Analysis of Variables **Pre**dicting **P**athological Complete Response and Immune related adverse events in Patients with Resectable Non-Small Cell **L**ung Cancer receiving **N**eoadjuvant Immunotherapy with Chemotherapy – A Prospective Cohort Study

'Pre-PLaN'

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1.0 INTRODUCTION

Lung cancer is the leading cause of cancer-related death in both men and women. Non-small cell lung cancer (NSCLC) accounts for 85% of lung cancers. About 30% present with 'early stage' lung cancer (stages I-IIIA). The implementation of screening programs for lung cancer will increase the incidence and proportion of patients with early-stage lung cancer. Surgical resection has traditionally been and continues to be the standard-of-care (SoC), yet most patients still relapse and die. Adjuvant (post-operative) chemotherapy offers modest additional survival benefit (1), and there remains equipoise in the relative value of adjuvant vs neoadjuvant (pre-operative) chemotherapy (2). Recently, Health Canada approved neoadjuvant immunotherapy (nivolumab, a type of immune checkpoint inhibitor (ICI)) with platinum-based chemotherapy in patients with resectable (IB-IIIA) NSCLC, based on a recent large prospective trial (CheckMate 816), showing better event-free survival (EFS) and overall survival (OS), and markedly increased pathological complete responses (pCR), vs chemotherapy alone (3,4) (Figure 1 and 2). Achievement of pCR resulted in markedly prolonged EFS (*i.e.,* a very low risk of relapse, hazard ratio (HR) 0.13) compared to any other response status; response status, therefore, seems to be a critically important early endpoint with considerable predictive power for the presence or absence of later events (Figure 3). Actually, pCR has been used as a surrogate of survival in neoadjuvant therapy trials in numerous disease sites. Further trials and updates (*e.g.,* NADIM II) have been confirmatory, which has propelled neoadjuvant chemoimmunotherapy towards becoming the new SoC (5–7). pCR rates in CM816 and NADIM II were 24% and 37% respectively with neoadjuvant nivolumab plus chemotherapy compared to 2.2% and 7% with neoadjuvant chemotherapy alone (3,5,7). This approach also led to improved major pathological responses (MPR), overall response rates (ORR), EFS and OS in CheckMate 816, and improved 3-year-progression free survival (PFS) and OS in NADIM II.

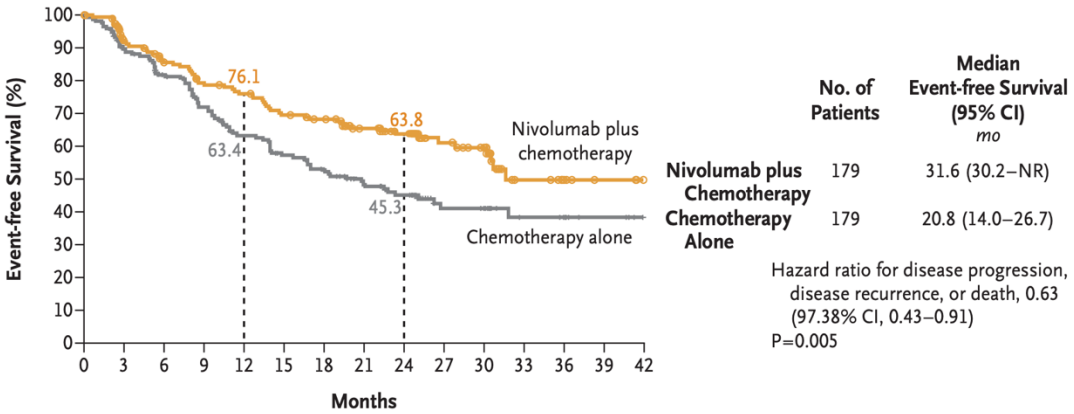

Figure 1. Event-free survival in patients in CM816 (3).

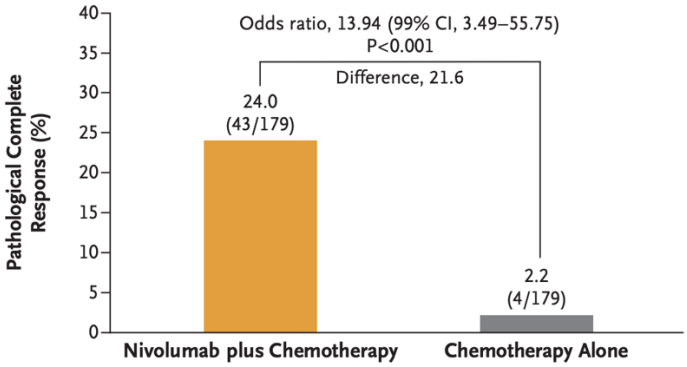

Figure 2. Pathological complete response in CM816 (3).

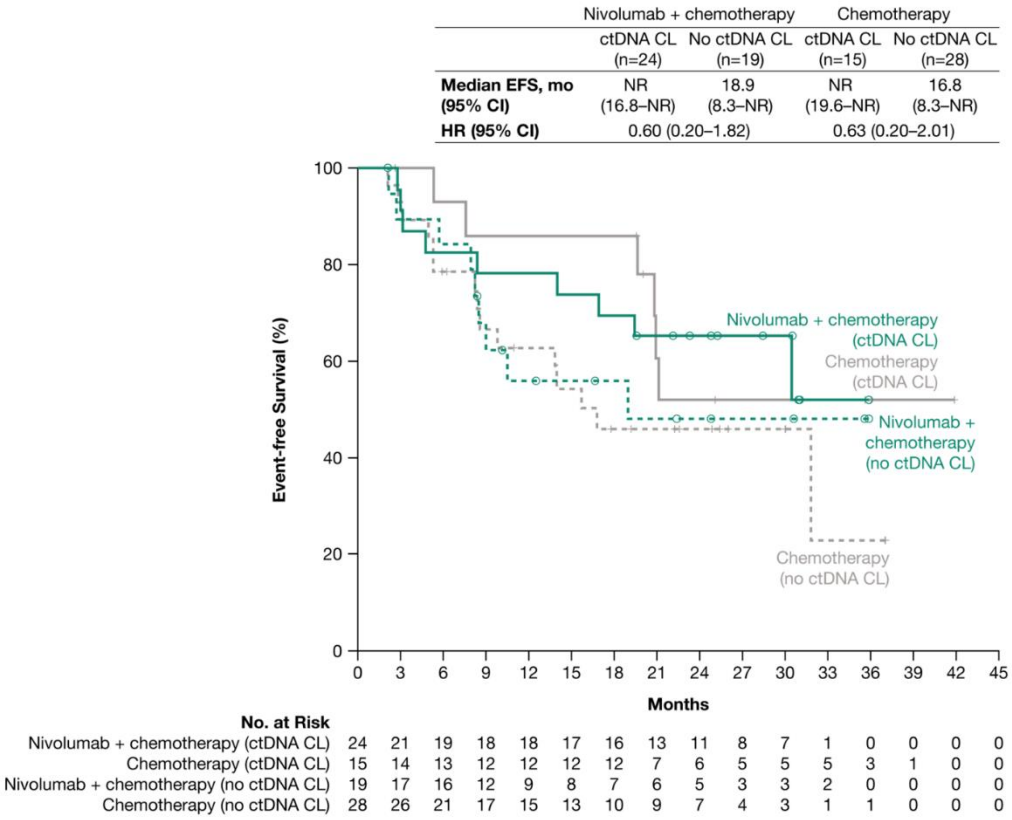

Figure 3. Event-free Survival in Patients with or without a Pathological Complete Response (3).

In NADIM II trial survival benefit is especially observed in patients with tumors with positive PD-L1 expression and in those patients achieving pCR. As an exploratory analysis in NADIM II (only 28 patients with full data), multimodal analysis of variables predicting the response to neoadjuvant treatment revealed a model which could predict pCR with an area under curve (AUC) of 0.76 (8). Notably their model

included histology, mutational profile, radiomics, and the neutrophil/lymphocyte ratio. Such a model is not sufficiently accurate to base clinical decisions on.

We propose to improve upon this, by means of a prospective single cohort study to analyze the predictors of pCR in a larger cohort of our patients on the same neoadjuvant immunotherapy plus chemotherapy but employing a range of parameters that are both much wider and considerably more sophisticated. We intend to make use of parameters that reflect both the tumor itself, as well as the integrity of immune system of the host, obviously a critical determinant of immune-mediated efficacy, yet strangely neglected in the literature. We will then seek to develop an initial integrated predictive model for pCR with a good sensitivity/specificity, as a prelude to testing (and if necessary, refining) the model in future work, with a much larger sample. If subsequently validated, such a model would have several important applications.

Noting that immune-related adverse events (irAEs) are strongly associated with efficacy in multiple trials across several disease sites (9–12), we will not only be including the emergence of on-treatment irAEs in our pCR modeling, but also develop an additional model as a subsidiary aim to predict irAEs themselves. The patient's baseline immune status is a critical and heretofore largely neglected common determinant of both efficacy and irAEs, and clearly deserves more attention. The incidence of ≥ grade 3 adverse events (AEs) was 33.5% in CheckMate 816, and there is currently no available way to predict their occurrence.

We would be doing a more comprehensive and sophisticated assessment of both the immune status and the cancer, pre-treatment, than has been reported thus far, and using cutting-edge genomic, flow cytometric, proteomic, pathologic and radiomic technology, will enable a much better predictive system of both benefit and harm, and reveal novel insights into host-tumor biology. This will include demographic, clinical and radiological (PET, radiomics (13), sarcopenia index) data. We would collect laboratory data including neutrophil/lymphocyte ratio, flow cytometry for neutrophils and lymphocyte subsets, gene expression profiling, circulating tumor cells (CTCs), baseline/serial ctDNA, LAG3-expressing T cells, lactate dehydrogenase (LDH) and iso-enzymes (for LDH-A), leukemia inhibitory factor (LIF) (14), cytokines especially interleukin-6 (IL-6) (15), interleukin-7 (16), serum protein electrophoresis, tumor markers and growth/differentiation factor-15 (GDF-15) (17). The LIPI score (Derived NLR (neutrophils/(leucocytes-neutrophils) ratio) and lactate dehydrogenase (LDH)) have been correlated with the efficacy of ICIs in lung cancer (18). The pathologic assessment will also include multiplex analysis for analysis of intra- and peri-tumoral microenvironment cell-type analysis, tumor-infiltrating lymphocyte

subsets e.g., killer CD8+, Tregs, etc., M1 vs M2 macrophages, N1 vs N2 neutrophils, dendritic cells, cancer-associated fibroblasts; angiogenesis score; spatial transcriptomics for highly focused and detailed gene expression analysis matched to cellular infiltrate (19,20) and immunohistochemistry (IHC) for suspected biomarkers (LSD1, CD47). A large next-generation sequencing (NGS) lung panel, specifically including the usual driver mutations, STK-11, and tumor mutation burden (TMB) would be done. Most of above parameters have a prognostic and/or predictive value in lung cancer.

At an institutional/systems level, this new standard of care involves complex interdisciplinary collaboration between thoracic surgeons, respirologists, medical oncologists, pathologists, radiologists, nuclear-medicine specialists, interventional radiologists, medical geneticists, and immunologists. The demands placed on the system will increase and the smooth patient flow through this novel care-path will be challenging. Previously, patients with early resectable lung cancer (based on imaging (PET-CT), were eligible for upfront surgery and then adjuvant chemotherapy. Now, ideally these patients should have an upfront biopsy (which may take few weeks for the intervention and another few weeks for the pathology and molecular results) before beginning neo-adjuvant chemotherapy and immunotherapy. With this neoadjuvant paradigm the standard of care demands interdisciplinary co-ordination to make decisions in the best interests of the patients, keeping in mind patient variables, tumor variables, as well as the complex logistics of waiting lists, biopsy, pathology/molecular result wait times etc. There should be a stress test of the care-delivery system, providing unprecedented opportunities to identify and fix emergent problems under a rigorously defined protocol designed to reflect future standard of care.

We propose a prospective single cohort study to analyze the predictors of pCR and irAEs in patients receiving neoadjuvant immunotherapy and chemotherapy before surgery. We will then develop a predictive model that can predict pCR with a good sensitivity and specificity. If validated in subsequent studies such a model could be used to select patients who are more likely to benefit with neoadjuvant immunotherapy and chemotherapy approach. Such decision support tool will help choose the right patients for treatment with neoadjuvant chemo-immunotherapy versus upfront surgery.

This project, besides having the scientific goals described in the paragraph above, will help improve the timeliness and coordination of care, and serve as a test-run for the future standardized implementation of this paradigm in our center and elsewhere. In summary, this study is a perfect example of coordination of ‘bench (para-clinical departments)’ and ‘bedside (clinical departments)’ working together in real time to improve clinical care and patient outcomes – and at the same time answering a very important clinical question of how to predict which resectable lung cancer patients can best benefit

from neoadjuvant treatment with immunotherapy and chemotherapy and which among them are more likely to have a serious immune related adverse event.

2.0 AIM

To develop a predictive model to predict which patients will develop irAEs and who will achieve a pCR with neoadjuvant chemoimmunotherapy treatment for resectable NSCLC.

3.0 HYPOTHESIS

A model combining predictive variables for pCR in patients with resectable NSCLC treated with neoadjuvant immunotherapy and chemotherapy can predict a pCR with an AUC of at least 0.8.

4.0 OBJECTIVES

4.1 Primary objectives

1. To explore a combination of baseline and treatment-emergent potentially predictive variables, relating to both the tumor and the patient's immune status, including clinicopathological, blood-based, tissue-based, molecular, gene-expression, and radiological, in patients with early-stage NSCLC participant to neoadjuvant immunotherapy and chemotherapy.
2. To curate these data by univariate and multivariate analyses, and to identify those variables independently useful in predicting pCR and occurrence irAEs in these patients.
3. To parsimoniously combine these independently predictive factors into integrated models with adequate sensitivity and specificity, enabling both clinical utility and enhanced scientific insights.

4.2. Secondary objectives

1. To assess pCR, MPR, objective response rates (ORRs), EFS, OS, chemotherapy related toxicity and irAEs in patients with resectable NSCLC treated with neoadjuvant immunotherapy and chemotherapy.
2. To evaluate exploratory and entirely novel potential biomarkers for predicting pCR, MPR, ORR, EFS and irAEs.
3. To assess if a post-treatment (pre-surgery) complete metabolic response by F18-FDG-PET-CT and a blood-only molecular residual disease assay can accurately predict a pCR.
4. To assess whether liquid biopsy for molecular residual disease during follow-up can predict a recurrence of lung cancer.

5. To document, understand, and resolve impediments to the smooth and timely flow of patients on this new standard of care path.

5.0 ENDPOINTS

5.1 Primary endpoints

1) Predictive power of various variables to predict pCR and irAEs.
2) To determine the predictive power (sensitivity, specificity, receiver operator characteristic (ROC) curves) of a model, combining predictive variables, in predicting pCR.
3) To determine whether a similar model can be constructed to predict irAEs.

5.2 Secondary endpoints

1) Assess pCR, MPR, objective response rates (ORR), 24-month EFS, 24-month OS and irAEs.
2) Exploratory novel potential biomarkers.
3) Predictive power of post-treatment (pre-surgery) complete metabolic response by F18-FDG-PET-CT and a blood-only molecular residual disease assay for predicting pCR.
4) To determine if a liquid biopsy for cancer detection, looking for molecular residual disease, during routine follow-up visits can predict a recurrence of lung cancer before or at the same time as conventional imaging.
5) 5a. Time from diagnosis to initiation of treatment
   5b. Time from date of 3rd cycle of chemo-immunotherapy to date of surgery

5.3 Definition of endpoints

- Pathologic Complete Response Rate - Pathological complete response (pCR) rate is defined as number of participants with absence of residual tumor in lung and lymph nodes at surgery, divided by the total number of participants. for each treatment group. Participants who are no longer eligible for surgery, or who are on alternative anticancer therapy before surgery, or who discontinue the study (e.g. withdraw consent) before surgery are all counted as non-responders.

For histologic assessment, all tumor and associated lymph node tissue should be sectioned at 1 cm intervals. For assessments of pathological response, the percentage of viable tumor cells in at least 1 section per centimeter of the tumor and lymph node tissue resected should be evaluated.

- Major Pathological Response Rate - Major pathological response (MPR) rate, defined as number of participants with ≤ 10% residual tumor in lung and lymph nodes at surgery, divided by the number of total participants. Viable tumors with only in situ carcinoma should not be included in MPR calculation. Participants who are no longer eligible for surgery, or who are on alternative

anti-cancer therapy, or who discontinue the study (e.g. withdraw consent) before surgery are all counted as non-responders.

- Event free survival – Event free survival (EFS) defined as the length of time from date of enrolment to any of the following events: progression of disease, recurrence disease, or death due to any cause. Progression/recurrence will be assessed as per RECIST 1.1.
- Overall Response Rate - Overall response rate (ORR) is defined as proportion of participants whose overall radiological response prior to definitive surgery is either a complete response (CR) or partial response (PR) per RECIST 1.1 criteria. Participants who received alternative anti-cancer therapy before the pre-surgery tumor assessment will be counted as non-responders.
- Overall Survival - Overall survival (OS) is defined as the time between the date of enrolment and the date of death due to any cause. For a participant without documentation of death, OS will be censored on the last date the participant was known to be alive.
- PD-L1 Protein Expression - PD-L1 expression is defined as the percent of tumor cells membrane staining in a minimum of 100 evaluable tumor cells per validated Dako PD-L1 immunohistochemistry (IHC) assay. This is referred to as quantifiable PD-L1 expression. If the PD-L1 staining could not be quantified, it is further classified as: 1) Indeterminate: Tumor cell membrane staining hampered for reasons attributed to the biology of the tumor tissue sample and not because of improper sample preparation or handling. 2) Not evaluable: Tumor tissue sample was not optimally collected or prepared and PD-L1 expression is neither quantifiable nor indeterminate. Not evaluable can be determined from H&E process before the tumor biopsy specimen is sent for PD-L1 evaluation or from the H&E process during PD-L1 evaluation. Participants with missing PD-L1 expression are participants with no tumor tissue sample available for evaluation.

6.0 STUDY POPULATION

6.1 Inclusion criteria

1. Patients 18 years of age or older.
2. Participants with histologically confirmed Stage IB (≥ 4 cm), II, IIIA (N2) NSCLC (as per the 8th American Joint Committee on Cancer (AJCC)) who are considered to have resectable disease.
3. Measurable disease according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1).
4. Participants must have tumor tissue available for PD-L1 immunohistochemical (IHC) testing.

5. Eastern Cooperative Group (ECOG) Performance Status 0-2.
6. Able to give informed consent.

6.2 Exclusion criteria

1. Presence of locally advanced, unresectable, or metastatic disease.
2. Participants with known EGFR mutations, ALK or ROS1 translocation.
3. Participants with active, known, or suspected autoimmune disease (except participants with type I diabetes mellitus, residual hypothyroidism due to autoimmune thyroiditis only requiring hormone replacement, skin disorders (such as vitiligo, psoriasis, or alopecia) not requiring systemic treatment).
4. Participants with a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone or equivalent) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids are permitted in the absence of active autoimmune disease.
5. Participants with previous malignancies are excluded unless a complete remission was achieved at least 3 years prior to study entry and no additional therapy is required or anticipated to be required during the study (non-melanoma skin cancer, low grade prostate cancer and other indolent malignancies not requiring any treatment and that are unlikely to affect blood-based biomarkers are allowed).

6.3 Participant discontinuation

Participants may voluntarily discontinue participation in the study at any time. Data previously analyzed will not be able to be withdrawn, however, any data not analyzed can be deleted and no further data or samples will be collected once a participant has discontinued on the study.

7.0 STUDY DESIGN

7.1 Study schema

This is a prospective cohort study. Consecutive patients with resectable NSCLC meeting eligibility criteria will be treated with neoadjuvant nivolumab and chemotherapy. Patients' demographic, tumor, investigation, and treatment data will be collected.

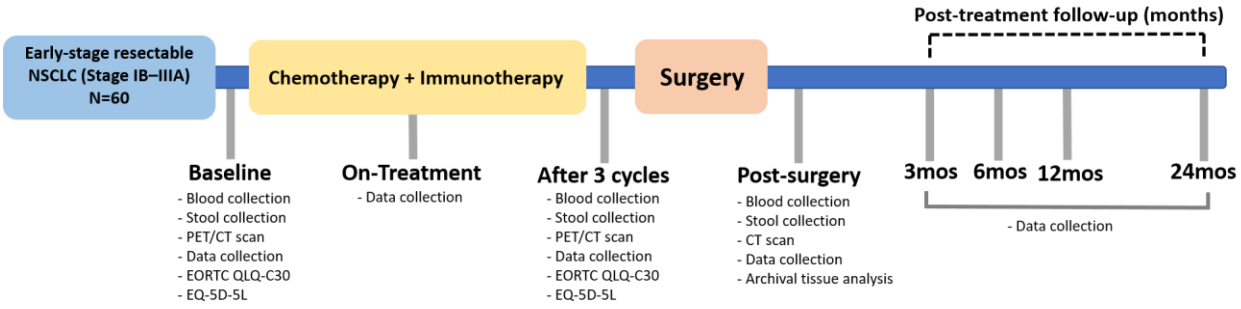

Figure 4. Study flow diagram.

Patients referred to the thoracic surgical component of the Thoracic mutidiciplinary team (MDT) who have NSCLC, stages IB – IIIA, will be worked up in the usual manner with routine biopsies and staging investigations; and in accordance with the new SOC, they will be offered neoadjuvant nivolumab and chemotherapy similar to that used in CM816 trial (i.e. histology-appropriate platinum doublets plus nivolumab for 3 cycles prior to definitive surgery). If they accept this general approach, and they do not have a known EGFR mutations, or ALK/ROS1 re-arrraangement, they will be offered accrual onto this study, in the knowledge that their therapy and subsequent management will not be impacted, except in the acquisition of some additional data at baseline and while on systemic therapy. Patients will be provided with an informed consent form that describes the data to be collected over and above SOC, the impact on their care path (essentially some additional bloodwork and radiology), and the purpose of the study. Patients will be compensated for additional travel and parking.

7.2 Data collection

Study data will be stored in an electronic REDCap database hosted by Lawson Health Research Institute. The collection of data will be oriented around a thorough assessment of the tumor, and the immune status of the host, inclusive of both directly and indirectly relevant factors, according to the following categorization: clinical variables (e.g. symptoms, performance status, recent medications etc.), radiological variables (e.g. results of routine imaging, sarcopenia index, radiomics etc.), hematological variables (e.g. complete blood count and differentials, neutrophil/lymphocyte and platelet/lymphocyte ratios, flow cytometry etc.), routine blood chemistry (e.g. electrolytes, tumor markers, cytokines etc.), pathological variables (e.g. routine H&E, immunohistochemistry, peri-tumoral microenvironment cell-type analysis, spatial transcriptomics etc.), molecular variables (e.g. next-generation sequencing, ctDNA etc.), and fecal microbiome data.

7.3 Data analysis

We will first perform univariable and multivariable Logistic regression model analysis of the features associated with the pCR and irAEs to evaluate their importance in predicting pCR and irAEs. The top predictors identified will be used as candidate predictors to create an interpretable multimodal deep learning model with a good sensitivity/specificity. This model will be able to integrate predictors and predict the pCR and irAEs. Model performance will be evaluated using receiver operating characteristic-area under the curve (AUC) and the precision-recall AUC. Other baseline models, such as random forest and LASSO will also be applied to compare model performance.

7.4 Sample size justification

Our planned accrual of 60 patients will result in approximately 15 pCRs, assuming an event rate of 25%. This will allow us to explore our potential predictors and their univariable associations with pCR and/or non-pCR outcomes. The top predictors identified in univariable analyses could be used as candidate predictors in a multivariable prediction model. Previous simulation studies have shown that scenarios with as few as 5 events per variable can produce estimates with valid confidence interval coverage, type I error rate, and relative bias (citation below). This framework would allow for an exploratory multivariable model predicting non-pCR with up to 9 of the top candidate predictors identified in univariable analyses, and/or up to 3- 4 of the top candidates predicting pCR. This assumes a two-sided type I error rate of 0.05 (21).

DEMOGRAPHIC	CLINICAL	RADIOLOGY	BLOOD-BASED	PATHOLOGY
BASELINE				
Age Sex/gender Ethnicity Smoking status Alcohol consumption Pneumococcal vaccination status COVID status Country of birth	Co-morbidities Medications ECOG PS Weight loss Stage (AJCC/TNM) Body composition Physical examination EORTC QLQ-C30 EQ-5D-5L	Sarcopenia Index CT scan Volumetrics SUV (PET/CT) Evidence of PE Radiomics	Routine CBCD N/L ratio Flow (cell subsets) Activation marker Routine chemistry LDH and iso's Protein electrophoresis CEA, CA199, CA125 LIF, CRP, IL6, IL7, GDF-15 ctDNA LIPI score Blood banking	Routine H&E, IHC (PDL1, ALK, ROS1) Histotype Mulitiplex IHC for TME cell types and proteins of interest (LAG3, LSD1, and CD47) (LHSC algorithm) NGS (TMB) Spatial transcriptomics Fecal microbiome (stool sample)
ON-TREATMENT				
Smoking status Vaccination COVID status	Rx-emergent AE's Type of chemo Dose intensity Use of G-CSF Use of PPIs/antibiotics Blood transfusions	Development of PE	CBCD nadir	
AFTER 3 CYCLES				
Smoking status Vaccination COVID status	Operative fitness Physical AE assessment QoL (global, symptoms)	CT scan SUV (PET/CT)	ctDNA Routine pre-op Blood banking	Biopsy of any suspected progression sites Fecal microbiome (stool sample)
AT AND AFTER SURGERY				
	Extent of surgery Complications Long-term EFS/OS	CT scan	ctDNA N/A Blood banking	Assessment of path response (pCR, MPR) IHC for cell types Fecal microbiome (stool sample)

Figure 5. Pre-PLaN study assessments and detailed data collection schematic [ECOG, Eastern Oncology Cooperative Group; PS, performance status; QoL, quality of life; CT, computed tomography, SUV, standardized uptake values; PE, pulmonary embolism; CBCD, CBC with differential, TME, tumor microenvironment; cell types: tumor cells, angiogenesis score, tumor-infiltrating lymphocytes (TILs) (CD8+, CD4+, Tregs), myeloid derived suppressor cells (neutrophil-type, monocyte -type including M1 and M2 macrophages); cancer-associated fibroblasts; natural killer (NK) cells, dendritic cells; G-CSF, granulocyte colony stimulating factor; GDF-15, growth-differentiation factor 15; PPIs, proton pump Inhibitors; PDL1, programmed death ligand-1; LAG3, ; Lymphocyte-activation gene 3; TLS, tertiary lymphocyte structures; LIF, Leukemia inhibitory factor; LSD1, Lysine-specific demethylase 1; CMR, complete metabolic response; MRD, molecular residual disease; STK11, serine-threonine kinase; TMB, tumor mutational burden]

7.5 Peripheral blood collection for ctDNA and biomarker analysis

Plasma will be collected for ctDNA and biomarker analysis at three time-points. At each time-point, four 10 mL (Paxgene ccfDNA, Streck BCT or K2EDTA) tubes will be drawn.

- Draw #1: Prior to start of neoadjuvant chemotherapy and immunotherapy (Day 1).
- Draw #2: After completion of neoadjuvant chemotherapy and immunotherapy, but before surgical resection (2-6 weeks after 3rd cycle).
- Draws #3: 4-6 weeks after surgery.

5-10 mL of plasma from each collection will be sent to a commercial liquid biopsy provider. The remaining 5-10 mL of plasma will be used for future confirmatory or correlative studies, which may include blood-based proteomics or other diagnostic liquid-biopsy testing platforms.

7.6 Treatment details

Patients with resectable stage Ib-IIIa NSCLC will be treated with standard of care (as per CheckMate 816), neoadjuvant nivolumab plus platinum doublet chemotherapy. It is possible that standard of care may evolve in future, but in principle will still broadly involve a combination of PD1/PDL1 inhibitor with chemotherapy.

Eligible participants will be receiving one of the following standard of care chemotherapy regimens:

Regimen 1 (non-squamous histology only):

− Pemetrexed 500 mg/m2 IV over 10 minutes or per institutional standard on Day 1

− Cisplatin 75 mg/m2 IV over 120 minutes or per institutional standard on Day 1, immediately following pemetrexed or Carboplatin AUC 5 or 6 IV over 30 minutes or per institutional standard on Day 1

Regimen 2 (any histology):

− Paclitaxel 175 or 200 mg/m2 IV over 180 minutes or per institutional standard on Day 1

− Carboplatin AUC 5 or 6 IV over 30 minutes or per institutional standard on Day 1, immediately following paclitaxel

Regimen 1 (squamous histology and patient cannot tolerate regimen 2):

− Gemcitabine 1000-1250 mg/m2/day on days 1 and 8 or per institutional standard on Day 1

− Cisplatin 75 mg/m2 IV over 120 minutes or per institutional standard on Day 1, immediately following pemetrexed or Carboplatin AUC 5 or 6 IV over 30 minutes or per institutional standard on Day 1

Following the completion of neoadjuvant treatment, all participants who remain operative candidates will undergo definitive surgery for their NSCLC within 6-8 weeks after completing neoadjuvant treatment.

Following definitive surgery, participants in each arm may receive an adjuvant immune check point inhibitor and/or adjuvant chemotherapy with or without radiation or no further treatment as per the evolving standard of care and institutional standard at the discretion of the investigator.

7.7 Feasibility

Our centre treats on an average 50 patients per year with resectable NSCLC who are eligible for neoadjuvant therapy. As such, we should be able to meet are enrolment goal in approximately 14 months.

7.8 Data safety monitoring committee

This trial does not include a data safety monitoring committee, since the only additional change from standard treatment is collection of blood samples.

8.0 EXPECTED OUTCOMES

Development of a large and highly detailed data bank containing baseline and on-treatment information, to be exploited for the parsimonious derivation of models accurately predicting pCR and IrAEs, and which will also be useful for highlighting novel opportunities for therapeutic improvement as well as providing an unprecedented 'deep dive' into the biology of the host-tumor relationship.

9.0 SIGNIFICANCE

- An improved model that can predict pCR could help in selecting patients who are most likely to benefit with neoadjuvant immunotherapy and chemotherapy. Concerning the model for predicting IrAE's, this is not to suggest such a model, if successful, should be used to deny such patients ICIs, but it would allow a more informed consent process, as well as more intensive pro-active monitoring to avoid the worst outcomes of serious irAEs (which are occasionally fatal) by early intervention.
- Identification of patients needing additional treatments (e.g., additional ICI's like CTLA4 inhibitors or LAG3 inhibitors, or radiotherapy)
- Acquisition of important scientific insights into the biological underpinnings of resistance
- Identification of novel targets or strategies to overcome resistance.

- Will determine if a blood MRD assay can determine recurrence earlier than conventional imaging on surveillance.

10.0 NOVELTY

The intention to develop models predicting pCR is not *per se* novel, and indeed we have quoted the partly successful but still simple and inadequate NADIM II model above; however, the substantially extended scope of the parameters, and their sophistication, which we will employ will elevate this exercise to a level that might well achieve both of the critical (and currently unmet) needs for utility in clinical decision-making and, of more fundamental importance, highlight the in-depth biological differences between those achieving pCR and the rest, such that by this winnowing process, the key and fundamental cause-and-effect relationships can be identified. This latter information could lead to the identification of new targets and/or novel opportunities for clinical intervention, to enable a further quantum leap in the rate of those achieving pCR. In summary, the vastly increased breadth and depth of parameters for model-building, the markedly enhanced likelihood they will achieve clinical and scientific utility, the subsidiary yet important focus on side effects as well as anti-cancer efficacy, and in particular the use of an in-depth assessment of the patient's immune status, are all individually novel, and in aggregate, unprecedented.

11.0 ETHICAL CONSIDERATIONS

The Principal Investigator will obtain ethical approval and clinical trial authorization by competent authorities according to local laws and regulations.

11.1 Institutional Review Board (IRB) / Research Ethics Board (REB)

The protocol (and any amendments), the informed consent form, and any other written information to be given to participants will be reviewed and approved by a properly constituted Institutional Review Board (IRB)/Research Ethics Board (REB), operating in accordance with the current federal regulations (e.g., Canadian Food and Drug Regulations (C.05.001); US Code of Federal Regulations (21CFR part 56)), ICH GCP and local regulatory requirements. A letter to the investigator documenting the date of the approval of the protocol and informed consent form will be obtained from the IRB/REB prior to initiating the study. Any institution opening this study will obtain REB IRB/REB approval prior to local initiation.

11.2 Informed Consent

The written informed consent form to be provided to potential study participants should be approved by the IRB/REB and adhere to ICH GCP and the ethical principles that have their origin in the Declaration of Helsinki. The investigator is responsible for obtaining written informed consent from each participant, or

if the participant is unable to provide informed consent, the participant's legally acceptable representative, prior to beginning any study procedures and treatment(s). The investigator should inform the participant, or the participant's legally acceptable representative, of all aspects of the study, including the potential risks and benefits involved. The participant should be given ample time and opportunity to ask questions prior to deciding about participating in the study and be informed that participation in the study is voluntary and that they are completely free to refuse to enter the study or to withdraw from it at any time, for any reason.

The informed consent must be signed and dated by the participant, or the participant's legally acceptable representative, and by the person who conducted the informed consent discussion. A copy of the signed and dated written informed consent form should be given to the participant or the participant's legally acceptable representative. The process of obtaining informed consent should be documented in the patient source documents.

11.3 Confidentiality of Participant Records

The names and personal information of study participants will be held in strict confidence and restricted to members of the study team. The data coordinator will maintain a confidential participant identification list (i.e. master list) during the study. Access to confidential information (i.e., source documents and patient records) is only permitted for direct participant management and for those involved in monitoring the conduct of the study (i.e., Sponsors, CRO's, representatives of the IRB/REB, and regulatory agencies). The participant's name will not be used in any public report of the study.

12.0 REFERENCES

1. Butts CA, Ding K, Seymour L, Twumasi-Ankrah P, Graham B, Gandara D, et al. Randomized phase III trial of vinorelbine plus cisplatin compared with observation in completely resected stage IB and II non-small-cell lung cancer: updated survival analysis of JBR-10. J Clin Oncol. 2010 Jan 1;28(1):29–34.

2. Brahmer JR, Ettinger DS. Non-small cell lung cancer: adjuvant and neo-adjuvant chemotherapy. Respirology. 2007 May;12(3):320–5.

3. Forde PM, Spicer J, Lu S, Provencio M, Mitsudomi T, Awad MM, et al. Neoadjuvant Nivolumab plus Chemotherapy in Resectable Lung Cancer. N Engl J Med. 2022 May 26;386(21):1973–85.

4. Forde PM, Spicer J, Girard N, Provencio M, Lu S, Wang C, et al. 84O Neoadjuvant nivolumab (N) + platinum-doublet chemotherapy (C) for resectable NSCLC: 3-y update from CheckMate 816. Journal of Thoracic Oncology. 2023 Apr 1;18(4):S89–90.

5. Provencio M, Serna R, Nadal E, Larriba JLG, Martínez-Martí A, Bernabé R, et al. PL03.12 Progression Free Survival and Overall Survival in NADIM II Study. Journal of Thoracic Oncology. 2022 Sep 1;17(9):S2–3.

6. Provencio M, Serna-Blasco R, Nadal E, Insa A, García-Campelo MR, Casal Rubio J, et al. Overall Survival and Biomarker Analysis of Neoadjuvant Nivolumab Plus Chemotherapy in Operable Stage IIIA Non–Small-Cell Lung Cancer (NADIM phase II trial). JCO. 2022 Sep;40(25):2924–33.

7. Provencio M, Nadal E, González-Larriba JL, Martínez-Martí A, Bernabé R, Bosch-Barrera J, et al. Perioperative Nivolumab and Chemotherapy in Stage III Non–Small-Cell Lung Cancer. N Engl J Med. 2023 Jun 28;NEJMoa2215530.

8. Ferrer L, Nadal E, Guidel F, Insa A, Menu P, Casal J, et al. Multimodal prediction of response to neoadjuvant nivolumab and chemotherapy for surgically resectable stage IIIA non–small cell lung cancer. JCO. 2022 Jun;40(16_suppl):8542–8542.

9. Paz-Ares LG, de Marinis F, Dediu M, Thomas M, Pujol JL, Bidoli P, et al. PARAMOUNT: Final overall survival results of the phase III study of maintenance pemetrexed versus placebo immediately after induction treatment with pemetrexed plus cisplatin for advanced nonsquamous non-small-cell lung cancer. J Clin Oncol. 2013 Aug 10;31(23):2895–902.

10. Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Cowey CL, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol. 2018 Nov;19(11):1480–92.

11. Tannir NM, Agarwal N, Pal SK, Cho DC, Formiga M, Guo J, et al. PIVOT-09: A phase III randomized open-label study of bempegaldesleukin (NKTR-214) plus nivolumab versus sunitinib or cabozantinib (investigator's choice) in patients with previously untreated advanced renal cell carcinoma (RCC). JCO. 2020 Feb 20;38(6_suppl):TPS763–TPS763.

12. Socinski MA, Jotte RM, Cappuzzo F, Nishio M, Mok TSK, Reck M, et al. Association of Immune-Related Adverse Events With Efficacy of Atezolizumab in Patients With Non–Small Cell Lung Cancer. JAMA Oncol. 2023 Feb 16;e227711.

13. Shur JD, Doran SJ, Kumar S, ap Dafydd D, Downey K, O'Connor JPB, et al. Radiomics in Oncology: A Practical Guide. RadioGraphics. 2021 Oct;41(6):1717–32.

14. Loriot Y, Marabelle A, Guégan JP, Danlos FX, Besse B, Chaput N, et al. Plasma proteomics identifies leukemia inhibitory factor (LIF) as a novel predictive biomarker of immune-checkpoint blockade resistance. Ann Oncol. 2021 Nov;32(11):1381–90.

15. Jones SA, Jenkins BJ. Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer. Nat Rev Immunol. 2018 Dec;18(12):773–89.

16. Robert C, Vagner S, Mariette X. Using genetics to predict toxicity of cancer immunotherapy. Nat Med. 2022 Dec;28(12):2471–2.

17. Wischhusen J, Melero I, Fridman WH. Growth/Differentiation Factor-15 (GDF-15): From Biomarker to Novel Targetable Immune Checkpoint. Front Immunol. 2020;11:951.

18. Mezquita L, Auclin E, Charrier M, Ferrara R, Masip JR, Planchard D, et al. The Lung Immune Prognostic Index (LIPI), a predictive score for immune checkpoint inhibitors in advanced non-small cell lung cancer (NSCLC) patients. Annals of Oncology. 2017 Sep 1;28:v473.

19. Maniatis S, Petrescu J, Phatnani H. Spatially resolved transcriptomics and its applications in cancer. Curr Opin Genet Dev. 2021 Feb;66:70–7.

20. Rao A, Barkley D, França GS, Yanai I. Exploring tissue architecture using spatial transcriptomics. Nature. 2021 Aug;596(7871):211–20.

21. Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. Am J Epidemiol. 2007 Mar 15;165(6):710–8.