SPECIAL FORMAT FOR REGISTRATION OF RESEARCH PROTOCOLS

RESEARCH AND ETHICS COMMITTEE
INSTITUTO NACIONAL DE CANCEROLOGÍA

I. GENERAL DATA

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Especiality	Medical Oncology
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Adscription	Research Department

2. PROJECT

Name of the project: **Effect of the use of metformin in combination with tyrosine kinase inhibitors on clinical, biochemical and nutritional parameters in patients with NSCLC: Randomized Clinical Trial.** Amendment 1, Version 1, September 14, 2016, English version.

Type of research (Mark with X)

X	Basic		Exploratory
X	Clinic		Experimental Proposal
	Mixed		Comparative

Source (Mark with X)

x	Internal
	External
	Degree Thesis

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(A letter of support from each collaborator must be attached)

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4. OTHER PARTICIPATING INSTITUTIONS

(A letter of support signed by the director of each participating institute must be attached)

Name Institution I	
Name Institution II	
Name Institution III	
Name Institution IV	
Name Institution V	

Type of assist

	Type of assist			
	Infrastructure	Personal	Material	Equipment
Name Institution I				
Name Institution II				
Name Institution III				
Name Institution IV				
Name Institution V				

II. PROJECT SUMMARY

Write a structured summary of the project (background, objective, hypothesis, methodology). May not exceed 1000 words

Treatment for Non-small cell Lung Cancer (NSCLC) patients with epidermal growth factor receptor (*EGFR*) mutations with specific tyrosine kinase domain inhibitors (TKIs) has led to high objective clinical responses, increased progression-free survival (PFS) compared with cytotoxic chemotherapy.

However, despite clinical success with different TKIs, most patients eventually develop acquired resistance to these agents after an average period of 10 months. Therefore, innovative treatment strategies are urgently needed to overcome resistance to EGFR-TKIs and improve the survival of patients with NSCLC.

Recently, metformin, which is an oral hypoglycemic agent, has been linked to a reduction in the overall risk of incidence and mortality of different types of cancer, by exerting antitumor properties. The role of metformin as a chemo-preventive drug and adjuvant in overcoming acquired resistance to chemotherapy, targeted therapy and immunotherapy in NSCLC remains debated. However, preclinical data support its role as an adjuvant drug in the treatment of NSCLC in combination with chemotherapy or TKIs.

This evidence led to examine the effects of metformin in combination with EGFR-TKIs, in a panel of NSCLC cell lines, obtaining a different sensitivity to treatment with EGFR-TKIs only. The combination of metformin with TKIs reduced the capacity of colony formation, proliferation, and induced a large proapoptotic effect of the NSCLC cell lines and resistance to EGFR-TKIs. This suggests that metformin can reverse the resistance to TKIs. We carried out a retrospective study with patients from the Instituto Nacional de Cancerología from 2008 to 2014, in this study, an important clinical benefit was found in those patients who were treated with metformin independently improving overall survival.

Based on these considerations, we propose this phase II randomized study to evaluate the safety and activity of metformin in combination with TKIs as second-line of treatment in patients with advanced NSCLC with *EGFR* mutations.

The primary objective is to evaluate the progression-free survival in patients with advanced non-small cell lung cancer treated with TKIs plus metformin versus TKIs alone. The secondary

objectives are the response rate, overall survival, quality of life (QoL), safety, as well as determining the affectation of the nutritional parameters associated with the combined use of TKIs and metformin.
In addition to secondary objectives, we want to explore potential biomarkers for tumor characteristics in order to predict antitumor activity, additionally, search for serum markers; we will analyze the type of mutations in *EGFR* (mutations in exon 18-21), serum levels of IL-6 and IGF-1. Besides, we will evaluate the expression of Liver Kinase B1 (LKB-1) in tumor tissue, in order to assess its prognostic role.

Hypothesis: The combined use of metformin with TKI will increase the progression-free survival compared with patients treated with TKIs alone.

Methodology: According to sample size calculation, 138 patients will be included in the study. The patients will be randomized to receive metformin plus the TKI of choice by the oncologist (experimental group) or the TKI alone (control group). Objective response evaluation will be performed by CT scans using the Response Evaluation Criteria in Solid Tumors (RECIST 1.1). Progression-free survival and overall survival differences between groups will be evaluated with the Kaplan Meier method using SPSS software ver. 21. IL-6 and IGF-1 will be quantified in plasma of the patients by the ELISA technique. The tumor tissue will be analyzed for intracellular LKB-1 expression using the Western Blot technique. *EGFR* mutations will be assessed using a Therascreen RGQ PCR Kit (QIAGEN, Scorpions ARMS method) and real-time PCR performed in a Rotor-Gene Q 5plex HRM (QIAGEN). The nutritional evaluation will be carried out through previously validated questionnaires for the determination of anorexia, subjective global assessment, food consumption, as well as measurements of weight, height, waist, hip and the evaluation of body composition using the Slice-O-Matic software 5.0.

In order to reduce the adverse effects of metformin and improve adherence to treatment, Extended-release Metformin (Dabex XR 500 mg and 1000) mg will be administered. Treatment will start with 1000 mg daily; the dose will be reduced to 500 mg in case of any adverse effect.

III. PROJECT BACKGROUND

May not exceed 5000 words

Lung cancer is the leading cause of cancer death in both men and women, with 28% and 26% of cancer deaths worldwide (1). Among patients with this neoplasm, at least 80% are affected by non-small cell lung cancer (NSCLC), and 65% of patients present with locally advanced or metastatic disease. Treatment schemes for NSCLC are dependent on the disease stage and may involve surgery, chemotherapy, radiotherapy, or a combination of these modalities. Survival rates at 5 years are poor for patients in stage II and III of the disease, ranging from 30% to a minimum of 5%, which implies the need for improvements in therapy (2).

Advances in understanding the molecular biology of cancer have allowed the discovery of several molecular targets and the development of new targeted therapies. The epidermal growth factor receptor (EGFR) is involved in the development and progression of several human cancers including NSCLC. However, despite clinical successes with different therapies based on tyrosine kinase domain inhibitors (TKIs), most cancer patients who initially respond to these drugs eventually develop acquired resistance to these agents after an average period of 10 months (3). Therefore, innovative treatment strategies are urgently needed to overcome the therapeutic resistance to EGFR-TKIs to improve the survival of patients with NSCLC.

Metformin is an oral hypoglycemic drug that was discovered in 1950 in Europe, it has been used universally in the treatment of patients with type 2 diabetes mellitus (DM), due to its adequate

safety profile (4). Metformin acts mainly in the liver, likely decreasing glycogenolysis, as well through reduction in the glucose uptake in peripheral tissues by inactivating hepatic LKB-1, which leads to decrease mitochondrial respiration and increase the synthesis of gluconeogenesis enzymes (5). Recently, metformin has been linked to a reduction in the overall risk of incidence and mortality of different types of cancer, by exerting antitumor properties (6). In selected types of cancer, retrospective studies have shown a clinical benefit of the use of metformin concurrently during cancer treatment.

Mechanisms of resistance to anti-EGFR treatment

NSCLC patients with EGFR mutations are highly sensitive to specific anti-EGFR TKIs, however, most patients who initially respond to these targeted therapies, subsequently experience disease progression during treatment; this is known as acquired resistance.

The acquired resistance to targeted therapies was first studied in chronic myeloid leukemia patients with BCR-ABL translocation treated with Imatinib, an inhibitor of aberrant BCR-ABL kinase. Mutations in the kinase domain of ABL were found in about 50% to 90% of patients who developed secondary resistance to Imatinib. These acquired mutations interfere with the binding of Imatinib to ABL, preventing its anti-tumor action. Through these investigations, *EGFR* mutations associated with treatment resistance to TKIs in NSCLC patients were discovered.
Additionally, other studies reported additional mutations in the kinase domain and in *KRAS* in patients with acquired resistance to Gefitinib or Erlotinib. Through the use of RT-PCR, it was found that 50% of the patients with resistance to TKIs develop a specific mutation in exon 20 (T790M) (6, 7). However, in the other 50% of patients who develop resistance to TKIs, the mechanism of resistance remains unknown. There are studies that have found focal amplification of the *MET* proto-oncogene in 22% of patients with acquired resistance to Gefitinib. The proposed mechanism is that *MET* amplification promotes resistance through the activation of the PI3K pathway dependent on HER3 (8). However, there are few studies with small series about the amplification of *MET* as a mechanism of resistance.

On the other hand, some patients have this resistance mutation from the moment of presentation, or *de novo*. The T790M resistance mutation may be present prior to exposure to TKIs, and is frequently found along with other activating mutations in *EGFR* (exon 19 deletion and L858R point mutation). The reported frequency of resistance mutations to diagnosis varies according to the literature, however it is found in <1% of all lung cancers and in 1-15% of NSCLC with *EGFR* mutations. A response rate of 8% has been reported in patients treated with gefitinib or erlotinib who carry a T790M mutation at diagnosis, with 2-month progression-free survival and a median overall survival of 16 months. In conclusion, these patients are not candidates for treatment with first and second generation TKIs, and should be treated with other agents (9,10).

Although advances in treatment have increased response rates and progression-free survival with TKIs in patients harboring *EGFR* mutations, most of them will develop resistance mutations (T790M) leading to disease progression. There is no standard of treatment in these patients who progress to first generation TKIs such as erlotinib and gefitinib (12). Some studies have used Afatinib as a second-line treatment in patients who progressed to Erlotinib or Gefitinib, finding a benefit in progression-free survival, disease control rates up to 58% and delayed development of symptoms associated with lung cancer, thus improving the QoL in patients treated with Afatinib (11). The acquired resistance develops in a median of 9-13 months, and in 50% -60% of cases it is secondary to the development of the resistance mutation T790M. When a patient with an *EGFR*-activating mutation is treated with a first-line TKI (erlotinib, Gefitinib) and presents disease progression, if the second-line treatment is platinum-based chemotherapy, the median survival is less than 2 years.

Rociletinib (CO-1686) is a novel TKI which has shown antitumor activity in patients with *EGFR* mutation, including exon 19 deletions, L858R and T790M mutations, with minimal activity against tumors without *EGFR* mutations. The reported Objective response rate (ORR) was 46% and disease control rate was 93%. Response rates were similar among patients with exon 19 deletions and L858R mutations. The progression-free survival reported was 13.1 months. The most important grade 3 adverse event was hyperglycemia in 22% of patients, regularly it is a well-tolerated drug and the toxicity is manageable with oral hypoglycemic agents (13).

Metformin and resistance to tyrosine kinase inhibitors

The molecular mechanisms which generate acquired resistance to EGFR-TKIs are not completely clear. We know that about 50% are due to an acquired mutation in T790M, and in a lower percentage due to *MET* oncogene amplification. However, other molecular mechanisms have been proposed, such as activation of the epithelium-mesenchyme transition. The epithelial-mesenchymal transition refers to the changes from the epithelial cell phenotype to cells with a mesenchymal phenotype, resulting in an increase in motility, invasion, proliferation and metastasis of tumor cells (15, 16). It has been proposed that the epithelial-mesenchymal transition (TEM) is related to sensitivity to both, chemotherapy and TKIs.

This transition occurs through TGF-β stimulation, which induces IL-6 activation by paracrine signaling through the IL-6 receptor, leading to JAK1/STAT3 activation. STAT is a family of transcription factors that play an important role in multiple cellular functions. STAT3 activation regulates the progression of the cell cycle, tumor invasion, metastasis and angiogenesis (17).

Pre-clinical studies with xenographic models have shown that one mechanism of resistance in tumor cells with T790M mutation is the activation of the IL-6/JAK1/STAT3 receptor, and that the inhibition of this signaling pathway increases TKIs sensitivity (18, 19). Therefore, the development of an effective therapy for patients who develop the T790M resistance mutation is priority in order to overcome resistance to first generation TKIs.

Afatinib, a second-generation TKI, has shown in some clinical studies a certain effect in patients with resistance mutations, nevertheless, the benefit is marginal (13). Other studies have shown that the binding of afatinib with the kinase domain in patients with resistance mutation is 100 times less potent than in cells with activating *EGFR* mutations (20). In preclinical studies, inhibition of IL-6 receptor activation and activation of the JAK1/STAT3 pathway has been shown to reverse resistance and re-sensitize cells with presence of resistance mutations (17).

Metformin is a drug that has been used for many years for the treatment of DM and metabolic syndrome, habitually, it is well tolerated. Several studies conducted since 1910 have suggested that patients with diabetes have an increased risk of developing cancer. The American Diabetes Association and the American Cancer Society have reached a consensus that suggests a clear association of an increased risk of cancer for diabetic patients. The most studied tumors in patients with diabetes are: colon, endometrium, rectum and breast cancer (26). Moreover, both epidemiological and case-control studies have suggested that the use of Metformin decreases the risk of developing cancer by up to 30%, with a HR of 0.77 (0.64-0.92) and also decreases the risk of cancer-specific death, with a HR of 0.67 (0.53-0.85) (21, 29). This protective effect has been observed in all types of cancer, but has been studied extensively in breast cancer, gastrointestinal tumors and lung cancer.

Metformin can enhance the effect of Chemotherapy

The effect of Metformin as chemo-prevention remains debated; however, there is more information about its adjuvant use in the lung cancer treatment, in combination with

chemotherapy or molecular targeted therapy (27). Preclinical studies in mice have shown that oral administration of Metformin can decrease the dose of chemotherapy needed and prolong the remission of the tumor (22). By inhibiting the repair and anti-apoptosis mechanisms, metformin increases the sensitivity to chemotherapy, particularly to platinum-based schemes. Studies with Metformin, paclitaxel, carboplatin and doxorubicin have shown an effect on tumor regression and prevention of recurrences up to four times higher compared with monotherapy, using xenographic models with lung and prostate cancer cell lines (27). In retrospective studies, a benefit in progression-free survival and overall survival has been found in diabetic patients with NSCLC treated with Metformin (30).

Metformin overcomes resistance to TKIs in NSCLC patients with T790M mutation

The T790M mutation and MET amplification are the main mechanisms of resistance to TKIs, another mechanism of resistance is the increase in the mesenchyme epithelium transition through TGF-β. TGF-β also induces IL-6 activation and paracrine activation of its receptor (IL-6R), which leads to activation of the JAK1 / STAT3 pathway and cell immortalization. Pre-clinical studies, with lung cancer cell lines with acquired resistance to anti-EGFR treatment, demonstrate that Metformin prevents the transcription of factors that activate the epithelium-mesenchyme transition, inhibiting TGF-β, thus inhibiting the activation of the pathway. IL-6R / JAK1 / STAT3, overcoming resistance to TKIs in patients with presence of T790M resistance mutation, both *in vitro* and *in vivo* models (23, 24). A recent study reports that the use of Metformin in combination with gefitinib can increase its efficacy, showing an anti-proliferative and pro-apoptotic effect in NSCLC cell lines (25). Other studies have shown through Western blot analysis, a decrease in phosphorylation levels and activation of MAPK, AKT and mTOR growth pathways with the use of Metformin in lung cancer cell lines. Based on the anti-proliferative effects of metformin found in preclinical studies, a phase I / II study is currently in progress to determine the effective dose, safety profile and subsequently activity of Metformin combined with erlotinib as a second-line treatment in patients with NSCLC without *EGFR* mutations (31).

Adverse effects related to the use of Metformin

Several adverse reactions associated with the use of metformin have been identified; however, the incidence of these reactions varies in each patient and the dose used. Among the adverse effects that have been related to the use of metformin are:

Gastrointestinal effects, which have been the most frequently encountered, among them, the most frequent is diarrhea (53.2% of patients). Other gastrointestinal adverse effects associated with the use of metformin are abdominal pain (6.4%), flatulence (12.1%), nausea and vomiting (25.5%) and indigestion (7.1%). However, it has been reported that dose reduction improves the manifestation of these reactions in 95% of patients (32).
Lactic acidosis is an adverse effect that is rarely detected, but when it occurs it can be fatal in up to 50% of cases. However, certain conditions must be fulfilled for this adverse effect to be found in patients, among which are type 2 diabetes mellitus, hypoxemia and tissue hypoperfusion. The incidence of lactic acidosis in patients due to the consumption of metformin has been very low (0.03 / 1000 cases per patient in a year), and has been found in patients who have significant renal failure and also in patients who consume many concomitant medications (32). Another adverse reaction found (even though is infrequent) with the use of metformin is the decreased absorption of vitamin B12. This reaction was found in 10-20% of patients who consumed metformin, nonetheless, this effect can be reversed with the use of calcium supplements (33). There are other associated reactions that occur very sporadically and in a very low percentage of patients, including leukocytoclastic vasculitis, cholestatic jaundice, allergic pneumonitis and hemolytic anemia (33). Hypoglycaemia is very uncommon with the use of metformin and has mostly been found in patients with impaired renal function (33).

If an unusual sign or symptom occurs, patients should be instructed to notify their doctor immediately in order to prescribe medication to relieve the side effects. Additionally, if a severe-intensity reaction to the medication occurs, the treating physician may interrupt the treatment temporarily or permanently, or may reduce the dose of one or more of the drugs. Close monitoring for possible side effects and supplemental studies is necessary, to ensure the best course of action in the event of a serious adverse event directly related to the study drug.

Nutritional aspect in the progression of the disease and use of TKI plus Metformin

Malnutrition can affect up to 80% of patients with advanced cancer and is associated with up to 20% of deaths (34). Malnutrition is associated with a greater risk in the development of complications and mortality, and can extend hospital stay by up to 90%, increasing the cost of treatment by 35-75% (34,35).
The main cause of involuntary weight loss in patients with cancer is the cachexia syndrome, which is a set of metabolic disorders that occur regularly with anorexia, which leads to a decrease in physical functionality. The causes of cachexia are complex and multifactorial, as is the release of catabolic tumor substances and pro-inflammatory cytokines.
The weight loss can be both fat mass and lean mass, and may be due to a decrease in caloric intake either by direct consequences (anorexia, dysphagia, dysgeusia, etc.) or indirect (pain, fatigue, etc.) (36). However, the loss of muscle mass is considered a distinctive feature of the cachexia syndrome. The loss of muscle mass has also been observed in patients with type 2 diabetes mellitus 2 (DM2), and it is recognized that in patients with DM2 and patients who develop cachexia, insulin resistance is present and limits muscle anabolism in several tissues. A high contribution of gluconeogenesis in the production of glucose could limit the availability of amino acids for the synthesis of muscle proteins, making it possible to aggravate the muscle loss.
Since it has shown antitumor properties, metformin has been suggested as a viable option to treat cancer patients, moreover, by reducing gluconeogenesis and improving insulin sensitivity, metformin is proposed as a possible agent in the treatment of cachexia syndrome.

IV. REFERENCES

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V. PROJECT CONTRIBUTION FOR THE ADVANCEMENT OF KNOWLEDGE IN ITS OWN SUBJECT AND AREA OF KNOWLEDGE

JUSTIFICATION:

The protocol is an original study that proposes, through a randomized clinical trial, to evaluate the effect of the use of metformin on the progression-free survival, overall survival as well as biochemical and nutritional parameters in patients with Non-small cell Lung Cancer harboring *EGFR* mutations who are treated with TKIs. Since this is an internal study, patients will be provided with medications, TKIs and metformin, whose cost will be covered by the research budget.

The use of metformin has been studied for several years, accrediting protective effects against several types of cancer, as well as increased response in standard treatment regimens for lung cancer, however, this has only been seen in retrospective studies, and at this time, there is no published prospective data.

A previous study from our research group reported that the population treated with standard dose metformin for type 2 diabetes mellitus has a greater overall survival compared with those who do not take metformin (25.6 vs. 18.3 months, p = 0.046), HR 0.54 ([0.34-0.85] p = 0.009). Another study suggests that the dose used in diabetic patients prolongs the progression-free survival in patients with lung cancer and concomitant diabetes (39).

Metformin is a drug used widely in the management of type 2 Diabetes Mellitus as the first-line of treatment in newly diagnosed patients, since it has few adverse effects and hypoglycemia is exceptional. Several preclinical trials have been conducted in which the effect of this drug alone and in combination with TKI on the proliferative activity of cell lines with and without *EGFR* mutation is tested.

VI. OBJECTIVES

Primary

- Assess the therapeutic effect of the concomitant use of TKIs and Metformin vs. TKIs alone, in terms of the progression-free survival in patients with non-small cell lung cancer.

Secondary

- To evaluate the functional status of patients receiving concomitant treatment with metformin and TKIs and those receiving treatment with TKIs alone, through outpatient evaluation, imaging and laboratory studies.
- To evaluate the effect of using metformin concomitantly with TKIs on overall survival compared with patients receiving TKIs alone.
- To evaluate the effect of the use of metformin on nutritional and body composition parameters in patients with NSCLC treated with TKIs and compare them with patients receiving TKIs alone.
- To evaluate the effect of using metformin on the quality of life of NSCLC patients treated with TKIs and compare it with patients treated with TKIs alone.
- To evaluate the effect of metformin treatment on the plasmatic levels of IGF-1 and IL-6 as possible prognostic markers for treatment response to the combination of TKIs plus metformin.
- To evaluate the expression of LKB-1 on tumor tissue as a prognostic marker.

VII. HYPOTHESES

- Patients with advanced stage NSCLC treated with TKIs plus metformin will have an increase in progression-free survival compared with patients treated with TKIs alone.
- Patients with advanced stage NSCLC treated with TKIs plus metformin will have a higher ORR compared with patients treated with TKIs alone.
- Patients with advanced stage NSCLC treated with TKIs plus metformin will have a longer OS compared with patients treated with TKIs alone.
- Patients with advanced stage NSCLC treated with TKIs plus metformin will have lower levels of IL-6 and IGF-1 in serum compared with patients treated with TKIs alone.

- Patients with advanced stage NSCLC treated with TKIs plus metformin will have a lower muscle mass loss and improved nutritional state compared with patients treated with TKIs alone.

- Patients with advanced stage NSCLC treated with TKIs plus metformin will have better quality of life compared with patients treated with TKIs alone.

VIII. GOALS PER YEAR

- During the first 12 months, 80% of the patients required for the sample size completion will be recruited at the thoracic oncology unit.
- At 12 months, a preliminary analysis of the acquired data will be presented.
- During months 12-24 the patient enrollment will be completed and follow-up will be continued.
- During months 20-24 the paper will be drafted using the final analysis.

X. RESEARCH AND METHODOLOGY STRATEGIES

Describe in full detail the research design, study universe, sample size computation, inclusion and exclusión criteria, randomization process, procedures, methods and techniques which will be used for the analysis and interpretation of data.

Design

Randomized clinical assay, which will evaluate the use of metformin in combination with EGFR-TKIs compared with treatment with TKIs alone, on several parameters including survival, nutrition, biochemical variables and quality of life in patients with advanced stage NSCLC.

Inclusion criteria

1.-Histologically confirmed diagnosis of advanced stage non-small cell lung cancer diagnosis, non-operable, recurrent or metastatic.

2.-Measurable disease: Lesions which can be measured in a precise and reproducible manner in at least one dimension. The smallest lesion is sized at 10 mm, lymph nodes are considered evaluable if their diameter is at least 15 mm.

3.-18 years of age or older.

4.-ECOG performance status 0-2.

5.-Life expectancy of at least 12 weeks.

6.-Patients with documented *EGFR* mutations.

7.- Patients who are naive to EGFR-TKI treatment.

8.- Patients who had received first-line treatment with any type of chemotherapy.

9.- Non-diabetic patients.

10.- Patients who have not received treatment with metformin.

11.-Granulocyte count ≥1.5x 109/L and platelet count >100x 109/L.

12.-Serum bilirubin at least ≤1.5 the upper normal limit.

13.-AST and/or ALT ≤ 2 the upper normal limit (or ≤ 5 x the upper normal limit when it is clearly caused by the presence of hepatic metastases).

14.-Serum creatinin ≤ 1.5 (upper limit) or creatinin clearance ≥ 60ml/min.

15.- Glycated hemoglobin < 5.5

16.-Ability to fulfill the study and follow-up procedures.

17.-A negative pregnancy test must be obtained from all potentially child-bearing women within the previous 72 hours before treatment start.

18.-All fertile patients must use a high-efficacy contraceptive method.

19.-Signed informed consent for participation in the study.

Exclusion criteria

Patients who fulfill any of the below mentioned exclusion criteria must not be recruited for this study.

1.-Presencce of any unstable disease (including but not limited to active infection, grade 4 hypertension, unstable angina, congestive heart failure, hepatic disease, renal disease).

2.-Patients diagnosed with type 2 diabetes mellitus or receiving treatment with metformin.

3.-Patients who have already received or are currently receiving treatment (in any line) with EGFR-TKIs.

4.-Previous history of any other malignant disease in the 5 years previous to study start (except *in situ* cervical cancer or basal cell carcinoma with adequate treatment.

5.-Patients with recently diagnosed brain metastases or spinal cord compression who have not been treated with surgery and/or radiotherapy; patients with these conditions who have received adequate treatment and have evidence of stable disease (clinically stable in imaging studies) for at least 2 months may be included in the study.

6.-Patients unable to receive oral medication, who require intravenous nutrition, or who have been subjected to surgical procedures which might impact gastrointestinal absorption, or who present an active peptic ulcer.

7.-Lactating women.

Elimination criteria

1.- Failure to follow the protocol norms.

2.- Loss to follow up.

3.- Patients who do not wish to continue participating in the study.

4.- Patients who present serious , uncontrollable, adverse events.

Study procedures

This study will recruit patients with advanced stage, non-operable, non-small cell lung cancer with documented *EGFR* mutations, according to the inclusion/exclusion criteria previously described.

Patients will receive an EGFR-TKI (choice of TKI will be at the discretion of the attending physician), and will be randomized into the control arm (TKI alone) or the experimental arm (TKI plus metformin).

All patients will be under treatment with one of the following EGFR-TKI: Gefitinib 250 mg once a day, oral route; Afatinib 50 mg once a day, oral route; erlotinib 150 mg once a day, oral route. Each treatment cycle will be 28 days in length; the study dose can be adjusted according to the toxicity criteria established by the medical oncologist.

Patients in the experimental arm will additionally receive treatment with metformin (500 mg. b.i.d.) since day one, and will continue with metformin treatment until serious toxicity.

Metformin will be administered in the form of tablets. Prolonged-release metformin will be used (Dabex XR; 500 mg) with the objective of reducing the adverse effects associated with metformin treatment and improve treatment adherence (40-42). Treatment will be started at 1000 mg each day; however a dose reduction might be considered to 500 mg each day when an adverse event is observed with the initial dose.

Patients will be followed at the end of each treatment cycle. Adverse events, dose adjustments, nutritional status, quality of life and blood samples for biochemical parameters will be obtained and recorded in the clinical file.

Possible biomarker evaluation

A blood sample consisting of 10 mL will be drawn using two EDTA tubes on day 1 of treatment start, after 2 cycles of treatment and upon TKI progression. Serum will be obtained in order to determine serum levels of IL-6 and IGF-1 using an ELISA-based method.

Assessment of mutations in tumor tissue

Tumor tissue will be obtained in order to determine the expression of LKB1 (Liver Kinase B1), since previous studies have identified that NSCLC patients with presence of the mutated LKB1 gene have a better prognosis. Lack of expression for this mutation has been associated with certain histological tumor subtypes, with a worse prognosis. This has also been associated with less-differentiated tumor cells and a lower overall survival.

Nutritional assessment:

Body weight and height measurements: These measures will be obtained using calibrated scale and stadiometer, without shoes and using a hospital gown.

Anorexia questionnaire ("Functional assessment of anorexia-cachexia" [FAACT]).

Previously validated evaluation instrument which takes into account patient perception of appetite, food intake, weight and overall health status and global functioning. Patients with a score ≤ 24 points, are diagnosed with anorexia. This questionnaire will be filled out via a patient interview.

Subjective Global Assessment (SGA):

Previously validated evaluation instrument for oncology patients, which takes into account the following parameters: change in body weight, changes in food intake, gastrointestinal symptoms, changes in Functional capacity; physical examination (loss of body fat, loss of muscle mass, edema, etc.). This assessment will be administered to the patient by a certified nutritionist and according to the global score patients will be classified as: (A) good nutritional status; (B) malnutrition risk or mild or moderate malnutrition; (C) serious malnutrition. This questionnaire will be administered to the patients via an interview. Body weight and body height will be obtained from the previous measurement.

Food consumption frequency questionnaire (SNUT)

The system for the Evaluation of Nutrition Habits and Food Consumption (SNUT in spanish) is a questionnaire which evaluates the frequency of food intake and has a complimentary software developed by the National Institute of Public Health in Mexico in 2003. It was designed to evaluate the long-term exposure in terms of different food types, and study their potential role as a risk factor for the development of cardiovascular disease. This questionnaire will be given to all study participants in order to assess how frequently they consume the food items listed along the instrument, giving 9 possible answers: never; less than once a month; 1-3 times a month; once a week; 2-4 times a week; 5-6 times a week; once a day; 2-3 times a day; 4-5 times a day; 6 times a day. Once these data are incorporated into the software, we will obtain the average intake of nutrients. This questionnaire will be given in an interview format.

Body composition evaluation

Imaging studies will be carried out using the Slice-O-Matic v. 4.3 software. We will select lumbar vertebrae L3 as an anatomical landmark. Different body compartments according to body composition will be identified through their anatomic characteristics, and will be quantified according to the pre-established values: muscle mass (-29 to +150 Houdsfield units [UH]), subcutaneous and intramuscular adipose tissue (190 to -30 UH), visceral adipose tissue (-150 to -50 UH). The nutritional assessment and body composition analyses will be performed monthly in both study groups.

Intervention

The original container with the medication will be given to each patient at each follow-up visit. The medication must be stored in its original package, in a clean and dry place (avoid humid environments) and with a temperature above 25° Celsius.

Sample calculation

The sample size was calculated for a two-sample comparison progression-free survival function with the Log-Rank test using the Freedman method, to observe an effect size correspondent to a HR of 0.47 between the therapeutic arms to prove the next hypothesis: Ho: S1(t) = S2(t). Additionally, the Type I Error (alpha) was set as 0.20. Therefore, the estimated sample number is 124. Finally, 14 (10%) patients were added to the whole sample size, to account for losses, with an expected simple of 138.

Hypothesis:

$$Ho: S1(t) = S2(t) \text{ vs. } S1(t) \neq S2(t)$$

Assumptions:

Using the Friedman method (1982), the following was considered for the sample calculation:

$$HR: S2(t) = \{S1(t)\}\ \Delta$$

Therapeutic group assignment

Candidate patients will be selected as those who fulfill the inclusion criteria. Patients will be invited to participate in the study. All study objectives and procedures will be extensively explained as by the informed consent document. If the patient agrees and signs the informed consent, he or she will be assigned to one of the two study arms in a 1:1 randomization which will be obtained using a random numbers table. The experimental group will receive treatment with standard EGFR-TKIs plus metformin 500 mg b.i.d, while the control group will receive EGFR-TKIs alone.

Duration of the study

The study will complete patient enrollment in the course of 18 months. Following, patients will be followed a minimum of 6 months longer in order to acquire data. The full study is planned for 24 months.

Statistical considerations and analyses plan

The sample size for this clinical trial is estimated at 138 patients, which will be randomized into two treatment arms (experimental arm: treatment with EGFR-TKIs plus metformin 500 mg b.i.d; control arm: treatment with EGFR-TKIs alone). The total duration for this study is estimated at 24 months.

All patients who receive at least one dose of metformin will be included in a descriptive safety analysis. Safety parameters will be presented in the form of tables, and will describe all safety considerations.

Continuous variables will be summarized as arithmetic means, with standard deviations or medians and range according to data distribution assessed with Kolmogorov-Smirnov test. For descriptive purposes, categorical variables will be summarized as frequencies and percentages. Cross-sectional inferential comparisons will be made using the Mann-Whitney U test. Paired comparisons will be performed using the Wilcoxon-rank test. The $\chi 2$ test or Fisher exact test were used for assessing the statistical significance of categorical variables. Both, OS and PFS will be analyzed with the Kaplan-Meier method. Comparisons among the subgroups will be analyzed using the log-rank test. Statistically significant and borderline significant variables ($p<0.1$) will be included for the adjustment in the multivariate Cox regression model and hazard ratios (HR) will be calculated alongside with their corresponding 95% CIs as a measure of association. Statistical significance is determined as $p<0.05$ using a 2-tailed test. SPSS software version 20 (SPSS Inc., Chicago, IL) will be used for statistical analysis and STATA and R will be used for plotting.

Variables and interpretation

Response rate or objective response

Each subject will be assessed and the best objective response will be recorded as per investigator decision (according to RECIST criteria previously described). This will be defined as the best response recorded since the beginning of treatment until disease progression/recurrence. For patients with a partial response (PR) or complete response (CR) status, changes in tumor size must be confirmed through sequential assessments which will need to be performed in no less than 4 weeks after the response criteria was met for the first time.

CT-scans will be performed every 2 months in order to evaluate treatment response. Objective response will be summarized in a descriptive manner.

Disease control rate

This is defined as the sum of PR, CR and stable disease (SD), excluding progressive disease (PD). All these are measured using the RECIST 1.1 criteria.

Progression-free Survival (PFS)

PFS is defined as the time since treatment start until the date of the first documented evidence of progression (according to RECIST 1.1 criteria) or the time of death for any reason in the absence of progressive disease. For patients who have not progressed or died at the time of the final analysis, the last follow-up date will be used.

Overall Survival (OS)

OS will be determined since the date of treatment start, until the date of death, independently of cause of death. For patients who have not died at the time of the final analysis, the last follow-up date will be used.

Observations for patients who did not experience either event (progression or death) will be censored at patient-specific last follow-up.

Both survival outcomes will be plotted using the Kaplan-Meier method.

FOLLOW-UP PLAN

Visit schedule

1.- Screening/inclusion: In this visit, the necessary information to assess whether the patient is eligible to participate in the study will be collected and made readily available to the attending physician in order to allow for patient inclusion should this be adequate. Laboratory information will be assessed, patients will be expressly asked concerning recent use of medication. The informed consent must be presented and signed at this time, and the patient record will be screened in order to include the following information in the study database:

Demographic data (sex, birthdate, race), history of substance abuse, data regarding the disease (date of histological diagnosis), site of the primary tumor, number and location of metastases, TNM staging, previous surgeries, previous therapies, general clinical history information, functional status, physical exploration findings, urine pregnancy test.

2.- Safety evaluation visits: Safety will be recorded on the day of recruitment, at 2 weeks, 4 weeks and every 4 weeks thereafter. This will be open to more visits in particular cases. Monitoring of adverse events as per CTCAE (reports of events, hospitalizations, emergency treatments, new medication and general patient state). Patients will be asked regarding therapeutic compliance.

3.- Study termination and patient follow-up: When persistent grade 3-4 toxicity which does not yield to metformin dose reductions and requires treatment withdrawal is documented, patient participation will be finalized. At this point, functional status, physical examination, vital signs and toxicity information will be collected. Follow-up will continue until death or loss to follow-up.

Laboratory and other assessments: Pregnancy tests, CBC with differential, PT, INR and aPTT, comprehensive serum chemistry panel, urianalysis, T2, FT4 and TSH, lipids panel will be performed every 28 days. Glycated hemoglobin will be evaluated every 3 months and imaging

studies using CT-scans will be performed every 2 months.

Quality of life assessment

Quality of life (QoL) may vary significantly in lung cancer patients due to several causes, including disease progression, treatment-related adverse effects and other variables. Therefore, it is recommended that QoL be assessed continuously in these patients.

A multidimensional evaluation is necessary in this disease entity, due to the fact that symptoms are extensive, and the toxicity which might be caused by some medications generates a wide array of effects. Because of tis, it is often the case that health personnel centers the quality of life evaluation on physical spheres alone, meanwhile it is known that lung cancer affects other areas, including the social, psychological and biological functioning of the patient and his or her surroundings.

QoL will be determined using the previously validated instrument “The European organization for research and treatment of cancer quality of life questionnaire (QLQ)-C30 and (QLQ)LC-13. Both of which have been previously validated in Mexican patients.

The QoL questionnaire will be given to patients every two months, and the data interpretation will be performed using a paired variable analysis.

Measure instrument: EORTC Study group on quality of life

Variable: Discontinuous, quantitative

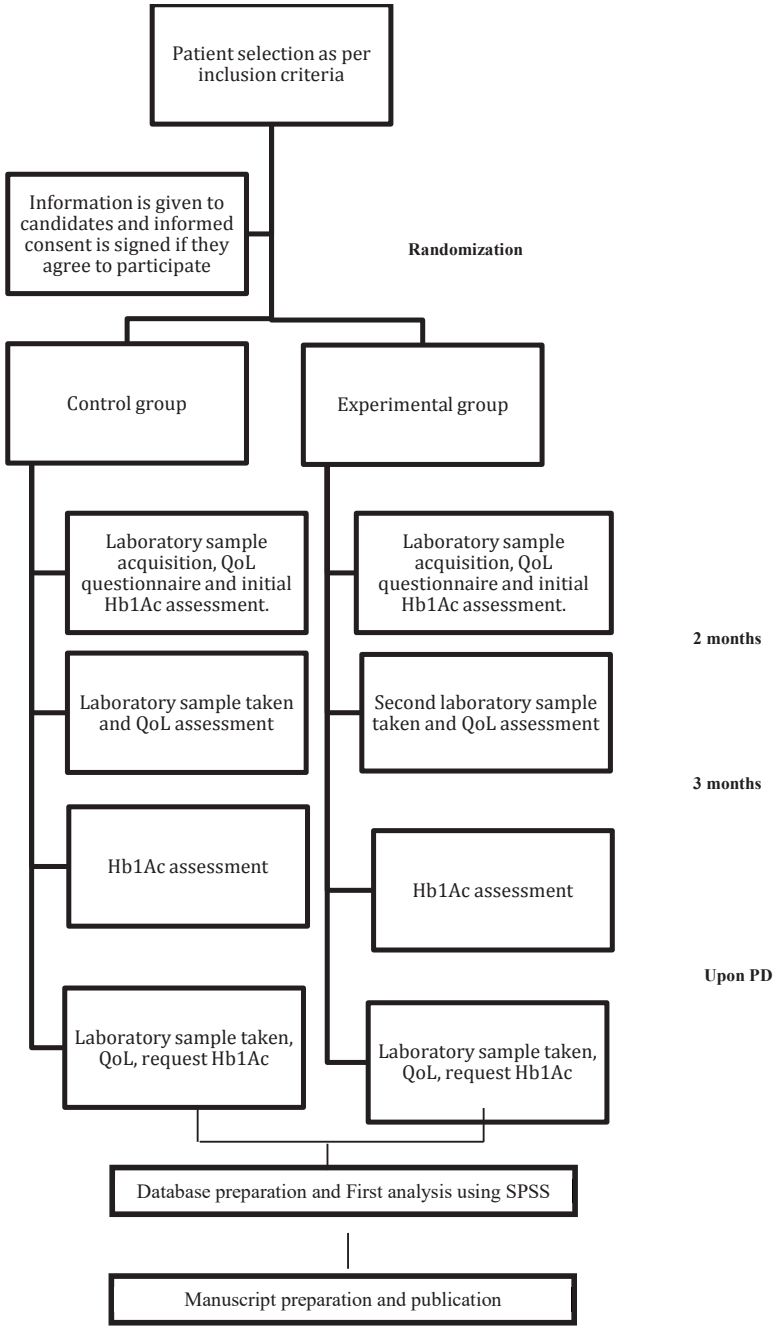
Interval: Score ranges from 1 to 100, the higher the score the better the QoL.

Toxicity assessment

The most common adverse events related to metformin use include: nausea, diarrhea, vomit, anorexia and gastritis. Management of these adverse events is symptomatic and ambulatory (ondansetron, loperamide, riopan), unless the patient presents with serious toxicity which will require treatment suspension and will be treated while the patient is hospitalized (i.e. IV fluids for the treatment of moderate-severe dehydration).

Allergic reactions (swelling, skin redness or itching and shortness of breath): In the case an allergic reaction to metformin presents in a patient, treatment will be definetly suspended and the patient will be managed using antihistamine drugs when the reaction is mild-moderate. The patient will be admitted to the hospital and treated with IV steroids or epinephrine in the case a severe allergic reaction or anaphylactic shock occurs.

As with new substances and new combinations, the use of metformin concomitantly with EGFR-TKIs might produce new and unknown adverse events, which will be closely monitored at each patient visit.



X. ETHICAL CONSIDERATIONS

Describe the ethical considerations of the study:

1. SAMPLE ACQUISITION

This study considers additional studies to the ones routinely performed in the laboratory of personalized medicine in the National Cancer Institute of Mexico. Samples will be taken according to the guidelines for Good Clinical Practice (GCP).

2. INFORMED CONSENT REQUEST

It will be the primary investigator´s (PI) responsibility (or a person specifically designated by the PI, if accepted by local regulation), to obtain each participant´s signature on the informed consent document, this only after an adequate and through explanation of the purpose, methods, objectives and potential risks of participating in this study. Similarly, all subjects must be made aware that they are completely free to refuse participation in this study or to withdraw their consent at any time point for any reason.

In the case that an eligible subject is not qualified or is incapable of giving legal consent, this written consent must be obtained from a legally acceptable representative. In the case when both the subject and his or her legal representative are unable to read and fully understand the informed consent document, an impartial witness must be present during the entire duration of the informed consent discussion. Once the subject and his or her legal representative have verbally accepted to participate in the study, the witness must sign the document in order to attest that the information contained in this document was precisely explained and fully understood by the participant. The PI or the designated representative will also explain that the subjects are completely free to refuse participation in this study, or to withdraw from the study at any time point and for any reason. The case report forms will contain a section to document that the informed consent for every subject must be fully and adequately filled out. If at any point the acquisition of new data regarding safety will consequentially bring significant changes in terms of risk/benefit for the patients, the new information will be taken into consideration in order to revise and update the informed consent, should it be necessary. This new information will be provided to all subjects (including those already under treatment), they will receive a new revised copy of the form and their signature will be requisite to continue participating in the study.

Should a situation arise which endangers the patient´s life, in which the patient is unconscious or is incapable of communicating for any other reason whatsoever, the emergency will be such that there may not be enough time to obtain the informed consent from the legal representative of the patient, and if there is no other or better available treatment, it is acceptable to treat the patient according to protocol with the consent of the PI as well as the consent of another physician who is not directly involved in the study. The necessary documents in this situation must be sent to the Institutional Review Board (IRB) within the next 5 days of the event. In the case that this cooperation is not immediately possible, a written evaluation from another independent physician must be obtained and sent to the IRB within 5 days of the treatment administration. Additionally, the patient and his or her legal representative must be informed of the procedure as soon as possible and must then agree to continue, by signing a written informed consent as previously described.

3. AGREEMENTS FOR PATIENT COMPENSATION FOR POTENTIAL DAMAGES DERIVED FROM PARTICIPATING IN THIS STUDY

This study protocol originates as a local initiative in our Thoracic Oncology Unit. In this study we aim to provide patients with treatment for their condition, and therefore any potential damage which is derived from the study intervention will be covered using the research budget.

1. DECLARATION OF HELSINKI

Clinical Research (Medical investigation combined with profesional attention)

1. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient’s best interest when providing medical care.”

2. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician’s knowledge and conscience are dedicated to the fulfillment of this duty.

3. Medical progress is based on research that ultimately must include studies involving human subjects.

4. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality

5. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

6. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

7. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

8. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

9. Medical research should be conducted in a manner that minimises possible harm to the environment.

10. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

11. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

12. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

13. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

14. In medical practice and in medical research, most interventions involve risks and burdens.

15. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

16. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

17. Measures to minimize the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study. En el tratamiento de la persona enferma, el médico debe tener la libertad de usar un nuevo método diagnóstico y terapéutico, Si a su juicio ofrece la esperanza de salvar una vida, restablecer la salud o aliviar el sufrimiento.

Mark with an X

[X] I accept
[ ] I do not accept

5. **INCOME DECLARATION OF PARTICIPATING PHYSISCIANS DERIVED FROM THE STUDY**

This study protocol will not interfere with routine institutional resources and procedures, it will provide patients with a therapeutic option not widely available in Mexico and therefore no payment, donation or special request will be obtained by any of the participating individuals.

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. *set more off
. *stset slp_139, failure (progres1=1)
. *stsum, by(brain_mets)
. *stci, by(brain_mets)
. *stcox i.brain_mets
. *sts test brain_mets
. *sts graph, by(brain_mets) risktable censored(single)
. *restore
. * 11 Liver metastases
. *preserve
. *set more off
. *stset slp_139, failure (progres1=1)
. *stsum, by(liver_mets)
. *stci, by(liver_mets)
. *stcox i.liver_mets
. *sts test liver_mets
. *sts graph, by(liver_mets) risktable censored(single)
. *restore
. * 12 Mutation status
. *preserve
. *set more off
. *stset slp_139, failure (progres1=1)
. *stsum, by(mut)
. *stci, by(mut)
. *stcox i.mut
. *sts test mut
. *sts graph, by(mut) risktable censored(single)
```

```
. *restore
. * 13 EGFR TKIs
. *preserve
. *set more off
. *stset slp_139, failure (progres1=1)
. *stsum, by(EGFR_TKI)
. *stci, by(EGFR_TKI)
. *stcox ib3.EGFR_TKI
. *sts test EGFR_TKI
. *sts graph, by(EGFR_TKI) risktable censored(single)
. *restore
. * 14 Objective Response Rate (ORR)
. *preserve
. *set more off
. *stset slp_139, failure (progres1=1)
. *stsum, by(orr)
. *stci, by(orr)
. *stcox i.orr
. *sts test orr
. *sts graph, by(orr) risktable censored(single)
. *restore
. * 15 Disease control rate (DCR)
. *preserve
. *set more off
. *stset slp_139, failure (progres1=1)
. *stsum, by(dcr)
. *stci, by(dcr)
. *stcox i.dcr
. *sts test dcr
. *sts graph, by(dcr) risktable censored(single)
. *restore
. * 16 Intervention (Therapeutic arm)
. *preserve
. *set more off
. *stset slp_139, failure (progres1=1)
. *stsum, by(intervention)
. *stci, by(intervention)
. *stcox i.intervention
. *sts test intervention
. *sts graph, by(intervention) risktable censored(single)
. *restore
. * 17 LKB-1
. *preserve
. *set more off
```

```
. *stset slp_139, failure (progres1=1)
. *stsum, by(lkb1)
. *stci, by(lkb1)
. *stcox i.lkb1
. *sts test lkb1
. *sts graph, by(lkb1) risktable censored(single)
. *restore
. * 18 Previous treatment
. *preserve
. *set more off
. *stset slp_139, failure (progres1==1)
. *stsum, by(tto_previo)
. *stci, by(tto_previo)
. *stcox i.tto_previo
. *sts test tto_previo
. *sts graph, by(tto_previo) risktable censored(single)
. *restore
. * 19 2nd Line
. *preserve
. *set more off
. *stset slp_139, failure (progres1=1)
. *stsum, by(segunda_linea)
. *stci, by(segunda_linea)
. *stcox i.segunda_linea
. *sts test segunda_linea
. *sts graph, by(segunda_linea) risktable censored(single)
. *restore
. * 20 3rd Line
. *preserve
. *set more off
. *stset slp_139, failure (progres1=1)
. *stsum, by(tercera_linea)
. *stci, by(tercera_linea)
. *stcox i.tercera_linea
. *sts test tercera_linea
. *sts graph, by(tercera_linea) risktable censored(single)
. *restore
.
. *preserve
. *set more off
. *stset slp_139, failure (progres1==1)
. *sw, pe (0.10)lockterm1:stcox intervention gender age ///,
> *smk wse adeno_gp bone_mets pleural_mets cl_mets ///,
> *adrenal_mets brain_mets liver_mets mut ///,
```

> *EGFR_TKI orr dcr segunda_linea tercera_linea
. *estat phtest
. *estat ic
. *restore
. *preserve
. *set more off
. *stset slp_139, failure (progres1==1)
. *sw, pe (0.05)lockterm1:stcox intervention gender age ///,
> *smk wse adeno_gp bone_mets pleural_mets cl_mets ///,
> *adrenal_mets brain_mets liver_mets mut ///,
> *EGFR_TKI orr dcr segunda_linea tercera_linea
. *estat phtest
. *estat ic
. *restore
. *preserve
. *set more off
. *stset slp_139, failure (progres1==1)
. *sw, pe (0.05):stcox intervention gender age ///,
> *smk wse adeno_gp bone_mets pleural_mets cl_mets ///,
> *adrenal_mets brain_mets liver_mets mut ///,
> *EGFR_TKI orr dcr segunda_linea tercera_linea
. *estat phtest
. *estat ic
. *restore
. * Note: LKB1 was excluded a priori from multivariable model
. * due to the low frequency of available data (n=24)
.
. *0 Overall
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum
. *stci
. *sts graph, risktable censored(single)
. *restore
. * 1 Sex
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(gender)
. *stci, by(gender)
. *stcox i.gender
. *sts test gender
. *sts graph, by(gender) risktable censored(single)

```
. *restore
. * 2 Age
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(age_groups)
. *stci, by(age_groups)
. *stcox c.age
. *streg age, dist(exponential) tr
. *sts test age
. *sts graph, by(age_groups) risktable censored(single)
. *restore
. * 3 Smoking status
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(smk)
. *stci, by(smk)
. *stcox i.smk
. *sts test smk
. *sts graph, by(smk) risktable censored(single)
. *restore
. * 4 Wood-smoke exposure
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(wse)
. *stci, by(wse)
. *stcox i.wse
. *sts test wse
. *sts graph, by(wse) risktable censored(single)
. *restore
. * 5 Adenocarcinoma Subtype
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(adeno_gp)
. *stci, by(adeno_gp)
. *stcox i.adeno_gp
. *sts test adeno_gp
. *sts graph, by(adeno_gp) risktable censored(single)
. *restore
. * 6 Bone metastases
. *preserve
```

```
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(bone_mets)
. *stci, by(bone_mets)
. *stcox i.bone_mets
. *sts test bone_mets
. *sts graph, by(bone_mets) risktable censored(single)
. *restore
. * 7 Pleural metastases
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(pleural_mets)
. *stci, by(pleural_mets)
. *stcox i.pleural_mets
. *sts test pleural_mets
. *sts graph, by(pleural_mets) risktable censored(single)
. *restore
. * 8 Contralateral metastases
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(cl_mets)
. *stci,by(cl_mets)
. *stcox i.cl_mets
. *sts test cl_mets
. *sts graph, by(cl_mets) risktable censored(single)
. *restore
. * 9 Adrenal metastases
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(adrenal_mets)
. *stci, by(adrenal_mets)
. *stcox i.adrenal_mets
. *sts test adrenal_mets
. *sts graph, by(adrenal_mets) risktable censored(single)
. *restore
. * 10 Brain metastases
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(brain_mets)
. *stci, by(brain_mets)
```

```
. *stcox i.brain_mets
. *sts test brain_mets
. *sts graph, by(brain_mets) risktable censored(single)
. *restore
. * 11 Liver metastases
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(liver_mets)
. *stci, by(liver_mets)
. *stcox i.liver_mets
. *sts test liver_mets
. *sts graph, by(liver_mets) risktable censored(single)
. *restore
. * 12 Mutation status
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(mut)
. *stci, by(mut)
. *stcox i.mut
. *sts test mut
. *sts graph, by(mut) risktable censored(single)
. *restore
. * 13 EGFR TKIs
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(EGFR_TKI)
. *stci, by(EGFR_TKI)
. *stcox ib3.EGFR_TKI
. *sts test EGFR_TKI
. *sts graph, by(EGFR_TKI) risktable censored(single)
. *restore
. * 14 Objective Response Rate (ORR)
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(orr)
. *stci, by(orr)
. *stcox i.orr
. *sts test orr
. *sts graph, by(orr) risktable censored(single)
. *restore
```

```
. * 15 Disease control rate (DCR)
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(dcr)
. *stci, by(dcr)
. *stcox i.dcr
. *sts test dcr
. *sts graph, by(dcr) risktable censored(single)
. *restore
. * 16 Intervention (Therapeutic arm)
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(intervention)
. *stci, by(intervention)
. *stcox i.intervention
. *sts test intervention
. *sts graph, by(intervention) risktable censored(single)
. *restore
. * 17 LKB-1
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(lkb1)
. *stci, by(lkb1)
. *stcox i.lkb1
. *sts test lkb1
. *sts graph, by(lkb1) risktable censored(single)
. *restore
. * 18 Previous treatment
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(tto_previo)
. *stci, by(tto_previo)
. *stcox i.tto_previo
. *sts test tto_previo
. *sts graph, by(tto_previo) risktable censored(single)
. *restore
. * 19 2nd Line
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
```

```
. *stsum, by(segunda_linea)
. *stci, by(segunda_linea)
. *stcox i.segunda_linea
. *sts test segunda_linea
. *sts graph, by(segunda_linea) risktable censored(single)
. *restore
. * 20 3rd Line
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *stsum, by(tercera_linea)
. *stci, by(tercera_linea)
. *stcox i.tercera_linea
. *sts test tercera_linea
. *sts graph, by(tercera_linea) risktable censored(single)
. *restore
.
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *sw, pe (0.10):stcox intervention gender age ///,
> *smk wse adeno_gp bone_mets pleural_mets cl_mets ///,
> *adrenal_mets brain_mets liver_mets mut ///,
> *EGFR_TKI orr dcr segunda_linea tercera_linea
. *estat phtest
. *estat ic
. *restore
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *sw, pe (0.05)lockterm1:stcox intervention gender age ///,
> *smk wse adeno_gp bone_mets pleural_mets cl_mets ///,
> *adrenal_mets brain_mets liver_mets mut ///,
> *EGFR_TKI orr dcr segunda_linea tercera_linea
. *estat phtest
. *estat ic
. *restore
. *preserve
. *set more off
. *stset sg_139, failure (status=1)
. *sw, pe (0.05):stcox intervention gender age ///,
> *smk wse adeno_gp bone_mets pleural_mets cl_mets ///,
> *adrenal_mets brain_mets liver_mets mut ///,
> *EGFR_TKI orr dcr segunda_linea tercera_linea
```

```
. *estat phtest
. *estat ic
. *restore
. * Note: LKB1 was excluded a priori from multivariable model
. * due to the low frequency of available data (n=24)
. *
. *END
. clear

. log close
      name:  <unnamed>
       log:  /Users/alejandra/Desktop/jama.log
  log type:  text
 closed on:   6 May 2019, 12:21:57
-------------------------------------------------------------------------------
```