A Phase II Trial Measuring the Integration of Stereotactic Radiotherapy *plus* Surgery in Early Non-Small Cell Lung Cancer

Supplemental Appendix

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A Phase II Trial Measuring the Integration of Stereotactic Radiotherapy *plus* Surgery in Early Non-Small Cell Lung Cancer

(MISSILE-NSCLC)

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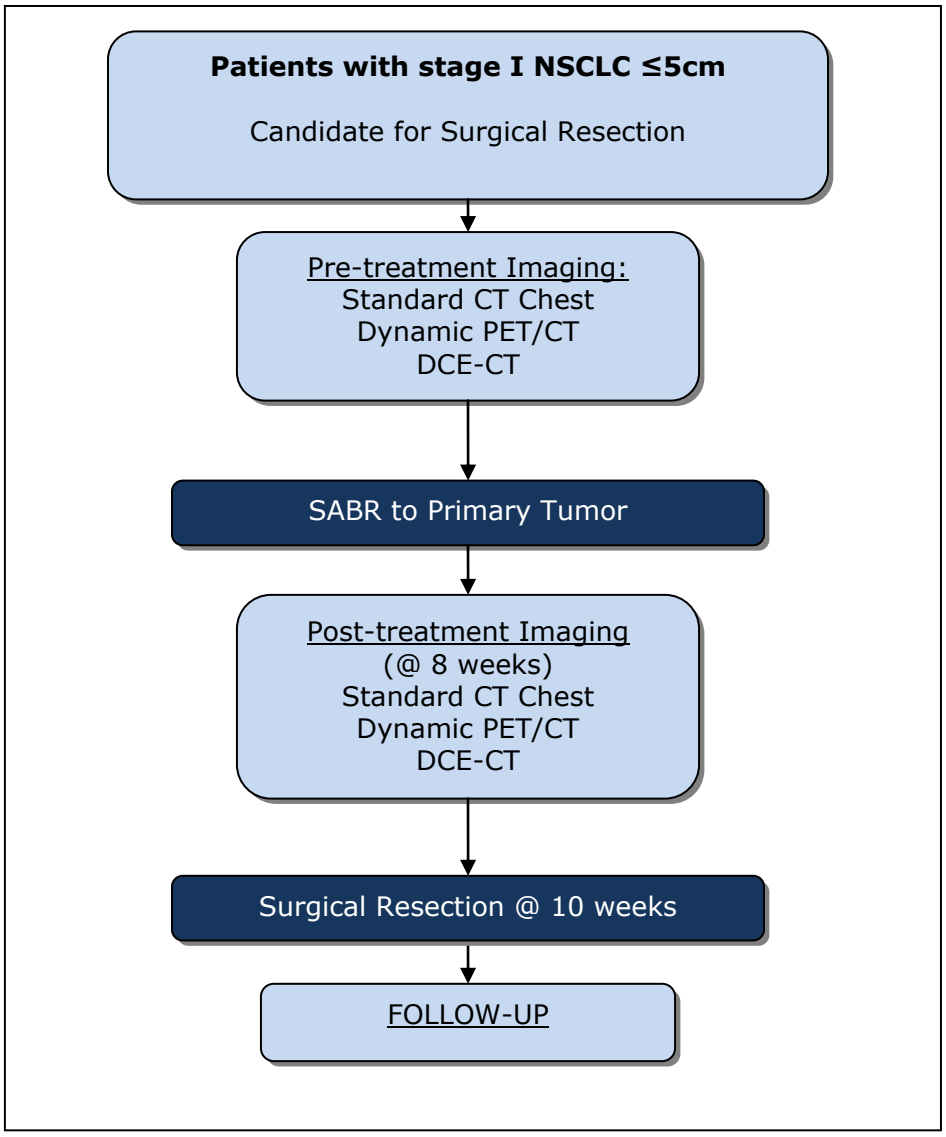
STUDY SCHEMA



1.0 INTRODUCTION

Non-small cell lung cancer (NSCLC) is a major public health problem: it is the leading cause of cancer death in men and women in Canada,[1] the United States,[2] and worldwide.[3] Due to the aging population demographics in many developed countries, the incidence of NSCLC is expected to increase further in the coming decades.[4-6] Approximately 20% of NSCLC patients present with early stage disease (T1N0 or T2N0), defined as tumors up to 5 cm in size without nodal metastases.[7]

Despite the apparent localized nature of early-stage NSCLC, long-term outcomes are suboptimal. Anatomic lobectomy is the standard of care for the treatment of T1/T2N0 NSCLC, and in the fittest patients who undergo lobectomy, 5-year survival is often 60-80%.[8] Survival is substantially lower in patients with comorbid conditions, <50% in some series.[9] For patients who are not candidates for lobectomy due to comorbidities, sublobar resection (segmentectomy or wedge resection) offers an alternative to anatomic lobectomy, but is associated with increased locoregional recurrence, and possibly inferior cancer-specific survival and overall survival, compared to lobectomy.[10]

Historically, alternatives to surgery have proven unsatisfactory. Most patients who were not candidates for surgery were treated with conventional radiotherapy, delivered as doses of 50-60 Gy in 4-6 weeks, with relatively rudimentary tumor targeting techniques. Conventional radiotherapy was associated with high rates of local recurrence, often 30-40% or higher, with no improvement in long-term survival compared to observation alone.[11,12]

In the past decade, the advent of stereotactic ablative radiotherapy (SABR, also known as “stereotactic body radiation therapy” [SBRT]) has provided a novel, promising treatment for patients with early-stage NSCLC.[13-18] SABR uses modern radiotherapy planning and targeting technologies to precisely deliver larger, ablative doses of radiotherapy (up to 60 Gy in 3-8 fractions, which may equate to as much as 150 Gy delivered in conventional fractions[19]). SABR has been associated with high rates of local control, and population-based comparisons suggest that SABR improves overall survival relative to conventional radiotherapy.[14,20] Many studies report 3-year local control of approximately 90% after SABR, comparable to results obtained with anatomic lobectomy.[8] Retrospective unmatched comparisons, propensity-score matched studies, and modeling studies have suggested that SABR may provide an alternative to surgery, but with potentially lower acute morbidity.[9,21-24]

Because of these promising outcomes, randomized trials comparing SABR with surgery were launched; however, all three such trials have closed due to poor accrual. A Dutch study, “Radiosurgery or Surgery for Operable Early Stage I Lung Cancer Study” (ROSEL) aimed to compare lobectomy vs. SABR for patients with T1N0 disease with a non-inferiority design, but closed after enrolling approximately 30 patients.[25] A large North American trial (ACOSOG4099/RTOG 1021) aimed to compare wedge resection (+/- brachytherapy) vs. SABR,[26] but was also unsuccessful in enrolling patients, as is often the

case with trials randomizing patients between surgical and non-surgical treatments. A third trial comparing SABR vs. lobectomy (NCT00840749) also recently closed in the United States, but accrual has been poor, and results are not expected in the near-term.[27]

Despite the promising results reported with SABR, an important confounder has been the difficulty in assessing response after treatment.[28] Due to the very high doses of radiotherapy delivered, nearly all patients develop evidence of local radiation induced lung injury (RILI) on CT, which is often asymptomatic (Figure 1). This RILI can persist and evolve for years after treatment, and in some cases can be difficult to distinguish from recurrence. Theoretically, in a SABR patient with a large volume of RILI, a small local recurrence could be obscured and remain undetected for a long period of time; in comparison, after surgery, a small local recurrence would be relatively easy to detect against a background of normal lung tissue. As such, there is a risk of bias in reporting local control rates after SABR based on imaging criteria only, and some have argued that SABR local control has been overestimated.[29] To date, no reported studies have addressed the true pathological outcomes in patients treated with SABR.[28]

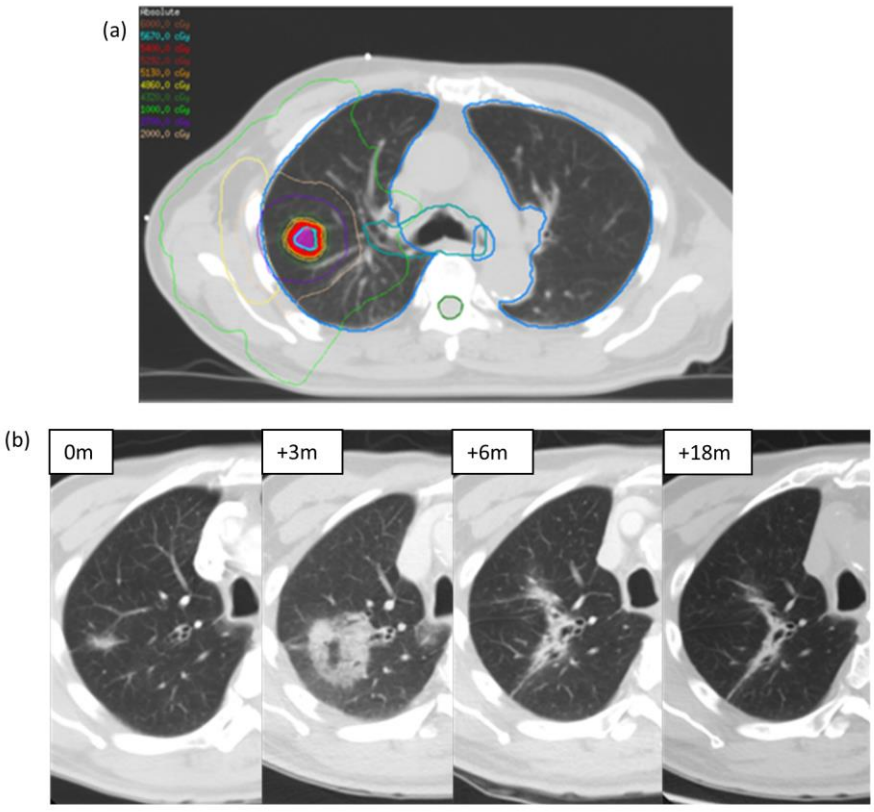


Figure 1. Radiological changes following SABR for an 85 year old gentleman with biopsy proven adenocarcinoma. This patient received 54 Gy in 3 fractions with the treatment plan shown in (a). Radiological changes are seen (b) where 0m indicates the pre-treatment lesion measuring 2.0 cm. At 3 months post-SABR, further enlargement of a ground-glass semi-solid opacity measuring 4.3 cm and at 6 months there is interval reduction in size and a decrease in ground-glass opacity, with ongoing reduction in size by 18 months.

Distinguishing a recurrent tumor from lung injury on CT can be challenging, as radiation-induced lung injury and recurrent disease follow a similar time course. Lung fibrosis continues to evolve beyond two years post-treatment, during which time most local recurrences occur.[30,31] Historically, lung injury after traditional radiotherapy was characterized by straight edges conforming to treatment portals [32] (Figure 2); in contrast, the pattern of lung injury on CT following SABR can be mass-like, due to the conformal nature of SABR.[30,33,34]

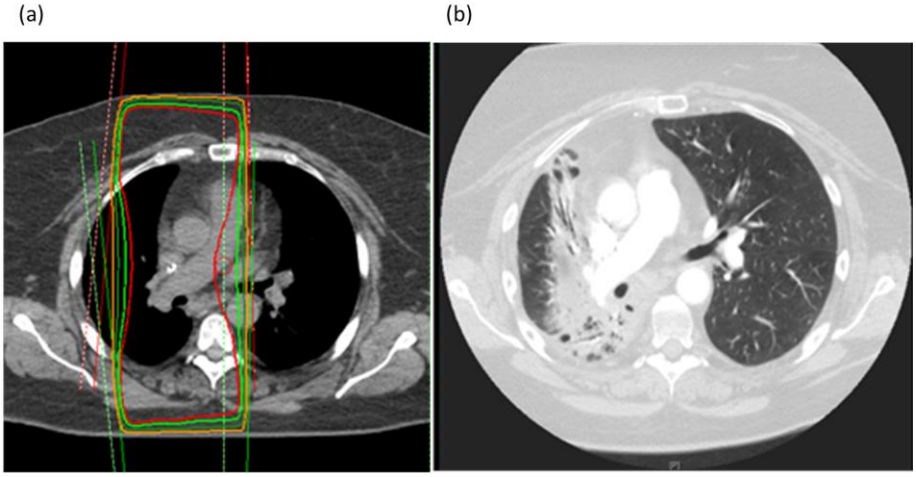
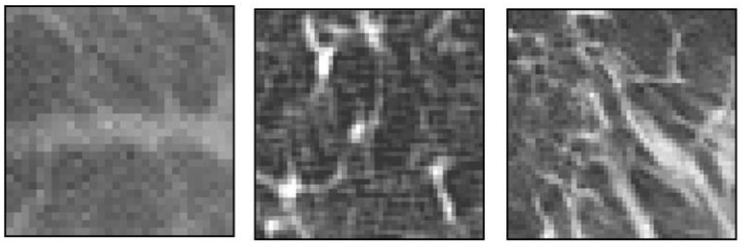


Figure 2. a) Radiation induced lung injury following a traditional anterior/posterior parallel opposed pair. b) The resulting benign injury conforms to the treatment portals and is easily distinguished by a straight line.

Accurate assessment of local recurrence post-SABR is of paramount importance. If a recurrence is misclassified as "benign fibrosis", the window of opportunity for curative-intent salvage treatment could be missed. Alternatively, if fibrosis is misclassified as a "recurrence", the patient would be exposed to unnecessary interventions and risks of morbidity, such as biopsy, imaging, chemotherapy, and even surgery.[33,35-38] As a growing number of fitter patients are being treated by SABR,[39] this clinical scenario will become more common.

Imaging-based biomarkers of response post-SABR are urgently needed, and although several promising modalities have been identified in preliminary studies, no such studies have correlated imaging findings to pathological response at the microscopic level.[28] As a result, the gold-standard definition of "recurrence" varies across studies, and many studies use imaging-based definitions of recurrence, rather than pathologic confirmation. Such imaging-based definitions of the endpoint may introduce substantial bias and create a self-fulfilling prophesy: if imaging features are used to define "recurrence" (e.g. sequential growth of lesion) and then the same features are assessed to predict these "recurrences", their performance may be artificially inflated. The majority of studies include only a small number of biopsy-proven recurrences (and resected recurrences are even more uncommon), with remainder of patients defined as recurrence an increase in tumor size on successive CT scans. [40-42]

Promising modalities for assessment of response after SABR include Dynamic Contrast-Enhanced Computed Tomography (DCE-CT), advanced CT-based image feature analysis, and dynamic functional imaging. DCE-CT may be useful as an imaging biomarker in SABR patients, as it allows for the characterization of the neovascularization patterns in tumors.[43] Preliminary studies have evaluated DCE-CT derived perfusion parameters in NSCLC patients undergoing radiotherapy or chemotherapy, and suggest that DCE-CT may be a valuable predictor of response, and may serve as a biomarker for tumor hypoxia.[44-46] CT-based quantitative image feature analysis extracts measurable information from within an image, such as intensities or densities, shape or morphology, or texture, the latter referring to a set of complex measurements which describe local brightness variation or the spatial arrangement of intensities in an image (Figure 3).[47,48] Preliminary data suggests that after SABR, image feature analysis may be able to distinguish recurrence from fibrosis much earlier than currently-used response metrics such as Response Criteria in Solid Tumors (RECIST).[49-51] FDG-PET has also been evaluated in preliminary studies for assessment of response after SABR, but the lack of true pathologic confirmation of recurrence (or lack thereof) in most studies precludes any definitive conclusions.[28] Dynamic PET is a novel approach that improves upon several issues inherent to the using the standard semi-quantitative maximum standardized uptake value (SUV_{max}) as a biomarker. Rather than obtaining a single measure of glucose uptake 60 minutes after injection of the tracer (FDG), dynamic PET obtains several repeated measures of glucose uptake during and after injection, allowing for quantitative measurements of several parameters of glucose kinetics that may be predictive of outcomes and response to treatment.[52-54]



	(a)	(b)	(c)
FIRST-ORDER APPEARENCE			
Mean Density	-677.8 HU	-794.5 HU	-661.2 HU
Standard Deviation of Density	114. 6 HU	225.6 HU	229.4 HU
SECOND-ORDER APPEARENCE			
Energy	0.0054	0.0028	0.0021
Entropy	8.06	9.03	9.59

Figure 4. Sample lung images showing the variations in two first-order appearance measures (mean density and standard deviation of density [first-order texture analysis]) and two second-order appearance measures, energy and entropy. (a) and (c) have similar mean densities, but are better differentiated by the first and second-order texture measures. (b) and (c) have similar first-order texture values, but are better differentiated by the second order measures.

The use of SABR as neoadjuvant therapy prior to surgery may provide a novel therapeutic opportunity. In oncology, the use of neoadjuvant radiotherapy or chemoradiotherapy prior to surgery has become widespread for several types of cancer, and in many instances improves local control and/or survival compared to surgery alone.[55] Neoadjuvant radiotherapy provides several theoretical advantages, including potentially decreasing the rate of positive margins, decreasing the size of the required resection, or by sterilizing the tumor to avoid seeding of circulating tumor cells during surgery.[55] In the setting of stage I NSCLC, the approach of neoadjuvant ablative treatment has been evaluated in the form of radiofrequency ablation (RFA); however, the high prevalence of viable tumor cells after RFA in this setting (62% of cases) has discouraged widespread adoption of this technique.[56] Combining SABR with surgery appears safe: at least 4 small studies have reported on patients who have undergone surgery for salvage in patients who have recurred after SABR.[57-60] Such surgery is generally well tolerated with a favorable toxicity profile, with only one patient sustaining a major toxicity (fistula requiring further surgery for correction).[59] To our knowledge, no study has employed an *a priori* planned combination of SABR + surgical resection with the goal of maximizing local control.

The goal of this study is to evaluate a novel treatment approach: the combination of neoadjuvant SABR followed by surgical resection in patients with T1T2N0 NSCLC, in order to measure the true pathologic rates of local control after SABR, to develop new imaging biomarkers of response, and to assess clinical outcomes, including toxicity, relapse patterns, and survival.

2.0 OBJECTIVES

1. To assess the true pathological rate of complete primary tumor response after SABR.

2. To evaluate imaging-based biomarkers of response using functional imaging (dynamic FDG-PET), image texture analysis, and dynamic contrast enhanced CT (DCE-CT), done pre- and post-SABR.

3. To correlate imaging findings with digital histopathology at the individual voxel level using deformable co-registration.

4. To assess local recurrence, regional recurrence, distant recurrence, overall survival, quality of life (QOL) and toxicity after a combined approach of SABR + surgical resection for stage I NSCLC.

5. To assess the immunological effects of SABR on the NSCLC tumor micro-environment

3.0 STUDY DESIGN

Single-arm cohort study.

4.0 PATIENT SELECTION

4.1 Inclusion Criteria

- Age 18 or older
- Willing to provide informed consent
- Histologically confirmed NSCLC
- Tumor stage T1 or T2a (≤5 cm)
- No evidence of nodal disease (N0)
- No evidence of distant metastases (M0)
- ECOG performance status 0-2
- Life expectancy >6 months
- adequate FEV1 for resection, defined as a predicted post-operative FEV1 of 30% or greater

4.2 Exclusion Criteria

- Serious medical comorbidities or other contraindications to radiotherapy or surgery
- Prior history of lung cancer within 5 years
- Prior thoracic radiation at any time

- Inability to attend full course of radiotherapy, surgery, or follow-up visits
- Contrast allergy
- Pregnant or lactating women

5.0 PRE-TREATMENT EVALUATION

- History and physical examination by a radiation oncologist and thoracic surgical oncologist within 8 weeks prior to enrollment onto study

- Histological confirmation of non-small cell carcinoma

- Standard staging within 12 weeks prior to enrollment including
    - CT chest and upper abdomen, acquired using a 6 mm slice thickness with isotropic 3-D reconstruction around the tumor
    - Whole body FDG-PET-CT scan
    - CT head or MRI head

- Staging of the mediastinum:
    - Mediastinoscopy is required for all patients, except for patients who have both a peripheral T1 lesion and no FDG-avid regional nodes on PET/CT.

    - Patients with regional nodes positive on PET/CT are eligible if surgical staging does not reveal evidence of nodal disease (e.g. EBUS/EUS/mediastinoscopy)

- Pulmonary function tests within 12 weeks of enrollment showing adequate FEV1 for resection, with a predicted post-operative FEV1 of 30% or greater.

- Pregnancy test for women of child-bearing age

- Informed consent required

6.0 TREATMENT PLAN

6.1 Pre-SABR and Pre-Surgical Imaging

Standard of care pre-treatment staging includes a thin-slice CT chest, and whole-body static PET/CT, done prior to enrollment. Additional pre-treatment imaging will include DCE-CT and dynamic PET/CT of the primary tumor, acquired in a single visit.

All scans (thin-slice CT chest, DCE-CT, dynamic PET-CT) will be repeated 8 weeks (+/- 2 weeks) post-SABR.

6.1.1 Dynamic contrast enhanced CT chest

The index lesion will be scanned repeatedly using 120 kVp, 50 mAs, 8 x 5 mm slices at intervals of 2.8 – 3.0 s for 3 min. Contrast (e.g. Visipaque 320) at a dosage of 0.7 ml/kg is injected at 4 ml/s through an antecubital vein after a delay of 6 s from start of scanning. The perfusion parameters derived from DCE-CT include blood flow (BF), blood volume (BV), mean transit time (MTT) and capillary permeability surface area product (PS, which measures the leakage rate of contrast from blood into the interstitial space).

6.1.2 Dynamic 18-FDG-PET

A dynamic 18-FDG PET will be acquired sequentially after single injection of FDG. Acquisition will include transmission scan to correct for photon attenuation. After the bolus of 18-FDG, a dynamic scan will begin with a duration of 60 min and a variable frame length (e.g. 6 x 5 s, 6x 10 s, 3 x 20 s, 5 x 30 s, 5 x 60 s, 8 x 150 s, and 6 x 300 s).[52]

6.2 Radiotherapy

SABR will be delivered as per a risk-adapted protocol, with the dose and number of fractions dependent on the size and location of the tumor.[61,62]

Treatment can be delivered using static beams (either 3D-conformal radiotherapy or intensity-modulated) or rotational therapy (volumetric modulated arc therapy, or tomotherapy). Dose constraints are listed in Appendix 1 for the 3-, 5-, and 8-fraction regimens.

Tumor Size and Location	Total Dose (Gy)	Number of fractions	Dose per fraction (Gy)	Frequency
Tumors 3 cm or less surrounded by lung parenchyma	54	3	18	Every second day
Abutting chest wall or >3 cm	55	5	11	Every second day
Within 2 cm of mediastinum or brachial plexus	60	8	7.5	Every second day

6.2.1 Immobilization, Imaging and Registration

Treatment will be set-up using reproducible positioning, verified using an on-line protocol, for all patients in this study. Immobilization may include a custom immobilization device, such as a vac-loc bag. All patients will undergo 4-D planning CT simulation.

Physics will review the 4D-CT images and will perform the following quality assurance procedures indicated on the 4D-CT template designed specifically for SABR:

1) Ensure all end inspiration (0%) tags exist and are in the right place. This ensures image integrity.

2) If the quality of the 4D-CT images are not sufficient (determined by Physics), then planning will be performed on the fast helical CT or Untagged Average CT.

3) Motion measurements in all 3 directions are performed.

    a) If the motion is less than or equal to 7 mm and the good quality images exist, then treatment planning may be performed on the Untagged Average CT with the 50% or 60% phase (End Expiration) and the 0% phase being fused to it.

b) If the motion is greater than 7 mm in any one direction, then respiratory-gated radiotherapy will be considered. In this case, treatment planning will be performed on a subset average CT dataset (usually labeled either 30%-60% Avg CT or 40%-70% Avg CT) generated by Physics. This is an average CT over the intended gated interval.

6.2.2 Volume Definitions and Prescription

The gross tumor volume (GTV) will be defined as the visible tumor on CT imaging +/- PET, and an internal GTV (iGTV) will be defined as the GTV from all phases of respiration, if gating is not used. No additional margin will be added for microscopic spread of disease. A Planning Target Volume (PTV) margin of 5 mm will be added Organs at risk visible in the planning CT scan will be contoured. Dose constraints are listed in Table 2.

Doses are prescribed to approximately the 80% isodose line surrounding the PTV, resulting in a hotspot of 120-140%; the latter should fall within the iGTV. 95% of the PTV should be covered by the prescription dose, and 99% of the PTV should be covered by 90% of the prescription dose. Several non-overlapping 6/10 MV beams (on the order of 7-11 beams) or 1-2 VMAT arcs combined possibly with a few non-coplanar beams should be utilized. Non-coplanar beams can be used to reduce 50% isodose volume for un-gated treatments.

6.2.3 Quality Assurance

In order to ensure patient safety and effective treatment delivery, a robust quality assurance protocol is incorporated. The following requirements must be completed for each patient:

- Prior to treatment, each patient must be discussed at quality assurance (QA) rounds.

- All radiotherapy plans must meet target dose levels for organs at risk (Appendix 1). Prior to plan approval, the dose to each organ at risk must be verified by the physicist or treating physician. It is strongly recommended that dose constraints not be exceeded.

- All dose delivery for intensity-modulated plans (including arc-based treatments) will be confirmed before treatment by physics staff.

- Cone-beam CT will be used to verify patient positioning immediately prior to treatment. Ideally, direct tumour localization should be performed for stereotactic treatments of soft tissues. For gated SBRT treatments, direct tumour localization will be performed by matching the tumour position with the ROI defined by IGTV_CBCT. This will be followed by a gated 2D-kV in the AP plane to verify the gating window. In the absence of direct tumour localization, reliable soft tissue

surrogates are recommended. A final CBCT may be done after completion of treatment.

6.3 Surgery and Post-Surgical Specimen Processing

Surgery will occur after the 2nd set of imaging, at 10 weeks following SABR (+/- 2 weeks), to allow sufficient time for a full pathological response. Surgery will consist of a lobectomy, or sublobar resection, and may employ either an open approach or a video-assisted thoracoscopic approach. Surgical sampling of the at-risk hilar and mediastinal nodes will be done at the time of resection.

6.3.1 Pathological Processing

After resection, the tumor is oriented by the surgeon and submitted to the pathology lab. Upon arrival in the pathology lab, it will undergo gross examination in the standard manner. The specimen will be submitted in total for microscopic examination, as follows: For sublobar resections, the staple line will be removed and the specimen will then be serially sectioned every 3-4 mm. For lobectomy, after the bronchial margin specimen is removed, the index lesion (+/- approximately 2 cm margin) will be excised and serially sectioned every 3-4 mm. Depending on the size of the sections they will be submitted as is or will be bisected. The serially sectioned slices will be submitted sequentially and in total for paraffin processing, in the standard manner. Glass slides will be created in the standard manner.

Digitized pathology slides will be co-registered with pre-treatment CT scans, and/or PET/CT, using a software method similar to that which we have previously developed for prostate cancer.[63,64] This approach uses deformable image registration techniques to reconstruct digitized pathology slides, computationally reconstituting them back into the 3D specimen context from which they were cut with 0.7 mm accuracy, and subsequently performs registration to in vivo prostate imaging with 1.1 mm accuracy.[64]

Viability of any visible cells will be assessed by H&E uptake. Additionally stains for viability may be subsequently used in a retrospective manner to validate H&E findings.

6.3 Adjuvant Treatment

Adjuvant chemotherapy will be delivered as per routine standard practice. Any patients with pathologic node-positive disease (N1, N2, or N3) will be referred for an opinion from a medical oncologist. For patients with N2 or N3 disease, adjuvant radiotherapy to the mediastinum may be considered as per institutional practice, provided there is minimal overlap with the SABR dose distribution.

7.0 ADVERSE EVENTS

7.1 Definitions

Adverse Event (AE) or reaction is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may *not* be considered related to the medical treatment or procedure.

Serious Adverse Event (SAE) or reaction as defined in the ICH Guideline: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, E2A Section IIB includes any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.)
- Results in persistent or significant disability/incapacity
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Is a congenital anomaly/birth defect

Important medical events that may not be immediately life-threatening or result in death or hospitalization may be considered a serious adverse event, when, based upon medical and scientific judgment, they may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

Unexpected adverse reaction is one that the nature and severity is not consistent with the applicable product information (e.g., Investigator's Brochure or Product Monograph, described in the REB/IRB approved research protocol or informed consent document), or occurs with more than expected frequency.

7.2 Causality (attribution)

An adverse event or reaction is considered **related** to the research intervention if there is a reasonable possibility that the reaction or event may have been caused by the research intervention (i.e. a causal relationship between the reaction and the research intervention cannot be ruled out by the investigator(s)).

The relationship of an AE to the study treatment (causality) will be described using the following definitions:

Unrelated: Any adverse event for which there is evidence that an alternative etiology exists or for which no timely relationship exists to the administration of the study treatment and the adverse event does not follow any previously documented pattern. The adverse event, after

careful consideration by the investigator, is clearly and incontrovertibly due to causes other than the intervention.

Unlikely: Any adverse event for which the time relationship between the study treatment and the event suggests that a causal relationship is unlikely and/or the event is more likely due to the subject's clinical condition or other therapies concomitantly administered to the subject.

Possible: Any adverse event occurring in a timely manner after the administration of the study treatment that follows a known pattern to the intervention and for which no other explanation is known. The adverse event, after careful consideration by the investigator, is considered to be unlikely related but cannot be ruled out with certainty.

Probable: Any adverse event occurring in a timely manner after the administration of the study treatment that follows a known pattern to the intervention and for which no other explanation is known. The adverse event, after careful consideration by the investigator, is believed with a high degree of certainty to be related to the intervention.

Definitely Related: Any adverse event occurring within a timely manner after administration of the study treatment that is a known sequela of the intervention and follows a previously documented pattern but for which no other explanation is known. The adverse event is believed by the investigator to be incontrovertibly related to the intervention.

7.3 Severity

The severity of adverse events will be evaluated using the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 grading scale (see http://ctep.cancer.gov).

Grade 1: Mild
Grade 2: Moderate
Grade 3: Severe
Grade 4: Life-threatening or disabling
Grade 5: Death

Note: The term "severe" is a measure of intensity: thus a severe adverse event is not necessarily **serious**. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious.

7.4 Immediately Reportable Adverse Events

Any grade 4 or 5 adverse reaction that is definitely, probably, or possibly the result of protocol treatment must be verbally reported to the Principal Investigator and Co-Investigators within 24 hours of discovery, and Office of Research Ethics as outlined below.

Events or Outcomes Not Qualifying as SAEs

Any sign, symptom, diagnosis, illness, and/or clinical laboratory abnormality that can be linked to the disease under study or disease progression and is not possibly attributable to study treatment, are not reported as SAEs even though such event or outcome may meet the definition of SAE.

Events that are exempt from reporting as SAEs include:

• Events emerging during the study that is part of the natural progression of the underlying cancer (including disease-related deaths) unless more severe than expected or not possibly attributable to study treatment. For example, hospitalization for the evaluation or treatment of signs and symptoms of disease progression that are not possibly attributable to study treatment will not be reported as an SAE.
• Serious Adverse Events that occur more than 30 days after the end of study treatment that are judged by the investigator to be unrelated to study treatment.

All **serious**, **unexpected** adverse events or reactions **regardless of causality** for subjects enrolled at the local site must be reported to the Office of Research Ethics, within **7** days of discovery of the event or reaction through the Local Adverse Events Report.

The Principal Investigator should also comply with the applicable regulatory requirement(s) related to the reporting of unexpected serious adverse drug reactions to the regulatory authority (ies).

8.0 SUBJECT DISCONTINUATION / WITHDRAWAL

Subjects may voluntarily discontinue participation in the study at any time. If a subject is removed from the study, the clinical and laboratory evaluations that would have been performed at the end of the study should be obtained. If a subject is removed because of an adverse event, they should remain under medical observation as long as deemed appropriate by the treating physician.

9.0 FOLLOW-UP EVALUATION AND ASSESSMENT OF EFFICACY

The follow-up schedule is as follows:

	Before Entry	Pre-SABR	8 weeks post-SABR*	3 Mo. Post-Surgery	q6 monthly for 2 years then annually until year 5 (from date of surgery)**
History and Physical	X			X	X
CT chest with isotropic 6 mm slice thickness through tumor	X		X		
Staging FDG-PET-CT scan	X				
CT head or MRI head	X				
Pulmonary function tests	X		X		
Pregnancy test for women of child-bearing age	X				
Biomarker Imaging: DCE-CT Dynamic FDG PET		X	X		
Toxicity Scoring and QOL	X		X	X	X***
Follow-up CT chest and upper abdomen					X

* +/- 2 weeks

**Follow-up appointments occurring 6 months after surgery and beyond may be conducted by the thoracic surgeon. Data will be submitted to the LRCP Clinical Research Unit by the Thoracics Clinical Research Unit.

***toxicity scoring will stop at one year, unless ongoing or new toxicity is related to treatment and grade 3 or higher. QOL scoring will stop at 2 years

10.0 Statistical Considerations and Sample Size Calculation

10.1 Analysis Plan

Primary Endpoint

The primary endpoint of this study will be the percentage of patients who exhibit a lack of viable tumor after surgical resection (e.g. a pathologic complete response [pCR]), which will be reported as the patients with a complete response, divided by the total number of patients undergoing resection, with a 95% confidence interval (CI).

Secondary Endpoints

Logistic regression will be used to evaluate the predictive value of the novel imaging biomarkers, with the dependent variable as pathologic outcome (complete response vs. non-complete response). From DCE-CT, changes in BF, BV, MTT and PS will be examined as independent predictors. From PET studies, parameters of FDG uptake (e.g. SUVmax) will be examined as independent predictors. For CT texture analysis, factors tested will include several first-order and second-order metrics; for dynamic PET/CT, kinetic analyses will be associated with pathologic response and long-term oncologic outcomes.

Local recurrence will be defined as any new tumor growth >5 mm within the involved lobe (post-sublobar resection) or at the resection margins (post-lobectomy). Regional recurrence will be defined as any recurrence in the hilar, mediastinal, or supraclavicular nodes. Distant recurrence will be defined as the development of hematogenous metastases. Time-to-event oncologic outcomes (overall survival, time to local-, regional- and distant- recurrence) will be measured from the date of enrollment and calculated using the Kaplan-Meier method.

Toxicity will be scored as per the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 grading scale (see http://ctep.cancer.gov), as described in Section 7.

QOL will be measured using the Functional Assessment of Cancer Therapy-Trial Outcome Index (TOI) for Lung Cancer, which is measured using the data from the FACT-Lung (FACT-L) questionnaire completed by patients. The FACT-TOI is a summary score derived from the FACT-L and is composed of 21 items, including physical well-being, functional well-being, and lung-cancer subscale questions).

10.2 Data Safety Monitoring Committee

Safety will be assessed by the data safety and monitoring committee (DSMC). The DSMC will meet semi-annually after study initiation to review toxicity outcomes. If any grade 5 toxicity is reported, the DSMC will review the case notes to determine if such toxicity is related to treatment. If the DSMC deems that toxicity rates are excessive (e.g. >5% grade 5 toxicity), then the DSMC can, at its discretion, recommend cessation or modification of the trial.

In addition, after 10 patients have been accrued and completed the surgery, an interim review of toxicity will be undertaken separately by the study team and DSMC; if these are deemed excessive, the trial may be modified.

10.3 Sample Size Calculation

The sample size is calculated to provide a true estimate of the rate of true pCR rate after SABR, within a 95% confidence interval (CI) of ± 10 percentage points. It is estimated that the rate of true pCR after SABR will be 90%. In order to restrict the 95% CI to ±10%, including an 8% dropout rate, a total of 40 patients would be required.[65]

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 subjects 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 subjects 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 subject, or if the subject is unable to provide informed consent, the subject's legally acceptable representative, prior to beginning any study procedures and treatment(s). The investigator should inform the subject, or the subject's legally acceptable representative, of all aspects of the study, including the potential risks and benefits involved. The subject 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 subject, or the subject'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 subject or the subject's legally acceptable representative. The process of obtaining informed consent should be documented in the patient source documents.

11.3 Confidentiality of Subject Records

The names and personal information of study participants will be held in strict confidence. All study records (CRFS, safety reports, correspondence, etc.) will only identify the subject by initials and the assigned study identification number. The data coordinator will maintain a confidential subject identification list (Master List) during the course of the study. Access to confidential information (i.e., source documents and patient records) is only permitted for direct subject 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 subject's name will not be used in any public report of the study.

11.4 Registration Procedure

Please call the data co-ordinator at the LRCP to notify of potential eligibility. Eligibility requirements, registration form, and signed letter of information are to be faxed to the co-ordinator at the LRCP. To complete the registration you must call the data co-ordinator immediately after faxing. If the patient is eligible the co-ordinator will confirm and provide a patient ID number.

12.0 BIOMARKER STUDIES

This patient population offers a great opportunity to assess the immunological effects of SABR on the NSCLC tumor micro-environment. This could also provide clues for the best future immunotherapy combinations. In collaboration with researchers at VU University Medical Center in Amsterdam Netherlands, multiplex (7-parameter) ICH will be performed on pre and post-treatment tumor biopsies.

Required Samples:

1) Core biopsy pre-treatment for all patients (10 slides, unstained, at 5um thickness)
2) Post-radiotherapy tumor tissue at resection and slides from the hilar lymph nodes, if possible. (10 slides, unstained, at 5um thickness)

Planned Analyses:

1) Multiplex (7-parameter) ICH with immune parameters
    - Panels:
        - Tumor panel: IDO, PDL1, PDL2, HCA2, HC10, B2m
        - T-cell panels: CD3, CD8, FoxP3, TBet, Ki67, GranzymeB; and CD3, CD8, PD1, Tim3, Lag3, GranzymeB

- APC Panels: CD14, CD163, CD83, CD1a, PDL1, CD83; and CD14, CD33, HLA-DR, CD11b, CD15, Arginase

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APPENDIX 1 – Radiotherapy Dose Constraints

A1.1 Normal tissue dose constraints for **THREE** fraction SABR regimens.[66]

Serial Tissue	Volume (mL)	Volume Max (Gy)	Max Point Dose (Gy)	Endpoint (≥Grade 3)
THREE-FRACTION TREATMENT				
Optic pathway	<0.2	15 (5 Gy/fx)	19.5 (6.5 Gy/fx)	Neuritis
Cochlea			20 (6.67 Gy/fx)	Hearing loss
Brainstem	<1	18 (6 Gy/fx)	23 (7.67 Gy/fx)	Cranial neuropathy
Spinal cord	<0.25	18 (6 Gy/fx)	22 (7.33 Gy/fx)	Myelitis
	<1.2	11.1 (3.7 Gy/fx)		
Cauda equina	<5	21.9 (7.3 Gy/fx)	24 (8 Gy/fx)	Neuritis
Sacral plexus	<3	22.5 (7.5 Gy/fx)	24 (8 Gy/fx)	Neuropathy
Esophagus*	<5	21 (7 Gy/fx)	27 (9 Gy/fx)	Stenosis/fistula
Ipsilateral brachial plexus	<3	22.5 (7.5 Gy/fx)	24 (8 Gy/fx)	Neuropathy
Heart/pericardium	<15	24 (8 Gy/fx)	30 (10 Gy/fx)	Pericarditis
Great vessels	<10	39 (13 Gy/fx)	45 (15 Gy/fx)	Aneurysm
Trachea and ipsilateral bronchus*	<4	15 (5 Gy/fx)	30 (10 Gy/fx)	Stenosis/fistula
Skin	<10	22.5 (7.5 Gy/fx)	24 (8 Gy/fx)	Ulceration
Stomach	<10	21 (7 Gy/fx)	24 (8 Gy/fx)	Ulceration/fistula
Duodenum*	<5	15 (5 Gy/fx)	24 (8 Gy/fx)	Ulceration
Jejunum/ileum*	<5	16.2 (5.4 Gy/fx)	27 (9 Gy/fx)	Enteritis/obstruction
Colon*	<20	20.4 (6.8 Gy/fx)	30 (10 Gy/fx)	Colitis/fistula
Rectum*	<20	20.4 (6.8 Gy/fx)	30 (10 Gy/fx)	Proctitis/fistula
Bladder wall	<15	15 (5 Gy/fx)	30 (10 Gy/fx)	Cystitis/fistula
Penile bulb	<3	21.9 (7.3 Gy/fx)	42 (14 Gy/fx)	Impotence
Femoral heads (right and left)	<10	21.9 (7.3 Gy/fx)		Necrosis
Renal hilum/vascular trunk	<2/3 volume	18.6 (6.2 Gy/fx)		Malignant hypertension

Parallel Tissue	Critical Volume (mL)	Critical Volume Dose Max (Gy)	Endpoint (≥Grade 3)
Lung (right and left)	1,500	10.5 (3.5 Gy/fx)	Basic lung function
Lung (right and left)	1,000	11.4 (3.8 Gy/fx)	Pneumonitis
Liver	700	17.1 (5.7 Gy/fx)	Basic liver function
Renal cortex (right and left)	200	14.4 (4.8 Gy/fx)	Basic renal function

A1.2 Normal tissue dose constraints for **FIVE** fraction SABR regimens.[66]

Serial Tissue	Volume (mL)	Volume Max (Gy)	Max Point Dose (Gy)	Endpoint (≥Grade 3)
FIVE-FRACTION TREATMENT				
Optic pathway	<0.2	20 (4 Gy/fx)	25 (5 Gy/fx)	Neuritis
Cochlea			27.5 (5.5 Gy/fx)	Hearing loss
Brainstem	<1	26 (5.2 Gy/fx)	31 (6.2 Gy/fx)	Cranial neuropathy
Spinal cord	<0.25	22.5 (4.5 Gy/fx)	30 (6 Gy/fx)	Myelitis
	<1.2	13.5 (2.7 Gy/fx)		
Cauda equina	<5	30 (6 Gy/fx)	34 (6.4 Gy/fx)	Neuritis
Sacral plexus	<3	30 (6 Gy/fx)	32 (6.4 Gy/fx)	Neuropathy
Esophagus*	<5	27.5 (5.5 Gy/fx)	35 (7 Gy/fx)	Stenosis/fistula
Ipsilateral brachial plexus	<3	30 (6 Gy/fx)	32 (6.4 Gy/fx)	Neuropathy
Heart/pericardium	<15	32 (6.4 Gy/fx)	38 (7.6 Gy/fx)	Pericarditis
Great vessels	<10	47 (9.4 Gy/fx)	53 (10.6 Gy/fx)	Aneurysm
Trachea and ipsilateral bronchus*	<4	18 (3.6 Gy/fx)	38 (7.6 Gy/fx)	Stenosis/fistula
Skin	<10	30 (6 Gy/fx)	32 (6.4 Gy/fx)	Ulceration
Stomach	<10	28 (5.6 Gy/fx)	32 (6.4 Gy/fx)	Ulceration/fistula
Duodenum*	<5	18 (3.6 Gy/fx)	32 (6.4 Gy/fx)	Ulceration
Jejunum/ileum*	<5	19.5 (3.9 Gy/fx)	35 (7 Gy/fx)	enteritis/obstruction
Colon*	<20	25 (5 Gy/fx)	38 (7.6 Gy/fx)	colitis/fistula
Rectum*	<20	25 (5 Gy/fx)	38 (7.6 Gy/fx)	proctitis/fistula
Bladder wall	<15	18.3 (3.65 Gy/fx)	38 (7.6 Gy/fx)	cystitis/fistula
Penile bulb	<3	30 (6 Gy/fx)	50 (10 Gy/fx)	Impotence
Femoral heads (right and left)	<10	30 (6 Gy/fx)		Necrosis
Renal hilum/vascular trunk	<2/3 volume	23 (4.6 Gy/fx)		Malignant hypertension

Parallel Tissue	Critical Volume (mL)	Critical Volume Dose Max (Gy)	Endpoint (≥Grade 3)
Lung (right and left)	1,500	12.5 (2.5 Gy/fx)	Basic lung function
Lung (right and left)	1000	13.5 (2.7 Gy/fx)	Pneumonitis
Liver	700	21 (4.2 Gy/fx)	Basic liver function
Renal cortex (right and left)	200	17.5 (3.5 Gy/fx)	Basic renal function

*Avoid circumferential irradiation.

A1.3. Dose constraints for **EIGHT**-fraction SABR regimens.

Note: for targets overlapping the stomach or esophagus, 12 fractions should be used, with a maximum dose of 48 Gy in 12 fractions for either organ. For any organs not listed, or for OARs for 12 fraction regimens, a biologically effective dose can be calculated using an alpha-beta ratio of 2.

Structure	Maximum Dose
Liver	At least 700 cc below 22 Gy (unless using NTCP calculation method)
Kidney (right and left)	At least 200 cc below 21 Gy
Spinal Cord	32 Gy point dose V(27 Gy) < 0.25 cc V(16 Gy) < 1.25 cc
Stomach	40 Gy point dose V(34 Gy) < 10 cc
Esophagus	40 Gy point dose V(33 Gy) < 5 cc
Great Vessels	65 Gy point dose V(58 Gy) < 10 cc
Trachea and Ipsilateral Mainstem Bronchus	40 Gy point dose V(21.5 Gy) < 4 cc
Ipsilateral Brachial Plexus	39 Gy point dose V(36.5 Gy) < 3 cc
Heart/Pericardium	46 Gy point dose V(39 Gy) < 15 cc
Duodenum	39 Gy point dose V(21.5 Gy) < 5 cc
Jejunum/Ileum	40 Gy point dose V(23 Gy) < 5 cc

V(X) Gy): volume of structure receiving X Gy or more (i.e. for the stomach, V34 Gy is the volume of stomach receiving 34 Gy or more).

A1.4. Dose conformality parameters for lung SABR treatments
From Hurkmans *et al,* 2010[67]

Table 1: Dose conformity requirements and definition of protocol deviations. $R_{100\%}$ and $R_{50\%}$ = ratio of respectively the 100% and 50% Prescription Isodose Volume to the PTV. $D_{2\ cm}$ = dose maximum at 2 cm from the PTV as percentage of the prescribed dose. $V_{20\ Gy}$ = Percent of lung receiving 20 Gy or more (both lungs minus GTV).

Type A models (standard algorithms)								
$R_{100\%}$		$R_{50\%}$		$D_{2\ cm}$ (%)		$V_{20\ Gy}$ (%)		PTV (cc)
Deviation		Deviation		Deviation		Deviation		
None	Minor	None	Minor	None	Minor	None	Minor	
<1.15	1.15–1.25	<8	8–10	<55	55–60	<4	4–6	0–20
<1.15	1.15–1.25	<7	7–8	<65	65–70	<6	6–8	20–40
<1.10	1.10–1.20	<6	6–6.5	<65	65–75	<8	8–10	>40
Type B models (more advanced algorithms)								
$R_{100\%}$		$R_{50\%}$		$D_{2\ cm}$ (%)		$V_{20\ Gy}$ (%)		PTV (cc)
Deviation		Deviation		Deviation		Deviation		
None	Minor	None	Minor	None	Minor	None	Minor	
<1.25	1.25–1.40	<12	12–14	<65	65–75	<5	5–8	0–20
<1.15	1.15–1.25	<9	9–11	<70	70–80	<6	6–10	20–40
<1.10	1.10–1.20	<6	6–8	<70	70–80	<10	10–15	>40

APPENDIX 2

LETTER OF INFORMATION [TEMPLATE]

A Phase II Trial Measuring the Integration of Stereotactic Radiotherapy plus Surgery in Early Non-Small Cell Lung Cancer

(MISSILE-NSCLC)

Introduction

You are being invited to participate in this study because you have a lung cancer (called non-small cell lung cancer) that is less than 5 cm in size and has not spread to any other areas of the body, such as the lymph nodes or other organs.

The standard treatment for a lung cancer such as yours is surgery. In healthy patients, this surgery removes a whole lobe of the lung (called a 'lobectomy'), and in less-health patients, the surgery removes less than a whole lobe of the lung (called a 'sub-lobar' surgery.

'Stereotactic ablative radiation' (called SABR) is a new radiation treatment that delivers high-dose, precise radiation to small tumors in 1-3 weeks of treatment. This new technique can potentially allow radiation treatments to be focused more precisely, and delivered more accurately than with older treatments. This improvement could help by reducing side effects and by improving the chance of controlling the cancer by more precisely treating the cancer.

The study combines both SABR and surgery to treat lung cancer. SABR will be done first, with the surgery done approximately 10 weeks later. There will be some extra imaging (described below) done before and after the SABR. The purpose of this study is to determine how effective SABR is in killing the cancer cells, and if SABR can help make surgery more effective.

The study starts with some additional scans to better understand your tumor. These scans will be done in a single day at St. Joseph's Health Centre, and include a scan called a 'Dynamic Contrast Enhanced CT scan (DCE-CT)', and a Positron-Emission Tomography (PET) scan.

After these scans, patients will receive SABR. SABR treatments will be given every other day, on weekdays, over 1-3 weeks, depending on the location of your tumor. A CT scan through the region being treated will be taken on the radiation unit prior to treatment each day and your position for the treatment adjusted if necessary. Once your positioning is confirmed, the treatment will be given.

The scans will be repeated 8 weeks after SABR, along with a PET/CT scan and a thin-slice CT scan looking at your tumor. Surgery will be done approximately 10 weeks after SABR.

You will be followed regularly by your cancer specialists before and after treatment for 5 years. The effects of the treatment and any side effects will be measured. You will also have follow-up scans to assess the effects of treatment. As part of the study, you will be asked to fill out questionnaires before and after treatment. These questionnaires can be expected to take 5-10 minutes to complete on each occasion.

Potential Benefits of Participating in the Study

Potential benefits of participating in the study include the possibility improving your chances of curing the cancer using SABR.

Risks and Discomforts of the Scans

If you participate in the study, you will have 3 extra scans prior to SABR, and the same 3 scans after SABR. The results of the scans will be used for research purposes only and will not affect your treatment.

These scans include CT scanning. Since CT scans use x-rays, an ionizing radiation, there is a small risk associated. The risk is that of developing another cancer. In comparison to the radiotherapy used for SABR, the amount of radiation from the extra CT scans as a result of participating in this study will be very minimal. The extra radiation dose from each CT scans is estimated at approximately 10 mSv. The additional risk of cancer from a single CT is adult is estimated at less than 0.005% (5 in 100,000). The DCE-CT requires injection of a contrast agent called Visopaque 320. These scans also include a positron emission tomography (PET) scan which involve the injection of a small amount of radioactive sugar into a vein.

Risks and discomforts of SABR

Potential side effects from radiation depend on the area being treated:

- Radiation treatments to the chest area may commonly cause fatigue, dry cough, sore throat or difficulty swallowing as well as mild sunburn of the skin. Delayed (late, >6 months post treatment) side effects from radiation treatments to the chest area may rarely cause new or persistent difficulties with swallowing; shortness of breath or cough.

- Radiation treatments are associated with a small risk of serious injury to tissues or organs that are included in the area being treated. This injury may show up months to years post treatment. In very rare instances, these side effects may result in death. Some of these side effects include (depending on whether these areas are being treated):
  - Spinal cord injury resulting in paraplegia
  - Lung injury resulting in shortness of breath
  - Esophagus injury resulting in difficulty swallowing
  - Heart injury resulting in a heart attack or fluid collection on the heart
  - Bone injury resulting in a broken bone

Your physician will monitor your therapy and make adjustments to your treatment or prescribe medicines in order to manage side effects that occur during treatment. The radiation technique, daily dose and total dose of radiation for your treatment will be prescribed by your physician in order to minimize the chance of late serious injury as outlined above.

Risks and discomforts of surgery

Common side effects after surgery include a prolonged need for chest tube drainage after surgery, persistent cough, shortness of breath or difficulty breathing.

Less common side effects include **l**ung infection or a pneumonia, a blood clot in the lung, a blood clot in a large vein (which can cause a stroke or a heart attack in some cases), prolonged intubation or repeat intubation (a tube placed in the airway to help breathing), infection, bleeding, leakage of air from the lung after your lung cancer, injury to the laryngeal nerves (may cause hoarseness or difficulty swallowing), or changes in lung function tests.

Rare side effects include a severe infection (called sepsis), heart attack, irregular or rapid heartbeat, severe inflammation of the lung that affects the ability to breathe, or severe bleeding.

Withdrawal from the study

Participation in this study is voluntary. You may withdraw from the study at any time and still continue under the care of your radiation oncologist.

Privacy and Confidentiality:

All data that will be collected from this study will be considered confidential. We will maintain your confidentiality by using a unique identifier number on all documents instead of your name. A separate secure document will contain the linkage between your name and identifier number in order to minimize the possibility of a breach of your privacy. Your research records will be stored in a locked cabinet at the clinical trials unit. Once the data has been put into the research database, any identifying information, apart from your initials and a unique study number, will be removed from the database in order to protect your confidentiality. If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published without your explicit consent. By signing this consent form; you hereby consent to participation in this study. By consenting to this study you agree to allow us to confidentially collect this data. If you do not consent to this data collection, then you cannot participate in this study. Representatives of your local Research Ethics Board and the research team at your hospital may contact you or require access to your study-related records to monitor the conduct of the research.

If, during the course of this study, new information becomes available that may relate to your willingness to continue to participate, this information will be provided to you by the investigator.

Patient Rights:

Your participation in this study is voluntary. You may refuse to participate, refuse to answer any questions, or you may withdraw from the study at any time with no effect on your future care. If you decide not to participate or if you withdraw from the study before it is completed, the alternative procedures or courses of action will be explained to you by your doctor. A Data Safety Monitoring Committee will be reviewing the data from this research on a regular basis throughout the study. This will ensure that the participants are not exposed to increased risks as part of the study. If you are already participating in another study at this time, please inform the study doctor right away to determine if it is appropriate for you to participate in this study. We will tell you about new information that may affect your health, welfare, or willingness to stay in this study. If the results of the study are published, your name will not be used. If you would like to receive a copy of the overall results of this study, please put your name and address on a blank piece of paper and give it to the Clinical Research Associate.

If you have any questions about your rights as a research participant or the conduct of the study you may contact VP Research, Chief Administrator Officer, Lawson Health Research Institute, 519-667-6649.

Compensation and Costs:

For taking part in this research study, the extra costs associated with parking to get the additional scans will be reimbursed (you will be provided with parking vouchers for those visits). Taking part in the study may result in added costs to you (e.g. travel to the cancer clinic, etc.). In the event you are injured as a consequence of participation in this study due to the administration of the study treatment and/or procedure(s), your medical condition will be evaluated and medical care will be provided by one of the investigators or you will be referred for appropriate treatment. Although no funds have been set aside to compensate you in the event of injury or illness related to the study treatment or procedures, you do no waive any or your legal rights for compensation by signing the consent form.

A copy of this letter is for you to keep.

CONSENT FORM

A Phase II Trial Measuring the Integration of Stereotactic Radiotherapy *plus* Surgery in Early Non-Small Cell Lung Cancer

(MISSILE-NSCLC)

I have read the accompanying letter of information and have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction. Upon signing this form I will receive a copy.

_____ _____

Date Patient's Signature

I certify that I have explained to the individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised and have witnessed the above signature.

_____ _____

Date Investigator's Signature

Summary of Changes from Original Protocol (May 2014) to Final Protocol (Sept 2017)

Dec 2015 Amendment

Section 6.0 Treatment Plan

• imaging at 8 weeks allows +/- 2 weeks for normal scheduling variability, holidays, and scanner shutdown

Section 9.0 - Follow-Up Evaluation and Assessment of Efficacy

• visits are now specified to be time from date of surgery- Clarification purpose
• stopping PAR at 1 year and QOL at 2 year - reduce questionnaire burden.

March 2016 Amendment

Section 9.0 Follow up Evaluation and Assessment of Efficacy

1. Removal of PFTs at 3 month post-surgery visit: not considered clinically indicated

2. Follow-up with surgeon: It is standard of care for patients to be followed by surgeons. The current protocol has introduced duplication, as the patient must visit the surgeon and also visit radiation oncologist separately only to have trial data collected.

September 2017 Amendment

Added new translational objective (page 9):

5. To assess the immunological effects of SABR on the NSCLC tumor micro-environment

Added new section: 12.0 BIOMARKER STUDIES

This patient population offers a great opportunity to assess the immunological effects of SABR on the NSCLC tumor micro-environment. This could also provide clues for the best future immunotherapy combinations. In collaboration with researchers at VU University Medical Center in Amsterdam Netherlands, multiplex (7-parameter) ICH will be performed on pre and post-treatment tumor biopsies.
Required Samples:

1) Core biopsy pre-treatment for all patients (10 slides, unstained, at 5um thickness)
2) Post-radiotherapy tumor tissue at resection and slides from the hilar lymph nodes, if possible. (10 slides, unstained, at 5um thickness)

Planned Analyses:

1) Multiplex (7-parameter) ICH with immune parameters
    - Panels:
        - Tumor panel: IDO, PDL1, PDL2, HCA2, HC10, B2m
        - T-cell panels: CD3, CD8, FoxP3, TBet, Ki67, GranzymeB; and CD3, CD8, PD1, Tim3, Lag3, GranzymeB