adjusting for sex, age and history of smoking, COPD is associated with a two-fold increase, and emphysema with a 3.5-fold increase in the LC risk [16,18,20,34,35].

INCLUSION AND PROCEDURES

Patients who meet all the inclusion criteria and none of the exclusion criteria will be enrolled by a designated investigator from each center, after signing written informed consent. Patient inclusion will be performed online (secured internet protocol) using a dedicated web based platform; the patient inclusion number will be allocated online with the format: 00-00/ center number/ number of inclusion. No randomization is planned in this study. Patients and investigators are blinded to the results of CTC analysis.

The study flow diagram is displayed in figure 1. After the assessment for eligibility, enrolled patients will undergo a LDCT and a blood test for CTC search (CTCs detected with the ISET technique (RARECELLS®)) once a year for 3 consecutive years. Patients will be excluded from further screening when diagnosed with a lung cancer after a LDCT. All patients will be followed for at least one year after the last screening tour.

For low dose chest computed tomography, multidetectors scanners (≥16 detectors) will be used to ensure that the whole chest can be scanned in a single maximal breath hold. Unenhanced acquisitions (i.e. without contrast media) will use a low radiation exposure protocol consistent with lung cancer screening protocols with flexibility for making adjustments based on the patients' body mass index (BMI). The tube voltage will range from 100 to 140 kVp and the effective tube current time product will range from 20 to 60 mAs, according to the patient's BMI and on the CT apparatus. Each low dose CT will result in an average effective dose ≤ 1.5 mSv [36-37], for an average 70 kg adult. T corresponds to a dose length product (DLP) of about 75 mGy.cm.

During each round, 20 ml of peripheral blood on Streck tubes (Cell-Free DNA BCT® tubes, Streck, Omaha, USA) will be taken from each patient. These tubes will be sent at ambient temperature, to the coordinator center (Nice Biobank) within 48 hours. ISET will be carried out as previously described [26]. To compare the immediate delayed filtration, during the first round, the coordinator center (Nice) and the 4 other centers equipped with the ISET machines (Strasbourg, Toulouse, Nancy, Grenoble) will collect an additional 10 ml

on an EDTA tube, which will be processed for immediate ISET filtration. The ISET non-colored filters, stored at 4°C, will be shipped later to the coordinator center for further CTC analysis.

MANAGEMENT OF RADIOLOGICALLY SIGNIFICANT INCIDENTAL FINDINGS

As in the NLST trial [7], no formal recommendation will be made as to how a suspicious lesion should be managed in each center. For the management of a suspicious lesion all the centers involved in the present study will follow the strategies recommended by the French thoracic oncology groups [35] and the Fleischner society [38]. However, specific clinical presentations may fall outside of this general approach and require specific discussion in a multidisciplinary team meeting (MDT), which is the standard of care in all the centers involved in the present study.

OUTCOMES

The primary end point will be evaluated at the end of the follow-up period and will be represented by the rate of detection of CTCs in patients for whom lung cancer is detected during the study.

The secondary end points will include: (1) rate of detection of CTCs in the whole study population, (2) predictive value of CTC detection for the diagnosis of LC in patients identified as having a pulmonary nodule, (3) time span between detection of CTC and detection of lung cancer with LDCT and vice versa, (4) microRNA and DNA methylation profile in the study population, (5) smoking cessation rate and (6) pre/post-test changes in the Hospital Anxiety and Depression (HAD) scale.

For patients for whom a suspicious lung nodule has been identified and in whom a final and definite diagnosis of cancer cannot be established because of premature death, a dedicated adjudication committee will review the entire medical chart and available documents to establish the probability of cancer.

Two interim analysis are planned, one at the end of the first screening tour and one at the end of the second one, in order to check whether the number of cases with CTCs is significantly higher than we would expect.

COLLECTION OF DATA AND MONITORING

The usual demographic data will be recorded. The COPD phenotype will be recorded and thus will include measurement of airflow limitation by pulmonary function tests and assessment of COPD severity by the ABCD grading system (i.e. the ABCD grading system considers COPD symptoms along with the exacerbation frequency and severity, A is better, D is worse) [39]. Professional exposure to carcinogens will be evaluated with the RECAP questionnaire [40]. Lastly, the HAD scale will be measured during each protocol visit.

An electronic case report form (eCRF) has been created and is connected to a dedicated web-based biobank management module (BiobanK 06, Nice and Naeka, Grenoble France).

All chest-CT will be anonymized and stored in electronic (DICOM) format for further centralized analysis.

Quality control will be done by clinical research monitors appointed by the sponsor. The nature and frequency of monitoring will be based on the rate of inclusion. They will check for the accuracy and completeness of the Case Report Form entries, source documents and other trial-related records, they will verify that written informed consent was obtained and that in each center, the trial is in compliance with the currently approved protocol/amendment(s), with good clinical practice, and with the applicable regulatory requirement(s).

ADVERSE EVENTS

An adverse event (AE) is defined as any untoward medical occurrence occurring during the participation of a subject in the trial, independently of the relationship with the study related interventions and procedures. A serious adverse event (SAE) is defined as any AE that results in death, is life-threatening, requiring in patient hospitalization or prolongation of an existing hospitalization, results in persistent or significant disability/incapacity. Each adverse event (AE) must be judged by the investigator and the sponsor, for assessment of the severity of the causal link between AE and the procedure and the character expected or unexpected. A SUSAR (Suspected Unexpected Severe Adverse Reaction) is an adverse reaction that is both unexpected (not consistent with the study related interventions and procedures) and also meets the definition of a SAE. According to the law of 9 August 2004 of the Code of Public Health any occurrence of a SAE will immediately be reported to the sponsor. The sponsor will declare SAE likely to be related to biomedical research to the EC and to the French Agence Nationale du Medicament et des produits de Santé (ANSM) without delay and no later than 7 calendar days in case of death or life-threatening and without delay and at the latest within 15 days for the other SAE.

STATISTICAL ANALYSIS AND SAMPLE SIZE

Statistical analyses will be performed on the whole study population, those with lung cancer (LC positives) and those without (LC negatives), detected by means of LDCT or by any others means. Data from both groups will be summarized separately for all criteria, demographics and baseline characteristics by descriptive statistics. A flow diagram presenting the progress of both groups throughout the study (enrollment, intervention allocation, follow-up and techniques for definite LC diagnosis) in each case will be displayed. The sensitivity and specificity of the ISET technique (detection of CTCs) for the detection of LC will be determined, and presented with their 95% CI based on the Wilson score calculation method.

Positive and negative predictive values (PPV, NPV) will be estimated assuming a 5% prevalence of LC in the studied population. The PPV and NPV of detection of CTCs for diagnosing LC in the participants for whom clinically significant incidental findings will be revealed during the study course will be calculated, assuming a 25-35% prevalence of such findings in the studied population. In the LC positives group, descriptive statistics describing the temporal relationship (first sign suggestive of LC) and positivity of CTC detection will be provided. Test assumptions will be verified before analyses. P-values below 0.05 will be considered to denote statistical significance. Based on the prevalence (baseline screening round) and incidence (repeat screening rounds) of LC in this high risk population and considering a 10% dropout rate, if we aim to study a total of 25-35 detected LC, including baseline and repeat screening rounds, we need to enroll a total of 600 patients.

ETHICAL AND REGULATORY CLEARANCES

Ethic Committee Approval, CPP Sud Méditerranée V and ANSM authorization were obtained on July 8 and 10, 2015. Liability Insurance: Hospital Mutual Insurance Company (SHAM n° 145.017). ClinicalTrial.gov N°: NCT02500693. ISET®-Rarecells® CE mark were obtained on May 12, 2013. A steering committee has been set up to monitor ethical aspects of the project.

DISCUSSION

The AIR Project is the first study to prospectively assess the value of CTC detection for lung cancer screening. We expect that in this high-risk population, CTC detection (via the ISET technology) will improve the detection rate of lung cancer and perhaps also reduce the rate of false positive chest computed tomography by distinguishing benign from malignant screen-detected pulmonary nodules in persons at high risk for LC. Concurrent biomarker analysis (miRNA and DNA methylation) in blood samples may also offer the opportunity to validate a multi-modal signature predictive of lung cancer in a high-risk populations with screen detected pulmonary nodules.

The main limitation of this study is that lung cancer related mortality will not be assessed as the primary outcome. Therefore, the power of this study may not be adequate to show a clinical benefit. Another limitation may be the detection of CTCs from a cancer developing in another organ (such as the bladder) in this high-risk population. However, ancillary methods are under development to better characterize the origin of the detected CTCs [33].

TABLES AND FIGURES

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. age ≥ 55 years 2. tobacco pack-years ≥ 30 3. if former smoker, quit within 15 years 4. presence of COPD[1] 5. affiliation to the French social security system	1. chest CT examination in the 12 months prior to eligibility assessment, except Chest CT made in the prior 6 weeks * 2. known pulmonary nodule or abnormality warranting radiological follow-up or further diagnostic work up 3. treatment for, or evidence of, any cancer other than skin basocellular carcinoma in the 5 previous years 4. acute respiratory tract infection treated with antibiotics in the previous 12 weeks 5. unexplained weight loss ≥ 10% in the previous 12 months 6. recent hemoptysis 7. history of lung volume reduction with coils, glue or valves 8. thoracic metallic implants or devices such as Harrington fixation rods 9. participation in another cancer screening trial 10. participation in a cancer prevention study, other than a smoking cessation study 11. vulnerable persons: adults under guardianship, adults under trusteeship or persons deprived of their liberty, patients under 18 years old , Medical and/or psychiatric problems
[1]compatible medical history AND fixed airflow limitation as defined by post-bronchodilator FEV1/FVC < 0.7 [39]	

Legend of figures

Figure 1. Flow chart of the AIR Project.

LDCT, low-dose computed tomography; CTC, circulating tumor

REFERENCES

1. INCA http://www.e-cancer.fr/Actualites-et-evenements/Actualites/Les-cancers-en-France-en-2016-l-essentiel-des-faits-et-chiffres
2. Malvezzi M, Bertuccio P, Rosso T, Rota M, Levi F, LaVecchia C, Negri E. European cancer mortality predictions for the year 2015: does lung cancer have the highest death rate in EU women? Ann Oncol 2015;26:779-86
3. Blanchon F, Grivaux M, Asselain B, Lebas FX, Orlando JP, Piquet J, Zureik M. 4-year mortality in patients with non-small-cell lungcancer: development and validation of a prognostic index. Lancet Oncol.2006;7:829-36.
4. Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2014, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site, April 2017.
5. Morgensztern D, Ng SH, Gao F, Govindan R. Trends in Stage Distribution for Patients with Non-small Cell Lung Cancer: a National CancerDatabase survey. J ThoracOncol.2010;5:29-33
6. Locher C, Debieuvre D, Coëtmeur D, Goupil F, Molinier O, Collon T, Dayen C, Le Treut J, Asselain B, Martin F, Blanchon F, Grivaux M. Major changes in lung cancer over the last ten years in France: the KBP-CPHG studies. Lung Cancer. 2013;81:32-8.
7. Aberle D, Adams A, Berg C, Black W, Clapp J, Fagerstrom, Gareen IF, Gatsonis C, Marcus PM, Sicks JD, National Lung Screening Trial Research Team. Reduced Lung-Cancer Mortality with Low-Dose Computed Tomographic Screening. N Engl J Med 2011;365:395–409.
8. Field JK, van Klaveren R, Pedersen JH, Pastorino U, Paci E, Becker N, Infante M, Oudkerk M, de Koning HJ; European Randomized Screening Trial Group. J Surg Oncol. 2013;108:280-6
9. Manser R, Lethaby A, Irving LB, Stone C, Byrnes G, Abramson MJ, Campbell D. Screening for lung cancer. Cochrane Database Syst Rev. 2013 Jun 21;6:CD001991
10. HAS http://www.has-sante.fr/portail/jcms/c_2001613/fr/pertinence-du-depistage-du-cancer-broncho-pulmonaire-en-france-point-de-situation-sur-les-donnees-disponibles-analyse-critique-des-etudes-controlees-randomisees
11. Bach PB, Mirkin JN, Oliver TK, Azzoli CG, Berry DA, Brawley OW, Byers T, Colditz GA, Gould MK, Jett JR, Sabichi AL, Smith-Bindman R, Wood DE, Qaseem A, Detterbeck FC. Benefits and Harms of CT Screening for Lung Cancer. A Systematic Review. JAMA 2012;307:2418-2429
12. Berg CD, Aberle DR, Wood DE. Lung Cancer Screening: Promise and Pitfalls. Am Soc Clin Oncol Educ Book. 2012:450-7
13. National Lung Screening Trial Research Team. Results of initial low-dose computed tomographic screening for lung cancer. N Engl J Med. 2013 ;368:1980-91
14. de Torres JP, Bastarrika G, Wisnivesky JP, Alcaide AB, Campo A, Seijo LM, Pueyo JC, Villanueva A, Lozano MD, Montes U, Montuenga L, Zulueta JJ. Assessing the relationship between lung cancer risk and emphysema detected on low-dose CT of the chest. Chest. 2007;132:1932-8
15. Li Y, Swensen SJ, Karabekmez LG, Marks RS, Stoddard SM, Jiang R, Worra JB, Zhang F, Midthun DE, de Andrade M, Song Y, Yang P. Effect of emphysema on lung cancer risk in smokers: a computed tomography-based assessment. Cancer Prev Res (Phila). 2011;4:43-50.
16. Young RP, Hopkins RJ. Diagnosing COPD and targeted lung cancer screening. Eur Respir J. 2012;40:1063-4.
17. Arenberg D. Searching for red shirts. Emphysema as a lung cancer screening criterion ? Am J Respir Crit Care Med. 2015;191:868-9.

18. de-Torres JP, Wilson DO, Sanchez-Salcedo P, Weissfeld JL, Berto J, Campo A, Alcaide AB, García-Granero M, Celli BR, Zulueta JJ. Lung Cancer in Patients with Chronic Obstructive Pulmonary Disease. Development and Validation of the COPD Lung Cancer Screening Score. Am J Respir Crit Care Med. 2015;191:285-91

19. Lowry KP, Gazelle GS, Gilmore ME, Johanson C, Munshi V, Choi SE, Tramontano AC, Kong CY, McMahon PM. Personalizing annual lung cancer screening for patients with chronic obstructive pulmonary disease: A decision analysis. Cancer. 2015 15;121:1556-62.

20. Sanchez-Salcedo P, Wilson DO, de-Torres JP, Weissfeld JL, Berto J, Campo A, Alcaide AB, Pueyo J, Bastarrika G, Seijo LM, Pajares MJ, Pio R, Montuenga LM, Zulueta JJ. Improving Selection Criteria for Lung Cancer Screening: the Potential Role of Emphysema. Am J Respir Crit Care Med. 2015;191:924-31

21. Katki HA, Kovalchik SA, Berg CD, Cheung LC, Chaturvedi A. Development and validation of risk models to select ever-smokers for CT lung cancer screening. JAMA 2016;315:2300-11

22. NCCN guidelines version 1.2017.Lung Cancer screening. https://www.nccn.org/professionals/physician_gls/pdf/lung_screening.pdf

23. Yip R, Henschke CI, Yankelevitz DF, Smith JP. CT Screening for Lung Cancer: Alternative Definitions of Positive Test Result Based on the National Lung Screening Trial and International Early Lung Cancer Action Program Databases. Radiology. 2014;273:591-6

24. Vachani A, Sequist LV, Spira A. AJRCCM: 100-Year Anniversary. The Shifting Landscape for Lung Cancer: Past, Present, and Future. Am J Respir Crit Care Med. 2017;195: 1150–1160

25. Ilie M, Hofman V, Long E, Bordone O, Selva E, Washetine K, Marquette CH, Hofman P. Current challenges for detection of circulating tumor cells and cell-free circulating nucleic acids, and their characterization in non-small cell lung carcinoma patients. What is the best blood substrate for personalized medicine? Ann Transl Med. 2014;2:107.

26. Ilie M (b), Hofman V, Long-Mira E, Selva E, Vignaud JM, Padovani B, Mouroux J, Marquette CH, Hofman P. "Sentinel'' Circulating Tumor Cells Allow Early Diagnosis of Lung Cancer in Patients with Chronic Obstructive Pulmonary Disease. PLoS One. 2014;9:e111597

27. Mazières J, Catherinne C, Delfour O, Gouin S, Rouquette I, Delisle MB, Prévot G, Escamilla R, Didier A, Persing DH, Bates M, Michot B. Alternative processing of the U2 small nuclear RNA produces a 19-22nt fragment with relevance for the detection of non-small cell lung cancer in human serum. PLoS One. 2013;8:e60134.

28. Montani F, Marzi MJ, Dezi F, Dama E, Carletti RM, Bonizzi G, Bertolotti R, Bellomi M, Rampinelli C, Maisonneuve P, Spaggiari L, Veronesi G, Nicassio F, Di Fiore PP, Bianchi F. miR-Test: A Blood Test for Lung Cancer Early Detection. J Natl Cancer Inst. 2015 ; 107:djv063.

29. Hofman P. Liquid biopsy for early detection of lung cancer. Curr Opin Oncol. 2017;29:73-78

30. Lu Y, Li S, Zhu S, Gong Y, Shi J, Xu L. Methylated DNA/RNA in Body Fluids as Biomarkers for Lung Cancer. Biol Proced Online. 2017 Mar 17;19:2. doi: 10.1186/s12575-017-0051-8.

31. Hofman V, Bonnetaud C, Ilie MI, Vielh P, Vignaud JM, Fléjou JF, Lantuejoul S, Piaton E, Mourad N, Butori C, Selva E, Poudenx M, Sibon S, Kelhef S, Vénissac N, Jais JP, Mouroux J, Molina TJ, Hofman P. Preoperative circulating tumor cell detection using the isolation by size of epithelial tumor cell method for patients with lung cancer is a new prognostic biomarker. Clin Cancer Res. 2011 ;17:827-35

32. Ilie M, Long E, Butori C, Hofman V, Coelle C, Mauro V, Zahaf K, Marquette CH, Mouroux J, Paterlini-Bréchot P, Hofman P. ALK-gene rearrangement: a comparative analysis on circulating tumour cells and tumour tissue from patients with lung adenocarcinoma. Ann Oncol. 2012;23:2907-13

33. Hofman VJ, Ilie M, Hofman PM. Detection and characterization of circulating tumor cells in lung cancer: Why and how? Cancer Cytopathol. 2016;124:380-7.

34. Wilson DO, Weissfeld JL, Balkan A, Schragin JG, Fuhrman CR, Fisher SN, Wilson J, Leader JK, Siegfried JM, Shapiro SD, Sciurba FC. Association of radiographic emphysema and airflow obstruction with lung cancer. Am J Respir Crit Care Med. 2008 ; 178:738-44

35. Smith BP, Pinto L, Ezer N, Sverzellati N, Muro S, Schwartzman K. Emphysema detected on computed tomography and risk of lung cancer: A systematic review and meta-analysis. Lung Cancer 2012;77:58-63

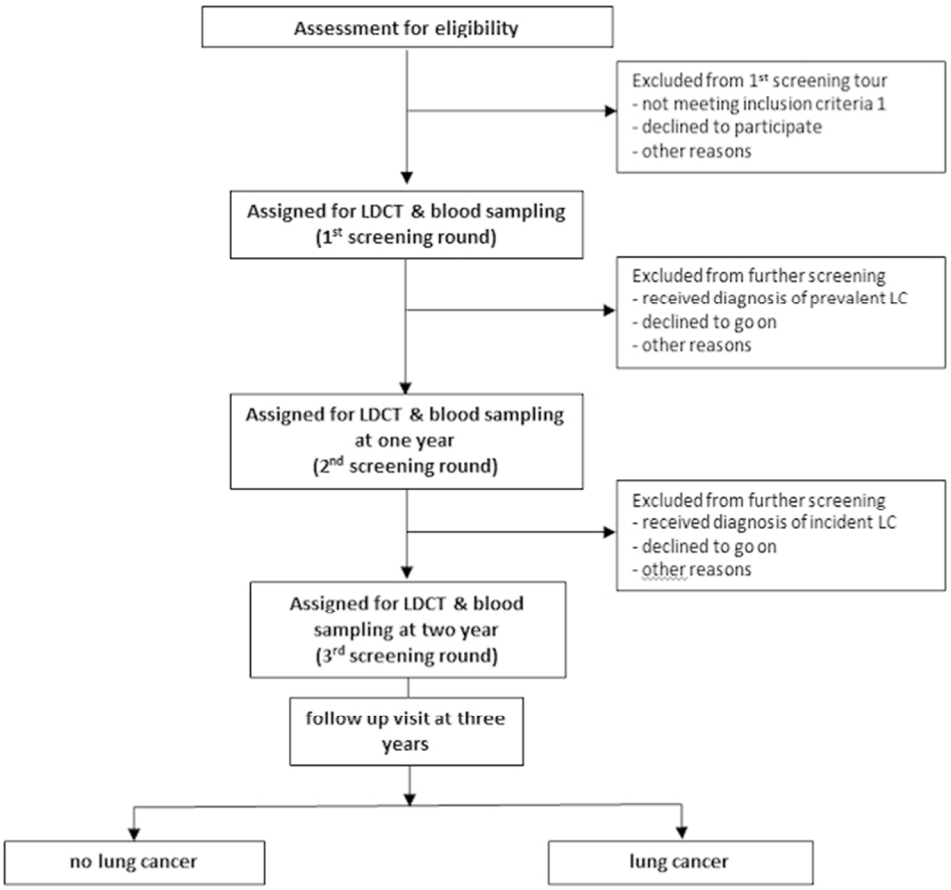
36. Couraud S, Cortot AB, Greillier L, Gounant V, Mennecier B, Girard N, Besse B, Brouchet L, Castelnau O, Frappé P, Ferretti GR, Guittet L, Khalil A, Lefebure P, Laurent F, Liebart S, Molinier O, Quoix E, Revel MP, Stach B, Souquet PJ, Thomas P, Trédaniel J, Lemarié E, Zalcman G, Barlési F, Milleron B; French lung cancer screening statement taskforce; groupe d'Oncologie de langue française. From randomized trials to the clinic: is it time to implement individual lung-cancer screening in clinical practice? A multidisciplinary statement from French experts on behalf of the French intergroup (IFCT) and the groupe d'Oncologie de langue française (GOLF). Ann Oncol 2013;24:586-97

37. Lederlin M, Revel MP, Khalil A, Ferretti G, Milleron B, Laurent F. Management strategy of pulmonary nodule in 2013. Diagn Interv Imaging. 2013;94:1081-94.

38. MacMahon H, Naidich DP, Goo JM, Lee KS, Leung AN, Mayo JR, Mehta AC, Ohno Y, Powell CA, Prokop M, Rubin GD, Schaefer-Prokop CM, Travis WD, Van Schil PE, Bankier AA. Guidelines for Management of Incidental Pulmonary Nodules Detected on CT Images: From the Fleischner Society 2017. Radiology. 2017 Feb 23:161659. doi: 0.1148/radiol.2017161659.

39. Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2017. Available from http://goldcopd.org.

40. Pélissier C, Dutertre V, Fournel P, Gendre I, Michel Vergnon J, Kalecinski J, Tinquaut F, Fontana L, Chauvin F. Design and validation of a self-administered questionnaire as an aid to detection of occupational exposure to lung carcinogens. Public Health. 2017;143:44-51.



Milestones

First study participant enrolled on October 30, 2015; last participant enrolled (cohort completed) on February 28, 2017. Last follow-up visit of the last enrolled volunteer: February 2020

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Circulating tumor cells as a potential screening tool for lung cancer (the AIR study). Protocol of a prospective multicenter cohort study in France

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Circulating tumor cells as a potential screening tool for lung cancer (the AIR study). Protocol of a prospective multicenter cohort study in France

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ABSTRACT

Lung cancer (LC) is the leading cause of death from cancer. Early diagnosis of LC is of paramount importance in terms of prognosis. The health authorities of most countries do not accept screening programs based on low dose chest computed tomography (LDCT), especially in Europe, since they are flawed by a high rate of false positive results, leading to a large number of invasive diagnostic procedures. These authorities advocated further research, including companion biological tests that could enhance the effectiveness of LC screening. The present project aims to validate early diagnosis of LC by detection and characterization of circulating tumor cells (CTCs) in a peripheral blood sample taken from a prospective cohort of persons at high-risk of lung cancer.

The AIR Project is a prospective, multicenter, double-blinded, cohort study conducted by a consortium of 21 French university centers. The primary objective is to determine the operational values of CTCs for the early detection of LC in a cohort of asymptomatic participants at high risk for LC, i.e. smokers and ex-smokers (≥ 30 PY, quitted ≤ 15 years), aged ≥ 55 years, with chronic obstructive pulmonary disease (COPD). The study participants will undergo yearly screening rounds for 3 years plus a one year follow-up. Each round will include LDCT plus peripheral blood sampling for CTC detection. Assuming 5% prevalence of LC in the studied population and a 10% dropout rate, a total of at least 600 volunteers will be enrolled.

Ethics and dissemination: The study sponsor is the University Hospital of Nice. The study was approved for France by the ethical committee Sud-Méditerranée V and the ANSM (Ministry of Health) in July 2015. The findings of the trial will be disseminated through peer-reviewed journals, and national and international conference presentations.

ClinicalTrials.gov identifier : NCT02500693

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study focuses on an unmet need, i.e. identification of persons at high risk for lung cancer (LC).
- Previous results from our group suggest that circulating tumor cells (CTCs) is an early event in LC and therefore could be used as a screening tool for LC.
- The main limitation is the insufficient power of this study to show a clinical benefit (i.e. reduction of LC mortality)
- Another limitation may be the detection of CTCs from a cancer developing in another organ (such as the bladder) in this high-risk population, although ancillary methods are under development to better characterize the origin of the detected CTCs

BACKGROUND AND RATIONALE

Lung cancer (LC) is a leading cause of death from cancer. In France, LC is the 1st and 2nd cause of cancer-related death in men and women, respectively, before prostate and colorectal cancers in men and after breast cancer in women [1]. In 2015 for the first time, the LC death rate in women overtook that of breast cancer in some European countries (UK & Poland) [2].

Despite recent progress in therapeutic strategies, overall survival marginally decreased in the past 30 years and almost all patients with a symptomatic LC die of their disease within 5 years of diagnosis [3-4]. The main reason is that surgery can be offered to less than 25% of patients since more than 75% of LC are diagnosed at advanced stages or are associated with comorbidities that contraindicate surgery [5-6].

Beside tobacco control, screening for early stage LC makes great sense. The National Lung Screening Trial (NLST), a methodologically rigorous trial conducted by the National Cancer Institute, provided evidence on the effect of low dose chest computed tomography (LDCT) screening on LC mortality [7]. This trial conducted in patients aged 55 to 74 years, current or former smokers (at least 30 pack-years) who quitted within fifteen years prior to recruitment, showed that LDCT screening decreased LC-specific mortality by at least 20% compared with chest X-ray screening. Several other LDCT screening trials are currently underway in Europe [8-9], but may not reach definite conclusions [10]

Implementation and generalization of LDCT screening generates a substantial number of patients seeking advice for lung nodules of an undetermined nature and is therefore under debate [10]. False positive tests (i.e. identified at least one nodule ≥ 4 mm in size), occur in close to one third of LDCT screening examinations and less than 3% of the patients who underwent the 3 rounds ultimately had a confirmed LC [7,11-13]. In addition to generating anxiety and being costly these “incidental” nodules lead to multiple additional investigations that may per se be harmful to the patients. Even in North America, where LDCT screening is recommended, there is large debate on improving the LC screening efficiency by: (1) enhanced risk-based approaches, i.e. focusing on the appropriate high-risk patients, such as patients with chronic obstructive pulmonary disease (COPD) [14-22], (2) setting more conservative thresholds for a positive nodule (e.g., ≥ 6 mm) instead of the current “minimal size” of 4 mm [23] or (3) development of novel biomarkers measured in different biological samples (e.g., blood, urine) that improve the ability to predict lung cancer risk [24] or to distinguish benign from malignant screen-detected nodules. Non-invasive biomarkers under investigation include circulating tumor cells (CTCs) [25-26], circulating specific non-protein-coding RNA [27-29] and methylation of circulating DNA [30]. Migration of CTCs into the blood stream is an early event of human carcinogenesis and tumors measuring around 300 microns can be associated with the presence of CTCs. Up to now, CTCs have been mainly analyzed in patients with an established diagnosis of cancer and used to monitor response to chemotherapy or to look for genomic alterations associated with a targeted therapy [25,31-32]. We recently showed that in high-risk patients, i.e. COPD patients, CTCs detected with the isolation by size of epithelial tumor cell (ISET) technique

(RARECELLS®), were present up to 4 years before LC was identified on LDCT [26]. The CTCs detected had a heterogeneous expression of epithelial and mesenchymal markers, which was similar to the corresponding lung tumor phenotype [33]. No CTCs were detected in control smoking and non-smoking healthy individuals. With these preliminary results we demonstrated for the first time, that in high-risk patients, CTCs can be detected very early in the course of lung cancer and therefore, could be used as a screening tool in high-risk patients or be a biomarker that helps focusing LDCT efforts on individuals who are at highest risk for LC. It also might help in distinguishing benign from malignant lesions in the patients who show lung nodules on LDCT in screening programs. To confirm these hypotheses, a national prospective cohort study will be run with high-risk participants.

STUDY DESIGN AND OBJECTIVES

The AIR Project is a prospective, non-randomized, multicenter, double-blinded, cohort study that evaluates CTCs as a screening tool for LC in a population at high-risk. Study participants and investigators will be blinded to the results of the CTC analysis. At inclusion in the trial study participants will undergo LDCT. In participants without prevalent cancer at baseline, 2 additional LDCT, one and two years after baseline will be offered for search of incidental cases. Search for CTC will be performed at baseline along with LDCT (± 1 months of LDCT) and then once a year for 2 years along with LDCT. The study sponsor is the University Hospital of Nice (Centre Hospitalier Universitaire de Nice). Twenty-one sites throughout France will participate in this study. The total duration is 4 years (inclusion period = 16 months; follow up = 1 to 4 years). ClinicalTrials.gov identifier NCT02500693.

The primary objective is to determine the sensitivity, specificity, PPV and NPV of CTCs for the early detection of LC in a cohort of asymptomatic participants at high risk for LC.

The secondary objectives are: i) to determine whether CTC and/or miRNA signature from plasma and/or DNA methylation from circulating free DNA can distinguish benign from malignant screen-detected pulmonary nodules in asymptomatic persons at high risk for LC, ii) to check whether the presence of CTCs should prompt a special follow-up in a population at high-risk of LC with negative LDCT screening, iii) to study the relationship between the severity and distribution of emphysema and LC development, iv) to study the exposure of the cohort to professional carcinogens and v) to study the effect of screening on smoking behavior in smokers and to study the psychological impact of LDCT detection of lung nodules.

STUDY POPULATION

The study population will consist of at least 600 participants who will be enrolled in 21 centers in France. Inclusion and exclusion criteria are detailed in table 1.

The main inclusion criteria combine the NLST criteria [7], i.e. age 55 years, tobacco pack-years ≥ 30, if a previous smoker, quit within 15 yr, and the presence of COPD.

We chose to add the "COPD" criteria since we aim to focus on a population at high-risk for LC. In fact, accumulating data suggest that the presence of COPD or emphysema, not considered in the NLST trial [7], dramatically improves the selection process for LDCT cancer screening. For instance it was shown that after adjusting for sex, age and history of smoking, COPD is associated with a two-fold increase, and emphysema with a three-fold increase in the LC risk [16,18,20,34,35].

INCLUSION AND PROCEDURES

Participants who meet all the inclusion criteria and none of the exclusion criteria will be enrolled by a designated investigator from each center, after signing written informed consent. Inclusion will be performed online (secured internet protocol) using a dedicated web based platform; the participant's inclusion number will be allocated online with the format: 00-00/ center number/ number of inclusion. No randomization is planned in this study. Participants and investigators are blinded to the results of CTC analysis.

The study flow diagram is displayed in figure 1. After the assessment for eligibility, enrolled participants will undergo a LDCT and a blood test for CTC search (CTCs detected with the ISET technique (RARECELLS®)) once a year for 3 consecutive years. Participants will be excluded from further screening when diagnosed with a lung cancer after a LDCT. All participants will be followed for at least one year after the last screening tour.

For low dose chest computed tomography, multidetectors scanners (≥16 detectors) will be used to ensure that the whole chest can be scanned in a single maximal breath hold. Unenhanced acquisitions (i.e. without contrast media) will use a low radiation exposure protocol consistent with lung cancer screening protocols with flexibility for making adjustments based on the participants' body mass index (BMI). The tube voltage will range from 100 to 140 kVp and the effective tube current time product will range from 20 to 60 mAs, according to the participants' BMI and on the CT apparatus. Each low dose CT will result in an average effective dose ≤ 1.5 mSv [36-37], for an average 70 kg adult. T corresponds to a dose length product (DLP) of about 75 mGy.cm.

During each round, 20 ml of peripheral blood on Streck tubes (Cell-Free DNA BCT® tubes, Streck, Omaha, USA) will be taken from each participant. These tubes will be sent at ambient temperature, to the coordinator center (Nice Biobank) within 48 hours. ISET will be carried out as previously described [26]. To compare the immediate delayed filtration, during the first round, the coordinator center (Nice) and the 4

other centers equipped with the ISET machines (Strasbourg, Toulouse, Nancy, Grenoble) will collect an additional 10 ml on an EDTA tube, which will be processed for immediate ISET filtration. The ISET non-colored filters, stored at 4°C, will be shipped later to the coordinator center for further CTC analysis.

MANAGEMENT OF RADIOLOGICALLY SIGNIFICANT INCIDENTAL FINDINGS

As in the NLST trial [7], no formal recommendation will be made as to how a suspicious lesion should be managed in each center. For the management of a suspicious lesion all the centers involved in the present study will follow the strategies recommended by the French thoracic oncology groups [35] and the Fleischner society [38]. However, specific clinical presentations may fall outside of this general approach and require specific discussion in a multidisciplinary team meeting (MDT), which is the standard of care in all the centers involved in the present study.

OUTCOMES

The primary end point will be evaluated at the end of the follow-up period and will be represented by the rate of detection of CTCs in participants for whom lung cancer is detected during the study.

The secondary end points will include: (1) rate of detection of CTCs in the whole study population, (2) predictive value of CTC detection for the diagnosis of LC in participants identified as having a pulmonary nodule, (3) time span between detection of CTC and detection of lung cancer with LDCT and vice versa, (4) microRNA and DNA methylation profile in the study population, (5) smoking cessation rate and (6) pre/post-test changes in the Hospital Anxiety and Depression (HAD) scale.

For participants for whom a suspicious lung nodule has been identified and in whom a final and definite diagnosis of cancer cannot be established because of premature death, a dedicated adjudication committee will review the entire medical chart and available documents to establish the probability of cancer.

Two interim analysis are planned, one at the end of the first screening tour and one at the end of the second one, in order to check whether the number of cases with CTCs is significantly higher than we would expect.

COLLECTION OF DATA AND MONITORING

The usual demographic data will be recorded. The COPD phenotype will be recorded and thus will include measurement of airflow limitation by pulmonary function tests and assessment of COPD severity by the ABCD grading system (i.e. the ABCD grading system considers COPD symptoms along with the exacerbation frequency and severity, A is better, D is worse) [39]. Professional exposure to carcinogens will be evaluated with the RECAP questionnaire [40]. Lastly, the HAD scale will be measured during each protocol visit.

An electronic case report form (eCRF) has been created and is connected to a dedicated web-based biobank management module (BiobanK 06, Nice and Naeka, Grenoble France).

All chest-CT will be anonymized and stored in electronic (DICOM) format for further centralized analysis.

Quality control will be done by clinical research monitors appointed by the sponsor. The nature and frequency of monitoring will be based on the rate of inclusion. They will check for the accuracy and completeness of the Case Report Form entries, source documents and other trial-related records, they will verify that written informed consent was obtained and that in each center, the trial is in compliance with the currently approved protocol/amendment(s), with good clinical practice, and with the applicable regulatory requirement(s).

ADVERSE EVENTS

An adverse event (AE) is defined as any untoward medical occurrence occurring during the participation of a subject in the trial, independently of the relationship with the study related interventions and procedures. A serious adverse event (SAE) is defined as any AE that results in death, is life-threatening, requiring in participants' hospitalization or prolongation of an existing hospitalization, results in persistent or significant disability/incapacity. Each adverse event (AE) must be judged by the investigator and the sponsor, for assessment of the severity of the causal link between AE and the procedure and the character expected or unexpected. A SUSAR (Suspected Unexpected Severe Adverse Reaction) is an adverse reaction that is both unexpected (not consistent with the study related interventions and procedures) and also meets the definition of a SAE. According to the law of 9 August 2004 of the Code of Public Health any occurrence of a SAE will immediately be reported to the sponsor. The sponsor will declare SAE likely to be related to biomedical research to the EC and to the French Agence Nationale du Medicament et des produits de Santé (ANSM) without delay and no later than 7 calendar days in case of death or life-threatening and without delay and at the latest within 15 days for the other SAE.

STATISTICAL ANALYSIS AND SAMPLE SIZE

Statistical analyses will be performed on the whole study population, those with lung cancer (LC positives) and those without (LC negatives), detected by means of LDCT or by any others means. Data from both groups will be summarized separately for all criteria, demographics and baseline characteristics by descriptive statistics. A flow diagram presenting the progress of both groups throughout the study (enrollment, intervention allocation, follow-up and techniques for definite LC diagnosis) in each case will be displayed. The sensitivity and specificity of the ISET technique (detection of CTCs) for the detection of LC will be determined, and presented with their 95% CI based on the Wilson score calculation method.

Positive and negative predictive values (PPV, NPV) will be estimated assuming a 5% prevalence of LC in the studied population. The PPV and NPV of detection of CTCs for diagnosing LC in the participants for whom clinically significant incidental findings will be revealed during the study course will be calculated, assuming a 25-35% prevalence of such findings in the studied population. In the LC positives group, descriptive statistics describing the temporal relationship (first sign suggestive of LC) and positivity of CTC detection will be provided. Test assumptions will be verified before analyses. P-values below 0.05 will be considered to denote statistical significance. Based on the prevalence (baseline screening round) and incidence (repeat screening rounds) of LC in this high risk population and considering a 10% dropout rate, if we aim to study a total of 25-35 detected LC, including baseline and repeat screening rounds, we need to enroll a total of 600 participants.

ETHICS, REGULATORY CLEARANCES AND DISSEMINATION

National ethic committee approval (CPP Sud Méditerranée V; registration # 15.072) and ANSM (Ministry of Health) authorization were obtained on July 8 and 10, 2015. Liability Insurance: Hospital Mutual Insurance Company (SHAM n° 145.017). ClinicalTrial.gov N°: NCT02500693. ISET®-Rarecells® CE mark were obtained on May 12, 2013. A steering committee has been set up to monitor ethical aspects of the project. The findings of the trial will be disseminated through peer-reviewed journals, and national and international conference presentations.

MILESTONES

The first study participant was enrolled on October 30, 2015; last participant was enrolled (cohort completed) on February 28, 2017. Last follow-up visit of the last enrolled participant: February 2020

DISCUSSION

The AIR Project is the first study to prospectively assess the value of CTC detection for lung cancer screening. We expect that in this high-risk population, CTC detection (via the ISET technology) will improve the detection rate of lung cancer and perhaps also reduce the rate of false positive chest computed tomography by distinguishing benign from malignant screen-detected pulmonary nodules in persons at high risk for LC. Concurrent biomarker analysis (miRNA and DNA methylation) in blood samples may also offer the opportunity to validate a multi-modal signature predictive of lung cancer in a high-risk populations with screen detected pulmonary nodules.

The main limitation of this study is that lung cancer related mortality will not be assessed as the primary outcome. Therefore, the power of this study may not be adequate to show a clinical benefit. Another limitation may be the detection of CTCs from a cancer developing in another organ (such as the bladder) in this high-risk population. However, ancillary methods are under development to better characterize the origin of the detected CTCs [33].

TABLES

Table 1. Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
1. age ≥ 55 years 2. tobacco pack-years ≥ 30 3. if former smoker, quit within 15 years 4. presence of COPD[1] 5. affiliation to the French social security system	1. chest CT examination in the 12 months prior to eligibility assessment, except Chest CT made in the prior 6 weeks * 2. known pulmonary nodule or abnormality warranting radiological follow-up or further diagnostic work up 3. treatment for, or evidence of, any cancer other than skin basocellular carcinoma in the 5 previous years 4. acute respiratory tract infection treated with antibiotics in the previous 12 weeks 5. unexplained weight loss ≥ 10% in the previous 12 months 6. recent hemoptysis 7. history of lung volume reduction with coils, glue or valves 8. thoracic metallic implants or devices such as Harrington fixation rods 9. participation in another cancer screening trial 10. participation in a cancer prevention study, other than a smoking cessation study 11. vulnerable persons: adults under guardianship, adults under trusteeship or persons deprived of their liberty, participants under 18 years old , Medical and/or psychiatric problems
[1]compatible medical history AND fixed airflow limitation as defined by post-bronchodilator FEV1/FVC < 0.7 [39]	

LEGEND OF FIGURES

Figure 1. Flow chart of the AIR Project.

REFERENCES

1. INCA http://www.e-cancer.fr/Actualites-et-evenements/Actualites/Les-cancers-en-France-en-2016-l-essentiel-des-faits-et-chiffres
2. Malvezzi M, Bertuccio P, Rosso T, Rota M, Levi F, LaVecchia C, Negri E. European cancer mortality predictions for the year 2015: does lung cancer have the highest death rate in EU women? Ann Oncol 2015;26:779-86
3. Blanchon F, Grivaux M, Asselain B, Lebas FX, Orlando JP, Piquet J, Zureik M. 4-year mortality in patients with non-small-cell lungcancer: development and validation of a prognostic index. Lancet Oncol.2006;7:829-36.
4. Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2014, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site, April 2017.
5. Morgensztern D, Ng SH, Gao F, Govindan R. Trends in Stage Distribution for Patients with Non-small Cell Lung Cancer: a National CancerDatabase survey. J ThoracOncol.2010;5:29-33
6. Locher C, Debieuvre D, Coëtmeur D, Goupil F, Molinier O, Collon T, Dayen C, Le Treut J, Asselain B, Martin F, Blanchon F, Grivaux M. Major changes in lung cancer over the last ten years in France: the KBP-CPHG studies. Lung Cancer. 2013;81:32-8.
7. Aberle D, Adams A, Berg C, Black W, Clapp J, Fagerstrom, Gareen IF, Gatsonis C, Marcus PM, Sicks JD, National Lung Screening Trial Research Team. Reduced Lung-Cancer Mortality with Low-Dose Computed Tomographic Screening. N Engl J Med 2011;365:395–409.
8. Field JK, van Klaveren R, Pedersen JH, Pastorino U, Paci E, Becker N, Infante M, Oudkerk M, de Koning HJ; European Randomized Screening Trial Group. J Surg Oncol. 2013;108:280-6
9. Manser R, Lethaby A, Irving LB, Stone C, Byrnes G, Abramson MJ, Campbell D. Screening for lung cancer. Cochrane Database Syst Rev. 2013 Jun 21;6:CD001991
10. HAS http://www.has-sante.fr/portail/jcms/c_2001613/fr/pertinence-du-depistage-du-cancer-broncho-pulmonaire-en-france-point-de-situation-sur-les-donnees-disponibles-analyse-critique-des-etudes-controlees-randomisees
11. Bach PB, Mirkin JN, Oliver TK, Azzoli CG, Berry DA, Brawley OW, Byers T, Colditz GA, Gould MK, Jett JR, Sabichi AL, Smith-Bindman R, Wood DE, Qaseem A, Detterbeck FC. Benefits and Harms of CT Screening for Lung Cancer. A Systematic Review. JAMA 2012;307:2418-2429
12. Berg CD, Aberle DR, Wood DE. Lung Cancer Screening: Promise and Pitfalls. Am Soc Clin Oncol Educ Book. 2012:450-7
13. National Lung Screening Trial Research Team. Results of initial low-dose computed tomographic screening for lung cancer. N Engl J Med. 2013 ;368:1980-91
14. de Torres JP, Bastarrika G, Wisnivesky JP, Alcaide AB, Campo A, Seijo LM, Pueyo JC, Villanueva A, Lozano MD, Montes U, Montuenga L, Zulueta JJ. Assessing the relationship between lung cancer risk and emphysema detected on low-dose CT of the chest. Chest. 2007;132:1932-8
15. Li Y, Swensen SJ, Karabekmez LG, Marks RS, Stoddard SM, Jiang R, Worra JB, Zhang F, Midthun DE, de Andrade M, Song Y, Yang P. Effect of emphysema on lung cancer risk in smokers: a computed tomography-based assessment. Cancer Prev Res (Phila). 2011;4:43-50.
16. Young RP, Hopkins RJ. Diagnosing COPD and targeted lung cancer screening. Eur Respir J. 2012;40:1063-4.
17. Arenberg D. Searching for red shirts. Emphysema as a lung cancer screening criterion ? Am J Respir Crit Care Med. 2015;191:868-9.

18. de-Torres JP, Wilson DO, Sanchez-Salcedo P, Weissfeld JL, Berto J, Campo A, Alcaide AB, García-Granero M, Celli BR, Zulueta JJ. Lung Cancer in Patients with Chronic Obstructive Pulmonary Disease. Development and Validation of the COPD Lung Cancer Screening Score. Am J Respir Crit Care Med. 2015;191:285-91

19. Lowry KP, Gazelle GS, Gilmore ME, Johanson C, Munshi V, Choi SE, Tramontano AC, Kong CY, McMahon PM. Personalizing annual lung cancer screening for patients with chronic obstructive pulmonary disease: A decision analysis. Cancer. 2015 15;121:1556-62.

20. Sanchez-Salcedo P, Wilson DO, de-Torres JP, Weissfeld JL, Berto J, Campo A, Alcaide AB, Pueyo J, Bastarrika G, Seijo LM, Pajares MJ, Pio R, Montuenga LM, Zulueta JJ. Improving Selection Criteria for Lung Cancer Screening: the Potential Role of Emphysema. Am J Respir Crit Care Med. 2015;191:924-31

21. Katki HA, Kovalchik SA, Berg CD, Cheung LC, Chaturvedi A. Development and validation of risk models to select ever-smokers for CT lung cancer screening. JAMA 2016;315:2300-11

22. NCCN guidelines version 1.2017.Lung Cancer screening. https://www.nccn.org/professionals/physician_gls/pdf/lung_screening.pdf

23. Yip R, Henschke CI, Yankelevitz DF, Smith JP. CT Screening for Lung Cancer: Alternative Definitions of Positive Test Result Based on the National Lung Screening Trial and International Early Lung Cancer Action Program Databases. Radiology. 2014;273:591-6

24. Vachani A, Sequist LV, Spira A. AJRCCM: 100-Year Anniversary. The Shifting Landscape for Lung Cancer: Past, Present, and Future. Am J Respir Crit Care Med. 2017;195: 1150–1160

25. Ilie M, Hofman V, Long E, Bordone O, Selva E, Washetine K, Marquette CH, Hofman P. Current challenges for detection of circulating tumor cells and cell-free circulating nucleic acids, and their characterization in non-small cell lung carcinoma patients. What is the best blood substrate for personalized medicine? Ann Transl Med. 2014;2:107.

26. Ilie M (b), Hofman V, Long-Mira E, Selva E, Vignaud JM, Padovani B, Mouroux J, Marquette CH, Hofman P. "Sentinel'' Circulating Tumor Cells Allow Early Diagnosis of Lung Cancer in Patients with Chronic Obstructive Pulmonary Disease. PLoS One. 2014;9:e111597

27. Mazières J, Catherinne C, Delfour O, Gouin S, Rouquette I, Delisle MB, Prévot G, Escamilla R, Didier A, Persing DH, Bates M, Michot B. Alternative processing of the U2 small nuclear RNA produces a 19-22nt fragment with relevance for the detection of non-small cell lung cancer in human serum. PLoS One. 2013;8:e60134.

28. Montani F, Marzi MJ, Dezi F, Dama E, Carletti RM, Bonizzi G, Bertolotti R, Bellomi M, Rampinelli C, Maisonneuve P, Spaggiari L, Veronesi G, Nicassio F, Di Fiore PP, Bianchi F. miR-Test: A Blood Test for Lung Cancer Early Detection. J Natl Cancer Inst. 2015 ; 107:djv063.

29. Hofman P. Liquid biopsy for early detection of lung cancer. Curr Opin Oncol. 2017;29:73-78

30. Lu Y, Li S, Zhu S, Gong Y, Shi J, Xu L. Methylated DNA/RNA in Body Fluids as Biomarkers for Lung Cancer. Biol Proced Online. 2017 Mar 17;19:2. doi: 10.1186/s12575-017-0051-8.

31. Hofman V, Bonnetaud C, Ilie MI, Vielh P, Vignaud JM, Fléjou JF, Lantuejoul S, Piaton E, Mourad N, Butori C, Selva E, Poudenx M, Sibon S, Kelhef S, Vénissac N, Jais JP, Mouroux J, Molina TJ, Hofman P. Preoperative circulating tumor cell detection using the isolation by size of epithelial tumor cell method for patients with lung cancer is a new prognostic biomarker. Clin Cancer Res. 2011 ;17:827-35

32. Ilie M, Long E, Butori C, Hofman V, Coelle C, Mauro V, Zahaf K, Marquette CH, Mouroux J, Paterlini-Bréchot P, Hofman P. ALK-gene rearrangement: a comparative analysis on circulating tumour cells and tumour tissue from patients with lung adenocarcinoma. Ann Oncol. 2012;23:2907-13

33. Hofman VJ, Ilie M, Hofman PM. Detection and characterization of circulating tumor cells in lung cancer: Why and how? Cancer Cytopathol. 2016;124:380-7.

34. Wilson DO, Weissfeld JL, Balkan A, Schragin JG, Fuhrman CR, Fisher SN, Wilson J, Leader JK, Siegfried JM, Shapiro SD, Sciurba FC. Association of radiographic emphysema and airflow obstruction with lung cancer. Am J Respir Crit Care Med. 2008 ; 178:738-44

35. Smith BP, Pinto L, Ezer N, Sverzellati N, Muro S, Schwartzman K. Emphysema detected on computed tomography and risk of lung cancer: A systematic review and meta-analysis. Lung Cancer 2012;77:58-63

36. Couraud S, Cortot AB, Greillier L, Gounant V, Mennecier B, Girard N, Besse B, Brouchet L, Castelnau O, Frappé P, Ferretti GR, Guittet L, Khalil A, Lefebure P, Laurent F, Liebart S, Molinier O, Quoix E, Revel MP, Stach B, Souquet PJ, Thomas P, Trédaniel J, Lemarié E, Zalcman G, Barlési F, Milleron B; French lung cancer screening statement taskforce; groupe d'Oncologie de langue française. From randomized trials to the clinic: is it time to implement individual lung-cancer screening in clinical practice? A multidisciplinary statement from French experts on behalf of the French intergroup (IFCT) and the groupe d'Oncologie de langue française (GOLF). Ann Oncol 2013;24:586-97

37. Lederlin M, Revel MP, Khalil A, Ferretti G, Milleron B, Laurent F. Management strategy of pulmonary nodule in 2013. Diagn Interv Imaging. 2013;94:1081-94.

38. MacMahon H, Naidich DP, Goo JM, Lee KS, Leung AN, Mayo JR, Mehta AC, Ohno Y, Powell CA, Prokop M, Rubin GD, Schaefer-Prokop CM, Travis WD, Van Schil PE, Bankier AA. Guidelines for Management of Incidental Pulmonary Nodules Detected on CT Images: From the Fleischner Society 2017. Radiology. 2017 Feb 23:161659. doi: 0.1148/radiol.2017161659.

39. Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2017. Available from http://goldcopd.org.

40. Pélissier C, Dutertre V, Fournel P, Gendre I, Michel Vergnon J, Kalecinski J, Tinquaut F, Fontana L, Chauvin F. Design and validation of a self-administered questionnaire as an aid to detection of occupational exposure to lung carcinogens. Public Health. 2017;143:44-51.

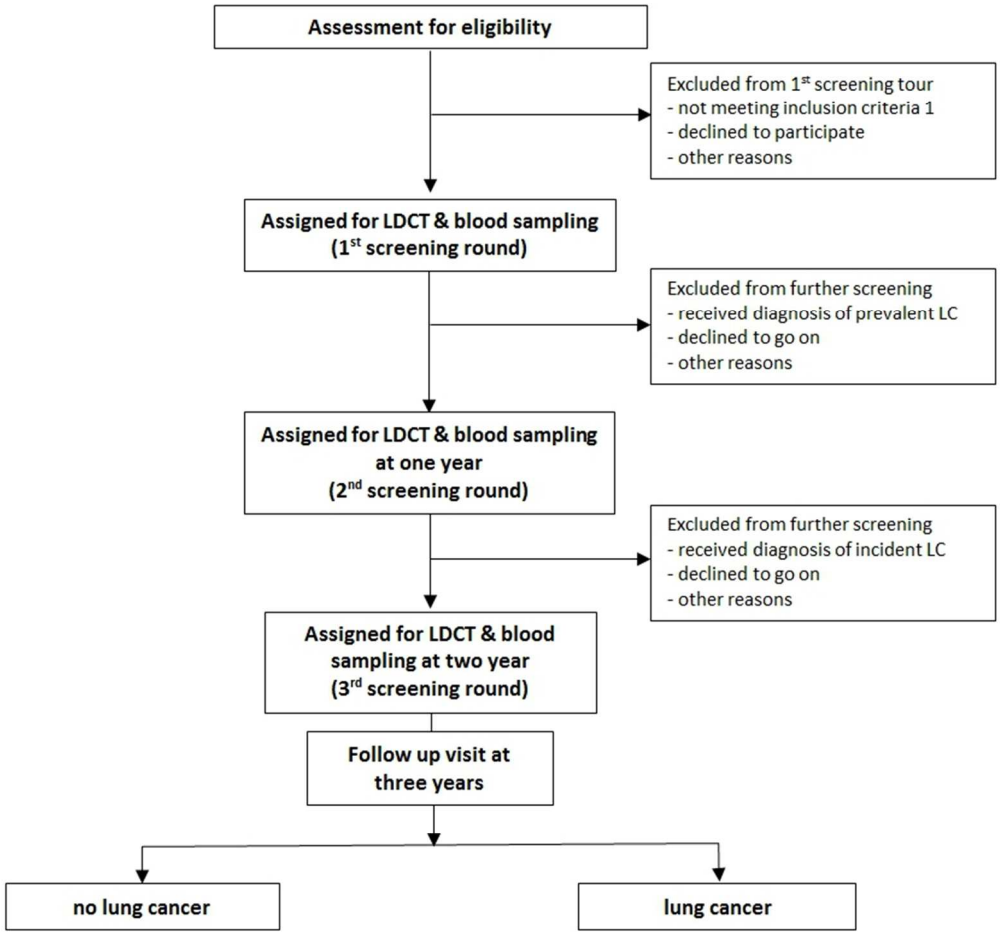

Figure 1. Flow chart of the AIR Project.

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