Synlogic Operating Company, Inc.
Protocol #: SYNB1891-CP-001

A Phase 1, Open-label, Multicenter Study of SYNB1891 Administered by Intratumoral Injection to Patients with Advanced/Metastatic Solid Tumors or Lymphoma, Alone and in Combination with Atezolizumab

Statistical Analysis Plan

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LIST OF ABBREVIATIONS

Abbreviation or Specialist Term	Explanation
AE	adverse event
AESI	adverse event of special interest
ANC	absolute neutrophil count
APC	antigen-presenting cell
BMI	Body mass index
CI	Confidence interval
CPI	checkpoint inhibitor
CR	complete response
CRA	clinical research associate
CRO	contract research organization
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DILI	drug-induced liver injury
DLT	dose-limiting toxicity
DOR	duration of response
ECG	electrocardiogram
ECHO	echocardiogram
*Ec*N	*Escherichia coli* Nissle 1917
eCRF	electronic case report form
EDC	electronic data capture
EOT	End of Treatment
°F	degree(s) Fahrenheit
FDA	Food and Drug Administration
FNA	fine needle aspirate
g	gram(s)
GCP	Good Clinical Practice
geoCV	geometric coefficient of variation
GLP	Good Laboratory Practice
h	hour
HIV	human immunodeficiency virus
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

Abbreviation or Specialist Term	Explanation
ICF	Informed consent form
IgG1	immunoglobulin G1
IFN	interferon
IL	interleukin
IL1Ra	interleukin 1 receptor agonist
IR	immune response
IRB	Institutional Review Board
iRECIST	immune-related Response Evaluation Criteria in Solid Tumors
i.t.	intratumoral
IV	intravenous
L	liter(s)
LVEF	left ventricular ejection fraction
LYRIC	Lymphoma Response to Immunomodulatory Therapy Criteria
m^2	meter(s) squared
mAb	monoclonal antibody
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
mM	millimolar(s)
MTD	maximum tolerated dose
mTPI	modified toxicity probability interval
MUGA	multi-gated acquisition scan
NCI	National Cancer Institute
NE	not evaluable
ORR	objective response rate
OS	overall survival
PD	progressive disease
PET	positron emission tomography
PFS	progression free survival
PK	pharmacokinetic(s)
PR	partial response
PT	preferred term
Q3W	every 3 weeks

Abbreviation or Specialist Term	Explanation
qPCR	quantitative polymerase chain reaction
QTcF	QT interval corrected for heart rate using Fridericia's formula
RP2D	recommended phase 2 dose
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	serious adverse event
SAP	statistical analysis plan
SD	stable disease
SOC	system organ class
STING	STimulator of INterferon Genes
TEAE	Treatment Emergent Adverse Events
TIL	tumor-infiltrating lymphocytes
TNFα	tumor necrosis factor α
TTP	time to progression
WHO-DD	World Health Organization Drug Dictionary

I. Introduction

A. Background

While immunotherapies have become the standard-of-care for numerous cancers, significant unmet medical need persists primarily for the 55-87% of patients failing to respond to checkpoint inhibitors (CPIs). However, one emerging biomarker correlated with response is intratumoral (i.t.) immune cell infiltration (Zou et al 2016) where patients with higher levels of immune cell infiltration prior to therapy exhibit higher response rates to CPIs. Combination therapies seek to expand response rates by driving higher levels of immune cell infiltration into tumors, triggering antigen-presenting cell (APC) activation and promoting productive tumor-antigen presentation to effector T cells (Zou et al 2016). To this end, an interest in innate immune agonists has emerged.

SYNB1891 is a strain of modified live probiotic bacterium (Escherichia coli [E. coli] Nissle 1917) (EcN) programmed as a live immuno-biotherapeutic agent designed to treat patients with cancer. From nonclinical studies, SYNB1891 demonstrated significant antitumor control and the ability to deliver a STING agonist. Activation of STING results in the induction of Type I IFNs, like IFNβ, and in the context of cancer can trigger the initiation of antitumor immunity through the activation of APCs and the presentation of tumor-antigens to effector T cells (Corrales et al 2015).

Atezolizumab is a humanized immunoglobulin G1 (IgG1) monoclonal antibody. It is currently approved in the United States as a single agent or as a component of combination therapy for several indications. Atezolizumab has demonstrated antitumor activity in both nonclinical models and cancer patients and is being investigated as a potential therapy in a wide variety of malignancies in addition to the approved indications.

This rationale lends support to the investigation of i.t. administration of SYNB1891 to activate the innate immune system which subsequently promotes the initiation of antitumor immune responses in the injected tumor, as well as the demonstration of long-term immunological memory. As the first clinical study of SYNB1891, study SYNB1891-CP-001 aims to determine the maximum tolerated dose (MTD) as a monotherapy and recommended Phase 2 dose (RP2D) of SYNB1891 in combination with atezolizumab in patients with advanced/metastatic solid tumors or lymphoma.

The protocol for Study SYNB1891-CP-001 describes the general approach to analysis of data from the study. This analysis plan describes additional detail needed to complete such an analysis. Appendix 5 provides the table of contents for summary tables, listings and figures based on this analysis plan.

B. Protocol and Amendment History

This Statistical Analysis Plan (SAP) is based on version 4.0 of Protocol Amendment SYNB1891-CP-001.

Version	Approval Date	Salient Changes, if any*
Protocol version 4.0	27 July 2020	
Protocol version 3.0	27 May 2020	
Protocol version 2.0	11 February 2020	
Protocol version 1.0	27 June 2019	

* Changes expected to require accommodation in analysis plan.

This SAP will govern the analysis of data from this study. Any deviations from the analysis plan will be documented as such in the study report.

II. Protocol Objectives and Analysis Endpoints

The overall objective is to evaluate the safety and tolerability of escalating doses of i.t. injections of SYNB1891 to determine the single-agent MTD as monotherapy and the RP2D in combination with atezolizumab.

Objective	Endpoints
Primary: • Incidence of dose-limiting toxicities (DLTs): Percentage of patients who experience a DLT	• The number and percentage of patients who experience a DLT through the end of Cycle 1
Secondary: • Nature, incidence, and severity of all adverse events (AEs) and serious adverse events (SAEs) according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 5.0 • Objective response rate (ORR) as determined by the Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1) (Eisenhauer et al 2009), immune-related RECIST (iRECIST) (Seymour et al 2017), and/or the Lymphoma Response to Immunomodulatory Therapy Criteria (LYRIC) (Cheson et al 2016).	• The number and frequency of AEs and SAEs. • The number and frequency of AEs and SAEs under each severity category. • Objective response rate (complete response and partial response) per RECIST, iRECIST and LYRIC at any time on study • Evaluations of the SYNB1891-injected tumor(s), including tumor size, tumor response, etc.

Exploratory: • Duration of response or time to progression for evaluated tumors as determined by RECIST 1.1, iRECIST, and/or LYRIC • Pharmacodynamic response biomarkers including tumor infiltrating lymphocytes (TIL) characterization, immunomodulatory cytokine gene expression and IFNβ responsive genes to be monitored in proximal and (if available) distal lesion biopsies at baseline and on study; systemic pharmacodynamic effects will be evaluated by assessment of changes in serum cytokine levels (including, but not limited to, tumor necrosis factor α [TNFα], interleukin [IL] 2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL1β, IL 1 receptor antagonist [IL1RA], IL 2 receptor alpha [IL2R α], and IFNγ) at baseline and while on study • Kinetics of SYNB1891 within the injected tumor will be assessed by quantitative polymerase chain reaction (qPCR) from predose (baseline) to Cycle 1 Day 8 from a fine needle aspirate (FNA); systemic kinetics of SYNB1891 will be monitored by qPCR from blood at 6 hours and 24 hours postdose in Cycle 1	• Time to progression (TTP); progression free survival (PFS); overall survival (OS); time to best response; duration of response (DOR) • Assessment of changes in biomarker and serum cytokine parameters at baseline and while on study. • Assessment of qPCR at each visit and changes from baseline

III. Study Design

A. Design Overview

This Phase 1, open-label, multicenter, 2-arm study will follow the design in Appendix 1.

Patients enrolled to Arm 1 may receive up to four 21-day cycles, of SYNB1891 monotherapy escalated until the target DLT range is determined. On Days 1, 8, and 15 of Cycle 1 and Day 1 of Cycles 2 through 4, patients will receive an i.t. injection of SYNB1891 into an eligible lesion. If the initial eligible lesion undergoes complete regression and is no longer injectable (at the discretion of the Investigator), a subsequent eligible lesion (until no more eligible lesions remain) may be injected. The starting dose of SYNB1891 in the first cohort of Arm 1 will be 1×10^6 live cells and will be increased in approximately 3-fold increments in subsequent cohorts until MTD determination. A mTPI dose-finding design (Ji et al 2013, see Appendix 2) with a target DLT rate of approximately 30% will be applied for dose escalation and confirmation to determine the MTD/RP2D. In Arm 1, predetermined dose levels of 1×10^6, $3 \times$

10^6, 1×10^7, 3×10^7, 1×10^8, 3×10^8, and 1×10^9 live cells will be explored independently. A de-escalation dose of 3×10^5 live cells is available if the starting dose is deemed not tolerable. All dose escalation and de-escalation decisions will be based on the occurrence of DLTs at a given dose during Cycle 1 and will be made jointly by the Investigators and the Sponsor. If the initial eligible lesion undergoes complete regression and is no longer injectable, subsequent eligible lesions (until no more eligible lesions remain) may be injected. At the end of Cycle 4, patients may receive additional cycles of SYNB1891; for details refer to protocol section 4. The dose selected as achieving the target DLT range will be considered the MTD.

After determination of the MTD in Arm 1, dosing will begin in Arm 2 at a 10-fold lower dose than the Arm 1 MTD and will be increased in approximately 3-fold increments in subsequent cohorts until RP2D determination. Patients will follow the same four 21-day cycles schedule as Arm 1 and SYNB1891 will be administered by i.t. injection into an eligible lesion on Days 1, 8, and 15 of Cycle 1 and Day 1 of Cycles 2 through 4. In addition, atezolizumab will be administered in accordance with its recommended dose and schedule (1200 mg IV Q3W) on Day 1 of each of the 4 planned cycles. Combination doses will not be escalated above the SYNB1891 single-agent MTD established in Arm 1. On days when atezolizumab and SYNB1891 are both administered, SYNB1891 will be administered first, followed by at least 1 hour of observation prior to the atezolizumab infusion. Patients in Arm 2 who do not have progressive disease at the end of Cycle 4 on combination therapy may receive additional cycles of SYNB1891 and atezolizumab for up to 24 months, details refer to protocol section 4.

Once the RP2D of Arm 2 is determined, up to 20 additional patients may be enrolled in the RP2D cohort to fully characterize the safety profile of SYNB1891 in combination with atezolizumab.

B. Study Population

This study comprises patients with advanced/metastatic solid tumors or lymphoma. Patients will be eligible for enrollment regardless of gender or race/ethnicity. Additional inclusion and exclusion criteria can be found in protocol section 5.

C. Sample Size Predictions

No formal sample size calculations were performed, as the study is primarily designed for empirical evaluation of safety and tolerability in patients with advanced solid tumors or lymphoma. The anticipated sample size is approximately 70 patients, which provides for 4 to 7 dose-finding cohorts in Arm 1, 1 to 5 cohorts in Arm 2, and replacement of 1 patient per cohort, as well up to 20 additional patients enrolled at the RP2D in Arm 2 to fully characterize the

safety profile of SYNB1891 in combination with atezolizumab. Additional cohorts may be required in either arm to determine the MTD/RP2D.

D. Assessment Schedule

The maximum time of study participation for a patient is up to 26 months, including the screening period (up to 28 days), treatment administration period (up to 24 months), and safety follow-up period (30 ± 5 days after the last dose). The various assessments that will be conducted during this study are summarized in Appendix 3.

IV. Interventions

A. Clinical Trial Material

SYNB1891 investigational drug product is formulated in buffer composed of 15% glycerol, 5% trehalose, 10 mM Tris at pH 7.5. SYNB1891 is packaged aseptically into AT-closed vials at a volume of 0.6 mL and labeled for clinical study use. The manufactured SYNB1891 drug product has a concentration of 1×10^{11} live cells/mL.

Atezolizumab is supplied by the manufacturer as a sterile liquid in a single-use, 20-mL glass vial and labeled and shipped to each investigational site. Atezolizumab (1200 mg/vial) is prepared in accordance with local prescribing information and administered as an IV infusion at a dose of 1200 mg Q3W.

The protocol provides additional product details in Section 6.

B. Study Procedures

Before recruitment of patients into the study, written Institutional Review Board (IRB) approval of the protocol, informed consent, and any additional patient information must be obtained. For both study arms, screening procedures must be performed to determine eligibility for enrollment within 28 days prior to the first dose of study treatment.

Patients will undergo safety assessment, efficacy assessment, pharmacodynamic assessments and follow-up assessment.

In safety assessment, patients will provide information on medical history, adverse event, vital signs, physical examination, laboratory measurements, Electrocardiograms, and Multi-gated Acquisition Scan or Echocardiogram.

Efficacy will be assessed in the SYNB1891-injected lesion(s) and an overall response of up to 5 noninjected target lesions determined using appropriate

imaging at screening and every 2 cycles on study (i.e., at the beginning of every odd-numbered cycle) for the duration of the treatment period and at the EOT visit (unless imaging has been performed within 8 weeks prior to the EOT visit).

Tumor biopsies and blood samples for qPCR will be collected during the first 24-hour period of Cycle 1 and will be analyzed to assess the pharmacodynamics of circulating SYNB1891.

All patients will attend a Safety Follow-up visit 30 ± 5 days after the last dose of study treatment. Within 14 days after the last dose of study treatment (or premature treatment discontinuation), an EOT visit will be performed.

The protocol provides additional details in Section 7.

V. General Analytical Considerations

A. Data Sources

All reported study data will be recorded on the electronic case report forms (eCRF) using Medidata Rave. During the data collection process, automated quality assurance programs will be used to identify missing data, out-of-range data, and other data inconsistencies. External data will be provided by external vendors. Central lab cytokines data from serum will come from Q2 Solutions; qPCR data from injected tumor FNA, tumor biopsies and blood samples will come from QPS; microbiology data will come from Laboratories ITNL Microbiology Studies (IHMA).

B. Definition of Baseline

Baseline is defined as the last measurement taken prior to the first dose of study medication. The average of the predose results will be considered as the baseline value for vital signs taken at different timepoints on the same day predose.

C. Missing Data

Due to the dose escalation design of the study, no imputation of missing values will be done for the primary objective. When relevant, sections below will address how missing data will be handled for the particular analysis.

Partial dates are allowed on the eCRF for prior disease therapy start and stop dates, adverse event (AE) onset and resolution dates, concomitant medication start and stop dates, and concomitant procedure, procedure dates. An entry for the year is required in the eCRF system for each of these dates. Only the month and day may be entered as unknown. Dates from these forms will be reported in listings as collected. Every effort will be made to query missing dates.

For records with missing AE onset date, the following procedure will be employed for use in determining whether the AE is treatment emergent:

- AE onset dates with missing day and non-missing month will be assumed to occur on the first day of the non-missing month, except for AEs occurring in the month and year of first dosing, in which case the date will be imputed using the date of first dosing.
- AE onset dates with missing day and month will be assumed to occur on the first day of the non-missing year (ie, January 1), except for AEs occurring in the year of first dosing, in which case the date will be imputed using the date of the first dosing.
- AEs that are not ongoing and have a resolution date with missing day and non-missing month, they will be assumed to occur on the last day of the month, except when the end of study date or date of death is prior to the last day of the month. The date will be imputed as end of study date, or death date, whichever happens the earliest.
- AEs that are not ongoing and have a resolution date with end date month missing, the imputed end date should be set to the last day of the year 31DECYYYY, except when the end of study date or date of death is prior to the last day of the year. The date will be imputed as end of study date, or death date, whichever happens the earliest.

For records with a missing medication start and/or stop date, the following procedure will be employed for use in determining whether the medication is prior or concomitant:

- Medication start dates with a missing day and non-missing month will be assumed to occur on the first day of the non-missing month, except for medications occurring in the month of first dosing, in which case the date will be the date of first dosing.
- Medication start dates with missing day and month will be assumed to occur on the first day of the non-missing year (ie, January 1), except for medications occurring in the year of first dosing, in which case the date will be the date of first dosing.
- Medications that are not ongoing and have a medication stop date with a missing day and non-missing month will be assumed to occur on the last day of the non-missing month.
- Medications that are not ongoing and have a medication stop date with missing day and month will be assumed to occur on the last day of the non-missing year (i.e., December 31).

For records with a missing procedure date, the following procedure will be employed for use in determining whether the procedure is prior or concomitant:

- Procedure dates with a missing day and non-missing month will be assumed to occur on the first day of the non-missing month, except for

procedures occurring in the month of first dosing, in which case the date will be the date of first dosing.
- Procedure dates with missing day and month will be assumed to occur on the first day of the non-missing year (i.e., January 1), except for procedures occurring in the year of first dosing, in which case the date will be the date of first dosing.

For records with a missing disease progression date from prior therapy, the following procedure will be employed for use in analyzing time to event endpoints:

- If the disease progression date is completely missing OR only the year is available, set as missing.
- If both year and month of disease progression date from are available and only day is missing, the date of disease progression will be imputed by the last day of the month.

For records with a partial death date, the following procedure will be employed for use in deriving time to event variables.

- The death date will be imputed by the earliest possible date that is not contradicting with any other available data in the database.

All other data will be reported as they are collected. No imputation methods will be used to replace missing data unless otherwise stated in this document.

D. Interim Analyses

This is a single-arm, open-label study, so interim analyses may be performed as the study progresses.

Details of analysis will follow each section described in this SAP, may including the following components:

- Disposition
- Protocol deviations
- Baseline characteristics: demographics, disease history, medical history, concomitant medication/procedure
- Key components from efficacy analysis
- Key components from safety analysis
- PK and pharmacodynamic data (as available)

E. Analysis Populations

1. Safety Population

The safety population includes participants who were enrolled, allocated to treatment, and received at least one dose of investigational product.

2. Efficacy Population

The efficacy population includes participants who received study drug and had an on-treatment response assessment (including "Not Evaluable") or who discontinued study before any response assessment performed due to disease progression.

3. PK/Pharmacodynamic Population

The PK/PD population includes participants who received study drug and had at least one PK or PD assessment.

F. Data Display Characteristics

All analyses will be performed using SAS statistical analysis software. Descriptive statistics for continuous variables will include the number of patients, arithmetic mean, standard deviation (SD), median, minimum, and maximum. Geometric mean and geometric coefficient of variation (geoCV) may be used for PK/PD analyses. Summary statistics for categorical variables will contain count and percentage based on the number of patients in a cohort and the selected analysis population. Percentages will be presented to one decimal, except for one hundred percent, which will be presented as 100%.

Data displays produced for this study will include three types: summary tables, data listings and figures. Unless stated otherwise, data listings will be produced for all recorded data. Data listings will list the data recorded on the eCRF or obtained from external vendor for each patient. They will be ordered by arm, dose level, site, subject number, and time of assessment. When expedient, additional levels of ordering hierarchy may reflect subsets of assessments within an individual patient. Data listings will not display patient initials. Additional data listings may be produced for outcome measures that involve extensive procedures to derive the analyzed outcomes.

Summary tables will be produced as specified in following sections. Summary tables will display summary statistics calculated for each arm, dose cohorts and all patients combined, unless described otherwise in the following sections. For most summary tables, the arm and dose group will be presented in a column and the summary statistics of interest will be presented in rows.

Efficacy analysis will be summarized according to the planned dosing schedule. Safety analysis will be summarized according to the actual dosing schedule. For patients who were assigned a wrong study dose, they would be recoded in protocol deviation and dosing compliance; if the wrong dose occurred only once, the safety analysis will use the patients' initial planned dose as summary cohorts. If more than one wrong dose occurred, the safety analysis will use patients'

actual dose as summary cohorts. If patients' doses were reduced, safety analysis will use the initial planned dose as summary cohorts.

Patients receiving different dosing schedules will be presented in the listings only. Figures will be produced when specified.

Dates in the clinical database are classified into the categories of procedure dates, log dates, milestone dates, outcome dates, and special dates.

- Procedure Dates are the dates on which given protocol-specified procedure are performed. They include the dates of laboratory testing, physical examinations, tumor scans, etc. They should be present whenever data for a protocol-specified procedure is present and should only be missing when a procedure is marked as NOT DONE in the database. Procedure dates will not be imputed.
- Log Dates are dates recorded in CRF data logs. Specifically, they are the start and end dates for adverse events and concomitant medications/procedures. They should not be missing unless an event or medication is marked as ongoing in the database. Otherwise, incomplete log dates will be imputed according to the rules in Section 5.3. However, in listings, log dates will be shown as recorded without imputation.
- Milestone Dates are dates of protocol milestones such as randomization, study drug start date, study drug termination date, study closure date, etc. They should not be missing if the milestone occurs for a subject. They will not be imputed.
- Outcome Dates are dates corresponding to study endpoints such as survival, progression, etc. In most cases they are derived either from a milestone (e.g., the survival date is derived from the death date), or a procedure date (e.g., the progression date is derived from the date of the tumor scan that was used to determine progression). They may be subject to endpoint-specific censoring rules if the outcome did not occur but are not otherwise subject to imputation.
- Special Dates cannot be classified in any of the above categories and they include the date of birth. They may be subject to variable-specific censoring and imputation rules.

Calculations using dates (e.g., subject's age or relative day after the first dose of study medication) will adhere to the following conventions:

- Trial Day 1 of Cycle 1 is defined as the day on which the first dose of SYNB1891 is administered. Study days after trial day 1 will be calculated as the difference between the date of interest and the first date of dosing of study medication plus 1 day. The generalized calculation algorithm for relative day: STUDY DAY = [(TARGET DATE – DSTART) + 1 where DSTART = the start day of study drug].
- For dates of interest before the first date of dosing of study medication, the study day will be calculated as: STUDY DAY = TARGET DATE – DSTART. Negative study days are reflective of observations obtained

prior to first study drug administration. Note: Partial date for the first study drug is not imputed in general. All effort should be tried to avoid incomplete study drug start date.

- Age (years) at informed consent will be calculated as: (Date of informed consent – date of birth+1)/365.25 and then truncated to an integer. In case of partial birth date: impute missing day as 15th of the month; impute missing month as July; set missing age for missing year.
- Duration will be calculated by the difference of start and stop date + 1. AE duration (days) = AE end date – AE start date +1; study drug exposure duration (weeks) = (date of the last dose – date of the first dose + 1)/7.
- Time since disease diagnosis (months) will be calculated as (Informed Consent Date – Diagnosis Date +1)/ 30.4167.
- The time since an event will be calculated as date of event minus reference date. The specific censoring rules on the endpoint will follow section VII.
- The following conversion factors will be used to convert days to months or years:
    - 1 month = 30.4167 days
    - 1 week = 7 days
    - 1 year = 365.25 days

- Multiple Assessments for the Same Assessment Time Point

In the case of multiple observations (e.g. scheduled vs. unscheduled) at a specific visit, the first non-missing measurement will be used for analysis, unless multiple study assessments are expected (e.g., pre-dose vs post-dose). When multiple study assessments are expected, the first non-missing measurement for the visit and assessment time point will be used for analysis.

VI. Subject Accountability

A. Disposition

Patients are considered enrolled when they pass screening and are assigned to a dose cohort. Screen failure is defined as a patient who signs informed consent but does not qualify for enrollment into the study. Screen failures and reasons will be reported on screen log and be summarized for all patients who sign informed consent.

A summary of patients in the Safety Population will display the numbers and percentages of patients in each dose escalation and the MTD expansion group, by arm, cohort and overall. End of Treatment form will record patients who completed or withdrew from the study drug and each of the reasons. End of Study form will record patients who completed or withdrew from the study and

each of the reasons. Counts and percentages will be calculated using the number of enrolled patients in the relevant cohort as the denominator.

All disposition data will be presented in a listing.

B. Protocol Deviations

A listing of protocol deviations will identify patients who were enrolled even though they did not meet one or more eligibility criteria, patients who received wrong doses or had missing assessments, unavoidable deviations due to COVID-19 illness or COVID-19 control measures or any other errors that were not compliant with the protocol.

The protocol deviations will be further classified into major and minor deviations. The clinical team will review periodically the protocol deviations and will finalize the deviations prior to the database lock.

C. Subject Characteristics

The subject characteristics defined below will be presented in summary tables and data listings, using the most recent data collected prior to the start of dosing for patients in the Safety Population. No formal statistical comparisons will be performed.

Demographic and Baseline Characteristics:

- Age (in years). Age will be calculated as the number of years elapsed between birth date and the date of informed consent. Age will also be categorized as groups: <65 years and ⩾65 years.
- Sex
- Childbearing potential (for females only)
- Race. If more than one race selected, summarize race as multiple.
- Ethnicity
- Height
- Weight
- BMI

Disease History

- Cancer type, grade, and status
- Stage at Initial Diagnosis

- TNM system: primary tumor, Regional lymph nodes and distant metastasis
- Time since initial diagnosis
- Ann Arbor Stage (for patients with lymphoma)

Medical History

Medical history will be summarized by history type: Non-Disease related Medical History, Non-Disease related Surgery, Prior Anti-Cancer Surgery and Transplant. They will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 22.0. System Organ Class (SOC) and Preferred Term (PT) will be summarized by number and percentage (%) of patients having at least one occurrence of a disease. All Medical history information, including diagnosis, start/end date will be listed.

Prior Anti-Cancer Systemic Therapy:

Prior anti-cancer systemic therapies will be coded using WHO Drug Dictionary (WHO-DD), version B3 Global March 2019. A summary table will be organized to display the therapeutic subgroup and preferred names of each coded medication. The summary table will display counts and percentages of patients who reported using at least one medication in each represented therapeutic subgroup and preferred term. All prior anti-cancer systemic therapies information, including type, setting, line of therapy, start/end date, best response will be listed.

Prior Anti-Cancer Radiotherapy:

Prior anti-cancer radiotherapy will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 22.0. System Organ Class (SOC) and Preferred Term (PT) will be summarized by number and percentage (%) of patients having at least one occurrence of radiotherapy. A listing will be provided to display information including therapy site, setting, start/end date.

Concomitant Medications

Concomitant medication is defined as any medication with a start date prior to the date of the first dose of study treatment and continuing after the first dose of study treatment or with a start date between the dates of the first and last dose of study treatment. Any medication with a start date after the date of the last dose of study treatment will not be considered a concomitant medication. Any medication with a stop date prior to the first dose of study treatment is defined as a prior medication.

All concomitant medications and dietary supplements received within 28 days prior to the first dose of study treatment through 30 days after the last dose of study treatment will be recorded in the eCRFs, using generic drug names when possible. Concomitant medications will be coded using World Health Organization (WHO) Drug version B3 Global March 2019 coding dictionaries and will be summarized by the anatomical main class (1st level) of each coded medication and, within that, the pharmacological subgroup (3rd level) of the coded medication. Patients are counted only once in each therapeutic class category. Concomitant medication will be summarized using the safety population and be tabulated for each dose level using frequencies and percentages. Percentages will be based on the number of patients in each dose level. A listing will be provided to display the indication, dose, units, frequency, route, start/end date of the medication.

Concomitant Procedure

Concomitant procedure is defined as any procedure with a start date prior to the date of the first dose of study treatment and continuing after the first dose of study treatment or with a start date between the dates of the first and last dose of study treatment. Any procedure with a start date after the date of the last dose of study treatment will not be considered a concomitant procedure. Any procedure with a stop date prior to the first dose of study treatment is defined as a prior procedure.

Concomitant procedure will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 22.0. System Organ Class (SOC) and Preferred Term (PT) will be summarized by number and percentage (%) of patients having at least one occurrence of a concomitant procedure. A listing will be provided to display the procedure reason, location, and time.

VII. Efficacy Analyses

Efficacy analyses will be performed on Efficacy Population. Efficacy analyses will be descriptive in nature. Summary tables and figures will be presented by dose cohorts within the dose escalation and investigational arms.

A. Definition of Efficacy Outcomes

1. Assessment Method

The response of SYNB1891-injected lesion(s) and an overall response of up to 5 non-injected target lesions will be assessed using RECIST 1.1 (Eisenhauer et al 2009), iRECIST (Seymour et al 2017), or LYRIC (Cheson et al 2016) at

screening, then end of even-numbered cycles, cycle 2, 4 and additional cycles, and within 14 days after last dose at end of treatment/early termination visit.

- RECIST v1.1 will be used to determine response for patients with solid tumors. The response categories of target lesions include complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), and not evaluable (NE). The response categories of non-target lesions include: CR, Non-CR/Non-PD, and PD. The appearance of new malignant lesions denotes disease progression. An overall response will be summarized taking all target lesion, non-target lesion, new lesion into consideration.

- iRECIST is an extension of RECIST 1.1 that allows for assessment of response in patients being treated with immunotherapeutic agents. Overall response per iRECIST will be captured in the eCRF. According to iRECIST (protocol appendix 2), target lesions, non-target lesion and new lesion will be continually measured per RECIST response criteria after disease progression. For patients who are assessed as PD by RECIST (Target or Non-target lesions), they will perform repeated response assessments by RECIST and the response will be collected according to the eCRF. If the repeated assessments show PD again, the patients are confirmed as PD in the first assessment. If the repeated assessments show CR, PR or SD, the patients are not confirmed as PD. The appearance of any new lesions is considered as disease progression per RECIST 1.1.

- LYRIC evaluates response in patients with lymphoma who are being treated with immunotherapeutic agents. The response of lymphoma will be assessed separately for CT-based assessment and PET-CT based assessment. CT-based assessment includes complete radiologic response, partial remission, stable disease, progressive disease, immune response 1, immune response 2, immune response 3 and not evaluable. PET-CT based assessment includes complete metabolic response, partial metabolic response, no metabolic response, progressive metabolic disease, immune metabolic response 1, immune metabolic response 2, immune metabolic response 3 and not evaluable. Combined Overall Response includes complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), immune response 1 (IR1), immune response 2 (IR2), immune response 3 (IR3), and not evaluable (NE).

- The size and change from baseline of injected lesions will be listed by patient and summarized in tables. A waterfall plot for the injected lesion size will be displayed. And the comparison between injected vs. non-

injected lesions will also be presented using waterfall plot. Details can be found in section VII.B.

2. Best Overall Response and Objective Response Rate

Baseline documentation of "target" and "non-target" lesions (lesion count and maximum lesion length) will be recorded in listings. For solid tumor, the sum of diameters (longest diameter for non-lymph node lesions, short axis for lymph node lesions) will be calculated for all target lesions at baseline and at each subsequent tumor assessment as a measure of tumor burden. For lymphoma, the sum of the product of diameters will be calculated for all target lesions at baseline and at each subsequent tumor assessment as a measure of tumor burden. The Deauville five-point scale using FDG PET-CT in the assessment of treatment response will also be listed. The baseline lesion size value will be used as reference by which to characterize the lesion response.

Other lesions (or sites of disease) not selected as target lesions (measurable or non-measurable), including pathological lymph nodes, should be identified as non-target lesions and should also be recorded during screening. Measurements of these lesions are not recorded, but the presence or absence of each, including any unequivocal progression, should be noted throughout the study. The appearance of new malignant lesions denotes disease progression.

Per RECIST criteria, target lesions considered 'too small to measure' will be assigned a default value of 5 mm and those identified as 'not present' will be assigned 0 mm for purposes of analysis.

Patients' best overall response will be selected based on the overall response order (CR, PR, SD, PD, NE for RECIST; CR, PR, SD, PD, IR1, IR2, IR3 for LYRIC) through the study. Objective Response Rate (ORR), defined as the proportion of patients' overall response achieving a PR or CR at any time on the study, will be summarized descriptively by arm and cohort. In addition, an exact 90% confidence interval (CI) will be presented for RP2D cohort in Arm 2. Note that the definition of the Efficacy Population includes all treated patients who have an on-treatment response assessment or who discontinued study early due to disease progression; therefore, all Efficacy Population patients will be included in the denominators for percentage even if the patient had a Not Evaluable response. The best overall response and objective response rate will be summarized separately for each assessment method (RECIST, iRECIST, or LYRIC).

Similarly, the proportion of patients with a PR and the proportion of patients with a CR at any time during the study will be summarized as part of the best

overall response. The sum of diameters and change from baseline will also be summarized, by arm and cohort at each assessment visit.

All overall tumor and lymphoma response data will be presented in patient listings.

3. Determination of Time to Event Endpoints

Below is a list of events definitions for the solid tumor patients with a confirmed response according to RECIST v1.1 and iRECIST, and lymphoma patients with response according to LYRIC. The number of events and the number of censored patients will be summarized. The median time to event (reported in days) and the 25th and 75th percentiles on the time to event for each arm and cohort will be summarized using Kaplan-Meier estimates. Progression-Free Survival graphs will also be provided.

When calculating the endpoint that involve PD diagnosis date, such as time to progression or progression free survival, below rules will apply to account for the repeated PD response assessment per iRECIST:

- When a patient has disease progression per RECIST, if the repeated assessment confirmed as disease progression according to iRECIST criteria, the first PD date will be used in the endpoint calculation.
- If the repeated assessment confirmed as no disease progression and later SD, PR or CR occur, the initial diagnosis of PD will not be considered as the endpoint. Patients will continue further assessments until consecutive disease progression assessments occur.
- If the repeated assessment confirmed no disease progression and no later SD, PR or CR occur, and patients stop treatment due to not clinically stable or the patients die from their cancer, then the first unconfirmed progression should still be used as the endpoint (Seymour et al 2017).
- If the patients do not do a repeat assessment due to any other reasons, the unconfirmed PD date will be used as the endpoint.

For patients with lymphoma and evaluated by LYRIC, if they are categorized as having any of the IR response, repeat imaging should be obtained after an additional 12 weeks.

- If a patient is assessed as having IR and then “true” progressive disease at a subsequent time point, the IR assessment should subsequently be corrected to progressive disease and the date will be used as the PD endpoint for the relevant time to event analysis,
- If repeated imaging could not be obtained, the IR category will still be used as the endpoint.

- **Time to Progression (TTP):** defined as the time from the first SYNB1891 injection (Day 1) to PD. Patients who complete the study without PD or death will be censored at the day of their last visit. Patients

who discontinue or die due to disease progression, the endpoint will be the last assessment date prior to discontinue or death. Patients who discontinue or die not due to disease progression will be censored at their last tumor assessment.

- **Progression Free Survival (PFS):** defined as the time from the first SYNB1891 injection (Day 1) to PD or death, whichever is first, from any cause. Patients who complete or discontinue the study without disease progression or death will be censored at the last Response assessment date.

- **Duration of Response:** defined as the time from the first day the patient achieves objective response (PR or CR) to the day the patient's response falls to PD. Patients who do not achieve an objective response of a PR or CR during the study are excluded from this analysis. Patients who achieve objective response and remain the response at the end of the study will be censored at their last tumor assessment visit. If patients discontinued study due to disease progression after achieving objective response, they will not be censored, and disease progression date will be the endpoint. If patients discontinued study not due to disease progression after achieving objective response, they will be censored at their last tumor response assessment date.

Waterfall plots for tumor response by individual will be presented. The plots will have one bar per subject sorted from worst to best response with color by dose administered. Tumor response will be calculated based on the type of cancer that the patient has with the appropriate measure using either RECIST or LYRIC; an indicator will be placed on the figure to show if the response for each subject is from "R", RECIST, or "L", LYRIC. One waterfall plot will indicate best tumor response (minimum percent change from baseline) at any time point for each subject. One waterfall plot will represent injected and non-injected tumors.

Spaghetti plots for tumor response by time will be presented. One line will be drawn per subject with color by response type (see Section VII.2). The horizontal axis will be time and the vertical axis will be percent change from baseline in tumor response. One spaghetti plot will be generated for all patients at all times. One set of spaghetti plots will be generated with one panel per analysis type (RECIST/ iRECIST, or LYRIC) and only patients who apply for the current analysis type will be shown.

Swimmer plots will be generated with panels by analysis type (RECIST/iRECIST, or LYRIC). The plots will have one horizontal bar per subject firstly sorted by best response type (see Section VII.2), and then sorted by from best to worst time to start that response; when best to worst time to start that response does not apply (e.g. NE for RECIST), patients will be sorted by

maximum observation duration. Symbols within the swimmer plot will indicate time to start and end each new level of response (if the end of one response is the start of a new type of response by definition, only the start will be shown). The plots will also show the length of treatment duration a patient had.

B. Efficacy Analysis on Injected Lesion

On Days 1, 8, and 15 of Cycle 1 and Day 1 of Cycles 2 through 4, patients will receive an i.t. injection of SYNB1891 into an eligible lesion. Disease response status will be assessed in the SYNB1891-injected lesion(s) using RECIST, iRECIST and LYRIC. If the initial eligible lesion undergoes complete regression and is no longer injectable, a subsequent eligible lesion may be injected (until no more eligible lesions remain). Under this situation, the injected lesion analysis will only present the initial lesion size change. The subsequent newly injected lesion will be removed from the non-injected lesion analysis.

For solid tumors evaluated using RECIST and iRECIST, the longest diameter for the injected non-lymph node lesions and the short axis for the injected lymph node lesions will be listed per patient at each assessment visit. The change in lesion size from baseline will be summarized descriptively by comparing each follow up measurement to the baseline values. The best (minimum) tumor burden ratio from baseline to the end of the study for the initially injected tumor will be displayed in waterfall plot. The tumor burden ratio from baseline to the end of the study for initial injected tumor will also be compared to the sum of diameter change in the non-injected lesions. A waterfall plot will display this comparison by presenting the percentage change of lesion size from baseline in injected and non-injected lesions by patient.

For lymphoma evaluated using LYRIC, the product of the perpendicular diameters (Longest Transverse Diameter× Shortest Axis Perpendicular to Node) of the injected node will be listed per patient at each assessment visit. The change in the product of the perpendicular diameters will be summarized descriptively by comparing each follow up measurement to the baseline values. In addition, the percentage change of the product of the perpendicular diameters from baseline to the end of study for injected lesions will be displayed in waterfall plot by patient. A comparison of the percentage change between injected lesions and non-injected lesions (change in sum of the product of the perpendicular diameters) will also be presented in a waterfall plot for each patient.

VIII. Safety Analyses

Safety analyses will use data from the Safety population.

A. Treatment Exposure and Dosing Compliance

Exposure to SYNB1891 and atezolizumab will be summarized with descriptive statistics for the number of doses received, the number of cycles received, duration of dosing (weeks), total cumulative dose (cells/mL for SYNB1891, mg for atezolizumab). Duration of dosing is defined as the time from first dose of study drug in cycle 1 to the last dose of study drug in the last cycle a patient had.

The number of drug administrations with dose reduced, interrupted, and delayed will also be summarized descriptively by arm, cohort. In addition, the planned dosing date and the actual dosing date will be listed. Listings will be provided with the information from all the study drug administration eCRFs.

B. Dose Limiting Toxicities

Patients must have received at least one dose of the investigational product to be considered evaluable for the DLT evaluation and must have received at least 2 of the 3 planned SYNB1891 injections to contribute to dose-escalation decisions for a given cohort. DLTs will be determined for each patient through the end of Cycle 1. AEs and SAEs will be determined to be dose-limiting if they occur in this timeframe and meet the described criteria. AEs or SAEs experienced after the end of Cycle 1 will not be reported as DLTs but will be documented. Patients who experience a DLT (other than grade 5) will remain on the study to complete the safety evaluations and follow-up until resolution or stabilization of the DLT.

DLT events will be included in the AE listings and the number and percentages of patients who experience DLT will be summarized. Detailed information about DLT is described in protocol section 4.2.4.

C. Adverse Events

Adverse events (AEs) will be documented on the AE eCRF and monitored continuously throughout the study. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 22.0, associating lower-level terms with preferred terms and system organ classes by the primary hierarchy. All adverse events will be included in the listings.

Treatment Emergent Adverse Events (TEAE) are defined as any event that occurs or worsens on or after the day study treatment SYNB1891 is initiated through the safety follow-up visit (30 ± 5 days after the last dose of study treatment) except the SAEs related to the study treatment after safety follow-up. The tables will display counts and percentages of patients who reported at least one treatment-emergent

adverse events (TEAE) in each system organ class represented in the AE data. Within each system organ class, the tables will display the counts and percentages of patients reporting at least one TEAE, as designated by the preferred terms.

Adverse events of special interest (AESIs) (serious or nonserious) are a subset of pre-specified adverse events including 1) cytokine release syndrome, 2) grade 3 or higher systemic infection and 3) grade 3 or higher local injection site reactions. Detailed definition about adverse events of special interest for SYNB1891 can be found in protocol section 9.3.

The severity of adverse events will be graded using the NCI Common Toxicity Criteria for Adverse Events (CTCAE version 5.0). The intensity is assigned a grade of 1 through 5 using the following CTCAE guidelines: Grade 1 – Mild; Grade 2 – Moderate; Grade 3 – Severe; Grade 4 – Life-threatening; Grade 5 – Death.

The causality of AE will be classified as either related to study drug (possibly, probably, or definitely related) or not related to study drug (unlikely, probably not related, or definitely not related).

If a patient has multiple occurrences of an adverse event, the strongest level of relationship to study drug and the worst toxicity grade a patient experiences for a given AE will be used in these tables. No imputation will be performed for missing toxicity grade or causality data. The number and percentage of adverse events without relationship or toxicity grade values will be summarized under "Missing" category.

Adverse events that have missing onset dates will be considered as treatment emergent unless the stop date is known to be prior to the first administration of the study medication. If the adverse event onset date is partial, the date will be correlated as far as possible with the date of first dose of study medication. Adverse events will be assumed to be treatment-emergent, unless there is clear evidence (through comparison of partial dates) to suggest that the adverse event started prior to the first dose of study medication. Detailed imputation rules can be found in section V. If patients died during the study and experienced AEs at the time of death, the AEs that lead to death will use death date as the AE end date; otherwise, AEs that do not lead to death will be reported as ongoing.

The following TEAE summaries will be produced by arm and dose cohort, and overall:

a. An overall summary of safety will summarize the numbers (and percentages) of patients with the following:
   - at least one AE and serious adverse event (SAE)
   - AEs related to each study drug
   - SAEs related to each study drug
   - AEs of Special Interest

- DLTs
- discontinued study drug (SYNB1891, atezolizumab, and overall) due to at least one AE
- AEs leading to death

b. Separate summaries of TEAEs by System Organ Class and Preferred Term, subset as follows:
   - All TEAEs
   - TEAEs related to each study drug separately (SYNB1891 and atezolizumab). This table will include TEAEs with a drug relationship of "Definitely Related", "Probably Related" or "Possibly Related". It will also include TEAEs with missing drug relationships. An AE reported by a patient more than once will be included in this table if at least one of the drug relationship is one of the categories listed here.
   - All Serious TEAEs
   - Serious TEAEs related to each study drug separately. This table will include Serious TEAEs with a drug relationship of "Definitely Related", "Probably Related" or "Possibly Related". It will also include Serious TEAEs with missing drug relationships. An SAE reported by a patient more than once will be included in this table if at least one of the drug relationship is one of the categories listed here.
   - All Grade 3 or above TEAEs
   - Grade 3 or above TEAEs related to each study drug separately. These tables will include Grade 3+ TEAEs with the same constraints as TEAEs related to study drug
   - All TEAEs leading to discontinuation of study treatment (SYNB1891, atezolizumab, and overall)
   - All TEAEs leading to dose interruptions (of SYNB1891 and atezolizumab separately)
   - All TEAEs leading to dose modifications (of SYNB1891 and atezolizumab separately)
   - All TEAEs leading to death
   - TEAEs of special interest (AESIs)

c. All AEs by System Organ Class, Preferred Term and CTCAE toxicity grade
d. All AEs by System Organ Class, Preferred Term and action taken with each drug separately (SYNB1891 and atezolizumab)
e. A separate summary of AEs by Preferred Term for all AEs will be provided.

The following AE listings (including all AEs - TEAEs and non-TEAEs) will be produced (sorted by dose cohort, patient, and time):

- All AEs
- All SAEs
- AESIs
- AEs leading to discontinuation of study treatment

- AEs leading to death

D. Clinical Laboratory Results

Clinical laboratory tests will be performed (predose unless otherwise specified) at the timepoints indicated in Appendix 3. Baseline laboratory measurements do not need to be repeated if screening measurements were performed within 3 days prior to Cycle 1 Day 1. Local laboratories may review all hematology, chemistry, thyroid function, coagulation and urinalysis. Central laboratories will review all cytokines, qPCR and tumor samples collected for exploratory analysis. All laboratory data will be converted to standardized conventional units for reporting purposes. Appendix 4 outlines the clinical laboratory measurements that will be reviewed at the local and central laboratory.

E. Vital Signs

Vital signs (systolic blood pressure, diastolic blood pressure, body temperature, pulse, and pulse oximetry) will be summarized with continuous descriptive statistics at screening, on Cycle 1 Day 1 (predose and 30 minutes, 1 hour, 2 hours, 3 hours, 6 hours, and 24 hours postdose), on all study treatment days (predose and 30 minutes, 1 hour, 2 hours, 3 hours, and 6 hours postdose), at EOT/Early Termination and Safety Follow up.

F. Physical Examination

Complete physical examinations will be performed at screening and Baseline, with symptom-directed physical examinations performed at subsequent time points (see Appendix 3). As a part of the physical examination and prior to each dose, a photograph of the visible injected and/or noninjected lesion(s) should be taken at the discretion of the Investigator. No information from the photo will be included into the analysis. A listing of patients' Physical Examination completion status will be provided.

G. Electrocardiograms

Semi-supine single 12-lead ECGs will be performed at screening and reviewed locally. An overall interpretation of the ECG results will be made and QT, corrected by Fridericia's formula (QTcF), intervals will be listed.

H. Multi-gated Acquisition Scan or Echocardiogram

MUGA or ECHO will be performed within 6 months prior to the first dose of study treatment provided the patient has not received any potential cardiotoxic agents in the intervening period. If potential cardiotoxic agents have been administered within 6 months, patients must undergo a MUGA/ECHO during the

screening period. Left Ventricular Ejection Fraction and an overall interpretation will be listed.

Assessments performed at screening visit, Virology (hepatitis B, C and HIV), ECGs, MUGA/ECHO, Urinalysis will be listed by patient. Clinical laboratory results, vital sign and Physical examination will be listed by patient and summarized by Arm, dose and visit in the Safety population. Summaries of baseline values and changes from baseline will be presented by dose level for each assessment time point. Shift tables summarizing the counts and percentages of patients who were normal at baseline, but who became abnormal subsequently, will also be displayed.

IX. Pharmacodynamics and Pharmacokinetics

PK and PD analysis may be reported in a separate report.

Kinetics of SYNB1891 within the injected tumor will be assessed by quantitative polymerase chain reaction (qPCR) at predose baseline (within 7 days prior to Cycle 1 Day 1) and predose Cycle 1 Day 8 from a fine needle aspirate (FNA); systemic kinetics of SYNB1891 will be monitored by qPCR from blood at predose (baseline) and 6 hours and 24 hours postdose in Cycle 1.

Core Biopsies of the intended SYNB1891-injected lesion will be performed predose on Cycle 1 Day 1 (within 7 days prior) and predose on Cycle 2 Day 1. To optimize the quality of the biopsy samples obtained, 3 core samples should be obtained at each time point for each lesion, when feasible in the opinion of the Investigator. Biopsy tissue will be evaluated at various time points for IFN-responsive cytokines, TILs, PD-1, and PD-L1.

Pharmacodynamic response biomarkers including tumor-infiltrating lymphocytes (TIL) characterization, immunomodulatory cytokine gene expression and interferon (IFN) β responsive genes to be monitored in proximal and (if available) distal lesion biopsies at baseline and on study; systemic pharmacodynamic effects will be summarized descriptively by arm, cohort and visit, including changes in serum cytokine levels (including but not limited to tumor necrosis factor α [TNFα], interleukin [IL] 2, IL-4, IL- 6, IL-8, IL-10, IL-12p70, IL-13, IL1β, and IL 1 receptor agonist [IL1Ra], IL 2 receptor alpha[IL2Ra], and IFNγ) at baseline and while on study. All pharmacodynamics results will be presented in a listing.

All PK results will be presented in a patient listing. A table of summary statistics by time point and specimen type (tumor aspirate or blood) will be generated. A figure for PK will be generated illustrating median pharmacokinetics by time (linear and semi-log scale) with one line per dose level in a single panel per specimen type. One figure per dose level will be generated illustrating individual PK by time (linear and

semi-log scale) with one line per subject and one panel per dose level and specimen type.

A. NanoString

NanoString data will be obtained from the core biopsies described above. The results will be summarized as ratio from baseline by arm, dose level, gene, and time. Doses levels with ≥10 subjects per treatment will be further summarized with the two-sided, Bonferroni-adjusted, *p*-value for ratio from baseline. The output table will be sorted by increasing *p*-value.

X. References

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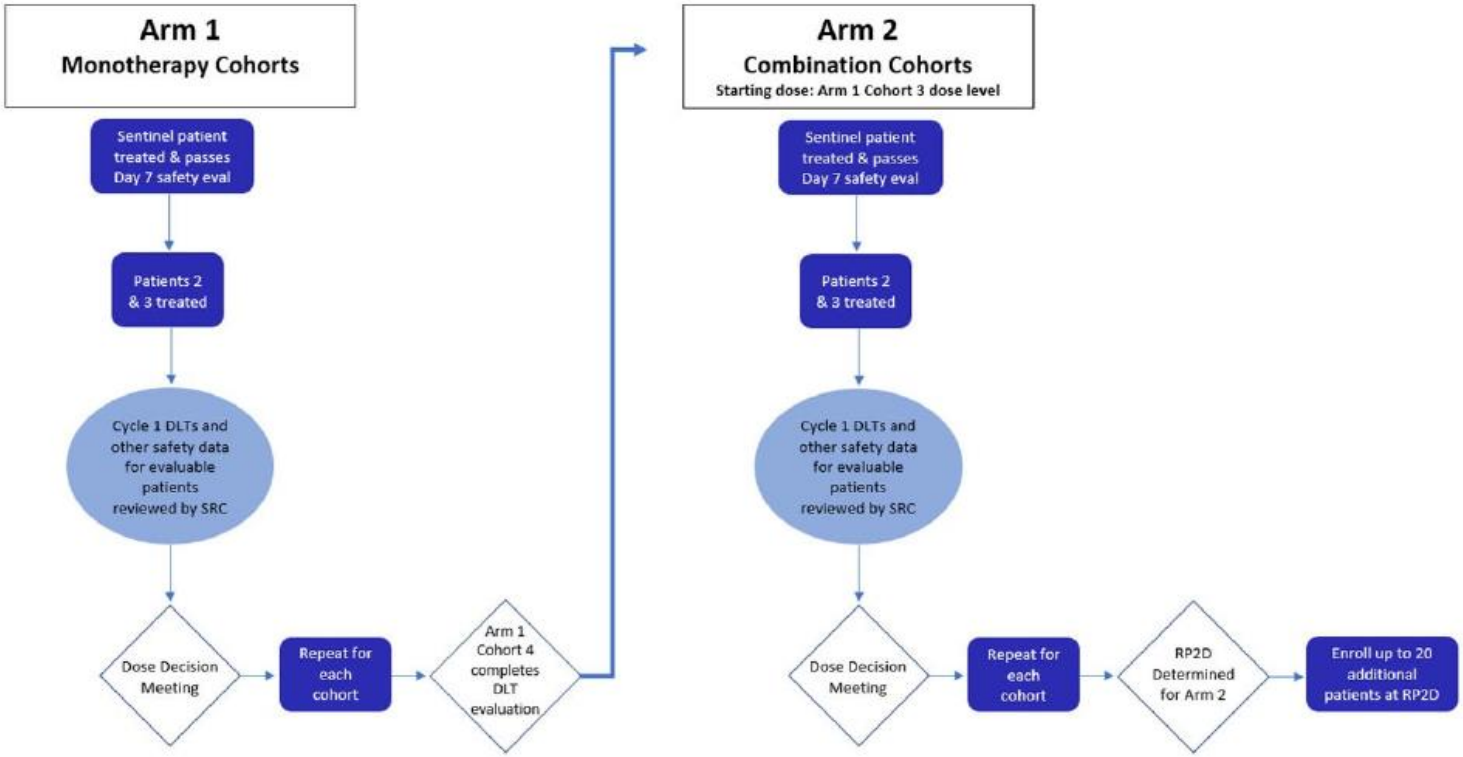
XI. Appendix 1: SYNB1891-CP-001 Study Design

Arm 1: SYNB1891 monotherapy:
- Safety and tolerability to determine single agent MTD
- Proof of mechanism by exploratory pharmacodynamic (PD) biomarkers
- mTPI dose escalation; enroll up to 28 patients

Arm 2: SYNB1891 combination with CPI:
- Safety and tolerability
- Proof of mechanism by exploratory biomarkers
- Enroll up to 20 patients, with potential to enroll up to 20 in extension



XII. Appendix 2: Dose-finding Rules using mTPI Design

Number of participants with at least 1 DLT	Number of Participants Evaluable for DLT at Current Dose											
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
0	E	E	E	E	E	E	E	E	E	E	E	E
1	S	S	S	E	E	E	E	E	E	E	E	E
2	D	S	S	S	S	S	S	S	E	E	E	E
3	DU	DU	D	S	S	S	S	S	S	S	S	S
4		DU	DU	DU	D	D	S	S	S	S	S	S
5			DU	DU	DU	DU	DU	D	S	S	S	S
6				DU	DU	DU	DU	DU	DU	D	S	S
7					DU	DU	DU	DU	DU	DU	DU	D
8						DU	DU	DU	DU	DU	DU	DU
9							DU	DU	DU	DU	DU	DU
10								DU	DU	DU	DU	DU
11									DU	DU	DU	DU
12										DU	DU	DU
13											DU	DU
14												DU

E = escalate to the next higher dose; S = stay at the current dose; D = de-escalate to the next lower dose;
DU = the current dose is unacceptably toxic.
Target toxicity rate = 30%
Flat noninformative prior Beta (1,1) is used as a prior and $\varepsilon1=\varepsilon2=0.03$

XIII. Appendix 3: Schedule of Events

Arm 1:

Study Period	Protocol Section	Screening	Cycle 1							Cycles 2-4	Additional Cycles[1]	EOT/Early Termination Visit	Safety Follow-up Visit
Study Day	7	-28 to 0	1			2 (24 ± 2 hours postdose)	3 (48 ± 2 hours postdose)	8 ± 1 day	15 ± 1 day	1 ± 1 day	1 ± 1 day	Within 14 days after last dose	30 ± 5 days after last dose
			Baseline (predose)	Dose	(6 ± 1 hours postdose)								
Informed consent/enrollment	7.1	●											
Medical history	7.2.1	●											
Physical examination	7.2.4	●	●			●		●	●	●	●	●	●
Height	7.2.4	●											
Weight	7.2.4	●	●			●				●	●	●	●
Vital signs (blood pressure, body temperature, pulse, and pulse oximetry) [2]	7.2.3	●	●		●	●		●	●	●	●	●	●
Screening for hepatitis B, C and HIV	7.2.5	●											
ECGs	7.2.6	●											
MUGA/ECHO	7.2.7	●											
Hematology (CBC with differential)	7.2.5.1	●	●					●	●	●	●	●	
Blood chemistry, including LFTs	7.2.5.1	●	●					●	●	●	●	●	
Coagulation (PT/aPTT)	7.2.5.1	●	●					●	●	●	●	●	
Thyroid function (FT4, TSH)	7.2.5.1	●								● [3]	● [3]	●	
Urinalysis	7.2.5.1	●											
Cytokine blood draw	7.2.5.2		●		●	●		● [4]	● [4]	● [4]	● [4]	●	
Blood preservation tube for exploratory analyses	7.2.5.2		●					● [5]		● [5,6]			
Kinetics of SYNB1891 (qPCR blood assay)	7.4		●		●	●							
Serum hCG (women of childbearing potential)	7.2.5	●											
Urine hCG (women of childbearing potential)	7.2.5		●							●	●	●	
Telephone call	7.2.2						●						

Study Period	Protocol Section	Screening	Cycle 1							Cycles 2-4	Additional Cycles[1]	EOT/Early Termination Visit	Safety Follow-up Visit
Study Day	7	-28 to 0	1			2 (24 ± 2 hours postdose)	3 (48 ± 2 hours postdose)	8 ± 1 day	15 ± 1 day	1 ± 1 day	1 ± 1 day	Within 14 days after last dose	30 ± 5 days after last dose
			Baseline (predose)	Dose	(6 ± 1 hours postdose)								
Fine needle aspirate of eligible lesion pre-injection	7.4		● [7]					● [5]					
Biopsy of eligible lesion	7.4		● [7]							● [8]		● [9]	
Biopsy of noninjected lesion (if applicable)	7.4		● [7]							● [8]		● [9]	
Administration of SYNB1891 intratumorally	4.2			●				●	●	●	●		
Tumor response using appropriate imaging	7.3	●								● [10]	● [10]	●	
Adverse event reporting	7.2.2	Continuously from the time of informed consent through the Safety Follow-up visit											
Monitoring for symptoms and signs of infection	7.2.2.1	Continuously from the time of informed consent through the Safety Follow-up visit											

Abbreviations: aPTT = activated partial thromboplastin time; CBC = complete blood count; ECG = electrocardiogram; ECHO = echocardiogram; EOT = end of treatment; FT4 = free thyroxine; hCG = human chorionic gonadotropin; HIV = human immunodeficiency virus; LFT = liver function test; MUGA = multi-gated acquisition; qPCR = quantitative polymerase chain reaction; PT = prothrombin time; TSH = thyroid stimulating hormone

[1] Patients who do not have progressive disease (i.e., those who achieve and sustain a complete/partial response or stable disease) may receive additional cycles of SYNB1891 given on Day 1 of each cycle for up to 24 months (i.e., Cycles 5 to 35) after the initial dose of study treatment until documentation of progressive disease or other discontinuation criteria, satisfaction of a predefined stopping rule, or no eligible lesions remain.

[2] Collected while patients are in a seated or supine position at screening, on Cycle 1 Day 1 (predose and 30 minutes, 1 hour, 2 hours, 3 hours, 6 hours, and 24 hours postdose), on all other study treatment days (predose and 30 minutes, 1 hour, 2 hours, 3 hours, and 6 hours postdose), at EOT/Early Termination and Safety Follow-up, and as clinically indicated.

[3] Day 1 of odd-numbered cycles only.

[4] Predose and 6 hours postdose (as indicated) after every SYNB1891 injection.

[5] Predose.

[6] Day 1 of Cycle 3 only.

[7] The predose (baseline) biopsies and fine needle aspirate may be performed up to 7 days prior to Cycle 1 Day 1.

[8] Core biopsies will be collected predose on Day 1 of Cycle 2 only (i.e., not Day 1 of Cycles 3 or 4).

[9] For patients who withdraw from the study prior to Cycle 2 Day 1, the second biopsy should be performed at the EOT visit.

[10] At the end of even-numbered cycles only.

Arm 2:

Study Period	Protocol Section	Screening	Cycle 1							Cycles 2-4	Additional Cycles [1]	EOT/Early Termination Visit	Safety Follow-up Visit
Study Day	7	-28 to 0	1			2 (24 ± 2 hours postdose)	3 (48 ± 2 hours postdose)	8 ± 1 day	15 ± 1 day	1 ± 1 day	1 ± 1 day	Within 14 days after last dose	30 ± 5 days after last dose
			Baseline (predose)	Dose	(6 ± 1 hours postdose)								
Informed consent/enrollment	7.1	●											
Medical history	7.2.1	●											
Physical examination	7.2.4	●	●			●		●	●	●	●	●	●
Height	7.2.4	●											
Weight	7.2.4	●	●							●	●	●	●
Vital signs (blood pressure, body temperature, pulse, and pulse oximetry) [2]	7.2.3	●	●		●	●		●	●	●	●	●	●
Screening for hepatitis B, C and HIV	7.2.5	●											
ECGs	7.2.6	●											
MUGA/ECHO	7.2.7	●											
Hematology (CBC with differential)	7.2.5.1	●	●					●	●	●	●	●	
Blood chemistry, including LFTs	7.2.5.1	●	●					●	●	●	●	●	
Coagulation (PT/aPTT)	7.2.5.1	●	●					●	●	●	●	●	
Thyroid function (FT4, TSH)	7.2.5.1	●								● [3]	● [3]	●	
Urinalysis	7.2.5.1	●											
Cytokine blood draw	7.2.5.2		●		●	●		● [4]	● [4]	● [4]	● [4]	●	
Blood preservation tube for exploratory analyses	7.2.5.2		●					● [5]		● [5,6]			
Kinetics of SYNB1891 (qPCR blood assay)	7.4		●		●	●							
Serum hCG (women of childbearing potential)	7.2.5	●											
Urine hCG (women of childbearing potential)	7.2.5		●							●	●	●	
Telephone call	7.2.2						●						
Fine needle aspirate of eligible lesion pre-injection	7.4		● [7]					● [5]					

Study Period	Protocol Section	Screening	Cycle 1							Cycles 2-4	Additional Cycles [1]	EOT/Early Termination Visit	Safety Follow-up Visit
Study Day	7	-28 to 0	1			2 (24 ± 2 hours postdose)	3 (48 ± 2 hours postdose)	8 ± 1 day	15 ± 1 day	1 ± 1 day	1 ± 1 day	Within 14 days after last dose	30 ± 5 days after last dose
			Baseline (predose)	Dose	(6 ± 1 hours postdose)								
Biopsy of eligible lesion	7.4		● [7]							● [8]		● [9]	
Biopsy of noninjected lesion (if applicable)	7.4		● [7]							● [8]		● [9]	
Administration of SYNB1891 intratumorally	4.2			●				●	●	●	●		
Administration of atezolizumab by IV infusion	4.2			●						●	●		
Tumor response using appropriate imaging	7.3	●								● [10]	● [10]	●	
Adverse event reporting	7.2.2	Continuously from the time of informed consent through the Safety Follow-up visit											
Monitoring for symptoms and signs of infection	7.2.2.1	Continuously from the time of informed consent through the Safety Follow-up visit											

Abbreviations: aPTT = activated partial thromboplastin time; CBC = complete blood count; ECG = electrocardiogram; ECHO = echocardiogram; EOT = end of treatment; FT4 = free thyroxine; hCG = human chorionic gonadotropin; HIV = human immunodeficiency virus; IV = intravenous; LFT = liver function test; MUGA = multi-gated acquisition; qPCR = quantitative polymerase chain reaction; PT = prothrombin time; TSH = thyroid stimulating hormone

[1] Patients who do not have progressive disease (i.e., those who achieve and sustain a complete/partial response or stable disease) at the end of Cycle 4 on combination therapy may receive additional cycles of study treatment for up to 24 months (i.e., Cycles 5 to 35) after the initial dose of study treatment until documentation of progressive disease or other discontinuation criteria, satisfaction of a predefined stopping rule, or no eligible lesions remain.

[2] Collected while patients are in a seated or supine position at screening, on Cycle 1 Day 1 (predose and 30 minutes, 1 hour, 2 hours, 3 hours, 6 hours, and 24 hours postdose), on all other study treatment days (predose and 30 minutes, 1 hour, 2 hours, 3 hours, and 6 hours postdose), at EOT/Early Termination and Safety Follow-up, and as clinically indicated.

[3] Day 1 of odd-numbered cycles only.

[4] Predose and 6 hours postdose (as indicated) after every SYNB1891 injection.

[5] Predose.

[6] Day 1 of Cycle 3 only.

[7] The predose (baseline) biopsies and fine needle aspirate may be performed up to 7 days prior to Cycle 1 Day 1.

[8] Core biopsies will be collected predose on Day 1 of Cycle 2 only (i.e., not Day 1 of Cycles 3 or 4).

[9] For patients who withdraw from the study prior to Cycle 2 Day 1, the second biopsy should be performed at the EOT visit.

[10] At the end of even-numbered cycles only.

XIV. Appendix 4: Local and Central Laboratory Parameters

Local Laboratory Parameters

Panel	Parameters	
Hematology	**Must be Reviewed Predose:**	
	Calculated absolute neutrophil count White blood cell Red blood cell Red cell indices Hemoglobin Hematocrit Platelet count	 Neutrophils Lymphocytes Monocytes Eosinophils Basophils
Chemistry	**Must be Reviewed Predose:**	
	Calcium Chloride Total protein Potassium Glucose Sodium Blood urea nitrogen Creatinine Bicarbonate Albumin Uric acid Phosphorus Magnesium	Alkaline phosphatase Aspartate aminotransferase Alanine aminotransferase Lactate dehydrogenase Direct bilirubin Indirect bilirubin Total bilirubin
Thyroid Function	**Must be Reviewed Predose:**	
	Free T4	Thyroid-stimulating hormone
Coagulation	**May be Reviewed Postdose:**	
	Prothrombin time and/or activated partial thromboplastin time International normalized ratio	
Urinalysis	**May be Reviewed Postdose:**	
	Dipstick: pH Glucose Protein Bilirubin Ketones Leukocytes Blood	Microscopic (if dipstick is positive): White blood cell count Red blood cell count Casts

Central Laboratory Parameters

Panel	Parameters
Cytokines	IL1RA, CXCL10, IFNγ, CXCL5, IL1a, IL6, CCL5, CCL20, TNFα, CCL4, CCL2, S100A8/9 (individual or both), CCL3
qPCR	• Tumor tissue quantitative polymerase chain reaction (qPCR) from fine needle aspirate • Whole blood qPCR • Whole blood qPCR from blood cultures
NanoString	• Tumor biopsy samples
Other	Blood samples collected for exploratory analyses in preservation tubes

XV. Appendix 5: Summary Tables, Listing and Figures

The following titles describe planned summary tables, listings, and figures. The final version of this list and additional details can be found in the separate Table, Listings and Figures shells document for Interim Analysis and Final Analysis. Tables may be combined or divided, and title details may be modified during development, if spacing and appearance require doing so. Additional summary tables may be produced if warranted by collected data. If a table in this appendix is not produced because it is not needed or it would not be informative (as, for example, when two analysis populations converge), that change in the set of data displays will updated in the TLF shells document.
As a general comment for the tables, all tables will show Arm 1 and Arm 2, separately.

	Table Title	Analysis Population	Interim Analysis (1st at the end of Arm 1 Cohort 4 [C4]; 2nd at the end of Arm 1 [E1]; or both [X])
1	Patient Disposition, Number (%) of Patients	Safety	X
2	Demographic and Baseline Characteristics	Safety	X
3	Subject Medical History, Number (%) of Patients	Safety	E1
4	Disease History, Number (%) of Patients	Safety	E1
5	Prior Anti-Cancer Systemic Therapy, Number (%) of Patients	Safety	X
6	Prior Anti-Cancer Radiotherapy Therapy, Number (%) of Patients	Safety	
7	Concomitant Medications, Number (%) of Patients	Safety	
8	Concomitant Procedure, Number (%) of Patients	Safety	
9	Exposure to Study Drug	Safety	X
10	Patients with Dose Modifications or Interruptions	Safety	E1
11	Summary of Tumor Response by RECIST 1.1/iRECIST	Efficacy	X
12	Summary of Lymphoma Response by LYRIC	Efficacy	X
13	Summary of Sum of Diameters of Target Lesion	Efficacy	E1
14	Summary of Injected Lesion	Efficacy	X
15	Summary of Time to Progression by RECIST 1.1	Efficacy	E1
16	Summary of Time to Progression by LYRIC	Efficacy	E1
17	Summary of Progression Free Survival by RECIST 1.1	Efficacy	E1
18	Summary of Progression Free Survival by LYRIC	Efficacy	E1
19	Summary of Duration of Response by RECIST 1.1	Efficacy	

20	Summary of Duration of Response by LYRIC	Efficacy	
21	Dose Limiting Toxicity (DLT), Number (%) of Patients	Safety	X
22	Overall Summary of Treatment Emergent Adverse Events (TEAE), Number (%) of Patients	Safety	X
23	Incidence of Treatment Emergent Adverse Events by Primary MedDRA System Organ Class and Preferred Term, Number (%) of Patients	Safety	E1
24	Incidence of Treatment Emergent Adverse Events by System Organ Class and Preferred Term – Related to SYNB1891, Number (%) of Patients	Safety	E1
25	Incidence of Treatment Emergent Adverse Events by System Organ Class and Preferred Term – Related to Atezolizumab, Number (%) of Patients	Safety	
26	Incidence of Serious Treatment Emergent Adverse Events by System Organ Class and Preferred Term, Number (%) of Patients	Safety	E1
27	Incidence of Serious Treatment Emergent Adverse Events by System Organ Class and Preferred Term – Related to SYNB1891, Number (%) of Patients	Safety	E1
28	Incidence of Serious Treatment Emergent Adverse Events by System Organ Class and Preferred Term – Related to Atezolizumab, Number (%) of Patients	Safety	
29	Incidence of Grade 3 or Greater Treatment Emergent Adverse Events by System Organ Class and Preferred Term, Number (%) of Patients	Safety	E1
30	Incidence of Grade 3 or Greater Treatment Emergent Adverse Events by System Organ Class and Preferred Term – Related to SYNB1891, Number (%) of Patients	Safety	E1
31	Incidence of Grade 3 or Greater Treatment Emergent Adverse Events by System Organ Class and Preferred Term – Related to Atezolizumab, Number (%) of Patients	Safety	
32	Incidence of Treatment Emergent Adverse Events Leading to Any Study Drug Discontinuation by System Organ Class and Preferred Term, Number (%) of Patients	Safety	
33	Incidence of Treatment Emergent Adverse Events Leading to SYNB1891 Discontinuation by System Organ Class and Preferred Term, Number (%) of Patients	Safety	
34	Incidence of Treatment Emergent Adverse Events Leading to Atezolizumab Discontinuation by System Organ Class and Preferred Term, Number (%) of Patients	Safety	

35	Incidence of Treatment Emergent Adverse Events Resulting in SYNB1891 Dose Interruption by System Organ Class and Preferred Term, Number (%) of Patients	Safety	
36	Incidence of Treatment Emergent Adverse Events Resulting in Atezolizumab Dose Interruption by System Organ Class and Preferred Term, Number (%) of Patients	Safety	
37	Incidence of Treatment Emergent Adverse Events Resulting in SYNB1891 Dose Modification by System Organ Class and Preferred Term, Number (%) of Patients	Safety	
38	Incidence of Treatment Emergent Adverse Events Resulting in Atezolizumab Dose Modification by System Organ Class and Preferred Term, Number (%) of Patients	Safety	
39	Incidence of Treatment Emergent Adverse Events Resulting in Death by System Organ Class and Preferred Term, Number (%) of Patients	Safety	
40	Incidence of Treatment Emergent Adverse Events of Special Interest by System Organ Class and Preferred Term, Number (%) of Patients	Safety	E1
41	Incidence of Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Maximum Severity, Number (%) of Patients	Safety	E1
42	Incidence of Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Action Taken, Number (%) of Patients	Safety	
43	Incidence of Treatment Emergent Adverse Events by Preferred Term, Number (%) of Patients	Safety	
44	Mean Change in Clinical Chemistry Parameters Over Time	Safety	
45	Mean Change in Hematology and Coagulation Parameters Over Time	Safety	
46	Mean Change in Thyroid Function Parameters Over Time	Safety	
47	Mean Change in Vital Signs Over Time	Safety	
48	Mean Change in Pharmacodynamic Over Time	PK/PD	
49	Mean Change in Pharmacokinetics Over Time	PK/PD	

	Listing Title	Analysis Population	Interim Analysis (1st at the end of Arm 1 Cohort 4 [C4]; 2nd at the end of Arm 1 [E1]; or both [X])	Comment
1	Patient Disposition	Safety	X	
2	Screening and Enrollment Status	Safety	X	
3	Protocol Deviations	Safety	X	
4	Demographic Characteristics	Safety		
5	Subject Medical History	Safety		
6	Disease History	Safety	X	
7	Prior Anti-Cancer Systemic Therapy	Safety	X	
8	Prior Anti-Cancer Radiotherapy	Safety		
9	Prior/Concomitant Medications	Safety		
10	Prior/Concomitant Procedures	Safety		
11	Intratumoral Administration of SYNB1891	Safety	X	
12	Administration of Atezolizumab by IV Infusion	Safety		
13	Dosing Compliance/Exposure	Safety		
14	Core Biopsy of Lesion	Safety		
15	RECIST 1.1 Target Lesions	Safety	E1	
16	RECIST 1.1 Non-Target Lesions	Safety	E1	
17	RECIST 1.1 New Lesions	Safety	E1	
18	RECIST 1.1 Injected Lesions	Safety	E1	
19	RECIST 1.1 Response Assessment	Safety	X	19.1. iRECIST
20	LYRIC Target Lesions	Safety	E1	
21	LYRIC Non-Target Lesions	Safety	E1	
22	LYRIC New Lesions	Safety	E1	
23	LYRIC Injected Lesions	Safety	E1	
24	LYRIC Response Assessment	Safety	X	
25	Adverse Events	Safety	X	
26	Serious Adverse Events	Safety	X	
27	Adverse Events of Special Interest	Safety		
28	Adverse Events Leading to Any Study Drug Discontinuation	Safety	X	
29	Adverse Events Leading to Death	Safety	X	

30	Clinical Chemistry	Safety	X	
31	Hematology and Coagulation	Safety	X	
32	Thyroid Function Tests	Safety		
33	Vital Signs	Safety	X	
34	Physical Examination	Safety		
35	12-Lead ECG	Safety		
36	Multi-gated Acquisition Scan or Echocardiogram	Safety		
37	Urinalysis	Safety		
38	Pregnancy Test	Safety		
39	Virology (Hepatitis B, C, and HIV)	Safety		
40	Pharmacodynamic	PK/PD		
41	Pharmacokinetics	PK/PD		
42	Core Biopsies	Safety		
43	Unscheduled Assessment	Safety		

As a general comment on the figures, all figures will combine RECIST and LYRIC.

	Figures Title	Analysis Population	Interim Analysis (1st at the end of Arm 1 Cohort 4 [C4]; 2nd at the end of Arm 1 [E1]; or both [X])
1	Waterfall Plot of Change in Tumor Size from Baseline to Patients' Best Tumor Response at Any Time Point	Efficacy	X
2	Waterfall Plot of Change in Tumor Size from Baseline to Patients' Best Tumor Response at Any Time Point Comparing Injected and Non-injected Lesions	Efficacy	X
3	Waterfall Plot of Change in Tumor Size from Baseline to Patients' Best Tumor Response at Any Time Point for Injected Lesions	Efficacy	X
4	Spaghetti Plot of Tumor Response	Efficacy	X
5	Swimmer Plot of Tumor Response	Efficacy	X
6	PK Linear and Semi-log Graph	PK/PD	(Optional)