CLINICAL STUDY SYNB1891-CP-001

Study Title:	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
Study Number:	SYNB1891-CP-001
Study Phase:	Phase 1
Product Name:	SYNB1891
Indication:	Advanced/metastatic solid tumors or lymphoma
Sponsor:	Synlogic Operating Company, Inc.
Protocol Version History:	Version 4: 27 July 2020 Version 3: 27 May 2020 Version 2: 11 February 2020 Version 1: 27 June 2019

Synlogic Operating Company, Inc.

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SIGNATURE PAGE (SPONSOR)

I have read and understand the contents of the clinical protocol for Clinical Study SYNB1891-CP-001 Version 4, dated 27 July 2020, and agree to meet all obligations of Synlogic Operating Company, Inc. as detailed in all applicable regulations and guidelines. In addition, I will ensure that the Principal Investigators are informed of all relevant information that becomes available during the conduct of this study.

_____ _____

[redacted] Date

Chief Medical Officer
Synlogic Operating Company, Inc.

PRINCIPAL INVESTIGATOR AGREEMENT

I have read and understand the contents of the clinical protocol for Clinical Study SYNB1891-CP-001 Version 4, dated 27 July 2020, and will adhere to the study requirements as presented, including all statements regarding confidentiality. In addition, I will conduct the study in accordance with the requirements of this protocol and also protect the rights, safety, privacy and well-being of study patients in accordance with the following:

- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Harmonised Tripartite Guideline for Good Clinical Practice (GCP) E6(R2).
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations.
- Requirements for reporting serious adverse events as defined in Section 9 of this protocol.
- Terms outlined in the Clinical Study Site Agreement.

My signature also acknowledges that:

- Neither my Sub-investigators nor I are members of the Institutional Review Board reviewing this protocol, or
- I and/or my Sub-investigators are members of the Institutional Review Board, but I/we will not participate in the initial review or continuing review of this study.

Name of Principal Investigator	Signature of Principal Investigator
Date	

PROTOCOL SYNOPSIS

Name of Sponsor/Company: Synlogic Operating Company, Inc.	
Name of Investigational Product: SYNB1891	
Name of Active Ingredient: SYNB1891	
Title of Study: 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	
Phase: 1 (first-in-human)	
Study Center: Approximately 8 study centers in the United States	
Number of Patients: Approximately 70	
Objectives and Endpoints	The overall objective is to evaluate the safety and tolerability of escalating doses of intratumoral (i.t.) injections of SYNB1891 to determine the single-agent maximum tolerated dose (MTD) as monotherapy and the recommended Phase 2 dose (RP2D) in combination with atezolizumab. Primary objective: • Incidence of dose-limiting toxicities (DLTs): Percentage of patients who experience a DLT Secondary objectives: • 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) in target lesions as determined by the Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1), immune-related RECIST (iRECIST), and/or the Lymphoma Response to Immunomodulatory Therapy Criteria (LYRIC), with evaluations of the SYNB1891-injected tumor(s) and an overall response of up to 5 noninjected target lesions Exploratory objectives: • Duration of response or time to progression for evaluated tumors as determined by RECIST 1.1, iRECIST, and/or LYRIC • Pharmacodynamic (PD) 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 PD 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 [IL2Rα], 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 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
Design	**Methodology** This Phase 1, open-label, multicenter, 2-arm study will be conducted using the modified toxicity probability interval (mTPI) dose-finding design. Cohorts of patients will be enrolled to receive escalating doses of SYNB1891 administered by i.t. injection until the target DLT range (approximately 30%) for SYNB1891 monotherapy is determined based on DLTs observed in Cycle 1 (Arm 1). The dose selected as achieving the target DLT range will be considered the MTD. After all patients in Arm 1 Cohort 4 have completed their Cycle 1 DLT safety evaluation, Arm 2 will enroll its first cohort starting at the Arm 1 Cohort 3 dose level, with SYNB1891 administered by i.t. injection in combination with atezolizumab administered as an intravenous (IV) infusion at a dose of 1200 mg every 3 weeks (Q3W) until the RP2D for the combination regimen is determined. The mTPI algorithm will be implemented separately in each arm. **Assessments** Eligible patients will be enrolled sequentially and will receive the first dose of study treatment within 28 days of screening. Safety will be monitored continuously by documentation of AEs, SAEs, clinical laboratory measurements, vital signs, and physical examinations. DLTs will be evaluated through the end of the first cycle. Disease status will be assessed by the local Investigator using appropriate imaging every 2 cycles for the duration of study treatment administration to evaluate the SYNB1891-injected lesion(s) and an overall response of up to 5 noninjected target lesions in accordance with RECIST 1.1, iRECIST, and/or LYRIC. Tumor biopsies and blood samples will be collected at baseline and at prespecified time points on study to evaluate the pharmacokinetics (PK), as measured by qPCR bacterial kinetics, and PD effects of study treatment.
Study Regimens	**Treatments Administered** Patients enrolled to Arm 1 may receive up to four 21-day cycles of SYNB1891 monotherapy. 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. At the end of Cycle 4, patients in Arm 1 who do not have progressive disease (i.e., those who achieve and sustain complete response [CR], partial response [PR], or stable disease [SD]) may receive additional cycles of SYNB1891 administered by i.t. injection 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 study stopping rule, or no eligible lesions remain. Patients enrolled to Arm 2 may receive up to four 21-day cycles with SYNB1891 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. 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 (i.e., those who achieve and sustain CR, PR, or SD) at the end of Cycle 4 on combination therapy may receive additional cycles of SYNB1891 and atezolizumab 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 study stopping rule, or no eligible lesions remain. Locating and/or injecting the lesion may be performed using guided imaging at the discretion of the Investigator. **Postdose Monitoring** The initial patient in each Arm 1 cohort (Sentinel patient) will be monitored for at least 6 hours following the first (Cycle 1 Day 1) i.t. injection of SYNB1891 (or overnight, at the discretion of the Investigator) for evaluation of any procedural complication or acute toxicity (i.e., injection reactions or cytokine release syndrome) and for collection of postdose blood samples. Safety data must be reviewed on or after Day 7 and deemed acceptable by the Investigator and the Sponsor and contract research organization (CRO) Medical Monitors before the Sentinel patient receives any further injections and before any subsequent patients are dosed in that cohort. For the remainder of Cycle 1 dosing for the Sentinel patient and for all dosing for non-Sentinel patients, SYNB1891 injections will be administered in the outpatient setting and patients will be closely observed at the clinic for at least 6 hours following each injection for evaluation of any procedural complication or acute toxicity (i.e., injection reactions or cytokine release syndrome) and for collection of postdose blood samples. All patients will be contacted by telephone on Cycle 1 Day 3 to monitor for signs of cytokine release syndrome. **Dose-finding Methodology** 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 in accordance with the mTPI algorithm. In Arm 2, dosing will begin after all patients in Arm 1 Cohort 4 have completed their Cycle 1 DLT safety evaluation. The starting dose of SYNB1891 in the first cohort of Arm 2 will be at SYNB1891 Arm 1 Cohort 3 dose level. Dosing in the Arm 2 cohorts will increase in approximately 3-fold increments in subsequent cohorts until RP2D determination. Once dosing in Arm 2 is initiated, patient allocation to cohorts will be managed by the Sponsor to ensure that a dosing level within Arm 2 is at least one dose level below the SYNB1891 monotherapy dose being evaluated in Arm 1. Combination doses will not be escalated above the SYNB1891 single-agent MTD established in Arm 1. The atezolizumab dose will remain constant at 1200 mg Q3W for each dose level in Arm 2.

	In both arms, the escalation schedule may be adjusted based on PK, PD, and safety data emerging throughout the study to determine the MTD/RP2D. In both study arms, each cohort will initially comprise 3 patients (a Sentinel patient and 2 non-Sentinel patients), with subsequent dose level investigation governed by the mTPI algorithm in accordance with protocol-mandated criteria. 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. **Definition of Dose-limiting Toxicity** The occurrence of protocol-specified toxicities during Cycle 1 (e.g., certain Grade 3/4 laboratory values; sepsis; or toxicity resulting in death, discontinuation of Cycle 1, or delay of Cycle 2; see Section 4.2.4) will be considered a DLT, if assessed by the Investigator to be possibly, probably, or definitely related to study treatment administration.
Duration of Study Participation	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). Patients in Arm 1 will receive an initial 4 cycles of SYNB1891. Patients who do not have progressive disease at the end of Cycle 4 may receive additional cycles of SYNB1891 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 study stopping rule, or no eligible lesions remain. Patients in Arm 2 will receive an initial 4 cycles of SYNB1891 and atezolizumab. Arm 2 patients 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 (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 study stopping rule, or no eligible lesions remain.
Eligibility Criteria	**Inclusion Criteria** Patients must meet all of the following inclusion criteria to be eligible: 1. Able and willing to voluntarily complete the informed consent process (patient or patient's representative). 2. Adults aged ≥ 18 years (on the day of signing informed consent) with histologically- or cytologically-confirmed stage III or IV advanced/metastatic solid tumor or lymphoma for which no therapeutic options are available to extend survival or for which the patient is not a candidate for standard-of-care therapy. 3. Eastern Cooperative Oncology Group performance status ≤ 1. 4. Life expectancy ≥ 3 months. 5. ≥ 1 injectable, measurable (≥ 10 mm in diameter, or ≥ 15 mm for nodal lesions), eligible lesion as defined by RECIST 1.1 (Eisenhauer et al 2009), iRECIST (Seymour et al 2017), and/or LYRIC (Cheson et al 2016) and as assessed by the Investigator. Eligible lesions must not be located in the thoracic cavity, spleen, pancreas, gastrointestinal tract (liver injection

allowed), or cranium and must be amenable to percutaneous injection and away from major blood vessels or neurological structures.

6. Able to provide biopsies for biomarker analysis from injected and (if available) noninjected lesions at baseline and other time points during the study.
7. Oxygen saturation > 90% without the use of supplemental oxygen.
8. Adequate cardiac function, defined as follows:
   a. Left ventricular ejection fraction (LVEF) > 50% by multi-gated acquisition (MUGA) scan or echocardiogram (ECHO) 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. Symptoms relating to left ventricular dysfunction, cardiac arrhythmia, or cardiac ischemia must all be Grade < 1 per NCI CTCAE version 5.0.
   b. QTc interval corrected for heart rate using Fridericia's formula (QTcF) < 480 msec at screening.
9. Laboratory values within the following ranges:
   - Absolute neutrophil count ≥ 1500/µL
   - Lymphocyte count ≥ 500/µL
   - Platelets ≥ 100,000/µL without transfusion
   - Hemoglobin ≥ 9.0 g/dL (patients may be transfused to meet this criterion)
   - Estimated glomerular filtration rate (eGFR) > 50 mL/min/1.73 m^2 per the Cockcroft-Gault formula
   - Total bilirubin ≤ 1.5 × upper limit of normal (ULN) OR direct bilirubin ≤ ULN for participants with total bilirubin levels > 1.5 × ULN or ≤ 3 × ULN for patients with Gilbert's syndrome
   - Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase ≤ 2.5 × ULN (AST and ALT ≤ 5 × ULN for participants with liver metastases and alkaline phosphatase ≤ 5 × ULN for participants with liver or bone metastases)
   - International normalized ratio (INR) or prothrombin time (PT) or activated partial thromboplastin time (aPTT) ≤ 1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants
10. Agree to use an acceptable method of contraception after informed consent, throughout the study, and for 5 months after the last dose of SYNB1891 or atezolizumab.

Additional inclusion criteria that apply only to Arm 2 study participants are as follows:

11. Thyroid-stimulating hormone (TSH) must be within the normal reference range or the patient must be receiving stable thyroid replacement therapy.
12. Patients must have received treatment with an anti-programmed cell death protein-1 (PD-1)/programmed death-ligand 1 (PD-L1) monoclonal antibody (mAb) administered either as monotherapy, or in combination

with other checkpoint inhibitors (CPIs) or other therapies, if indicated (if CPI therapy is not indicated as a part of standard-of-care therapy, patients may be CPI naïve).

Additional inclusion criteria that apply only to the Arm 2 <u>expansion</u> study participants are as follows:

13. Patients must have progressed on treatment with an anti-PD-1/PD-L1 mAb administered either as monotherapy, or in combination with other CPIs or other therapies, if indicated (if CPI therapy is not indicated as a part of standard-of-care therapy, patients may be CPI naïve).

    PD-1/L1 treatment progression is defined by meeting all of the following criteria:

    a. Has received at least 2 doses of an approved anti-PD-1/L1 mAb.
    b. Has demonstrated disease progression after PD-1/L1 as defined by RECIST 1.1, iRECIST, and/or LYRIC. The initial evidence of disease progression is to be confirmed by a second assessment no less than 4 weeks from the date of the first documented disease progression, in the absence of rapid clinical progression as determined by the Investigator. Once disease progression is confirmed, the initial date of disease progression documentation will be considered the date of disease progression.
    c. Progressive disease has been documented within 12 weeks from the last dose of anti-PD-1/L1 mAb.

Exclusion Criteria

1. Chemotherapy, radiation, or biological cancer therapy within 14 days prior to the first dose of study treatment, or failure of any AEs to recover to Baseline or NCI CTCAE version 5.0 Grade 1, except for any grade of alopecia caused by cancer therapeutics administered > 28 days earlier.
2. Systemic immunostimulatory agents (including, but not limited to, IFN and IL-2) within 28 days or 5 drug elimination half-lives (whichever is longer) prior to initiation of study treatment. Checkpoint inhibitors are not considered systemic immunostimulatory agents for this criterion and are subject to the washout period listed in exclusion criterion #1.
3. Allogeneic hematopoietic stem cell transplantation that requires current use of immunosuppressors.
4. Receipt of a live vaccine within 90 days prior to the first dose of study treatment or anticipation of a need for such a vaccine during treatment.
5. Receipt of antibiotics within 7 days prior to the first dose of study treatment.
6. Participation in a study of an investigational agent and receipt of study therapy or use of an investigational device within 28 days prior to the first dose of study treatment.
7. Diagnosed immunodeficiency or current use of chronic systemic steroid therapy in excess of replacement doses (prednisone ≤ 10 mg/day or equivalent is acceptable) or any other form of immunosuppressive medication within 7 days prior to the first dose of study treatment.
8. For injection and biopsy of visceral (deep) lesions: patients must not be on long-acting antiplatelet agents such as aspirin or clopidogrel or on

	therapeutic doses of anticoagulants, with the exception of patients receiving a preventative dose of low molecular weight heparin. In patients receiving preventative low molecular weight heparin, treatment must be stopped 24 hours before the intratumoral injection and resumed again 24 hours after the injection (Marabelle et al 2018). 9. Previous or concurrent malignancy, with the exception of: a. Adequately treated basal or squamous cell carcinoma, in situ carcinoma of the cervix, or ductal carcinoma in situ of the breast; b. Localized prostate cancer definitively treated with surgery or radiation or stable on hormone therapy; c. Other cancer from which the patient has been disease free for at least 2 years. 10. Clinically active central nervous system (CNS) metastases and/or carcinomatous meningitis (treated brain metastases are permitted if radiologically stable for ≥ 28 days prior to the first dose of study treatment). 11. Allergy to antibiotics that precludes treatment for infection with *E. coli* Nissle 1917 (see Appendix 4 for common antibiotics to which *E. coli* Nissle is sensitive). 12. Hepatitis B or C infection(s) unless screening tests indicate a negative viral load, and/or human immunodeficiency virus (HIV) infection unless screening tests indicate a negative or below the limit of quantitation viral load. 13. Known active tuberculosis. 14. Grade 3 or higher infection according to NCI CTCAE within 28 days prior to initiation of study treatment, including, but not limited to, hospitalization for complications of infection, bacteremia, or severe pneumonia. 15. Failure to fully recover from the effects of major surgery. Surgeries that required general anesthesia must be completed > 14 days before the first dose of study drug. Surgery requiring regional/epidural anesthesia must be completed > 72 hours before the first dose of study treatment and participants should be recovered. 16. Heart failure of New York Heart Association Class 3 or greater, restrictive cardiomyopathy, or unstable angina or recent myocardial infarction within 3 months prior to the first dose of study treatment. 17. Any other medical condition that might confound the results of the study, interfere with the participant's participation, or is not in the best interest of the participant, in the opinion of the treating Investigator. 18. Pregnant or breastfeeding or anticipated conception or fathering of children within the projected duration of the study and for 5 months after the last dose of SYNB1891 or atezolizumab. **Additional exclusion criteria that apply only to Arm 2 study participants are as follows:** 19. History of immune-mediated AEs on prior PD-1/PD-L1 therapies requiring discontinuation or immunosuppressor therapy, excluding endocrinopathies.

	20. Active or history of underlying autoimmune disease or immune deficiency, including, but not limited to, myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, antiphospholipid antibody syndrome, Wegener granulomatosis, Sjögren syndrome, Guillain-Barré syndrome, or multiple sclerosis, with the following exceptions: a. Patients with a history of autoimmune-related hypothyroidism who are on thyroid replacement hormone are eligible for the study. b. Patients with controlled Type 1 diabetes mellitus who are on an insulin regimen are eligible for the study. c. Patients with eczema, psoriasis, lichen simplex chronicus, or vitiligo with dermatologic manifestations only (e.g., patients with psoriatic arthritis are excluded) are eligible for the study provided all of following conditions are met: − Rash must cover < 10% of body surface area; − Disease is well controlled at baseline and requires only low-potency topical corticosteroids; − No occurrence of acute exacerbations of the underlying condition requiring psoralen plus ultraviolet A radiation, methotrexate, retinoids, biologic agents, oral calcineurin inhibitors, or high potency or oral corticosteroids within the previous 12 months. 21. History of a Grade ≥ 3 allergic anaphylactic reaction following treatment with a PD-1 mAb or to chimeric, human, or humanized antibodies or fusion proteins. 22. History of idiopathic pulmonary fibrosis, organizing pneumonia (e.g., bronchiolitis obliterans), drug-induced pneumonitis requiring treatment, or idiopathic pneumonitis, or evidence of active pneumonitis. 23. Known hypersensitivity to Chinese hamster ovary cell products or any component of the atezolizumab formulation.
Analytical and Statistical Methods	**Sample Size Determination** 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. Additional cohorts may be required in either arm to determine the MTD/RP2D. **Safety Analysis** DLTs will be determined for each patient through the end of Cycle 1. This timeframe will enable identification of any acute, dose-limiting events that will determine suitability for dose escalation. Patients who experience a DLT will remain on the study to complete the safety evaluations and follow-up until resolution or stabilization of the DLT. AEs and SAEs will be determined to be dose-limiting if they occur during the described 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.

	All AEs and SAEs will be determined and coded using the Medical Dictionary for Regulatory Activities (MedDRA), and severity of AEs and laboratory abnormalities will be graded using the NCI CTCAE, version 5.0. Adverse events will be tabulated by system organ class (SOC) and preferred term. Incidence tables of patients with AEs, SAEs, and DLTs will be presented by maximum severity. **Efficacy Analysis** Efficacy analyses using RECIST 1.1, iRECIST, and/or LYRIC will be descriptive in nature. The response of SYNB1891-injected lesion(s) and an overall response of up to 5 noninjected target lesions will be assessed. Exploratory evaluations for proof-of-concept (biopsy-based PD markers) will include change from baseline assessment. The duration of any response or lack of progression for all evaluated tumors using RECIST 1.1, iRECIST, and/or LYRIC will also be assessed.

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Abbreviation or Specialist Term	Explanation
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
ANC	absolute neutrophil count
APC	antigen-presenting cell
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
ATP	adenosine triphosphate
BAL	bronchoscopic alveolar lavage
°C	degree(s) Celsius
CBC	complete blood count
CD	cluster of differentiation
CDA	cyclic-di-AMP
CDN	cyclic dinucleotide
CNS	central nervous system
CPI	checkpoint inhibitor
CR	complete response
CRA	clinical research associate
CRO	contract research organization
CRS	cytokine release syndrome
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CV	curriculum vitae
dacA or DacA	di-adenylate cyclase *gene* or enzyme
DAP	diaminopimelic acid
DILI	drug-induced liver injury
DLT	dose-limiting toxicity
dTMP	2'-deoxythymidine-5'-monophosphate
dUMP	2'-deoxyuridine-5'-monophosphate
ECG	electrocardiogram
ECHO	echocardiogram
ECMO	extracorporeal membrane oxygenation
*Ec*N	*Escherichia coli* Nissle 1917

Abbreviation or Specialist Term	Explanation
eCRF	electronic case report form
EDC	electronic data capture
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
EOT	End of Treatment
°F	degree(s) Fahrenheit
FDA	Food and Drug Administration
FNA	fine needle aspirate
FNR	fumarate and nitrate reductase
FT4	free thyroxine
g	gram(s)
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GM-CSF	granulocyte-macrophage colony-stimulating factor
h	hour
hCG	human chorionic gonadotropin
HIV	human immunodeficiency virus
HLH	hemophagocytic lymphohistiocytosis
HNSTD	highest nonseverely toxic dose
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICU	intensive care unit
IgG1	immunoglobulin G1
IFN	interferon
IL	interleukin
IL1RA	interleukin 1 receptor antagonist
IL2Rα	interleukin 2 receptor alpha
INR	International normalized ratio
IRB	Institutional Review Board
iRECIST	immune-related Response Evaluation Criteria in Solid Tumors
IRR	infusion-related reaction
i.t.	intratumoral
IV	intravenous
kb	kilobase
kg	kilogram

Abbreviation or Specialist Term	Explanation
L	liter(s)
LC-MS/MS	liquid chromatography/tandem mass-spectrometry
LDH	lactate dehydrogenase
LFT	liver function test
LPS	lipopolysaccharide
LVEF	left ventricular ejection fraction
LYRIC	Lymphoma Response to Immunomodulatory Therapy Criteria
m^2	meter(s) squared
mAb	monoclonal antibody
MAS	macrophage activation syndrome
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
mM	millimolar(s)
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
mTPI	modified toxicity probability interval
MUGA	multi-gated acquisition scan
NCI	National Cancer Institute
NSAID	nonsteroid anti-inflammatory drug
ORR	objective response rate
PD	pharmacodynamic(s)
PD-1	programmed cell death protein-1
P_{fnrS}	fumarate and nitrate reductase regulator promoter [
PK	pharmacokinetic(s)
PD-L1	programmed death-ligand 1
PR	partial response
PRR	pattern recognition receptor
PT	prothrombin time
Q3W	every 3 weeks
qPCR	quantitative polymerase chain reaction
QTcF	QT interval corrected for heart rate using Fridericia's formula
RAW	immortalized murine macrophage cells

Abbreviation or Specialist Term	Explanation
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
SRC	Safety Review Committee
STING	STimulator of INterferon Genes
SUSAR	serious, unexpected, suspected adverse reaction
THY	thymidine
TIL	tumor-infiltrating lymphocytes
TLR	toll-like receptor
TME	tumor microenvironment
TNFα	tumor necrosis factor α
TSH	thyroid-stimulating hormone
ULN	upper limit of normal
US	United States
VAD	ventricular assist device

1. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The Principal Investigator is the person responsible for the conduct of the study at the investigational site. A Sub-investigator is any member of the clinical study team designated and supervised by the Principal Investigator to perform critical study-related procedures and/or to make important study-related decisions.

Prior to study initiation, the Principal Investigator at each site must provide to Synlogic Operating Company, Inc. (also referred to as "Synlogic" or "Sponsor") a signed protocol signature page, a fully executed and signed United States (US) Food and Drug Administration (FDA) Form 1572, a current curriculum vitae (CV) and medical license and a financial disclosure form. Financial disclosure forms, current CVs and medical licenses must also be provided for all Sub-investigators listed on Form 1572 who will be directly involved in the evaluation of patients.

The study will be administered and monitored by employees or representatives of Synlogic in accordance with all applicable regulations. Clinical research associates (CRAs) will monitor each site on a periodic basis and perform verification of source documentation for each patient. Synlogic or designee will be responsible for ensuring timely reporting of expedited serious adverse event (SAE) reports to regulatory authorities and Investigators.

2. INTRODUCTION

SYNB1891 is being developed for the treatment of patients with advanced/metastatic solid tumors or lymphoma.

2.1. Targeted Immunotherapy in Advanced Cancer

As the immuno-oncology field matures and as more therapies are evaluated in the clinic, our appreciation for the complexity of tumor-immune cell interactions deepens. 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) (Darvin et al 2018). Addressing the question of why some patients respond to checkpoint inhibition while others do not is currently an active area of investigation. However, one emerging biomarker correlated with response is intratumoral 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, such as the use of toll-like receptor (TLR) agonists (Adams 2009; Iribarren et al 2016) and STimulator of INterferon Genes (STING) agonists (Fu et al 2015; Corrales et al 2015; Woo et al 2014).

The crucial role that Type 1 inteferons (IFNs) play in infection and inflammation has been known for decades (Isaacs et al 1957), and the many pathways that revolve around this first described cytokine (now known as IFN-β) have been determined over time (Ivashkiv et al 2014). These findings provide some insight as to why initial work with intratumoral injections of active bacteria (Coley's toxin) led to many anecdotal responses in malignant tumors, defining the link between infection, inflammation and cancer. Since the "treatment era" that began in 1891, the importance of lipopolysaccharide (LPS), endotoxin and ultimately Type 1 IFN in adaptive immunity, tumor immunology and immune surveillance has been elucidated (Trinchieri 2010). Recent tumor immunology work has directly related Type 1 IFN-related gene expression signatures to the amount of T-cell infiltration in tumors (Fuertes et al 2011), leading investigators to use the cytokine to turn immune-unreactive tumors without lymphocyte infiltration (cold) into immunoreactive tumors (hot). The observation that Type 1 IFN signatures were associated with tumors in ipilimumab-responding melanoma patients (Chiappinelli et al 2015) was an important link in the chain, and there is currently little doubt that the Type 1 IFN pathway is essential for the efficacy of cancer immunotherapy (Zamarin et al 2014).

Over the last decade, several modern therapeutic strategies designed to upregulate the Type 1 IFN pathway as a potential cancer treatment have been devised. Because the STING pathway is required in immune recognition and elimination of tumors through Type 1 IFN (Woo et al 2014), agonists of STING provide a promising approach to elicit Type 1 IFN production (Ishikawa et al 2008). Unique nucleic acids called cyclic dinucleotides (CDNs), like cyclic-di-AMP (CDA), which function as signaling molecules in bacteria, have also been shown to induce a STING-dependent Type 1 IFN response intracellularly (Corrales et al 2015) and thus small molecule analogs were developed, with some evaluated clinically. While small molecule STING agonists have been shown to be potent inducers of antitumor immunity (Corrales et al 2015; Fu et al 2015; Woo et al 2014), "off-target" activation of STING in effector T cells can lead to

T cell apoptosis (Larkin et al 2017) and impede the establishment of immunological memory (Sivick et al 2018). To reduce nontargeted, systemic effects and improve "on-target" efficacy, new means of localized and targeted STING activation are needed.

2.2. Investigational Medicinal Product

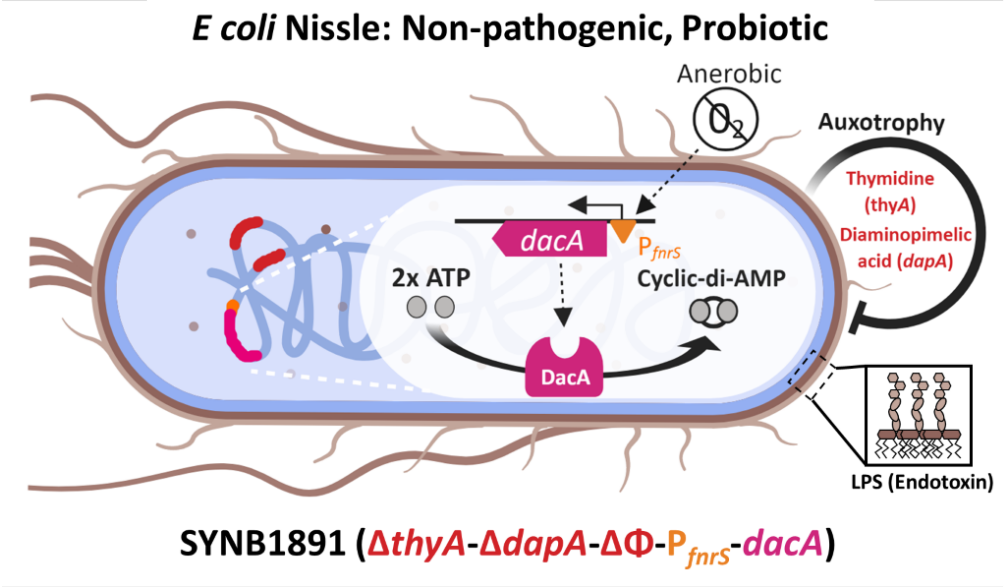
SYNB1891 is a strain of modified live probiotic bacterium (*Escherichia coli* [*E. coli*] Nissle 1917) (*Ec*N) programmed as a live immuno-biotherapeutic agent designed to treat patients with cancer. It is a genetically modified strain of *Ec*N that, under hypoxic conditions, is designed to produce CDA, which functions as a STING agonist. Figure 1 presents the structure of SYNB1891.

Cyclic di-AMP functions as 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). In addition to the production of CDA to stimulate APCs and innate immunity, the bacterial chassis itself provides supplementary immune stimulation within the TME via activation of pattern recognition receptors (PRRs) such as the activation of TLR4 by the gram-negative bacterial cell wall component endotoxin or LPS. Upon i.t. administration, SYNB1891 is taken up by APCs, such as dendritic cells and macrophages, within the TME. Once the SYNB1891 bacteria are phagocytosed by APCs, this leads to the intracellular release of the CDA contained inside of the SYNB1891 bacteria, which activates the STING pathway and leads to upregulation of Type I IFNs. In addition, parallel pathways of innate immune activation are triggered through PRRs, such as endotoxin stimulation of TLR4, which lead to the release of additional cytokines. The release of these pro-inflammatory cytokines and activation of APCs subsequently initiates and promotes antitumor immunity and immune rejection of the tumor.

SYNB1891 was engineered with several biocontainment and safety features to target the production of CDA to the TME and inhibit the proliferation of the bacterial chassis both inside and outside the tumor. Genomic insertion of a gene encoding DacA, a di-adenylate cyclase from the bacterium *Listeria monocytogenes*, was made within the *Ec*N chromosome. The DacA enzyme catalyzes the production of CDA from two molecules of adenosine triphosphate (ATP). The *dacA* gene was placed under the regulatory control of an anaerobic-inducible promoter (the fumarate and nitrate reductase regulator promoter [P_{fnrS}]) and the anaerobic-responsive transcriptional activator fumarate and nitrate reductase (FNR). These regulatory components are meant to ensure that the generation of CDA only takes place in the anoxic environment of the tumor. From a safety and control perspective, SYNB1891 was engineered to be an auxotrophic strain through a deletion of the *dapA* gene encoding 4-hydroxy-tetrahydropicolinate synthase and a *thyA* gene encoding the thymidylate synthase. *DapA* deletion renders SYNB1891 unable to synthesize diaminopimelic acid (DAP), thereby preventing the proper formation of the bacterial cell wall unless the strain is supplemented with DAP exogenously. Thymidylate synthase catalyzes the reductive methylation of 2'-deoxyuridine-5'-monophosphate (dUMP) to 2'-deoxythymidine-5'-monophosphate (dTMP) and without that, SYNB1891 can only grow in a thymidine-rich environment. For contract manufacturing facility access, SYNB1891 was also modified with a deletion of an approximately 9 kilobase (kb) pair segment of an endogenous prophage sequence, *Φ*, which prevents the cells from being able to express infectious bacteriophage particles. Finally, all antibiotic resistance genes utilized during the engineering

and development of SYNB1891 were removed to ensure antibiotic sensitivity and enable clearance of bacteria by available treatments.

Figure 1: Design of the Engineered *E. coli* Nissle Strain SYNB1891



Abbreviations: ΔΦ = deletion of the prophage sequence endogenous to *E. coli* Nissle; ATP = adenosine triphosphate; CDA = cyclic-di-AMP; *dacA* or DacA = di-adenylate cyclase *gene* or enzyme; *ΔdapA* = deletion of *dapA* gene leading to diaminopimelate auxotrophy; LPS = lipopolysaccharide or endotoxin; P_{fnrs} = fumarate and nitrate reductase regulator promoter; PRR = pattern recognition receptor; *ΔthyA* = deletion of *thyA* gene leading to thymidine auxotrophy; TME = tumor microenvironment

The combination of a genetically modified strain of bacteria engineered to deliver a STING agonist (like CDA) would be a strategy that marries both the targeted delivery of STING agonists to phagocytic APCs and the complimentary stimulation of the innate immune system through the activation of PRRs. The utilization of microbial based therapies for the treatment of human cancer can be charted back to the work of Dr. William B. Coley in 1891 when he began treating patients with living bacterial cultures after observing spontaneous regressions in patients with streptococcal infections (McCarthy 2006). Over the following 125 years, numerous bacterial based therapies for cancer have been investigated, with the majority utilizing attenuated pathogens such as *Salmonella typhimurium*, *Clostridium novyi*, and *Listeria monocytogenes* (Felgner et al 2016). Unfortunately, efforts to improve safety via strain attenuation often led to reductions in efficacy and attempts to improve efficacy by engineering in additional functions are often limited due to a lack of tools and approaches for genetic manipulation. Synthetic biology represents a promising means to develop novel therapies with combinatorial, rationally designed functionalities and safety features (Bashor et al 2018; Kitada et al 2018).

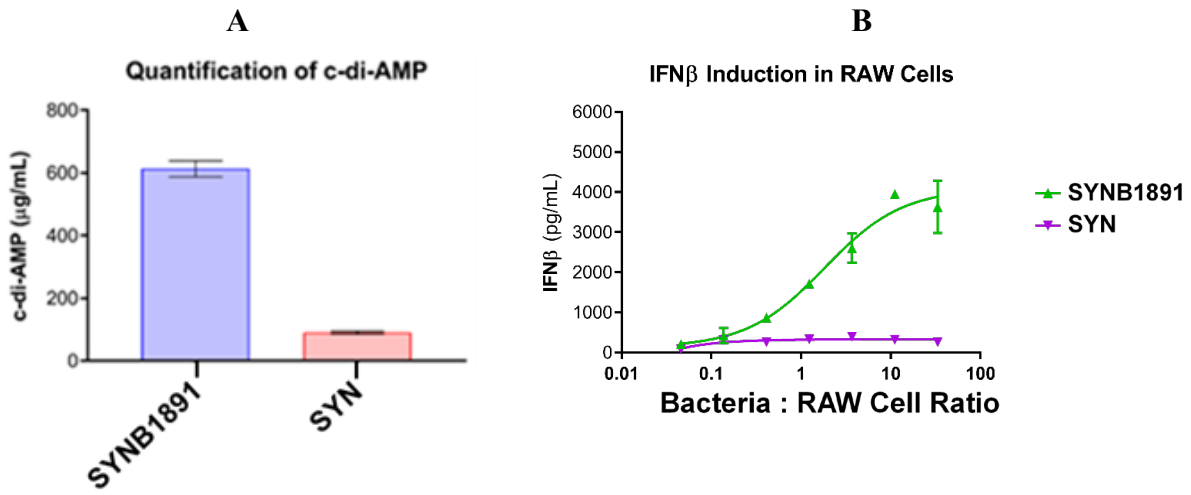
With these consideration in mind, the rationale for the development of SYNB1891 was to utilize synthetic biology techniques to create a live biotherapeutic that provides multi-pathway innate immune activation localized to the TME and STING pathway induction targeted to phagocytic APCs. By utilizing the nonpathogenic *Ec*N as a "vector" for the delivery of the STING agonist CDA, SYNB1891 has both broad innate immune activating and APC target STING activating functionalities. From a safety and control perspective, SYNB1891 was engineered to produce CDA under the hypoxic conditions found in tumors, contains dual DAP and thymidine

auxotrophies to inhibit bacterial proliferation and does not contain any antibiotic resistance genes. The administration of SYNB1891 as an immunotherapy for cancer is a promising approach and has shown activity and safety in several experimental models of cancer.

2.2.1. Nonclinical Studies

To evaluate the in vitro activity of the engineered bacteria SYNB1891, bacterial cells were expanded under anaerobic conditions to induce its agonist-producing genetic circuit. After 4 hours of culture, bacterial cell pellets were harvested, and intracellular CDA levels were analyzed by liquid chromatography/tandem mass-spectrometry (LC-MS/MS). The data shown in Figure 2A are representative of these types of studies and demonstrate the capacity of the SYNB1891 to produce high levels of the STING-agonist molecule, CDA, as compared to nonengineered bacteria (SYN differs from SYNB1891 in that it lacks the dacA gene insertion). To evaluate the biological activity of SYNB1891, bacteria were expanded in vitro and induced under anaerobic conditions as above to express CDA. Immortalized murine macrophage cells (RAW cells) were co-cultured at various ratios of bacteria-to-mammalian cells with either a nonengineered bacteria (SYN) or SYNB1891. Two hours post-treatment, culture supernatants were harvested and IFNβ was quantified by an enzyme-linked immunosorbent assay (ELISA). The data shown in Figure 2B are representative of these types of studies and demonstrate dose-dependent biological activity of SYNB1891 for the activation of the STING pathway in a biologically relevant antigen presenting cell and subsequent downstream production of IFNβ.

Figure 2: In Vitro CDA Production and Biological Activity of SYNB1891

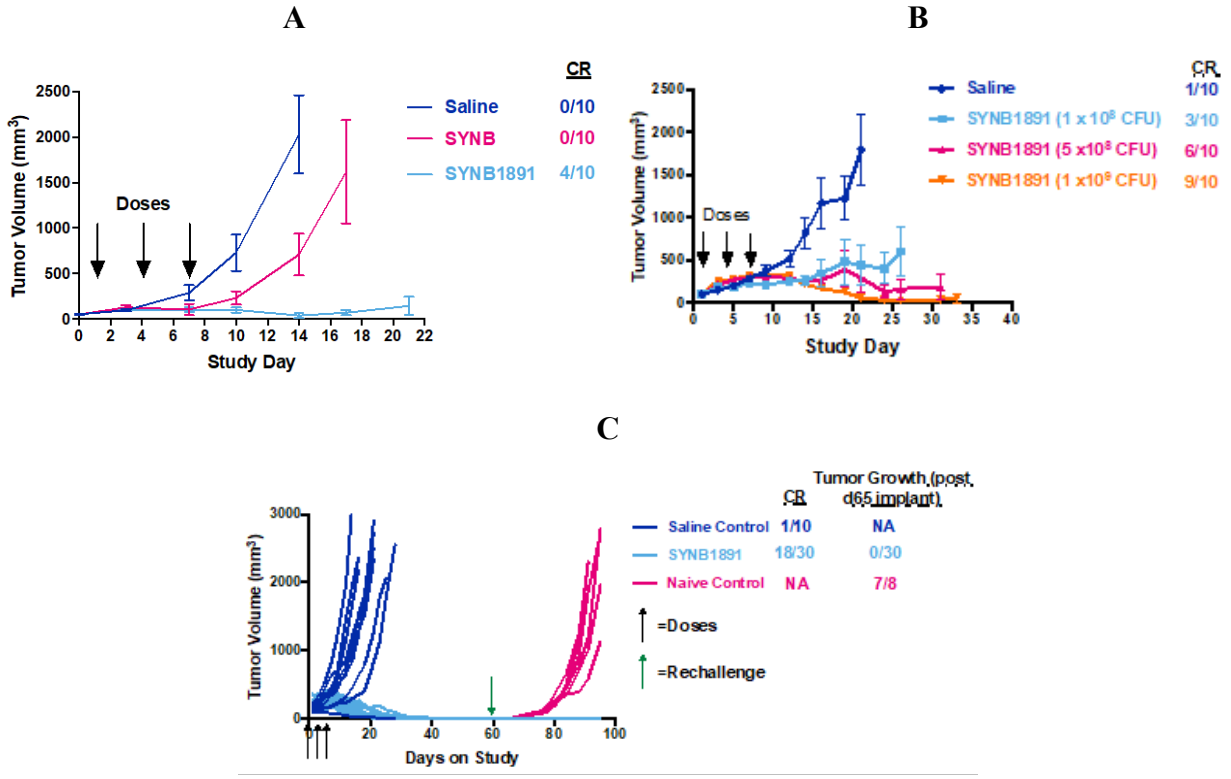


To demonstrate the antitumor activity of SYNB1891 in vivo, we employed two different syngeneic mouse models of cancer: the B16.F10 melanoma model and the A20 lymphoma model. These tumor models are representative of tumors lacking significant immune infiltrate and are known as “cold tumors”. They are also representative of the tumor types we intend to treat in the Phase 1 clinical trial. In data not shown here, the pharmacokinetic and pharmacodynamic activity of SYNB1891 in B16.F10 tumors after a single i.t. administration were evaluated. In these studies, dose and time dependent increases in tumoral levels of CDA, IFNα and β (IFNα1 and IFNβ1) and other pro-inflammatory cytokines (IL-6, granulocyte-

macrophage colony-stimulating factor [GM-CSF]) were observed after a single i.t. administration of SYNB1891. Moreover, there was a dose- and time-dependent profile observed for the bacterial persistence of SYNB1891 in the tumor, with lower doses of SYNB1891 being cleared from the tumor more rapidly than higher doses. In addition, blood analysis demonstrated no detectable SYNB1891 present at any dose or time point evaluated in this study. These experiments demonstrated that SYNB1891 can produce CDA in vivo in the tumor, this CDA is biologically active and capable of activation of the STING pathway and additionally, the *Ec*N bacterial chassis elicits further innate immune pathway activation (through for example LPS-mediated activation of TLR4), as expected.

To assess the antitumor activity of SYNB1891, B16.F10 tumor bearing mice were established and treated with three (3) doses separated by 3 days (Q3D × 3) of either of SYNB1891 or unengineered *Ec*N (1×10^9 live cells/dose), or saline controls and tumor growth was monitored over the timeframe of the study (21 days). The data in Figure 3A show that compared to both the saline and unengineered *Ec*N controls (SYNB), SYNB1891 administration results in significant antitumor control. Moreover, only SYNB1891 treatment led to complete tumor regressions (CRs). However, the administration of the unengineered *Ec*N control still led to some level of tumor growth inhibition, supporting in vivo the contribution of chassis-mediated activation of PRRs to the antitumor activity of SYNB1891. To explore the pharmacologic activity of SYNB1891, we established A20 lymphoma tumors in mice and then treated them with 3 different dose levels of SYNB1891 (1×10^8, 5×10^8, 1×10^9 live cells/dose; every 3 days × 3) and monitored tumor growth throughout the time frame of the study. The results shown in Figure 3B show that administration of SYNB1891 demonstrates dose-dependent antitumor activity and importantly, dose-dependent generation of CRs. Finally, to investigate the potential for SYNB1891 to systemic antitumor immunity, we treated A20 tumor bearing mice with either SYNB1891 or saline control. Mice that underwent complete tumor regression in response to SYNB1891 treatment (CRs) were then monitored for additional ~35 days after regression to verify CRs and then these animals were re-challenged with A20 tumor cells and tumor growth after re-challenge was compared to tumor growth in naïve age-matched controls. Figure 3C shows that the re-challenge of mice that were previously cured of their tumors by SYNB1891 treatment were immune to tumor re-establishment whereas naïve, age-matched control animals demonstrated robust tumor growth similar to what had been observed with the initial saline control treated animals.

Figure 3: Antitumor, Pharmacologic and Systemic Immunity Activity of SYNB1891



From a safety perspective, as mentioned above, SYNB1891 has been engineered with dual auxotrophies, deletion of *dapA* and *thyA* genes (*ΔdapA, ΔthyA*), as biosafety and control features. These genetic alterations render SYNB1891 unable to proliferate in absence of exogenous addition of the products of *dapA* and *thyA* (dipimelate and thymidine, respectively). These molecules are found at extremely low to negligible levels in tissues and systemic circulation as well as the external environment. Studies of in vitro bacterial cell cultures and in vivo in mice have demonstrated the inability of SYNB1891 to grow without the addition of these molecules. Additionally, SYNB1891 also lacks any antibiotic resistance cassettes and has been shown to be sensitive to a broad panel of antibiotics, further strengthening the safety control over SYNB1891.

A Good Laboratory Practice (GLP) toxicology study was performed in A20 tumor-bearing male and female mice. Intratumoral administration of SYNB1891 was given weekly for 4 weeks at doses of 4.7×10^7, 4.7×10^8, and 4.7×10^9 live cells/dose. SYNB1891was well tolerated in mice at the dose level of 4.7×10^7 live cells/dose. Based on the SYNB1891-related toxicological profile (mild injection site reactions and elevations in serum cytokine levels, no significant changes in body weight, slight increases in spleen weights and splenic extramedullary hematopoiesis), the biodistribution observations (no SYNB1891 was found in any tissue or body fluid examined for the 4.7×10^7 live cell/dose level) and the lack of mortality, the low dose of 4.7×10^7 live cells/dose was considered the highest nonseverely toxic dose (HNSTD) of SYNB1891. This dose, 4.7×10^7, is 47 times higher than the proposed starting dose of SYNB1891 (1×10^6 live cells/dose) in the Phase 1 study.

2.2.1.1. Summary of the Nonclinical Development Program

In summary, SYNB1891 is an engineered strain of *Ec*N that was designed to efficiently deliver the STING pathway agonist CDA to the TME and elicit activation of innate immune system. In vitro experiments demonstrate that CDA is produced at high levels under anaerobic conditions and that the CDA is biologically active in a macrophage cell-based assay, respectively. Studies in tumor-bearing mice demonstrate that SYNB1891 delivered by intratumoral administration is able to produce CDA locally within the TME leading to acute increases in tumoral levels of both IFNβ and pro-inflammatory cytokines. These increases in innate immune signaling correlate with dose dependent antitumor activity and complete tumor regressions. Importantly, the complete tumor regressions elicited by SYNB1891 led to the generation of long-term systemic immunity as evidenced by tumor rejections upon re-challenge in mice that had previously undergone complete regressions.

Finally, a GLP toxicology study in tumor-bearing mice did not reveal any adverse findings at the dose of 4.7×10^7 live cells/dose given once weekly for 24 days; therefore, this dose level was determined to be the HNSTD of SYNB1891. The preclinical development program therefore supports the further development of SYNB1891 as an immunotherapeutic treatment for cancer, as outlined further in Section 2.4.

2.3. Background of Atezolizumab

Atezolizumab is a humanized immunoglobulin G1 (IgG1) monoclonal antibody that targets PD-L1 and inhibits the interaction between programmed death-ligand 1 (PD-L1) and its receptors, programmed cell death protein-1 (PD-1) and B7-1 (also known as cluster of differentiation [CD] 80), both of which function as inhibitory receptors expressed on T cells. Therapeutic blockade of PD-L1 binding by atezolizumab has been shown to enhance the magnitude and quality of tumor specific T-cell responses, resulting in improved antitumor activity (Fehrenbacher et al 2016; Rosenberg et al 2016). Atezolizumab has minimal binding to Fc receptors, thus eliminating detectable Fc effector function and associated antibody-mediated clearance of activated effector T cells.

Atezolizumab is currently approved in the United States as a single agent or as a component of combination therapy for several indications, including urothelial carcinoma, nonsmall cell lung cancer, triple-negative breast cancer, and small cell lung cancer. Atezolizumab has demonstrated antitumor activity in both nonclinical models and cancer patients and is being investigated as a potential therapy (as a single agent or in combination with chemotherapy, targeted therapy, or immunotherapy) in a wide variety of malignancies in addition to the approved indications.

The most frequently reported adverse reactions (≥ 20% of patients) observed with atezolizumab as a single agent have included fatigue/asthenia, nausea, cough, dyspnea, and decreased appetite. Alopecia, constipation, and diarrhea have also been observed in ≥ 20% of patients when atezolizumab is administered in combination with other agents. Adverse events with potentially immune-related causes consistent with an immunotherapeutic agent, including rash, influenza-like illness, endocrinopathies, hepatitis or transaminitis, pneumonitis, colitis, and myasthenia gravis, have been observed. To date, these events have been manageable with treatment and/or interruption of atezolizumab treatment.

Refer to the atezolizumab Investigator's Brochure for nonclinical and clinical study results and detailed safety information.

2.4. Rationale for the Study

As outlined in Section 2.1, the ability to engineer a modified strain of nonpathological bacteria (*Ec*N with DAP and thymidine [THY] auxotrophies, and no antibiotic resistance genes) with the ability to deliver a STING agonist (c-di-AMP) that is active only under hypoxic conditions (like a tumor, not in normal tissue or blood) is a strategy that combines both the known stimulation of the innate immune system with a target important to the release of Type 1 IFN.

This rationale lends support to the investigation of i.t. administration of SYNB1891, which in preclinical studies in mouse tumor models, is taken up by APCs leading to CDN production, STING activation and the release of type 1 interferons (IFN-α1 and IFN-β1). In addition, chassis-mediated engagement of PRRs on APCs (through for example the interaction of bacterial-derived LPS with its cognate receptor TLR4) further stimulates innate immunity. This ability of SYNB1891 to dually activate the innate immune system subsequently promotes the initiation of antitumor immune responses in the injected tumor, as well as the demonstration of long-term immunological memory.

The strategy of the current clinical development plan for SYNB1891 is based on an initial priming of the tumor microenvironment with SYNB1891, followed by targeted checkpoint inhibition with atezolizumab administered concurrently in continuous cycles to induce an optimal tumor response. Agonism of the STING pathway (and other PRRs) by SYNB1891 will lead to productive T cell priming and trafficking, but sustained antitumor activity is likely to require inhibition of the PD-1/PD-L1 pathway to address the T cell "exhaustion" that develops after priming. Therefore, the scientific rationale underlying the combination of SYNB1891 with atezolizumab is to stimulate multiple components of the immune response to cancer; innate immune activation, effector T cell activation, reinvigorating exhausted tumor-associated T cells, and prevention of further T cell exhaustion.

Study SYNB1891-CP-001 represents the first clinical study of SYNB1891. As such, a modified toxicity probability interval (mTPI) dose-finding algorithm (Ji et al 2013) will be implemented to determine the maximum tolerated dose (MTD) of single-agent therapy with SYNB1891 and recommended Phase 2 dose (RP2D) of SYNB1891 in combination with atezolizumab in patients with advanced/metastatic solid tumors or lymphoma. In preclinical toxicology studies, SYNB1891 was well tolerated in mice at doses up to 4.7×10^7 live cells per dose, which was considered to be the HNSTD of SYNB1891. This HNSTD is 47 times higher than the proposed starting dose of SYNB1891 (1×10^6 live cells/dose) in this first-in-human Phase 1 study. Upon demonstration of tolerability in accordance with the mTPI algorithm, the dose of SYNB1891 in Arm 1 will be subsequently escalated in approximately 3-fold increments.

After all patients in Arm 1 Cohort 4 have completed their Cycle 1 DLT safety evaluation, Arm 2 will investigate SYNB1891 starting at the Arm 1 Cohort 3 dose level, when given in combination with atezolizumab (1200 mg IV Q3W) in patients with advanced/metastatic solid tumors or lymphoma. Although the primary purpose of the study is to define the MTD/RP2D of study treatment, the broad patient population and standard frequency of disease assessments (every 2 cycles on treatment) may also enable the evaluation of any preliminary clinical activity elicited by the single-agent and/or combination regimens.

3. STUDY OBJECTIVES AND 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.

3.1. Primary

Incidence of dose-limiting toxicities (DLTs): Percentage of patients who experience a DLT

3.2. Secondary

Secondary objectives include evaluation of the following:

- Nature, incidence, and severity of all adverse events (AEs) and SAEs according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 5.0
- Objective response rate (ORR) in target lesions 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), with evaluations of the SYNB1891-injected tumor(s) and an overall response of up to 5 noninjected target lesions

3.3. Exploratory

Exploratory objectives include evaluation of the following:

- Duration of response or time to progression for evaluated tumors as determined by RECIST 1.1, iRECIST, and/or LYRIC
- Pharmacodynamic (PD) 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 PD 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 [IL2Rα], 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 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

4. STUDY DESIGN

4.1. Study Design Overview

This Phase 1, open-label, multicenter, 2-arm study will be conducting using the mTPI dose-finding design (Ji et al 2013). Cohorts of patients will be enrolled to receive escalating doses of SYNB1891 administered by i.t. injection until the target DLT range (approximately 30%) for SYNB1891 monotherapy is determined based on DLTs observed in Cycle 1 (Arm 1). The dose selected as achieving the target DLT range will be considered the MTD. After all patients in Arm 1 Cohort 4 have completed their Cycle 1 DLT safety evaluation, Arm 2 will enroll its first cohort starting at the Arm 1 Cohort 3 dose level, with SYNB1891 administered by i.t. injection in combination with atezolizumab administered as an intravenous (IV) infusion at a dose of 1200 mg every 3 weeks (Q3W) until the RP2D for the combination regimen is determined (see Figure 4).

The mTPI algorithm (as further discussed in Section 4.2.3) will be implemented separately in each arm.

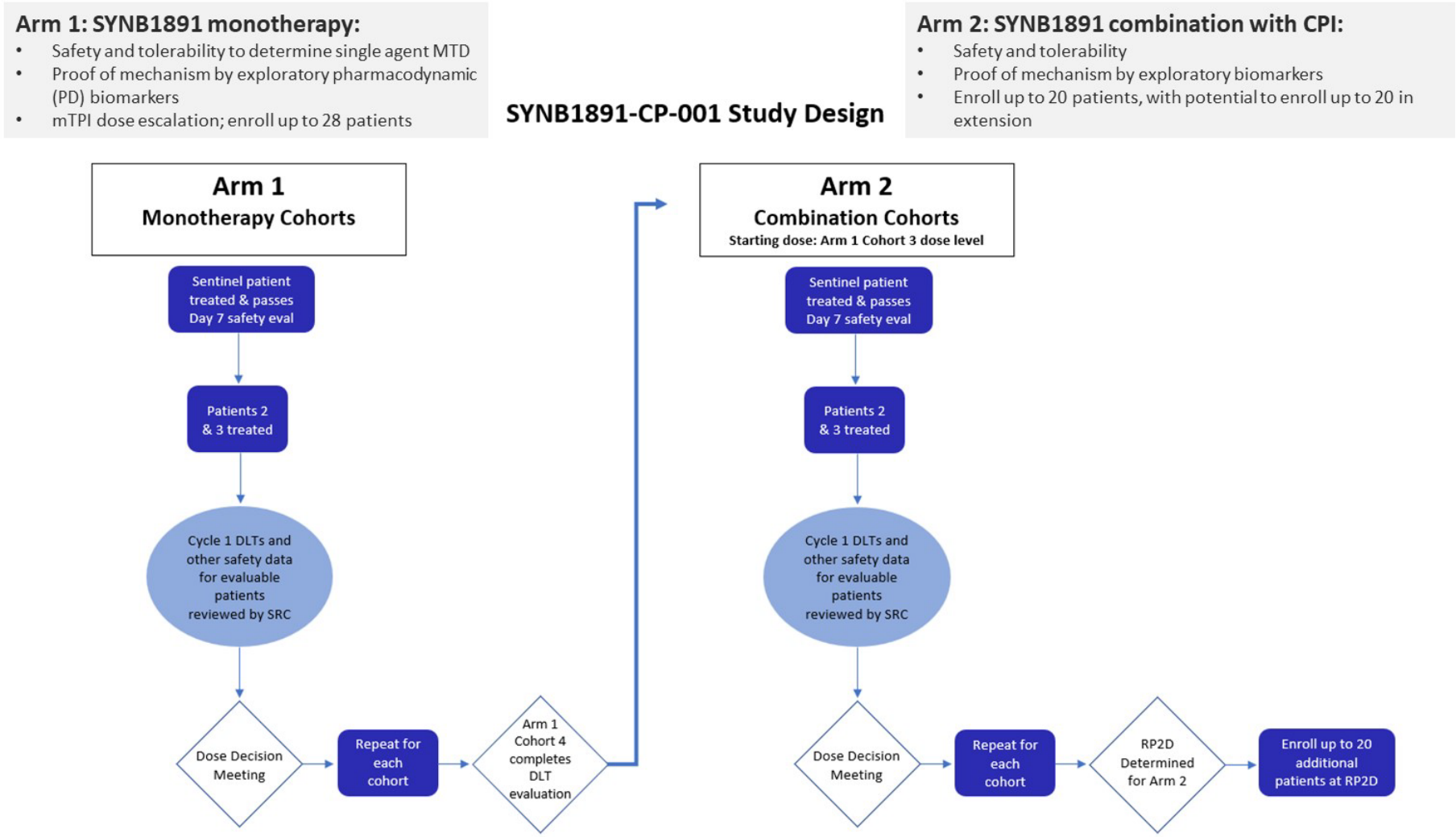
Eligible patients will be enrolled sequentially and will receive the first dose of study treatment within 28 days of screening.

Safety will be monitored continuously by documentation of AEs, SAEs, clinical laboratory measurements, vital signs, and physical examinations. DLTs, as defined in Section 4.2.4, will be evaluated through the end of the first cycle.

Disease status will be assessed by the local Investigator using appropriate imaging every 2 cycles for the duration of study treatment administration to evaluate the SYNB1891-injected lesion(s) and an overall response of up to 5 noninjected target lesions in accordance with RECIST 1.1 (Eisenhauer et al 2009 and Appendix 1), iRECIST (Seymour et al 2017 and Appendix 2), and/or LYRIC (Cheson et al 2016 and Appendix 3).

Tumor biopsies and blood samples will be collected at baseline and at prespecified time points on study to evaluate the pharmacokinetics (PK), as measured by qPCR bacterial kinetics, and PD effects of study treatment.

Figure 4: SYNB1891-CP-001 Study Design



Abbreviations: CPI = checkpoint inhibitor; DLT = dose-limiting toxicity; MTD = maximum tolerated dose; mTPI = modified toxicity probability interval; RP2D = recommended Phase 2 dose; SRC = Safety Review Committee

4.2. Study Dosing Regimens

4.2.1. Treatments Administered

Patients enrolled to Arm 1 may receive up to four 21-day cycles of SYNB1891 monotherapy. 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.

At the end of Cycle 4, patients in Arm 1 who do not have progressive disease (i.e., those who achieve and sustain complete response [CR], partial response [PR], or stable disease [SD]) may receive additional cycles of SYNB1891 administered by i.t. injection 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 study stopping rule, or no eligible lesions remain.

Patients enrolled to Arm 2 may receive up to four 21-day cycles with SYNB1891 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. 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 (i.e., those who achieve and sustain CR, PR, or SD) at the end of Cycle 4 on combination therapy may receive additional cycles of SYNB1891 and atezolizumab 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 study stopping rule, or no eligible lesions remain.

Locating and/or injecting the lesion may be performed using guided imaging at the discretion of the Investigator.

4.2.2. Postdose Monitoring

The initial patient in each Arm 1 cohort (Sentinel patient) will be monitored for at least 6 hours following the first (Cycle 1 Day 1) i.t. injection of SYNB1891 (or overnight, at the discretion of the Investigator) for evaluation of any procedural complication or acute toxicity (i.e., injection reactions or cytokine release syndrome) and for collection of postdose blood samples. Safety data must be reviewed on or after Day 7 and deemed acceptable by the Investigator and the Sponsor and contract research organization (CRO) Medical Monitors before the Sentinel patient receives any further injections and before any subsequent patients are dosed in that cohort.

For the remainder of Cycle 1 dosing for the Sentinel patient and for all dosing for non-Sentinel patients, SYNB1891 injections will be administered in the outpatient setting and patients will be closely observed at the clinic for at least 6 hours following each injection for evaluation of any procedural complication or acute toxicity (i.e., injection reactions or cytokine release syndrome) and for collection of postdose blood samples. All patients will be contacted by telephone on Cycle 1 Day 3 to monitor for signs of cytokine release syndrome (see Section 4.4).

4.2.3. Dose-finding with the mTPI Algorithm

In both study arms, each cohort will initially comprise 3 patients (a Sentinel patient and 2 non-Sentinel patients), with subsequent dose escalation governed by an mTPI design (Ji et al 2013).

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. If no MTD is identified, then the MTD will be considered the maximum dose at which a sufficient number of patients have been treated such that if 14 patients were administered that dose then either escalation or staying at the current dose would be recommended according to Table 1. For example, if 8 patients were administered a dose level with 0 DLTs observed, even if all remaining 6 patients had a DLT, Table 1 would indicate "stay at the current dose"; thus, the remaining 6 patients are not required to evaluate the MTD.

A mTPI design 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, 3×10^6, 1×10^7, 3×10^7, 1×10^8, 3×10^8, and 1×10^9 live cells will be explored independently, with additional dose levels as necessary to determine the MTD. 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.

In Table 1, the number of patients treated is indicated in the columns and the number of patients who experienced a DLT is indicated in the rows. Dosing decisions include escalate to the next higher dose (E), stay at the current dose (S), de-escalate to the next lower dose (D), and de-escalate to a lower dose and never test this dose again (i.e., unacceptably toxic dose; DU). During dose escalation, a minimum of 3 patients are required at each dose. Depending on accrual rate and occurrence of DLTs, 3, 4, 5 or 6 patients may be enrolled at each new dose until the last of those patients completes the Cycle 1 DLT assessment period. For example, the dose escalation rules will proceed as follows if 3 patients are enrolled: if 0 of the first 3 patients at a given dose level develops a DLT, then the dose can be escalated to the next level without further expansion. If 1 of the first 3 patients at a given dose level develops a DLT, no more than an additional 3 patients should be enrolled at this dose level until additional DLT data are available since this dose would be considered unacceptably toxic if all 3 of the additional patients experience a DLT (i.e., 4 of 6 patients). If 2 of the first 3 patients at a given dose level develop a DLT, the dose will be de-escalated to the next lower level. If 3 of the first 3 patients at a given dose level develop a DLT, this dose will be considered unacceptably toxic (i.e., the dose will be de-escalated and never re-escalated to that dose again). The same principle will be applied whether 3, 4, 5 or 6 patients are enrolled in the same dose cohort according to Table 1.

Based on the mTPI design, the number of patients who are enrolled at a dose, but are not yet fully evaluable for DLT assessment, may not exceed the number of remaining patients who are at risk of developing a DLT before the dose would be considered unacceptably toxic (denoted as DU in Table 1). To determine how many more patients can be enrolled at a dose level, one can count steps in a diagonal direction (down and to the right) from the current cell to the first cell marked DU. In total, 3 to 14 patients may be enrolled at a given dose level.

Dose escalation and confirmation will end after 14 patients have been treated at any of the selected doses. The pool-adjacent-violators-algorithm (Ji et al 2013) will be used to estimate the

DLT rates across doses. The dose with an estimated DLT rate closest to 30% may be treated as a preliminary MTD. Using the mTPI algorithm, the totality of the data will be considered before a dose is selected to carry forward to the next cohort, and the escalation schedule may be adjusted based on PK, PD, and safety data emerging throughout the study to determine the MTD/RP2D. Note that while 30% was the target toxicity rate used to generate the guidelines in Table 1, the observed rates of patients with DLTs at the MTD may be slightly above or below 30%.

Table 1: Dose-finding Rules per 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 $\varepsilon1=\varepsilon2=0.03$

After all patients in Arm 1 Cohort 4 have completed their Cycle 1 DLT safety evaluation, dosing will begin in Arm 2 starting at the Arm 1 Cohort 3 dose level. Dosing in the Arm 2 cohorts will increase in approximately 3-fold increments in subsequent cohorts until RP2D determination. Combination doses will not be escalated above the SYNB1891 single-agent MTD established in Arm 1. Once dosing in Arm 2 is initiated, patient allocation to cohorts will be managed by the Sponsor to ensure that a dosing level within Arm 2 is at least one dose level below the SYNB1891 monotherapy dose being evaluated in Arm 1. The atezolizumab dose will remain constant at 1200 mg Q3W for each dose level in Arm 2.

In both study arms, enrollment will proceed in accordance with the following rules:

- Review of the safety data for the first patient in a cohort (Sentinel patient) must be completed on or after Day 7 before the remaining 2 patients (non-Sentinel) in that cohort may be dosed (these 2 patients may be dosed concurrently). Observation of DLT(s) in a Sentinel patient will not necessarily preclude enrollment of subsequent

patients at the same dose level; rather, DLTs occurring in Sentinel patients will be reviewed individually by the SRC (consisting of the Sponsor Medical Monitor, the CRO Medical Monitor, all active Investigators, and other study personnel as needed) to determine appropriate dosing for subsequent patients.

- After all patients in a cohort have been assessed for DLTs (i.e., after each patient either experiences a DLT or completes the end of Cycle 1 safety assessment, whichever occurs first), the SRC will review all available safety data from the cohort and determine whether to escalate to the next higher SYNB1891 dose, enroll additional patients at the current dose, de-escalate to the next lower dose, or declare that the study objectives or stopping rules have been met. The decision will be informed by the mTPI algorithm recommendation to achieve the targeted toxicity interval.
- Patients must either experience a DLT during Cycle 1 or receive at least 2 of the 3 planned SYNB1891 injections to be considered evaluable for DLT evaluations and contribute to dose-escalation decisions for a given cohort. Patients who withdraw from the study for any reason other than DLTs prior to receiving the second injection of SYNB1891 in Cycle 1 may be replaced.

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.

4.2.4. Definition of Dose-limiting Toxicity

The occurrence of any of the following toxicities during Cycle 1 will be considered a DLT, if assessed by the Investigator to be possibly, probably, or definitely related to study treatment administration.

1. Grade 4 nonhematologic toxicity (not laboratory)
2. Grade 4 hematologic toxicity lasting ≥ 7 days, except thrombocytopenia:
   - Grade 4 thrombocytopenia of any duration
   - Grade 3 thrombocytopenia associated with clinically significant bleeding
3. Any nonhematologic AE Grade ≥ 3 in severity should be considered a DLT, with the following exceptions: Grade 3 fatigue lasting ≤ 3 days; Grade 3 diarrhea, nausea, or vomiting without use of antiemetics or antidiarrheals per standard of care; Grade 3 rash without use of corticosteroids or anti-inflammatory agents per standard of care
4. Any Grade 3 or Grade 4 nonhematologic laboratory value if:
   - Clinically significant medical intervention is required to treat the patient, or
   - The abnormality leads to hospitalization, or
   - The abnormality persists for > 1 week, or
   - The abnormality results in a drug-induced liver injury (DILI)
   - Exceptions: clinically nonsignificant, treatable, or reversible laboratory abnormalities including liver function tests, uric acid, etc.
5. Febrile neutropenia Grade 3 or Grade 4:

- Grade 3 is defined as absolute neutrophil count (ANC) < 1000/mm^3 with a single temperature of > 38.3°C (101°F) or a sustained temperature of ≥ 38°C (100.4°F) for more than 1 hour
- Grade 4 is defined as ANC < 1000/mm^3 with a single temperature of > 38.3°C (101°F) or a sustained temperature of ≥ 38°C (100.4°F) for more than 1 hour, with life-threatening consequences and urgent intervention indicated

6. Sepsis, severe abscesses and/or ulcerations requiring surgical management
7. Prolonged delay (>2 weeks) in initiating Cycle 2 due to treatment-related toxicity
8. Investigator decision to discontinue study treatment for a treatment-related toxicity during Cycle 1
9. Missing > 33% of study drug doses as a result of Grade 3 drug-related AE(s) during the first cycle
10. Grade 5 toxicity

4.3. Intrapatient Dose Modification

Every effort should be made to administer study treatment on the planned dose and schedule.

4.3.1. Dosing Delays and Interruptions

Study treatment dosing delays will be permitted in any treatment cycle if unforeseen circumstances prevent the patient from returning to the clinic on the scheduled day(s). A maximum delay of 2 days in Cycle 1 will be permitted. If administration of study treatment is delayed by > 2 days in Cycle 1, that dose is considered missed and the patient should receive the next dose at the next visit.

If a patient misses ≥ 2 SYNB1891 doses in Cycle 1, then the patient will be discontinued permanently from treatment and may be replaced (see Section 8.2.1). In Cycle 2 and beyond, if a patient misses the dose of SYNB1891, then the patient may be discontinued permanently from treatment, unless the Investigator's assessment of the benefit/risk suggests otherwise (after consultation with Sponsor). If treatment is permanently discontinued, patients will continue to be followed for safety as indicated for end of treatment and follow-up.

SYNB1891 treatment interruption can occur due to treatment-related toxicity, including Grade 3 or higher infections or local inflammation requiring antibiotics, and the start of a new cycle may not occur until the following criteria have been met:

- Nonhematologic toxicities have returned to either baseline or Grade ≤ 1, or at the Investigator's discretion, Grade ≤ 2 if not considered a safety risk. This condition must be met within 14 days of the initial treatment interruption.
- Hematologic toxicities: ANC ≥ 1000/mm^3 or have returned to baseline levels; platelet count ≥ 50,000/ mm^3 or has returned to baseline levels. This condition must be met within 14 days of the initial treatment interruption.
- If a patient requires treatment with a systemic antibiotic on study, SYNB1891 should be held for the duration of the antibiotic regimen and for 5 half-lives after the last dose of antibiotics. Once the patient is apyretic after completion of the antibiotic washout period, SYNB1891 may resume.

If the patient cannot meet the above conditions within the specified time frame, then study treatment should be permanently discontinued, unless the Investigator's assessment of the benefit/risk suggests otherwise (after consultation with Sponsor). The patient may be replaced (see Section 8.2.1).

Atezolizumab treatment may be temporarily suspended in patients experiencing toxicity considered to be related to study treatment. If corticosteroids are initiated for treatment of immune-mediated toxicity, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed. If atezolizumab is withheld for > 12 weeks after event onset, the patient will be discontinued from atezolizumab. However, atezolizumab may be withheld for > 12 weeks to allow for patients to taper off corticosteroids prior to resuming treatment. Atezolizumab can be resumed after being withheld for > 12 weeks if the Medical Monitor(s) agrees that the patient is likely to derive clinical benefit. Atezolizumab treatment may be suspended for reasons other than toxicity (e.g., surgical procedures) with Medical Monitor approval. The Investigator and the Medical Monitor(s) will determine the acceptable length of treatment interruption.

4.3.2. Dose Reductions

4.3.2.1. Dose Reductions for SYNB1891

No dose reductions of SYNB1891 will be permitted in Cycle 1; any patient who requires such a modification will be permanently discontinued and may be replaced (see Section 8.2.1). After Cycle 1, SYNB1891 dose reductions are permitted in approximately 3-fold decrements after consultation with the Sponsor. While no specific dose reductions are required for Grade 1 or 2 treatment-related toxicities, the Investigator should always manage patients according to medical judgment and assessment of risk/benefit for that patient. Patients experiencing recurrent or intolerable Grade 2 toxicity may resume dosing at the next lower dose level once the toxicity has resolved or recovery to Grade 1 or baseline has occurred.

If more than 1 dose reduction is required, then the patient(s) are permanently discontinued from treatment but will be followed for end of treatment and follow-up assessments.

Once a dose has been reduced, the patient should continue to receive that dose for all subsequent doses. In this setting, intrapatient dose escalation is not permitted.

4.3.2.2. Dose Reductions for Atezolizumab

No dose reductions of atezolizumab will be permitted in this study.

4.4. Toxicity Management

Participants should receive appropriate supportive care measures as deemed necessary by the treating Investigator.

4.4.1. Adverse Events Associated with SYNB1891

Guidelines for the management of patients who experience AEs considered to be potentially associated with SYNB1891 are provided in Table 2.

Table 2: Toxicity Management for Adverse Events Considered Potentially Associated with SYNB1891

Event	NCI CTCAE Grade	Recommended Medical Management	Premedication and Subsequent Dosing
Cytokine release syndrome (may include associated AEs, e.g., fever, tachypnea, headache, tachycardia, hypotension, rash, hypoxia)	Grade 1 Fever with or without constitutional symptoms	Increase vital sign and oxygen saturation monitoring as medically indicated until the patient is deemed medically stable in the opinion of the Investigator.	Administer as planned
	Grade 2 Hypotension responding to fluids; hypoxia responding to < 40% oxygen	Increase vital sign and oxygen saturation monitoring as medically indicated until the patient is deemed medically stable in the opinion of the Investigator. Additional appropriate therapy may include, but is not limited to: IV fluids NSAIDs Acetaminophen Narcotics Oxygen Evaluate for infectious etiologies and treat neutropenia if present.	Patient may be premedicated 1.5 hours (± 30 minutes) prior to study treatment administration with oral acetaminophen (500 to 1000 mg) or equivalent dose of antipyretics.
	Grade 3 Hypotension managed with one pressor; hypoxia requiring ≥ 40% oxygen	Appropriate therapy may include, but is not limited to: IV fluids NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Anti-IL6 (e.g., tocilizumab) Empiric antibiotics Monitor patients closely; consider an intensive care setting	Discuss with the Sponsor prior to the resumption of study treatment. Upon approval by the Sponsor, resume SYNB1891 at a reduced dose upon AE resolution to baseline or Grade 1. Patient may be premedicated 1.5 hours (± 30 minutes) prior to study treatment administration with oral acetaminophen (500 to 1000 mg) or equivalent dose of antipyretics.

Event	NCI CTCAE Grade	Recommended Medical Management	Premedication and Subsequent Dosing
	Grade 4 Life-threatening consequences; urgent intervention indicated	Appropriate therapy may include, but is not limited to: IV fluids NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Anti-IL6 (e.g., tocilizumab) Empiric antibiotics Monitor patients closely; consider an intensive care setting.	Permanently discontinue study treatment.
Systemic infection	Grade 3 or higher	Appropriate therapy may include, but is not limited to: IV fluids NSAIDs Acetaminophen Narcotics Oxygen Pressors Empiric antibiotics Refer to Appendix 4 for *Ec*N-sensitive antibiotics Perform local blood and urine cultures and sensitivities. Perform serum qPCR specific for *Ec*N.	Patients who require systemic antibiotic treatment should postpone dosing with SYNB1891 for the duration of the antibiotic regimen and for 5 half-lives after the last dose of antibiotics and until the patient is apyretic.
Local injection site reactions (may include associated AEs, e.g., abscess, infection, ulceration or necrosis, or severe tissue damage)	Grade 3 or higher	Consider surgical intervention as needed in addition to and in accordance with local standard of care, which may include, but is not limited to: NSAIDs Acetaminophen Antibiotics	Permanently discontinue study treatment.

Abbreviations: AE = adverse event; CTCAE = Common Terminology Criteria for Adverse Events; *Ec*N = *E. coli* Nissle; IL = interleukin; IV = intravenous; NCI = National Cancer Institute; NSAID = nonsteroidal anti-inflammatory drug; qPCR = quantitative polymerase chain reaction

4.4.2. Adverse Events Associated with Atezolizumab

4.4.2.1. Pulmonary Events

Dyspnea, cough, fatigue, hypoxia, pneumonitis, and pulmonary infiltrates have been associated with the administration of atezolizumab. Patients will be assessed for pulmonary signs and symptoms throughout the study and will have imaging (e.g., computed tomography [CT] scans) of the chest performed at every tumor assessment.

All pulmonary events should be thoroughly evaluated for other commonly reported etiologies such as pneumonia or other infection, lymphangitic carcinomatosis, pulmonary embolism, heart failure, chronic obstructive pulmonary disease, or pulmonary hypertension. Management guidelines for pulmonary events are provided in Table 3.

Table 3: Management Guidelines for Pulmonary Events, Including Pneumonitis

Event	NCI CTCAE Grade	Recommended Medical Management
Pulmonary event	Grade 1	• Continue atezolizumab and monitor closely. • Re-evaluate on serial imaging. • Consider patient referral to pulmonary specialist.
	Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to pulmonary and infectious disease specialists and consider bronchoscopy or BAL. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s). • For recurrent events, treat as a Grade 3 or 4 event.
	Grade 3 or higher	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Bronchoscopy or BAL is recommended. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

Abbreviations: BAL = bronchoscopic alveolar lavage; CTCAE = Common Terminology Criteria for Adverse Events; NCI = National Cancer Institute

[a] Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤ 10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the Investigator and the Medical Monitor(s).

[b] If corticosteroids have been initiated, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed.

4.4.2.2. Hepatic Events

Immune-mediated hepatitis has been associated with the administration of atezolizumab. Eligible patients must have adequate liver function, as manifested by measurements of total bilirubin and hepatic transaminases, and liver function will be monitored throughout study treatment. Management guidelines for hepatic events are provided in Table 4.

Patients with right upper-quadrant abdominal pain and/or unexplained nausea or vomiting should have liver function tests (LFTs) performed immediately and reviewed before administration of the next dose of study drug.

For patients with elevated LFTs, concurrent medication, viral hepatitis, and toxic or neoplastic etiologies should be considered and addressed, as appropriate.

Table 4: Management Guidelines for Hepatic Events

Event	NCI CTCAE Grade	Recommended Medical Management
Hepatic event	Grade 1	• Continue atezolizumab. • Monitor LFTs until values resolve to within normal limits or to baseline values.
	Grade 2	**All events:** • Monitor LFTs more frequently until return to baseline values. **Events of >5 days duration:** • Withhold atezolizumab for up to 12 weeks after event onset. [a] • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s).
	Grade 3 or higher	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Consider patient referral to gastrointestinal specialist for evaluation and liver biopsy to establish etiology of hepatic injury. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; LFT = liver function test; NCI = National Cancer Institute

[a] Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤ 10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the Investigator and the Medical Monitor(s).

[b] If corticosteroids have been initiated, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed.

4.4.2.3. Gastrointestinal Events

Immune-mediated colitis has been associated with the administration of atezolizumab. Management guidelines for diarrhea or colitis are provided in Table 5.

All events of diarrhea or colitis should be thoroughly evaluated for other more common etiologies. For events of significant duration or magnitude or associated with signs of systemic inflammation or acute-phase reactants (e.g., increased C-reactive protein, platelet count, or bandemia): perform sigmoidoscopy (or colonoscopy, if appropriate) with colonic biopsy, with 3 to 5 specimens for standard paraffin block to check for inflammation and lymphocytic infiltrates to confirm colitis diagnosis.

Table 5: Management Guidelines for Gastrointestinal Events

Event	NCI CTCAE Grade	Recommended Medical Management
Diarrhea or colitis	Grade 1	• Continue atezolizumab. • Initiate symptomatic treatment. • Endoscopy is recommended if symptoms persist for > 7 days. • Monitor closely.
	Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Initiate symptomatic treatment. • Patient referral to GI specialist is recommended. • For recurrent events or events that persist > 5 days, initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s).
	Grade 3	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to GI specialist for evaluation and confirmatory biopsy. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s).
	Grade 4	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Refer patient to GI specialist for evaluation and confirmation biopsy. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; GI = gastrointestinal; IV = intravenous; NCI = National Cancer Institute

[a] Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤ 10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the Investigator and the Medical Monitor(s).

[b] If corticosteroids have been initiated, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed.

4.4.2.4. Endocrine Events

Thyroid disorders, adrenal insufficiency, diabetes mellitus, and pituitary disorders have been associated with the administration of atezolizumab. Management guidelines for endocrine events are provided in Table 6.

Patients with unexplained symptoms such as headache, fatigue, myalgias, impotence, constipation, or mental status changes should be investigated for the presence of thyroid, pituitary, or adrenal endocrinopathies. The patient should be referred to an endocrinologist if an endocrinopathy is suspected. Thyroid-stimulating hormone (TSH) and triiodothyronine and

thyroxine levels should be measured to determine whether thyroid abnormalities are present. Pituitary hormone levels and function tests (e.g., TSH, growth hormone, luteinizing hormone, follicle-stimulating hormone, testosterone, prolactin, adrenocorticotropic hormone levels, and adrenocorticotropic hormone stimulation test) and magnetic resonance imaging (MRI) of the brain (with detailed pituitary sections) may help to differentiate primary pituitary insufficiency from primary adrenal insufficiency.

Table 6: Management Guidelines for Endocrine Events

Event	NCI CTCAE Grade	Recommended Medical Management
Hypothyroidism (Asymptomatic)	Not applicable	• Continue atezolizumab. • Initiate treatment with thyroid replacement hormone. • Monitor TSH weekly.
Hypothyroidism (Symptomatic)	Not applicable	• Withhold atezolizumab. • Initiate treatment with thyroid replacement hormone. • Monitor TSH weekly. • Consider patient referral to endocrinologist. • Resume atezolizumab when symptoms are controlled and thyroid function is improving.
Hyperthyroidism (Asymptomatic)	Not applicable	**TSH ≥ 0.1 mU/L and < 0.5 mU/L:** • Continue atezolizumab. • Monitor TSH every 4 weeks. **TSH < 0.1 mU/L:** • Follow guidelines for symptomatic hyperthyroidism.
Hyperthyroidism (Symptomatic)	Not applicable	• Withhold atezolizumab. • Initiate treatment with antithyroid drug such as methimazole or carbimazole as needed. • Consider patient referral to endocrinologist. • Resume atezolizumab when symptoms are controlled and thyroid function is improving. • Permanently discontinue atezolizumab and contact Medical Monitor(s) for life-threatening immune-mediated hyperthyroidism.

Event	NCI CTCAE Grade	Recommended Medical Management
Symptomatic adrenal insufficiency	Grade 2 or higher	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to endocrinologist. • Perform appropriate imaging. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event resolves to Grade 1 or better and patient is stable on replacement therapy, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better or patient is not stable on replacement therapy while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s).
Hyperglycemia	Grade 1 or 2	• Continue atezolizumab. • Investigate for diabetes. If patient has Type 1 diabetes, treat as a Grade 3 event. If patient does not have Type 1 diabetes, treat as per institutional guidelines. • Monitor for glucose control.
	Grade 3 or higher	• Withhold atezolizumab. • Initiate treatment with insulin. • Monitor for glucose control. • Resume atezolizumab when symptoms resolve and glucose levels are stable.
Hypophysitis (pan-hypopituitarism)	Grade 2 or 3	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to endocrinologist. • Perform brain MRI (pituitary protocol). • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • Initiate hormone replacement if clinically indicated. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s). • For recurrent hypophysitis, treat as a Grade 4 event.

Event	NCI CTCAE Grade	Recommended Medical Management
Hypophysitis (pan-hypopituitarism)	Grade 4	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Refer patient to endocrinologist. • Perform brain MRI (pituitary protocol). • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • Initiate hormone replacement if clinically indicated.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; IV = intravenous; MRI = magnetic resonance imaging; NCI = National Cancer Institute; TSH = thyroid-stimulating hormone

[a] Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤ 10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the Investigator and the Medical Monitor(s).

[b] If corticosteroids have been initiated, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed.

4.4.2.5. Ocular Events

An ophthalmologist should evaluate visual complaints (e.g., uveitis, retinal events). Management guidelines for ocular events are provided in Table 7.

Table 7: Management Guidelines for Ocular Events

Event	NCI CTCAE Grade	Recommended Medical Management
Ocular event	Grade 1	• Continue atezolizumab. • Patient referral to ophthalmologist is strongly recommended. • Initiate treatment with topical corticosteroid eye drops and topical immunosuppressive therapy. • If symptoms persist, treat as a Grade 2 event.
	Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Patient referral to ophthalmologist is strongly recommended. • Initiate treatment with topical corticosteroid eye drops and topical immunosuppressive therapy. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s).
	Grade 3 or higher	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Refer patient to ophthalmologist. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; NCI = National Cancer Institute

[a] Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤ 10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the Investigator and the Medical Monitor(s).

[b] If corticosteroids have been initiated, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed.

4.4.2.6. Immune-mediated Myocarditis

Immune-mediated myocarditis has been associated with the administration of atezolizumab. Immune-mediated myocarditis should be suspected in any patient presenting with signs or symptoms suggestive of myocarditis, including, but not limited to, laboratory (e.g., B-type natriuretic peptide) or cardiac imaging abnormalities, dyspnea, chest pain, palpitations, fatigue, decreased exercise tolerance, or syncope. Immune-mediated myocarditis needs to be distinguished from myocarditis resulting from infection (commonly viral, e.g., in a patient who reports a recent history of gastrointestinal illness), ischemic events, underlying arrhythmias, exacerbation of preexisting cardiac conditions, or progression of malignancy.

All patients with possible myocarditis should be urgently evaluated by performing cardiac enzyme assessment, an electrocardiogram (ECG), a chest X-ray, an echocardiogram, and a cardiac MRI as appropriate per institutional guidelines. A cardiologist should be consulted. An endomyocardial biopsy may be considered to enable a definitive diagnosis and appropriate treatment, if clinically indicated.

Patients with signs and symptoms of myocarditis, in the absence of an identified alternate etiology, should be treated according to the guidelines in Table 8.

Table 8: Management Guidelines for Immune-mediated Myocarditis

Event	NCI CTCAE Grade	Recommended Medical Management
Immune-mediated myocarditis	Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset [a] and contact Medical Monitor(s). • Refer patient to cardiologist. • Initiate treatment as per institutional guidelines and consider antiarrhythmic drugs, temporary pacemaker, ECMO, or VAD as appropriate. • Consider treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s).
	Grade 3 or higher	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Refer patient to cardiologist. • Initiate treatment as per institutional guidelines and consider antiarrhythmic drugs, temporary pacemaker, ECMO, or VAD as appropriate. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; ECMO = extracorporeal membrane oxygenation; IV = intravenous; NCI = National Cancer Institute; VAD = ventricular assist device

[a] Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤ 10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the Investigator and the Medical Monitor(s).

[b] If corticosteroids have been initiated, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed.

4.4.2.7. Infusion-related Reactions and Cytokine Release Syndrome

No premedication is indicated for the administration of Cycle 1 of atezolizumab. However, patients who experience an infusion-related reaction (IRR) or cytokine release syndrome with atezolizumab may receive premedication with antihistamines, anti-pyretics, and/or analgesics (e.g., acetaminophen) for subsequent infusions. Metamizole (dipyrone) is prohibited in treating atezolizumab-associated IRRs because of its potential for causing agranulocytosis.

IRRs are known to occur with the administration of monoclonal antibodies and have been reported with atezolizumab. These reactions, which are thought to be due to release of cytokines

and/or other chemical mediators, occur within 24 hours of atezolizumab administration and are generally mild to moderate in severity.

Cytokine release syndrome is defined as a supraphysiologic response following administration of any immune therapy that results in activation or engagement of endogenous or infused T cells and/or other immune effector cells. Symptoms can be progressive, always include fever at the onset, and may include hypotension, capillary leak (hypoxia), and end-organ dysfunction (Lee et al 2019). Cytokine release syndrome has been well documented with chimeric antigen receptor T-cell therapies and bispecific T-cell engager antibody therapies but has also been reported with immunotherapies that target PD-1 or PD-L1 (Rotz et al 2017; Adashek et al 2019), including atezolizumab.

There may be significant overlap in signs and symptoms of IRRs and cytokine release syndrome, and in recognition of the challenges in clinically distinguishing between the two, consolidated guidelines for medical management of IRRs and cytokine release syndrome are provided in Table 9.

Table 9: Management Guidelines for Infusion-related Reactions and Cytokine Release Syndrome

Event	NCI CTCAE Grade	Recommended Medical Management
Fever[a] with or without constitutional symptoms	Grade 1[b]	• Immediately interrupt infusion • Upon symptom resolution, wait for 30 minutes and then restart infusion at half the rate being given at the time of event onset. • If the infusion is tolerated at the reduced rate for 30 minutes, the infusion rate may be increased to the original rate. • If symptoms recur, discontinue infusion of this dose. • Administer symptomatic treatment,[c] including maintenance of IV fluids for hydration. • In case of rapid decline or prolonged CRS (> 2 days) or in patients with significant symptoms and/or comorbidities, consider managing as per Grade 2. • For subsequent infusions, consider administration of oral premedication with antihistamines, anti-pyretics, and/or analgesics, and monitor closely for IRRs and/or CRS.
Fever[a] with hypotension not requiring vasopressors and/or Hypoxia requiring low-flow oxygen[d] by nasal cannula or blow-by	Grade 2[b]	• Immediately interrupt infusion. • Upon symptom resolution, wait for 30 minutes and then restart infusion at half the rate being given at the time of event onset. • If symptoms recur, discontinue infusion of this dose. • Administer symptomatic treatment.[c] • For hypotension, administer IV fluid bolus as needed. • Monitor cardiopulmonary and other organ function closely (in the ICU, if appropriate). Administer IV fluids as clinically indicated, and manage constitutional symptoms and organ toxicities as per institutional practice. • Rule out other inflammatory conditions that can mimic CRS (e.g., sepsis). If no improvement within 24 hours, initiate workup and assess for signs and symptoms of HLH or MAS. • Consider IV corticosteroids (e.g., methylprednisolone 2 mg/kg/day or dexamethasone 10 mg every 6 hours).

Event	NCI CTCAE Grade	Recommended Medical Management
		• Consider anti-cytokine therapy.[e] • Consider hospitalization until complete resolution of symptoms. If no improvement within 24 hours, manage as per Grade 3, that is, hospitalize patient (monitoring in the ICU is recommended), permanently discontinue atezolizumab, and contact Medical Monitor. • If symptoms resolve to Grade 1 or better for 3 consecutive days, the next dose of atezolizumab may be administered. For subsequent infusions, consider administration of oral premedication with antihistamines, antipyretics, and/or analgesics and monitor closely for IRRs and/or CRS. • If symptoms do not resolve to Grade 1 or better for 3 consecutive days, inform the Medical Monitor.
Fever[a] with hypotension requiring a vasopressor (with or without vasopressin) and/or Hypoxia requiring high-flow oxygen[d] by nasal cannula, face mask, non rebreather mask, or venturi mask	Grade 3[b]	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Administer symptomatic treatment.[c] • For hypotension, administer IV fluid bolus and vasopressor as needed. • Monitor cardiopulmonary and other organ function closely; monitoring in the ICU is recommended. Administer IV fluids as clinically indicated and manage constitutional symptoms and organ toxicities as per institutional practice. • Rule out other inflammatory conditions that can mimic CRS (e.g., sepsis). If no improvement within 24 hours, initiate workup and assess for signs and symptoms of HLH or MAS. • Administer IV corticosteroids (e.g., methylprednisolone 2 mg/kg/day or dexamethasone 10 mg every 6 hours). • Consider anti-cytokine therapy.[e] • Hospitalize patient until complete resolution of symptoms. If no improvement within 24 hours, manage as per Grade 4, that is, admit patient to ICU and initiate hemodynamic monitoring, mechanical ventilation, and/or IV fluids and vasopressors as needed; for patients who are refractory to anti-cytokine therapy, experimental treatments may be considered at the discretion of the investigator and in consultation with the Medical Monitor.
Fever[a] with hypotension requiring multiple vasopressors (excluding vasopressin) and/or Hypoxia requiring oxygen by positive pressure (e.g., continuous positive airway pressure, bi-level	Grade 4[b]	• Permanently discontinue atezolizumab and contact Medical Monitor. • Administer symptomatic treatment.[c] • Admit patient to ICU and initiate hemodynamic monitoring, mechanical ventilation, and/or IV fluids and vasopressors as needed. Monitor other organ function closely. Manage constitutional symptoms and organ toxicities as per institutional practice. • Rule out other inflammatory conditions that can mimic CRS (e.g., sepsis). If no improvement within 24 hours, initiate workup and assess for signs and symptoms of HLH or MAS. • Administer IV corticosteroids (e.g., methylprednisolone 2 mg/kg/day or dexamethasone 10 mg every 6 hours).

Event	NCI CTCAE Grade	Recommended Medical Management
positive airway pressure, intubation and mechanical ventilation)		• Consider anti-cytokine therapy.[e] For patients who are refractory to anti-cytokine therapy, experimental treatments[f] may be considered at the discretion of the investigator (inform the Medical Monitor of such treatments). • Hospitalize patient until complete resolution of symptoms.

Abbreviations: CRS = cytokine release syndrome; CTCAE = Common Terminology Criteria for Adverse Events; HLH = hemophagocytic lymphohistiocytosis; ICU = intensive care unit; IRR = infusion-related reaction; IV = intravenous; MAS = macrophage activation syndrome; NCI = National Cancer Institute

Note: The management guidelines have been adapted from National Comprehensive Cancer Network guidelines for management of chimeric antigen receptor T-cell-related toxicities (Version 2.2019).

[a] Fever is defined as temperature ≥ 38°C not attributable to any other cause. In patients who develop CRS and then receive anti-pyretic, anti-cytokine, or corticosteroid therapy, fever is no longer required when subsequently determining event severity (grade). In this case, the grade is driven by the presence of hypotension and/or hypoxia.

[b] Grading system for management guidelines is based on American Society for Transplantation and Cellular Therapy consensus grading for CRS. NCI CTCAE version 5.0 should be used when reporting severity of IRRs, CRS, or organ toxicities associated with CRS. Organ toxicities associated with CRS should not influence overall CRS grading.

[c] Symptomatic treatment may include oral or IV antihistamines, anti-pyretics, analgesics, bronchodilators, and/or oxygen. For bronchospasm, urticaria, or dyspnea, additional treatment may be administered as per institutional practice.

[d] Low flow is defined as oxygen delivered at ≤ 6 L/min, and high flow is defined as oxygen delivered at > 6 L/min.

[e] There are case reports where anti-cytokine therapy has been used for treatment of CRS with immune checkpoint inhibitors (Rotz et al 2017; Adashek et al 2019), but data are limited, and the role of such treatment in the setting of antibody-associated CRS has not been established.

[f] Refer to Riegler et al (2019) for information on experimental treatments for CRS.

4.4.2.8. Pancreatic Events

Symptoms of abdominal pain associated with elevations of amylase and lipase, suggestive of pancreatitis, have been associated with the administration of atezolizumab. The differential diagnosis of acute abdominal pain should include pancreatitis. Appropriate workup should include an evaluation for ductal obstruction, as well as serum amylase and lipase tests. Management guidelines for pancreatic events, including pancreatitis, are provided in Table 10.

Table 10: Management Guidelines for Pancreatic Events, Including Pancreatitis

Event	NCI CTCAE Grade	Recommended Medical Management
Amylase and/or lipase elevation	Grade 2	**Amylase and/or lipase > 1.5 – 2.0 × ULN:** • Continue atezolizumab. • Monitor amylase and lipase weekly. • For prolonged elevation (e.g., > 3 weeks), consider treatment with corticosteroids equivalent to 10 mg/day oral prednisone. **Asymptomatic with amylase and/or lipase > 2.0 – 5.0 × ULN:** • Treat as a Grade 3 event.
	Grade 3 or higher	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to GI specialist. • Monitor amylase and lipase every other day. • If no improvement, consider treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s). • For recurrent events, permanently discontinue atezolizumab and contact Medical Monitor(s).
Immune-mediated pancreatitis	Grade 2 or 3	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to GI specialist. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s). • For recurrent events, permanently discontinue atezolizumab and contact Medical Monitor(s).
	Grade 4	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Refer patient to GI specialist. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; GI = gastrointestinal; IV = intravenous; NCI = National Cancer Institute; ULN = upper limit of normal

[a] Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤ 10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the Investigator and the Medical Monitor(s).

[b] If corticosteroids have been initiated, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed.

4.4.2.9. Dermatologic Events

Treatment-emergent rash has been associated with atezolizumab. The majority of cases of rash were mild in severity and self-limiting, with or without pruritus. A dermatologist should evaluate persistent and/or severe rash or pruritus. A biopsy should be considered unless contraindicated. Management guidelines for dermatologic events are provided in Table 11.

Table 11: Management Guidelines for Dermatologic Events

Event	NCI CTCAE Grade	Recommended Medical Management
Dermatologic event	Grade 1	• Continue atezolizumab. • Consider treatment with topical corticosteroids and/or other symptomatic therapy (e.g., antihistamines).
	Grade 2	• Continue atezolizumab. • Consider patient referral to dermatologist. • Initiate treatment with topical corticosteroids. • Consider treatment with higher-potency topical corticosteroids if event does not improve.
	Grade 3	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to dermatologist. • Initiate treatment with corticosteroids equivalent to 10 mg/day oral prednisone, increasing dose to 1–2 mg/kg/day if event does not improve within 48–72 hours. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s).
	Grade 4	• Permanently discontinue atezolizumab and contact Medical Monitor(s).

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; NCI = National Cancer Institute

[a] Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤ 10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the Investigator and the Medical Monitor(s).

[b] If corticosteroids have been initiated, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed.

4.4.2.10. Neurologic Disorders

Myasthenia gravis and Guillain-Barré syndrome have been observed with single-agent atezolizumab. Patients may present with signs and symptoms of sensory and/or motor neuropathy. Diagnostic workup is essential for an accurate characterization to differentiate between alternative etiologies. Management guidelines for neurologic disorders are provided in Table 12.

Table 12: Management Guidelines for Neurologic Disorders

Event	NCI CTCAE Grade	Recommended Medical Management
Immune-mediated neuropathy	Grade 1	• Continue atezolizumab. • Investigate etiology.
	Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Investigate etiology. • Initiate treatment as per institutional guidelines. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s).
	Grade 3 or higher	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Initiate treatment as per institutional guidelines.
Myasthenia gravis and Guillain-Barré syndrome	Any grade	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Refer patient to neurologist. • Initiate treatment as per institutional guidelines. • Consider initiation of corticosteroids equivalent to 1–2 mg/kg/day oral or IV prednisone.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; IV = intravenous; NCI = National Cancer Institute

[a] Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤ 10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the Investigator and the Medical Monitor(s).

[b] If corticosteroids have been initiated, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed.

4.4.2.11. Immune-mediated Meningoencephalitis

Immune-mediated meningoencephalitis is an identified risk associated with the administration of atezolizumab. Immune-mediated meningoencephalitis should be suspected in any patient presenting with signs or symptoms suggestive of meningitis or encephalitis, including, but not limited to, headache, neck pain, confusion, seizure, motor or sensory dysfunction, and altered or depressed level of consciousness. Encephalopathy from metabolic or electrolyte imbalances needs to be distinguished from potential meningoencephalitis resulting from infection (bacterial, viral, or fungal) or progression of malignancy, or secondary to a paraneoplastic process.

All patients being considered for meningoencephalitis should be urgently evaluated with a CT scan and/or MRI scan of the brain to evaluate for metastasis, inflammation, or edema. If deemed safe by the treating physician, a lumbar puncture should be performed and a neurologist should be consulted.

Patients with signs and symptoms of meningoencephalitis, in the absence of an identified alternate etiology, should be treated according to the guidelines in Table 13.

Table 13: Management Guidelines for Immune-mediated Meningoencephalitis

Event	NCI CTCAE Grade	Recommended Medical Management
Immune-mediated meningoencephalitis	All grades	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Refer patient to neurologist. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; IV = intravenous; NCI = National Cancer Institute

4.4.2.12. Renal Events

Immune-mediated nephritis has been associated with the administration of atezolizumab. Eligible patients must have adequate renal function, and renal function, including serum creatinine, should be monitored throughout study treatment. Patients with abnormal renal function should be evaluated and treated for other more common etiologies (including prerenal and postrenal causes, and concomitant medications such as nonsteroidal anti-inflammatory drugs). Refer the patient to a renal specialist if clinically indicated. A renal biopsy may be required to enable a definitive diagnosis and appropriate treatment.

Patients with signs and symptoms of nephritis, in the absence of an identified alternate etiology, should be treated according to the guidelines in Table 14.

Table 14: Management Guidelines for Renal Events

Event	NCI CTCAE Grade	Recommended Medical Management
Renal event	Grade 1	• Continue atezolizumab. • Monitor kidney function, including creatinine, closely until values resolve to within normal limits or to baseline values.
	Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to renal specialist. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s).
	Grade 3 or higher	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Refer patient to renal specialist and consider renal biopsy. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; NCI = National Cancer Institute

[a] Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤ 10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the Investigator and the Medical Monitor(s).

[b] If corticosteroids have been initiated, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed.

4.4.2.13. Immune-mediated Myositis

Immune-mediated myositis has been associated with the administration of atezolizumab. Myositis or inflammatory myopathies are a group of disorders sharing the common feature of inflammatory muscle injury; dermatomyositis and polymyositis are among the most common disorders. Initial diagnosis is based on clinical (muscle weakness, muscle pain, skin rash in dermatomyositis), biochemical (serum creatine kinase increase), and imaging (electromyography/MRI) features, and is confirmed with a muscle biopsy.

Patients with signs and symptoms of myositis, in the absence of an identified alternate etiology, should be treated according to the guidelines in Table 15.

Table 15: Management Guidelines for Immune-mediated Myositis

Event	NCI CTCAE Grade	Recommended Medical Management
Immune-mediated myositis	Grade 1	• Continue atezolizumab. • Refer patient to rheumatologist or neurologist. • Initiate treatment as per institutional guidelines.
	Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset [a] and contact Medical Monitor(s). • Refer patient to rheumatologist or neurologist. • Initiate treatment as per institutional guidelines. • Consider treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If corticosteroids are initiated and event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s).
	Grade 3	• Withhold atezolizumab for up to 12 weeks after event onset [a] and contact Medical Monitor(s). • Refer patient to rheumatologist or neurologist. • Initiate treatment as per institutional guidelines. Respiratory support may be required in more severe cases. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone, or higher-dose bolus if patient is severely compromised (e.g., cardiac or respiratory symptoms, dysphagia, or weakness that severely limits mobility); convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor(s). • For recurrent events, treat as a Grade 4 event.

Event	NCI CTCAE Grade	Recommended Medical Management
Immune-mediated myositis	Grade 4	• Permanently discontinue atezolizumab and contact Medical Monitor(s). • Refer patient to rheumatologist or neurologist. • Initiate treatment as per institutional guidelines. Respiratory support may be required in more severe cases. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone, or higher-dose bolus if patient is severely compromised (e.g., cardiac or respiratory symptoms, dysphagia, or weakness that severely limits mobility); convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; IV = intravenous; NCI = National Cancer Institute

[a] Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤ 10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the Investigator and the Medical Monitor(s).

[b] If corticosteroids have been initiated, they must be tapered over ≥ 1 month to the equivalent of ≤ 10 mg/day oral prednisone before atezolizumab can be resumed.

4.4.2.14. Systemic Immune Activation

Systemic immune activation is a rare condition characterized by an excessive immune response. Given the mechanism of action of atezolizumab, systemic immune activation is considered a potential risk for atezolizumab.

Recommendations regarding early identification and management of systemic immune activation are provided below. In the event of suspected systemic immune activation, atezolizumab should be withheld and clinical specialists (e.g., rheumatology, clinical immunology, or solid organ or hematopoietic stem cell transplant specialists) and the Medical Monitor(s) should be informed.

Early disease recognition is critical, and systemic immune activation should be suspected if, in the absence of an alternative etiology, the patient meets two or more of the following criteria:

- Hypotension that is refractory to aggressive IV fluid challenge (vasopressor support may be required).
- Respiratory distress that requires aggressive supportive care (supplemental oxygen and intubation may be required).
- Fever > 38.5°C
- Acute renal or hepatic failure
- Bleeding from coagulopathy
- Any of the following unexplained laboratory abnormalities (change from baseline): cytopenias (in two or more lineages), significant transaminitis, or coagulopathy

For patients with suspected systemic immune activation, an initial evaluation should include the following:

- Complete blood count with peripheral smear
- Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and D-dimer
- Ferritin
- Soluble IL-2 receptor (soluble CD25)
- Triglycerides
- Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and direct bilirubin
- Lactate dehydrogenase (LDH)
- Complete neurologic and abdominal examination (assess for hepatosplenomegaly)

Laboratory tests with normal results should be repeated frequently in patients for whom a high clinical suspicion of systemic immune activation exists.

If neurologic abnormalities are present, consider cerebrospinal fluid analysis and/or an MRI of the brain.

If cytopenias are present (Grade ≥ 2 in two or more lineages) or ferritin is ≥ 3000 ng/mL, the following evaluations should also be performed:

- Bone marrow biopsy and aspirate (assess for evidence of hemophagocytosis)
- Adenovirus, cytomegalovirus, Epstein Barr virus, herpes simplex virus, and human herpes virus 6, 7, and 8 evaluation (for reactivated or active disease)

Diagnostic criteria and recommended management for systemic immune activation are provided in Table 16. The diagnostic criteria apply only when alternative etiologies have been excluded.

An AE of systemic immune activation should be reported on the Adverse Event electronic case report form (eCRF) if the event meets the criteria for “consistent with systemic immune activation” or “probable systemic immune activation” as outlined in Table 16.

Table 16: Systemic Immune Activation Diagnosis and Management

Systemic Immune Activation Diagnostic Criteria (Applicable Only When Alternative Etiologies are Excluded)	
Major Criteria	**Minor Criteria**
• Fever ≥ 38.5°C on more than one occasion • Ferritin ≥ 3000 ng/mL • Cytopenias (Grade ≥ 2 in two or more lineages) • Age-adjusted soluble interleukin-2 receptor elevated by ≥ 2 standard deviations • Severe (Grade ≥ 3) or progressive dysfunction in two or more organs • Decreased fibrinogen	• Splenomegaly • Hemophagocytosis in bone marrow, spleen, or lymph nodes • Elevated gamma-glutamyl transpeptidase or liver function tests (aspartate aminotransferase, alanine aminotranferase, or direct bilirubin) • Elevated triglycerides • Elevated lactate dehydrogenase • Decreased natural killer cell activity

Diagnosis and Management of Systemic Immune Activation		
Number of Criteria	**Diagnosis**	**Action to Be Taken**
≥ 4 major criteria	Consistent with systemic immune activation	• Permanently discontinue atezolizumab. • Consider treatment with an immunosuppressive agent (i.e., cytokine inhibitors) and intravenous corticosteroids (i.e., methylprednisolone 1 g once daily or equivalent, or dexamethasone ≥ 10 mg/m^2 once daily if neurologic abnormalities are present). • Inform the Medical Monitor(s). • Consider HLH-2004 protocol (Henter et al 2007) if there is no clinical improvement.

3 major criteria OR 2 major plus ≥ 3 minor criteria	Probable systemic immune activation	• Depending on clinical severity, follow guidelines for "Consistent with systemic immune activation" or "Possible systemic immune activation" diagnosis. • Clinical specialists may be contacted for recommendations and the Medical Monitor(s) should be informed.
2 major plus ≤ 2 minor criteria OR 1 major plus ≥ 4 minor criteria	Possible systemic immune activation	• Withhold atezolizumab. • Consider treatment with intravenous corticosteroids. • Clinical specialists may be contacted for additional recommendations and the Medical Monitor(s) should be informed. • Follow guidelines for "Consistent with systemic immune activation" diagnosis if there is no clinical improvement or if clinical worsening occurs. • If clinical improvement occurs, atezolizumab may be resumed following a benefit-risk assessment by the Medical Monitor(s).

Notes: Criteria are adapted from a Delphi Survey of 26 experts who provided helpful criteria in the positive diagnosis of hemophagocytic syndrome in adult patients (Hejblum et al 2014).

Grades are based on National Cancer Institute Common Terminology Criteria for Adverse Events.

These recommendations do not replace clinical judgment and are intended as suggested guidance.

4.5. Stopping Rules

The Sponsor may stop this study at any time. Investigators will be notified by the Sponsor or its designee if the study is stopped. The occurrence of the following events will require that further enrollment in the study be stopped:

- Grade 3 or higher systemic infection assessed as definitely related to SYNB1891.
- Death assessed as at least possibly related to study treatment.
- The Investigator, Medical Monitor(s), and/or Sponsor determines that an event or current data warrant stopping the study.

The SRC will review the data concerning these event(s), along with all other available data. Based on the results of their investigation, the SRC will determine appropriate follow-up and decide whether the study should be continued, amended or stopped.

If the study is stopped, the events will be investigated, and the study will be closed to further enrollment. Patients already participating in the study at the time the study is stopped will not receive further study treatment and the evaluations for the End-of-treatment (EOT)/Early Termination visit will be performed.

4.6. Duration of Study Participation

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; see Section 4.2.1), and safety follow-up period (30 ± 5 days after the last dose).

5. PATIENT POPULATION

5.1. Number of Patients

Approximately 70 patients are anticipated for enrollment, 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. Additional cohorts may be required in either arm to determine the MTD/RP2D.

5.2. Selection of Patients

5.2.1. Eligibility Criteria

This study will comprise patients with advanced/metastatic solid tumors or lymphoma. Patients will be eligible for enrollment regardless of gender or race/ethnicity.

5.2.1.1. Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible:

1. Able and willing to voluntarily complete the informed consent process (patient or patient's representative).
2. Adults aged ≥ 18 years (on the day of signing informed consent) with histologically- or cytologically-confirmed stage III or IV advanced/metastatic solid tumor or lymphoma for which no therapeutic options are available to extend survival or for which the patient is not a candidate for standard-of-care therapy.
3. Eastern Cooperative Oncology Group performance status ≤ 1.
4. Life expectancy ≥ 3 months.
5. ≥ 1 injectable, measurable (≥ 10 mm in diameter, or ≥ 15 mm for nodal lesions), eligible lesion as defined by RECIST 1.1 (Eisenhauer et al 2009), iRECIST (Seymour et al 2017), and/or LYRIC (Cheson et al 2016) and as assessed by the Investigator. Eligible lesions must not be located in the thoracic cavity, spleen, pancreas, gastrointestinal tract (liver injection allowed), or cranium and must be amenable to percutaneous injection and away from major blood vessels or neurological structures.
6. Able to provide biopsies for biomarker analysis from injected and (if available) noninjected lesions at baseline and other time points during the study.
7. Oxygen saturation > 90% without the use of supplemental oxygen.
8. Adequate cardiac function, defined as follows:
    a. Left ventricular ejection fraction (LVEF) > 50% by multi-gated acquisition (MUGA) scan or echocardiogram (ECHO) 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. Symptoms relating to left ventricular dysfunction, cardiac arrhythmia, or cardiac ischemia must all be Grade < 1 per NCI CTCAE version 5.0.
    b. QTc interval corrected for heart rate using Fridericia's formula (QTcF) < 480 msec at screening.

9. Laboratory values within the following ranges:
   - Absolute neutrophil count ≥ 1500/μL
   - Lymphocyte count ≥ 500/μL
   - Platelets ≥ 100,000/μL without transfusion
   - Hemoglobin ≥ 9.0 g/dL (patients may be transfused to meet this criterion)
   - Estimated glomerular filtration rate (eGFR) > 50 mL/min/1.73 m^2 per the Cockcroft-Gault formula
   - Total bilirubin ≤ 1.5 × the upper limit of normal (ULN) OR direct bilirubin ≤ ULN for participants with total bilirubin levels > 1.5 × ULN or ≤ 3 × ULN for patients with Gilbert's syndrome
   - AST, ALT, and alkaline phosphatase ≤ 2.5 × ULN (AST and ALT ≤ 5 × ULN for participants with liver metastases and alkaline phosphatase ≤ 5 × ULN for participants with liver or bone metastases)
   - International normalized ratio (INR) or PT or aPTT ≤ 1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants
10. Agree to use an acceptable method of contraception after informed consent, throughout the study, and for 5 months after the last dose of SYNB1891 or atezolizumab.

Additional inclusion criteria that apply only to Arm 2 study participants are as follows:

11. TSH must be within the normal reference range or the patient must be receiving stable thyroid replacement therapy.
12. Patients must have received treatment with an anti-PD-1/L1 monoclonal antibody (mAb) administered either as monotherapy, or in combination with other CPIs or other therapies, if indicated (if CPI therapy is not indicated as a part of standard-of-care therapy, patients may be CPI naïve).

Additional inclusion criteria that apply only to the Arm 2 <u>expansion</u> study participants are as follows:

13. Patients must have progressed on treatment with an anti-PD-1/L1 mAb administered either as monotherapy, or in combination with other CPIs or other therapies, if indicated (if CPI therapy is not indicated as a part of standard-of-care therapy, patients may be CPI naïve).

    PD-1/L1 treatment progression is defined by meeting all of the following criteria:

    a. Has received at least 2 doses of an approved anti-PD-1/L1 mAb.
    b. Has demonstrated disease progression after PD-1/L1 as defined by RECIST 1.1, iRECIST, and/or LYRIC. The initial evidence of disease progression is to be confirmed by a second assessment no less than 4 weeks from the date of the first documented disease progression, in the absence of rapid clinical progression as determined by the Investigator. Once disease progression is confirmed, the initial date

of disease progression documentation will be considered the date of disease progression.

c. Progressive disease has been documented within 12 weeks from the last dose of anti-PD-1/L1 mAb.

5.2.1.2. Exclusion Criteria

Patients meeting any of the following criteria are not eligible for study enrollment:

1. Chemotherapy, radiation, or biological cancer therapy within 14 days prior to the first dose of study treatment, or failure of any AEs to recover to Baseline or NCI CTCAE version 5.0 Grade 1, except for any grade of alopecia caused by cancer therapeutics administered > 28 days earlier.
2. Systemic immunostimulatory agents (including, but not limited to, IFN and IL-2) within 28 days or 5 drug elimination half-lives (whichever is longer) prior to initiation of study treatment. Checkpoint inhibitors are not considered systemic immunostimulatory agents for this criterion and are subject to the washout period listed in exclusion criterion #1.
3. Allogeneic hematopoietic stem cell transplantation that requires current use of immunosuppressors.
4. Receipt of a live vaccine within 90 days prior to the first dose of study treatment or anticipation of a need for such a vaccine during treatment.
5. Receipt of antibiotics within 7 days prior to the first dose of study treatment.
6. Participation in a study of an investigational agent and receipt of study therapy or use of an investigational device within 28 days prior to the first dose of study treatment.
7. Diagnosed immunodeficiency or current use of chronic systemic steroid therapy in excess of replacement doses (prednisone ≤ 10 mg/day or equivalent is acceptable) or any other form of immunosuppressive medication within 7 days prior to the first dose of study treatment.
8. For injection and biopsy of visceral (deep) lesions: patients must not be on long-acting antiplatelet agents such as aspirin or clopidogrel or on therapeutic doses of anticoagulants, with the exception of patients receiving a preventative dose of low molecular weight heparin. In patients receiving preventative low molecular weight heparin, treatment must be stopped 24 hours before the intratumoral injection and resumed again 24 hours after the injection (Marabelle et al 2018).
9. Previous or concurrent malignancy, with the exception of:
    a. Adequately treated basal or squamous cell carcinoma, in situ carcinoma of the cervix, or ductal carcinoma in situ of the breast;
    b. Localized prostate cancer definitively treated with surgery or radiation or stable on hormone therapy;
    c. Other cancer from which the patient has been disease free for at least 2 years.
10. Clinically active central nervous system (CNS) metastases and/or carcinomatous meningitis (treated brain metastases are permitted if radiologically stable for ≥ 28 days prior to the first dose of study treatment).

11. Allergy to antibiotics that precludes treatment for infection with *E. coli* Nissle 1917 (see Appendix 4 for common antibiotics to which *E. coli* Nissle is sensitive).
12. Hepatitis B or C infection(s) unless screening tests indicate a negative viral load, and/or human immunodeficiency virus (HIV) infection unless screening tests indicate a negative or below the limit of quantitation viral load.
13. Known active tuberculosis.
14. Grade 3 or higher infection according to NCI CTCAE within 28 days prior to initiation of study treatment, including, but not limited to, hospitalization for complications of infection, bacteremia, or severe pneumonia.
15. Failure to fully recover from the effects of major surgery. Surgeries that required general anesthesia must be completed > 14 days before the first dose of study drug. Surgery requiring regional/epidural anesthesia must be completed > 72 hours before the first dose of study treatment and participants should be recovered.
16. Heart failure of New York Heart Association Class 3 or greater, restrictive cardiomyopathy, or unstable angina or recent myocardial infarction within 3 months prior to the first dose of study treatment.
17. Any other medical condition that might confound the results of the study, interfere with the participant's participation, or is not in the best interest of the participant, in the opinion of the treating Investigator.
18. Pregnant or breastfeeding or anticipated conception or fathering of children within the projected duration of the study and for 5 months after the last dose of SYNB1891 or atezolizumab.

Additional exclusion criteria that apply only to Arm 2 study participants are as follows:

19. History of immune-mediated AEs on prior PD-1/PD-L1 therapies requiring discontinuation or immunosuppressor therapy, excluding endocrinopathies.
20. Active or history of underlying autoimmune disease or immune deficiency, including, but not limited to, myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, antiphospholipid antibody syndrome, Wegener granulomatosis, Sjögren syndrome, Guillain-Barré syndrome, or multiple sclerosis, with the following exceptions:
    a. Patients with a history of autoimmune-related hypothyroidism who are on thyroid replacement hormone are eligible for the study.
    b. Patients with controlled Type 1 diabetes mellitus who are on an insulin regimen are eligible for the study.
    c. Patients with eczema, psoriasis, lichen simplex chronicus, or vitiligo with dermatologic manifestations only (e.g., patients with psoriatic arthritis are excluded) are eligible for the study provided all of following conditions are met:
        - Rash must cover < 10% of body surface area;
        - Disease is well controlled at baseline and requires only low-potency topical corticosteroids;

- No occurrence of acute exacerbations of the underlying condition requiring psoralen plus ultraviolet A radiation, methotrexate, retinoids, biologic agents, oral calcineurin inhibitors, or high potency or oral corticosteroids within the previous 12 months.

21. History of a Grade ≥ 3 allergic anaphylactic reaction following treatment with a PD-1 mAb or to chimeric, human, or humanized antibodies or fusion proteins.
22. History of idiopathic pulmonary fibrosis, organizing pneumonia (e.g., bronchiolitis obliterans), drug-induced pneumonitis requiring treatment, or idiopathic pneumonitis, or evidence of active pneumonitis.
23. Known hypersensitivity to Chinese hamster ovary cell products or any component of the atezolizumab formulation.

6. INVESTIGATIONAL MEDICINAL PRODUCT AND CONCOMITANT MEDICATIONS

6.1. Investigational Product Supply and Administration

Within this protocol, "study treatment" refers to SYNB1891 and/or atezolizumab.

6.1.1. SYNB1891

Detailed instructions for the storage, handling, and administration of SYNB1891 will be provided in the Pharmacy Manual and may override instructions provided in the following protocol sections.

6.1.1.1. Manufacturing and Formulation

SYNB1891 investigational drug product is formulated in buffer composed of 15% glycerol, 5% trehalose, 10 mM Tris at pH 7.5.

6.1.1.2. Packaging and Labeling

SYNB1891 is packaged aseptically into AT-closed vials at a volume of 0.6 mL and will be labeled for clinical study use.

6.1.1.3. Shipping, Storage, and Handling

Labeled, packaged SYNB1891 will be shipped from Synlogic's manufacturing site to a central drug vendor. SYNB1891 must be stored at ≤ -65°C in a secured freezer with controlled access.

6.1.1.4. Dispensing and Administration

The manufactured SYNB1891 drug product has a concentration of 1×10^{11} live cells/mL. To obtain appropriate doses, vial(s) of concentrated drug product will be thawed and appropriately diluted to a volume of 5 mL in room temperature 5% dextrose in water for injection and held at room temperature while handling prior to administration. The time between the thaw of vials containing SYNB1891 and injection should not exceed 2 hours. If the 2-hour stability window is exceeded, discard and prepare another dose.

The preparation of SYNB1891 doses must be performed in a biosafety cabinet while wearing sterile sleeves, sterile gloves, and a facemask to minimize contamination.

Once the dilutions have been prepared, withdraw 0.3 mL of the prepared cell concentration, change the needle to the appropriate length for the tumor to be treated prior to injection, and follow local protocol to obtain the final dose volume of 0.1 mL.

6.1.2. Atezolizumab

Atezolizumab will be 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) will be prepared in accordance with the current Investigator's Brochure and administered as an IV infusion at a dose of 1200 mg Q3W.

6.2. Accountability and Dosing Compliance

All doses of SYNB1891 and atezolizumab will be administered in the clinic by trained study personnel. Dosing details (e.g., lot/batch number, date/time of administration) will be documented in the eCRF.

Unless other arrangements are agreed to in writing, all unused clinical trial material should be returned to Synlogic at the completion of the study. Interim returns are permissible after drug accountability has been performed by the monitor and as agreed upon by the Sponsor.

6.3. Concomitant Medications

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.

No restrictions on concomitant medications will be implemented, other than those mentioned in the eligibility criteria (see Section 5.2.1) or the toxicity management guidelines (see Section 4.4). Concomitant medications can be administered at the Investigator's discretion to conform to standard practice during the dosing period; however, Investigators should use caution when prescribing concomitant medications, as SYNB1891 has not been previously investigated in the clinical setting. Investigators should contact the Medical Monitor(s) when they are unsure whether a drug should be prescribed to a patient. If there is a clinical indication for any medication or vaccination specifically prohibited during the study, discontinuation from study treatment may be required at the discretion of the Medical Monitor(s).

7. STUDY PROCEDURES AND ASSESSMENTS

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.

The various assessments that will be conducted during this study are summarized in Table 19 (Schedule of Events for Arm 1) and Table 20 (Schedule of Events for Arm 2).

Detailed instructions regarding sample collection, shipping, and handling, as well as all laboratory procedures, will be provided to the study site in separate study manuals. Site-specific handling instructions for hematology and clinical chemistry samples should be followed.

7.1. Screening

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. If a particular assessment is repeated, the results obtained closest to the first dose of study treatment should be used to assess eligibility.

During screening, a unique identification number will be assigned to each patient who signs informed consent for the study. If a patient meets all the inclusion criteria and none of the exclusion criteria and is selected to participate in the study, s/he will be enrolled and only be identified by the unique identification number.

The Investigator or designee is responsible for verifying that the patient is eligible before requesting an identification number. At the site, the Investigator will maintain a log for all screened patients (including patients who failed screening after signing informed consent) and all enrolled patients. Patients may be re-screened. Eligibility assessments from the initial screening may only be used for re-screening if performed within the screening period as defined in the protocol or within 14 days following the screening period only after consultation with the medical monitor.

Patients must be dosed within 5 calendar days of the registration/enrollment date (i.e., the date that a patient is assigned to a given dose cohort).

7.2. Safety Assessments

7.2.1. Medical History

Past and present medical history, including prior cancer diagnoses, will be recorded. Any ongoing condition and signs and symptoms observed prior to signing of the informed consent should be recorded as medical history.

7.2.2. Adverse Events

Adverse events will be assessed continuously by direct observation and patient interviews. The severity of AEs will be evaluated using the NCI CTCAE, version 5.0 (US DHHS 2017). All AEs occurring from the time a patient signs informed consent through the Safety Follow-up visit (30 ± 5 days after the last dose of study treatment) will be recorded, regardless of causality. After the Safety Follow-up visit, only SAEs that are considered related to study treatment will be recorded, if clinically indicated.

Postdose monitoring for AEs will be performed as described in Section 4.2.2, including telephone contact on Cycle 1 Day 3 to monitor for signs of cytokine release syndrome (see Section 4.4). If these symptoms are present, patients will be instructed on the appropriate next steps.

7.2.2.1. Monitoring for Signs and Symptoms of Infection

If clinical presentation warrants, including but not limited to cases in which patients develop persistent (> 24 hours) fever or a change in vital signs suggestive of clinical infection resulting from a study treatment or procedure, then blood and urine cultures (performed and analyzed locally) will be performed and weight will be recorded.

If a patient is treated for systemic infection such that blood culture tubes are drawn locally, an additional blood culture tube should be drawn for analysis for the presence of SYNB1891, if possible. For this purpose, patients will be provided with an identification card containing study contact information (see Central Laboratory manual for collection, shipping, and other processes).

7.2.3. Vital Signs

Vital signs (systolic blood pressure, diastolic blood pressure, body temperature, pulse, and pulse oximetry) will be 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 (see Table 19 and Table 20). A ± 15-minute window is permitted at all time points.

7.2.4. Physical Examination

Complete physical examinations will be performed by trained medical personnel at screening and Baseline, with symptom-directed physical examinations performed at subsequent time points (see Table 19 and Table 20). Height will be collected at screening only, and weight will be collected at the time points indicated in Table 19 and Table 20. Any abnormal findings will be recorded as AEs.

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.

7.2.5. Clinical Laboratory Measurements

Clinical laboratory tests will be performed (predose unless otherwise specified) at the time points indicated in Table 19 and Table 20. Baseline laboratory measurements do not need to be repeated if screening measurements were performed within 3 days prior to Cycle 1 Day 1.

Screening for HIV and hepatitis B and C will also be performed prior to study dosing. Serum pregnancy testing will be performed at screening, with urine pregnancy testing performed at subsequent time points (see Table 19 and Table 20).

Screening results will be assessed by the Investigator for inclusion of patients in the study. Additionally, unscheduled clinical laboratory tests can be obtained at any time during the study

at the Investigator's discretion. The diagnosis corresponding to any clinically significant abnormality, or abnormality requiring treatment/intervention, must be recorded as an AE.

7.2.5.1. Local Laboratory Measurements

Table 17 outlines the clinical laboratory measurements that will be reviewed at the local laboratory.

On dosing days, prior to receiving study treatment, patients must meet the laboratory value criteria outlined in Section 5.2.1.1, with the exception that platelets may be ≥ 75,000/µL without transfusion and hemoglobin may be ≥ 8.5 g/dL (patients may be transfused to meet this criterion).

Table 17: Local Laboratory Parameters

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

7.2.5.2. Central Laboratory Measurements

Table 18 outlines the clinical laboratory measurements that will be reviewed at the central laboratory as specified in the Central Laboratory Manual.

Table 18: Central Laboratory Parameters

Panel	Parameters
Cytokines	TNFα, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL1β, IL1RA, IL2Rα, and IFNγ
qPCR	• Tumor tissue quantitative polymerase chain reaction (qPCR) from fine needle aspirate and whole blood • qPCR from blood cultures
Other	Blood samples collected for exploratory analyses in preservation tubes

7.2.6. Electrocardiograms

Semi-supine single 12-lead ECGs will be performed at screening and reviewed locally.

7.2.7. 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.

7.3. Efficacy Assessments

Disease response status will be assessed in the SYNB1891-injected lesion(s), and an overall analysis of potential responses in up to 5 noninjected target lesions will be performed using appropriate imaging at screening and every 2 cycles on study (i.e., at the end of every even-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). Every effort should be made to assess each lesion that is measured at screening using the same imaging method(s) throughout the study to enable a consistent comparison. Injected lesions that are reduced in size on study to the extent that they are no longer able to sustain injections will continue to be monitored for response.

Tumor response will be evaluated by the local Investigator using appropriate imaging in accordance with RECIST 1.1 (Eisenhauer et al 2009 and Appendix 1), iRECIST (Seymour et al 2017 and Appendix 2), and/or LYRIC (Cheson et al 2016 and Appendix 3).

7.4. Pharmacodynamic Assessments

Blood samples for qPCR will be collected during the first 24-hour period of Cycle 1 and will be analyzed to assess for the presence of circulating SYNB1891.

An FNA of the eligible lesion will be performed predose on Cycle 1 Day 1 (within 7 days prior to Cycle 1 Day 1) and predose on Cycle 1 Day 8 and analyzed for bacterial kinetics by qPCR.

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 (within 3 days prior). For patients who withdraw from the study prior to Cycle 2 Day 1, the second biopsy should be performed at the EOT visit (see Section 7.5.1). While not required for study entry, second biopsiable lesions, if present, will undergo a mandatory biopsy at the same time points.

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. Refer to the study manuals for details on the procedures for collecting, shipping, and handling biopsy material.

7.5. Follow-up Assessments

7.5.1. End-of-Treatment Visit

Within 14 days after the last dose of study treatment (or premature treatment discontinuation), an EOT visit will be performed and every effort should be made to perform the procedures specified in Table 19 and Table 20.

7.5.2. Safety Follow-up Visit

All patients will attend a Safety Follow-up visit 30 ± 5 days after the last dose of study treatment.

7.6. Sample Retention for Exploratory Analyses

De-identified blood samples may be stored for future analyses related to cancer and immune responses for up to 5 years from the time the sample is collected. Any unused samples will be destroyed.

Table 19: 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]		●	
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			●				●	●	●	●		
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. **NOTE**: A ± 15-minute window is permitted for each timepoint.

[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 (or up to 3 days prior to) 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.

Table 20: Schedule of Events (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]		●	
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]					
Biopsy of eligible lesion	7.4		● [7]							● [8]		● [9]	

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 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. **NOTE**: A ± 15-minute window is permitted for each timepoint.

[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 (or up to 3 days prior to) 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.

8. STUDY DISCONTINUATION

8.1. Sponsor Discontinuation Criteria

This study may be discontinued at any time due to safety concerns (including, but not limited to, the stopping rules described in Section 4.5), failure to meet expected enrollment goals, administrative reasons, or at the discretion of the Sponsor. Should the study be terminated prematurely, the Sponsor will provide written notification to the Investigator and regulatory authorities and will specify the reason(s) for early termination. The Investigator must inform the IRB promptly and provide the reason(s) for the termination.

8.2. Study Discontinuation for Individual Patients

Determination of whether a patient should discontinue further study treatment administration and withdraw from the study will depend on whether one of the following qualifying events (except for withdrawal of consent) occurs prior to or after the patient receives SYNB1891:

- The patient withdraws consent.
- The Investigator or Sponsor notes a significant noncompliance with protocol procedures.
- The Investigator determines that the patient must discontinue further study dosing for medical reasons.
- Discontinuation of treatment with SYNB1891 may be considered for participants in whom all eligible injected lesion have undergone complete regression and are no longer injectable (at the discretion of the Investigator). In Arm 1, patients will continue in follow-up and in Arm 2, patients may continue on atezolizumab.
- Discontinuation of treatment with atezolizumab may be considered for participants who have attained a confirmed CR and have been treated for at least 8 cycles (at least 24 weeks), receiving at least 2 doses of atezolizumab beyond the date when the initial CR was declared. It is anticipated that these participants will have discontinued SYNB1891.

In patients who meet the criteria for permanent discontinuation due to intolerable toxicity, resumption of study treatment may be considered if the patient is deriving benefit and has fully recovered from the immune-related event. Patients can be re-challenged with study treatment only after approval has been documented by both the Investigator (or an appropriate delegate) and the Sponsor.

Patients may withdraw their consent at any time for any reason without prejudice to their future medical care by the physician or at the institution. If a patient withdraws consent, the date and stated reason for consent withdrawal should be documented. Patient data collected up to the date of consent withdrawal will be included in the analyses.

Patients who withdraw from the study prior to receiving the first dose of study treatment are not required to complete any further visits. Patients who withdraw from the study after receiving the first dose of study treatment must complete the EOT and Safety Follow-up visits, unless consent for follow-up is withdrawn. Patients who discontinue study treatment and withdraw from the

study will not be allowed to enroll again at a later date. If a patient who withdraws from the study has an ongoing SAE, every effort will be made to follow the SAE until the event has resolved, stabilized, or returned to baseline status. The Sponsor must be notified of all study withdrawals within 24 hours.

8.2.1. Patient Replacement

Patients who terminate participation in the study for any reason other than DLTs prior to receiving the second injection of SYNB1891 in Cycle 1 will be considered ineligible for the safety assessment required for dose-escalation decisions and may be replaced.

9. ADVERSE EVENTS

9.1. Adverse Event

An AE is any untoward medical occurrence, including the exacerbation of a pre-existing condition, in a patient administered a pharmaceutical product, regardless of causality.

9.1.1. Assessment of Severity

The severity rating of an AE refers to its intensity. The severity of each AE will be categorized using the NCI CTCAE, version 5.0 (US DHHS 2017). For any term that is not specifically listed in the CTCAE scale, intensity should be assigned a grade of 1 through 5 using the following CTCAE guidelines:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living

Grade 4: Life-threatening consequences; urgent intervention indicated

Grade 5: Death related to AE

9.1.2. Assessment of Causality

Medical judgment should be used to determine the cause of the AE, considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to study treatment (with separate causality assessments for SYNB1891 and atezolizumab), and de-challenge or re-challenge.

Yes (possibly, probably, or definitely related): there is a reasonable possibility that study treatment caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of study treatment.
- The event could not be reasonably attributed to the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
- The event follows a known pattern of response to study treatment.
- The event disappears or decreases on cessation or reduction in dose. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study dosing despite other clear indications of relatedness).

No (unlikely, probably not related, or definitely not related): there is no reasonable possibility that study treatment caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of study treatment.
- The event could be reasonably attributed to the known characteristics of the patient's clinical state, concurrent illness, environmental or toxic factors, or other modes of therapy administered to the patient.
- The event does not follow a known pattern of response to study treatment.
- The event does not disappear or decrease on cessation or reduction in dose(ing), and it does not reappear or worsen when dosing is resumed.

9.2. Serious Adverse Event

An SAE is any untoward medical occurrence that meets any of the following criteria:

- Results in death.
- Is immediately life-threatening (refers to an event in which the patient is 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).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Based on appropriate medical judgment, represents an important medical event that may jeopardize the patient or may require intervention to prevent one of the other outcomes described above.

9.2.1. Clarification of Serious Adverse Event Definition

- Progression of malignancy should be documented through appropriate methodology (e.g., as per iRECIST) and designated as progression of disease in the disease assessment eCRF, but should not be reported as an AE/SAE unless a causal relationship to study treatment is suspected.
- Death is an outcome of an SAE and not an SAE in itself. When death is an outcome, the event(s) resulting in death should be reported (e.g., "pulmonary embolism" with a fatal outcome). The appropriate diagnosis or term should be recorded and assigned severity Grade 5.
- In instances of death due ultimately to the underlying disease, the cause of death should be indicated as the specific event or condition resulting in death to the extent possible. If no appropriate term with a Grade 5 severity in the CTCAE can be identified, then a term should be selected from the CTCAE category "Death".

- "Life-threatening" means that the patient was at immediate risk of death from the event as it occurred. This does not include an event that might have led to death if it had occurred with greater severity. Grade 4 events (e.g., thrombocytopenia) are not always serious unless they have life-threatening consequences or result in hospitalization.
- Preplanned or elective hospitalizations including social and/or convenience situations (e.g., respite care) are excluded from SAE reporting. In addition, "admissions" under 23-hour observation or emergency room visits are excluded from SAE reporting; however, such events should still be reported on the appropriate eCRF page.
- Overdose of either study treatment or concomitant medication without any overdose signs or symptoms unless the event meets SAE criteria (e.g., hospitalization) are excluded from SAE reporting; however, such events should still be reported on the appropriate eCRF page.
- SAEs that occur within the safety follow-up period but are related to subsequent therapies are excluded from SAE reporting:
  - Patients who have completed study treatment administration or who terminate from the study and then undergo subsequent therapies during the safety follow-up period and experience an SAE specifically related to the administration of the subsequent therapy will not have those events reported as SAEs. This exclusion will include "elective" hospitalizations necessary for the administration of such therapies.

9.2.2. Serious, Unexpected, Suspected Adverse Reactions

In accordance with regulatory requirements, the Sponsor or designee will notify regulatory authorities as required per local law (i.e., 21 CFR 312.32 in the United States). The Sponsor or designee will also notify the Investigators, who will in turn notify their IRB as necessary, of any AE associated with study treatment administration or study procedures that is a serious, unexpected, suspected adverse reaction (SUSAR) or any finding from tests in laboratory animals that suggests a significant risk for human patients, including reports of mutagenicity, teratogenicity, or carcinogenicity. An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been previously observed.

9.3. Adverse Events of Special Interest

Adverse events of special interest (AESIs) (serious or nonserious) are a subset of events to monitor scientific and medical concern, for which ongoing monitoring by the Investigator to the Sponsor is required. An AESI may warrant further investigation and reporting in accordance with local regulatory requirements.

Adverse events of special interest for SYNB1891 are as follows:

- Cytokine release syndrome (may include associated AEs, e.g., fever, tachypnea, headache, tachycardia, hypotension, rash, hypoxia) graded in accordance with NCI CTCAE, version 5.0
- Systemic infection (Grade 3 or higher in accordance with NCI CTCAE, version 5.0)
- Local injection site reactions (Grade 3 or higher; may include associated AEs, e.g., abscess, infection, ulceration or necrosis, or severe tissue damage) graded in accordance with NCI CTCAE, version 5.0

9.4. Reporting of Adverse Events

All AEs, serious and nonserious, will be fully documented on the appropriate eCRF. For each AE, the Investigator must provide its duration (start and end dates or ongoing), intensity, assessment of causality, and whether specific action or therapy was required.

All AEs occurring from the time a patient signs informed consent through the Safety Follow-up visit must be recorded on the eCRF.

All SAEs, regardless of relationship to study treatment, must be reported to the Sponsor within 24 hours of the Investigator's knowledge. This should be done by faxing the completed SAE Report Form to the Sponsor at the number provided on the SAE Report Form. After the Safety Follow-up visit, only SAEs that are considered related to study treatment will be recorded, if clinically indicated.

Investigators must follow patients with AEs/SAEs until event resolution or stabilization, withdrawal of consent, patient loss to follow-up, or death, whichever occurs first.

9.5. Pregnancy

If a patient becomes pregnant during the study, study treatment administration is to be discontinued immediately.

Pregnancies (both those of female patients and female partners of male patients) must be reported to the Sponsor or designee within 24 hours of the Investigator's knowledge using the designated pregnancy reporting form. Investigators should make every effort to obtain permission to follow and report the outcome of the pregnancy to the Sponsor.

Pregnancies themselves are not considered AEs or SAEs. However, any AEs or SAEs occurring during pregnancy (or the outcome of the pregnancy) are to be reported following AE and SAE reporting guidelines.

9.6. Clinical Laboratory Abnormalities

It is the responsibility of the Investigator to assess the clinical significance of all abnormal laboratory values as defined by the appropriate reference ranges. All abnormal values assessed to be of clinical concern and at least possibly related to study treatment or of uncertain causality should be repeated. Persistent abnormal values and changes of possible clinical concern that remain within the normal range should be followed at the discretion of the Investigator.

An abnormal laboratory value that is not already associated with an AE is to be recorded as an AE if an action on study treatment dosing is made as a result of the abnormality, if intervention for management of the abnormality is required, or at the discretion of the Investigator.

9.7. Review of Safety Data

The Sponsor Medical Monitor in collaboration with the CRO Medical Monitor will be responsible for the ongoing review and evaluation of safety data, including AEs, clinical laboratory data, and any other safety evaluations, for the duration of the study.

The Sponsor will host Investigator teleconferences on a regular basis during the study. In addition, the Safety Review Committee (SRC; consisting of the Sponsor Medical Monitor, the CRO Medical Monitor, all active Investigators, and other study personnel as needed) will convene when all patients have completed the first cycle of study treatment in a given cohort and review all available safety information, including DLTs and all NCI CTCAE Grade 2 or higher toxicity data, as well as any other (e.g., PK/PD) data as indicated. Updated safety data on other ongoing patients, including data from later cycles, will be discussed as well.

10. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

All evaluations and tabulations will be carried out as described in detail in a statistical analysis plan (SAP), which will be finalized and approved prior to database lock.

10.1. Populations for Analysis

The following populations will be defined:

- The safety population includes participants who were enrolled, allocated to treatment, and received at least one dose of investigational product.
- 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 was performed due to disease progression.
- The PK/PD population includes participants who received study drug and had at least one PK or PD assessment.

Additional populations may be defined in the SAP, as required.

10.2. Safety Variables

Patients in the safety population will be included in safety analyses.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), and the severity of AEs and laboratory abnormalities will be graded using the NCI CTCAE, version 5.0 (US DHHS 2017). AEs will be tabulated by system organ class (SOC) and preferred term. Incidence tables of patients with AEs, SAEs, and DLTs will be presented by maximum severity.

Clinical laboratory data, ECGs, and other safety assessments will be presented in by-patient listings and will be presented in tabular format, including absolute values and changes from baseline (if applicable), by dose cohort, cycle, and study day.

10.3. Efficacy Variables

Efficacy analyses will be descriptive in nature. The response of SYNB1891-injected lesion(s) and an overall response of up to 5 noninjected target lesions will be assessed. Exploratory evaluations for proof-of-concept (biopsy-based PD markers) will include change from baseline assessment.

The duration of any response or lack of progression for all evaluated tumors will also be assessed using RECIST 1.1 (Eisenhauer et al 2009 and Appendix 1), iRECIST (Seymour et al 2017 and Appendix 2), and/or LYRIC (Cheson et al 2016 and Appendix 3).

Results will be presented in by-patient listings and will be presented in tabular format, including absolute values and changes from baseline (if applicable), by dose cohort, cycle, and study day.

10.4. Pharmacodynamic Variables

Pharmacodynamic data will be presented in by-patient listings and will be presented in tabular and/or graphical format, including absolute values and changes from baseline, by dose cohort and study day.

10.5. Kinetic Variables

Kinetics of SYNB1618 will be presented in by-patient listings and will be presented in tabular format and figures by dose cohort, cycle, and study day.

10.6. Determination of Sample Size

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.

10.7. Randomization and Blinding

This first-in-human, dose-finding study will be nonrandomized with open-label study treatment.

10.8. Changes in the Conduct of the Study or Planned Analysis

Only the Sponsor may modify the protocol. Amendments to the protocol will be made only after consultation and agreement between the Sponsor and the Investigator. The only exception is when the Investigator considers that a patient's safety is compromised without immediate action. In these circumstances, the Investigator should inform the Sponsor and the full IRB within 1 working day after the emergency occurred. All amendments that have an impact on patient risk or the study objectives or require revision of the informed consent document must receive approval from the IRB prior to implementation.

11. DATA RECORDING, RETENTION AND MONITORING

11.1. Case Report Forms

Data will be collected using source documents through an electronic data capture (EDC) system at the clinical site. The Investigator or designee will record data specified in the protocol using eCRFs. Changes or corrections to eCRFs will be made by the Investigator or an authorized member of the study staff according to the eCRF completion guidelines. It is expected that the site will enter all data within 5 days of a patient visit.

It is the Investigator's responsibility to ensure eCRFs are complete and accurate, regardless of whether this responsibility has been delegated in whole or in part. Following review and approval, the Investigator or designee will electronically sign and date the pages, which certifies that the Investigator has thoroughly reviewed and confirmed all data on the eCRF.

11.2. Data Retention

Data retention practices will follow International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, which note that essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of SYNB1891. However, these documents should be retained for a longer period if required by the applicable legal requirements.

11.3. Data Monitoring

This study will be closely monitored by representatives of the Sponsor or designee throughout its duration. Monitoring will include personal visits with the Investigator and study staff as well as appropriate communications by telephone, fax, mail, email, or use of the EDC system, as applicable. It is the monitor's responsibility to inspect eCRFs at regular intervals throughout the study to verify the completeness, accuracy, and consistency of the data and to confirm adherence to the study protocol and to Good Clinical Practice (GCP) guidelines. The Investigator agrees to cooperate with the monitor to ensure that any problems detected during the course of this study are resolved promptly. The Investigator and site will permit study-related monitoring, audits, IRB review, and regulatory inspection, including direct access to source documents.

It is understood that study monitors and any other personnel authorized by the Sponsor may contact and visit the Investigator and will be permitted to inspect all study records (including eCRFs and other pertinent data) on request, provided that patient confidentiality is maintained and that the inspection is conducted in accordance with local regulations.

Every effort will be made to maintain the anonymity and confidentiality of patients during this study. However, because of the experimental nature of SYNB1891, the Investigator agrees to allow representatives of the Sponsor and authorized representatives of regulatory authorities to inspect the facilities used in the conduct of this study and to inspect, for purposes of verification, the hospital or clinic records of all patients enrolled in the study.

11.4. Quality Control and Quality Assurance

Quality control procedures will be conducted according to the Sponsor and CRO's internal procedures. The study site may be audited by a quality assurance representative of the Sponsor. All necessary data and documents will be made available for inspection.

12. REGULATORY, ETHICAL, AND LEGAL OBLIGATIONS

12.1. Good Clinical Practice

The study will be performed in accordance with the protocol, guidelines for GCP established by the ICH and applicable local regulatory requirements and laws.

12.2. Institutional Review Board Approval

The Investigator must inform and obtain approval from the IRB for the conduct of the study at named sites, the protocol, informed consent documents, and any other written information that will be provided to the patients and any advertisements that will be used. Written approval must be obtained prior to recruitment of patients into the study and shipment of SYNB1891/atezolizumab.

Proposed amendments to the protocol (see Section 10.8) and aforementioned documents must be submitted to the Sponsor for review and approval, then to the IRB. Amendments may be implemented only after a copy of the approval letter from the IRB has been transmitted to the Sponsor.

Per GCP guidelines, the Investigator will be responsible for ensuring that an annual update is provided to the IRB until the study is completed (i.e., finalization of the clinical study report) to facilitate continuing review of the study and that the IRB is informed about the end of the study. Copies of the update, subsequent approvals, and final letter must be sent to the Sponsor.

12.3. Regulatory Authority Approval

The study will be performed in accordance with the requirements of the US FDA and will also meet all of the requirements of ICH GCP guidance. Amendments to the protocol will be submitted to the FDA prior to implementation in accordance with applicable regulations.

12.4. Other Required Approvals

In addition to IRB and regulatory authority approval, all other required approvals (e.g., approval from the local research and development board or scientific committee) will be obtained prior to recruitment of patients into the study and shipment of SYNB1891/atezolizumab.

12.5. Informed Consent

Informed consent is initiated prior to the patient's agreeing to participate in the study and continues throughout the patient's study participation. It is the Investigator's responsibility (or designee) to obtain written informed consent from each patient after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any study procedures are initiated. Each patient should be given a copy of the informed consent document and associated materials. The original copy of the signed and dated informed consent document must be retained at the site and is patient to inspection by representatives of the Sponsor or regulatory authorities. If any amendments occur throughout the course of the study that affect the informed consent form (i.e., when new study procedures or assessments have been added), all active patients should be reconsented using the same process for the initial consent.

12.6. Patient Confidentiality

The Investigator must ensure that the patient's privacy is maintained. On eCRFs and other documents submitted to the Sponsor, patients will be identified by their assigned patient number. Documents that are not submitted to the Sponsor (e.g., signed informed consent documents) should be kept in a confidential file by the Principal Investigator.

The Investigator shall permit authorized representatives of the Sponsor, regulatory authorities, and Ethics Committees to review the portion of the patient's medical record that is directly related to the study. As part of the required content of informed consent documents, the patient must be informed that his/her records will be reviewed in this manner.

12.7. Disclosure of Information

Information concerning the study, patent applications, processes, scientific data, or other pertinent information is confidential and remains the property of the Sponsor. The Principal Investigator may use this information for the purposes of the study only.

It is understood by the Principal Investigator that the Sponsor will use information obtained in this clinical study in connection with the clinical development program, and therefore may disclose it as required to other clinical Investigators and to regulatory authorities. In order to allow the use of the information derived from this clinical study, the Principal Investigator understands that he/she has an obligation to provide complete test results and all data obtained during this study to the Sponsor.

Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor.

12.8. Publication of Study Data

The Sponsor encourages the scientific publication of data from clinical research studies. However, Investigators may not present or publish partial or complete study results individually without participation of the Sponsor. The Principal Investigator and the Sponsor may propose appropriate scientific manuscripts or abstracts from the study data. All proposed publications must be reviewed and commented on by the Sponsor before submission for publication. The detailed procedures for the review of publications are set out in the clinical trial agreement entered into with the Sponsor in connection with this study. These procedures are in place to ensure coordination of study data publication and adequate review of data for publication against the validated study database for accuracy.

Qualification of authorship will follow the requirements of the International Committee of Medical Editors (www.icmje.org). The names of Investigators and Sponsor representatives responsible for designing the study and analyzing the results will be included in the publication(s). This custom can be adjusted upon mutual agreement of the authors and Synlogic. In addition, other than clinical pharmacology studies in healthy volunteers or Phase 1 trials, all clinical trials must be registered with ClinicalTrials.gov.

12.9. Ethical Standards

We are committed to designing, implementing, conducting, analyzing and reporting these trials in compliance with the highest ethical and scientific standards. Protection of patient safety is the overriding concern in the design of clinical trials. In all cases, Synlogic clinical trials will be conducted in compliance with local and/or national regulations and in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

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APPENDIX 1. RESPONSE EVALUATION CRITERIA IN SOLID TUMORS, VERSION 1.1

The following immune-related response criteria, adapted from the RECIST, version 1.1, guidelines (Eisenhauer et al 2009), should be implemented to assess disease status for patients with solid tumors.

Tumor Measurability

At baseline, tumor lesions/lymph nodes will be categorized as measurable or nonmeasurable as described below. All measurable and nonmeasurable lesions should be assessed at screening and at subsequent protocol-specified tumor assessment time points. Additional assessments may be performed as clinically indicated for suspicion of progression.

Definition of Measurable Lesions

Tumor Lesions

Tumor lesions must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size as follows:

- 10 mm by computed tomography (CT) or magnetic resonance imaging (MRI) scan (CT/MRI scan slice thickness/interval ≤ 5 mm)
- 10-mm caliper measurement by clinical examination (lesions that cannot be accurately measured with calipers should be recorded as nonmeasurable)
- 20 mm by chest X-ray

Malignant Lymph Nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in the short axis when assessed by CT scan (CT scan slice thickness recommended to be ≤ 5 mm). At baseline and follow-up, only the short axis will be measured and followed. Additional information on lymph node measurement is provided below (see "Identification of Target and Nontarget Lesions" and "Calculation of Sum of Diameters").

Definition of Nonmeasurable Lesions

Nonmeasurable tumor lesions encompass small lesions (longest diameter < 10 mm or pathological lymph nodes with short axis ≥ 10 mm but ≤ 15 mm) as well as truly nonmeasurable lesions. Lesions considered truly nonmeasurable include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, peritoneal spread, and abdominal mass/abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques.

Special Considerations Regarding Lesion Measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment, as outlined below.

Bone Lesions:

- Technetium-99m bone scans, sodium fluoride positron emission tomography scans, and plain films are not considered adequate imaging techniques for measuring bone lesions.

However, these techniques can be used to confirm the presence or disappearance of bone lesions.

- Lytic bone lesions or mixed lytic-blastic lesions with identifiable soft tissue components that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered measurable lesions if the soft tissue component meets the definition of measurability described above.
- Blastic bone lesions are nonmeasurable.

Cystic Lesions:

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered malignant lesions (neither measurable nor nonmeasurable) since they are, by definition, simple cysts.
- Cystic lesions thought to represent cystic metastases can be considered measurable lesions if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with Prior Local Treatment:

- Tumor lesions situated in a previously irradiated area or in an area subjected to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression in the lesion.

Methods For Assessing Lesions

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during the study. Imaging-based evaluation should always be the preferred option.

Clinical Lesions

Clinical lesions will only be considered measurable when they are superficial and ≥ 10 mm in diameter as assessed using calipers (e.g., skin nodules). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is suggested.

Chest X-Ray

Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT and MRI Scans

CT is the best currently available and reproducible method to measure lesions selected for response assessment. In this guideline, the definition of measurability of lesions on CT scan is based on the assumption that CT slice thickness is ≤ 5 mm. When CT scans have slice thickness of > 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable.

If prior to enrollment it is known that a patient is unable to undergo CT scans with intravenous (IV) contrast because of allergy or renal insufficiency, the decision as to whether a noncontrast CT or MRI (without IV contrast) will be used to evaluate the patient at baseline and during the study should be guided by the tumor type under investigation and the anatomic location of the disease. For patients who develop contraindications to contrast after baseline contrast CT is done, the decision as to whether noncontrast CT or MRI (enhanced or nonenhanced) will be performed should also be based on the tumor type and the anatomic location of the disease, and should be optimized to allow for comparison with the prior studies if possible. Each case should be discussed with the radiologist to determine if substitution of these other approaches is possible and, if not, the patient should be considered not evaluable from that point forward. Care must be taken in measurement of target lesions and interpretation of nontarget disease or new lesions on a different modality, since the same lesion may appear to have a different size using a new modality.

Endoscopy, Laparoscopy, Ultrasound, Tumor Markers, Cytology, Histology

Endoscopy, laparoscopy, ultrasound, tumor markers, cytology, and histology cannot be used for objective tumor evaluation .

Assessment of Tumor Burden

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements.

Identification of Target and Nontarget Lesions

When more than one measurable lesion is present at baseline, all lesions up to a maximum of 5 lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. This means that, for instances in which patients have only one or two organ sites involved, a maximum of two lesions (one site) and four lesions (two sites), respectively, will be recorded. Other lesions (albeit measurable) in those organs will be considered nontarget lesions.

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and should be representative of all involved organs, but in addition should lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement, in which circumstance the next largest lesion that can be measured reproducibly should be selected.

Lymph nodes merit special mention since they are normal anatomical structures that may be visible by imaging even if not involved by tumor. As noted above, pathological nodes that are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥ 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Lymph node size is normally reported as two dimensions in the plane in

which the image is obtained (for CT, this is almost always the axial plane; for MRI, the plane of acquisition may be axial, sagittal, or coronal). The smaller of these measures is the short axis. For example, an abdominal node that is reported as being 20 mm × 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement. All other pathological nodes (those with short axis ≥ 10 mm but ≤ 15 mm) should be considered nontarget lesions. Nodes that have a short axis of < 10 mm are considered nonpathological and should not be recorded or followed.

All lesions (or sites of disease) not selected as target lesions (measurable or nonmeasurable), including pathological lymph nodes, should be identified as nontarget lesions and should also be recorded at baseline. Measurements are not required. It is possible to record multiple nontarget lesions involving the same organ as a single item on the eCRF (e.g., "multiple enlarged pelvic lymph nodes" or "multiple liver metastases").

Calculation of Sum of Diameters

A sum of the diameters (longest diameter for nonlymph 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.

Measuring Lymph Nodes

Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the node regresses to < 10 mm during the study. Thus, when lymph nodes are included as target lesions, the sum of diameters may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of < 10 mm.

Measuring Lesions That Become Too Small to Measure

During the study, all target lesions (lymph node and nonlymph node) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g., 2 mm). However, sometimes lesions or lymph nodes that are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measurement and may report them as being too small to measure. When this occurs, it is important that a value be recorded on the eCRF, as follows:

- If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm.
- If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned and "too small to measure" should be ticked. (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well and "too small to measure" should also be ticked).

<u>To reiterate, however, if the radiologist is able to provide an actual measurement, that should be recorded, even if it is < 5 mm, and in that case "too small to measure" should not be ticked.</u>

Measuring Lesions That Split or Coalesce on Treatment

When nonlymph node lesions fragment, the longest diameters of the fragmented portions should be added together to calculate the sum of diameters. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximum longest diameter for the coalesced lesion.

Evaluation of Nontarget Lesions

Measurements are not required for nontarget lesions, except that malignant lymph node nontarget lesions should be monitored for reduction to < 10 mm in short axis. Nontarget lesions should be noted at baseline and should be identified as "present" or "absent" and (in rare cases) may be noted as "indicative of progression" at subsequent evaluations. In addition, if a lymph node lesion shrinks to a nonmalignant size (short axis < 10 mm), this should be captured on the eCRF as part of the assessment of nontarget lesions.

Response Criteria

Criteria for Target Lesions

Definitions of the criteria used to determine objective tumor response for target lesions are provided below:

- Complete response (CR): Disappearance of all target lesions
  - Any pathological lymph nodes must have reduction in short axis to < 10 mm.
- Partial response (PR): At least a 30% decrease in the sum of diameters of all target lesions, taking as reference the baseline sum of diameters, in the absence of CR
- Progressive disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum of diameters at prior time points (including baseline)
  - In addition to the relative increase of 20%, the sum of diameters must also demonstrate an absolute increase of ≥ 5 mm.
- Stable disease (SD): Neither sufficient shrinkage to qualify for CR or PR nor sufficient increase to qualify for PD

Criteria for Nontarget Lesions

Definitions of the criteria used to determine the tumor response for the group of nontarget lesions are provided below. While some nontarget lesions may actually be measurable, they need not be measured and instead should be assessed only qualitatively at the time points specified in the schedule of activities.

- CR: Disappearance of all nontarget lesions and (if applicable) normalization of tumor marker level
  - All lymph nodes must be nonpathological in size (< 10 mm short axis).
- Non-CR/Non-PD: Persistence of one or more nontarget lesions and/or (if applicable) maintenance of tumor marker level above the normal limits

- PD: Unequivocal progression of existing nontarget lesions

Special Notes on Assessment of Progression of Nontarget Lesions

For patients with both measurable and nonmeasurable disease to achieve unequivocal progression on the basis of the nontarget lesions, there must be an overall level of substantial worsening in nontarget lesions in a magnitude that, even in the presence of SD or PR in target lesions, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest increase in the size of one or more nontarget lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in nontarget lesions in the face of SD or PR in target lesions will therefore be extremely rare.

New Lesions

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal, that is, not attributable to differences in scanning technique, change in imaging modality, or findings thought to represent something other than tumor (for example, some “new” bone lesions may be simply healing or flare of preexisting lesions). This is particularly important when the patient's baseline lesions show PR or CR. For example, necrosis of a liver lesion may be reported on a CT scan report as a “new” cystic lesion, which it is not.

A lesion identified during the study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, progression should be declared using the date of the initial scan.

Criteria for Overall Response at a Single Time point

Table 21 provides a summary of the overall response status calculation at each response assessment time point for patients who have measurable disease at baseline.

Table 21: Criteria for Overall Response at a Single Time Point: Patients with Target Lesions (with or without Nontarget Lesions)

Target Lesions	Nontarget Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not all evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

Abbreviations: CR = complete response; NE = not evaluable; PD = progressive disease; PR = partial response; SD = stable disease

Missing Assessments and Not-Evaluable Designation

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable at that time point. If measurements are made on only a subset of target lesions at a time point, usually the case is also considered not evaluable at that time point, unless a convincing argument can be made that the contribution of the individual missing lesions would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50 mm with three measured lesions and during the study only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

Special Notes on Response Assessment

Patients with a global deterioration in health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration." Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response; it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and nontarget lesions as shown in Table 21.

For equivocal findings of progression (e.g., very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

APPENDIX 2. IMMUNE-RELATED RESPONSE EVALUATION CRITERIA IN SOLID TUMORS

Conventional response criteria may not be adequate to characterize the antitumor activity of immunotherapeutic agents, which can produce delayed responses that may be preceded by initial apparent radiographic progression, including the appearance of new lesions. Therefore, immunotherapy-specific response criteria adaptations to RECIST 1.1 (Eisenhauer et al 2009) have been developed to allow for unconventional response and progression patterns. These include modified RECIST 1.1 for immune-based therapeutics (iRECIST; Seymour et al 2017), which was developed by the RECIST working group in an effort to create a common set of criteria that the cancer immunotherapy field could apply to clinical trials.

Response evaluation through use of iRECIST requires collection of tumor assessment data after radiographic progression per RECIST 1.1. Details regarding lesion evaluation are described below. When not otherwise specified, RECIST 1.1 conventions will apply.

Criteria for determining overall response at a single time point per iRECIST are also summarized below. Of note, overall response per iRECIST will not be captured in the eCRF, but will instead be calculated programmatically by the Sponsor on the basis of Investigator-assessed individual lesion data recorded in the eCRF.

iRECIST response status is not a specific component of treatment discontinuation criteria, including decisions about whether to continue treatment beyond progression per RECIST 1.1. Investigators should instead take into account radiologic data and clinical status in making such decisions.

Evaluation of Lesions to Support IRECIST Response Assessment after Disease Progression per RECIST 1.1

iRECIST is an extension of RECIST 1.1 that allows for response assessment following disease progression per RECIST 1.1. RECIST 1.1 rules for categorizing lesions as measurable or nonmeasurable and measuring lesions (see Appendix 1) also apply to iRECIST. After disease progression per RECIST 1.1, the same target and nontarget lesions selected at baseline will continue to be followed, along with any new lesions that develop, to support iRECIST response evaluations, as described below and summarized in Table 22. Once a lesion has been categorized as a target, nontarget, or new lesion, it will remain classified as such.

Target Lesions

The target lesions selected at baseline should continue to be measured at all tumor assessment time points after disease progression per RECIST 1.1, according to RECIST 1.1 conventions.

Nontarget Lesions

Nontarget lesions selected at baseline should continue to be followed at all tumor assessment time points after disease progression per RECIST 1.1. At each time point, nontarget lesions should continue to be categorized as “absent” (complete response [CR]), “unequivocal progression” relative to baseline (progressive disease [PD]), or “present without unequivocal progression” (non-CR/non-PD), as defined by RECIST 1.1. In addition, any nontarget lesions that were categorized as PD at the previous time point should be evaluated to determine whether there has been any further increase in size.

New Lesions

New lesions identified after baseline will be evaluated for measurability with use of the same criteria applied to prospective target lesions at baseline per RECIST 1.1 (e.g., nonlymph node lesions must be ≥ 10 mm on the longest diameter; new lymph nodes must be ≥ 15 mm on the short axis [see note below]). All new lesions (measurable or nonmeasurable) must be assessed and recorded at the time of identification and at all subsequent tumor assessment time points.

Up to a maximum of 5 measurable new lesions total (with a maximum of two lesions per organ) should be selected and measured at each time point. New lesions that are not measurable at first appearance but meet measurability criteria at a subsequent time point should be measured from that point on, if the maximum number of measurable new lesions has not been reached. However, for calculation of the sum of diameters for new lesions, iRECIST excludes measurements from new lesions that were not measurable at first appearance.

All nonmeasurable new lesions (including those that subsequently become measurable) and additional measurable new lesions (in excess of 5 total or two per organ) should be assessed to determine whether there is any increase in size relative to the previous assessment time point.

Note regarding new lymph node lesions: If at first appearance the short axis of a lymph node lesion is ≥ 15 mm, it will be considered a measurable new lesion. If at first appearance the short axis of a lymph node lesion is ≥ 10 mm and < 15 mm, the lymph node will not be considered measurable but will still be considered a new lesion and should be identified as a nonmeasurable new lesion. If at first appearance the short axis of a lymph node is < 10 mm, the lymph node should not be considered pathological and should not be considered a new lesion. A lymph node can subsequently become measurable, when the short axis is ≥ 15 mm. Measurable new lymph node lesions should continue to be measured at all subsequent time points, even if the short axis decreases to < 15 mm (or even < 10 mm).

Table 22: Guidelines for Evaluation of Lesions to Support iRECIST Response Assessment after Disease Progression per RECIST 1.1

Lesion Type	Evaluation of Lesions to Support iRECIST Response Assessment after Disease Progression per RECIST 1.1
Target lesions	• Measurements should be continued according to RECIST 1.1 conventions.
Nontarget lesions	• Nontarget lesions should continue to be categorized as absent (CR), unequivocal progression (PD), or present without unequivocal progression (non-CR/non-PD), as defined by RECIST 1.1. In addition, any nontarget lesions that were categorized as PD at the previous time point should be evaluated to determine whether there has been any further increase in size.
New lesions	• New lesions should be evaluated for measurability per RECIST 1.1. • All new lesions (measurable or nonmeasurable) must be assessed and recorded at the time of identification and at all subsequent tumor assessment time points. • Up to a maximum of five measurable new lesions total (with a maximum of two lesions per organ) should be selected and measured at each time point. • All nonmeasurable new lesions (including those that subsequently become measurable) and additional measurable new lesions (in excess of five total or two per organ) should be assessed to determine whether there is any increase in size relative to the previous assessment time point.

Abbreviations: CR = complete response; iRECIST = modified RECIST 1.1 for immune-based therapeutics; PD = progressive disease; RECIST 1.1 = Response Evaluation Criteria in Solid Tumors, Version 1.1

APPENDIX 3. LYMPHOMA RESPONSE TO IMMUNOMODULATORY THERAPY CRITERIA

Immunomodulatory agents may be associated with clinical and imaging findings during treatment that are suggestive of progressive disease despite evidence of clinical benefit (e.g., tumor flare or pseudo-progression). The response criteria outlined below, which have been adapted from the Lymphoma Response to Immunomodulatory Therapy Criteria (LYRIC) (Cheson et al 2016), should be implemented to assess disease status for patients with lymphoma who receive immunomodulatory agents.

Table 23: Guidelines for the Classification of Disease Status According to LYRIC

Complete Response	Partial Response	Progressive Disease
PET-CT score 1, 2, or 3* with or without a residual mass on 5PS† OR On CT, target nodes/nodal masses must regress to ≤ 1.5 cm in LDi.	PET-CT score 4 or 5 with reduced uptake compared with baseline and residual mass(es) of any size. OR On CT, ≥ 50% decrease in SPD of up to 6 target measurable nodes and extranodal sites.	PET-CT score 4 or 5 with an increase in intensity of uptake from baseline and/or new FDG-avid foci consistent with lymphoma at interim or end-of-treatment assessment. OR On CT, an individual node/lesion must be abnormal with: LDi > 1.5 cm and increase by ≥ 50% from PPD nadir and an increase in LDi or SDi from nadir 0.5 cm for lesions > 2 cm 1.0 cm for lesions > 2 cm In the setting of splenomegaly, the splenic length must increase by > 50% of the extent of its prior increase beyond baseline (e.g., a 15-cm spleen must increase to > 16 cm). If no prior splenomegaly, must increase by ≥ 2 cm from baseline. New or recurrent splenomegaly New or clear progression of pre-existing nonmeasured lesions Regrowth of previously resolved lesions A new node > 1.5 cm in any axis or a new extranodal site > 1.0 cm in any axis; if < 1.0 cm in any axis, its presence must be unequivocal and must be attributable to lymphoma Assessable disease of any size unequivocally attributable to lymphoma AND/OR new or recurrent involvement of the bone marrow **The above criteria apply with the following exceptions for IR:** IR(1): ≥ 50% increase in SPD in first 12 weeks of therapy IR(2): < 50% increase in SPD with a. New lesion(s), or b. ≥ 50% increase in PPD of a lesion or set of lesions at any time during treatment IR(3): Increase in FDG uptake without a concomitant increase in lesion size or number meeting criteria for PD

Abbreviations: CT = computed tomography; FDG = fluorodeoxyglucose; IR = immune response; LDi = longest diameter; PET = positron emission tomography; PPD = product of the perpendicular diameters; SDi = short diameter; SPD = sum of the product of diameters; 5PS = 5-point scale.

*A score of 3 in many patients indicates a good prognosis with standard treatment, especially if at the time of an interim scan. However, in trials involving PET where de-escalation is investigated, it may be preferable to consider a score of 3 as inadequate response (to avoid undertreatment).

†PET 5PS: 1 = no uptake above background; 2 = uptake ≤ mediastinum; 3 = uptake > mediastinum but ≤ liver; 4 = uptake greater than liver; 5 = uptake markedly higher than liver (2-3 times SUVmax in normal liver) and/or new lesions; X = new areas of uptake unlikely to be related to lymphoma.

The term "immune response" (IR) does not make direct reference to the underlying mechanism, recognizing that a delayed response and an immune-mediated flare can both occur in the early treatment period and may be difficult to distinguish from progression by physical examination or imaging alone. Moreover, the term provides the flexibility to allow patients to continue treatment past IR in some circumstances with a subsequent evaluation within 12 weeks to confirm or refute true progressive disease. A patient will be considered to have IR in 1 or more of the 3 following circumstances.

1. **Increase in overall tumor burden (as assessed by sum of the product of the diameters [SPD]) of ≥ 50% of up to 6 measurable lesions in the first 12 weeks of therapy, without clinical deterioration [IR(1)]**. This pattern may be seen as a consequence of either delayed response or early immune-mediated flare. At least within the context of clinical trials, a biopsy is encouraged in this case because this may help to distinguish the two and, if positive, will confirm the impression of progressive disease. However, if negative for lymphoma, it will support the concept of pseudo-progression and contribute to our understanding of this phenomenon. When such a biopsy is neither safe nor feasible, decisions should be based on a repeat scan 12 weeks after the initial determination of IR.

   It is recognized that "clinical deterioration" is subjective. In some cases, the simple growth of a nodal or tumor mass could worsen the symptoms mechanically related to that mass, such as pain at the tumor site, compression of adjacent structures, etc. Such an increase in symptoms that can be directly attributed to the size of the tumor mass may not be considered as clinical deterioration in this context. However, in most cases, patients should be experiencing clinical stability or improvement by Investigator assessment to be considered as having IR, and in all cases, the patient must be considered likely to tolerate continued treatment and not at risk of serious complications should further tumor growth occur.

2. **Appearance of new lesions or growth of one or more existing lesion(s) ≥ 50% at any time during treatment; occurring in the context of lack of overall progression (< 50% increase) of overall tumor burden, as measured by SPD of up to 6 lesions at any time during the treatment [IR(2)]**. This phenomenon may occur early or late in the treatment course, and therefore, unlike IR(1), is not defined by its temporal relationship to treatment initiation. Both within and outside the context of clinical trials, a biopsy is strongly encouraged in such cases. If the biopsy does not confirm the presence of viable tumor in the new or enlarging lesion(s), then the lesion(s) are not considered active disease and should not be used in subsequent SPD assessments.

3. **Increase in FDG uptake of 1 or more lesion(s) without a concomitant increase in lesion size or number [IR(3)]**. Increased immune activity at the site of tumor may manifest as an increase in FDG uptake. Therefore, by itself, changes in uptake should not trigger an assignment of progressive disease with checkpoint inhibitors. The magnitude of increase in uptake in an immune-mediated flare compared with that in true tumor progression is not yet known. It is important to investigate this finding, especially in conjunction with biopsies of the lesion in question.

An increase in FDG avidity of 1 or more lesions suggestive of lymphoma, without a concomitant increase in size of those lesions meeting progressive disease criteria does not constitute progressive disease.

It is possible that, at a single time point, a patient could fulfill criteria for both IR(1) or IR(2) and IR(3): for example, there could be a new FDG avid lesion in the absence of overall progression [IR(2)], and, at the same time, increase in FDG uptake of a separate lesion [IR(3)]. In such cases, the designation of IR(1 or 2) should take priority [e.g., IR (2)] in the above example.

In patients categorized as having any of the above types of IR, repeat imaging should be obtained after an additional 12 weeks (or earlier if clinically indicated). At that time, response should be re-evaluated, and the patient should be considered to have true progressive disease if the SPD of target lesion has increased further, with the considerations below:

- In the case of IR(1), the comparison should be between the first IR(1) and the current SPD, with an increase of ≥ 10% constituting progressive disease. In addition, there should be an increase of ≥ 5 mm (in either dimension) of ≥ 1 lesion for lesions ≤ 2 cm and 10 mm for lesions > 2 cm. The 10% threshold is empiric but designed to account for variability in measurement, especially when taken along with the minimum increase. If the target SPD increase is < 10%, the response would still be categorized as IR(1), and the patient could continue treatment until a subsequent scan shows either true progressive disease [≥ 10% increase from first IR(1) time point and an increase of > 5 mm in either dimension of ≥ 1 lesion] or response (≥ 50% decrease from baseline). In this situation, it is reasonable to repeat imaging in 4 to 8 weeks of the original IR(1) time point to ensure absence of significant further increase.
- In the case of IR(2), the new or growing lesion(s) (unless biopsy proven to be benign) should be added to the target lesion(s), up to a total of no more than 6 total lesions. If the SPD of the newly defined set of target lesions has increased ≥ 50% from their nadir value (which may precede the IR time point), the patient should be considered to have progressive disease.
- In the case of IR(3), because inflammatory responses may result in an increase in the standardized uptake value of a lesion, the patient will not be considered to have PD unless there is evidence of PD by an increase in lesion size or the development of new lesions, as noted above.

Importantly, if a patient is assessed as having IR and then “true” progressive disease at a subsequent time point (without an intervening objective response between IR and progressive disease), the IR assessment should subsequently be corrected to progressive disease for reporting purposes to the date of the prior designation of IR. While these lesions may remain stable during the time of observation, the initial designation of IR should be changed to progressive disease.

APPENDIX 4. *E. COLI* NISSLE SENSITIVITY AND RESISTANCE AGAINST COMMON ANTIBIOTICS

***E. coli* Nissle sensitive to**		***E. coli* Nissle resistant against [a]**
Amikacin	Imipenem	Cefsulodin
Amoxicillin/clavulanic acid	Latamoxef	Clindamycin
Ampicillin	Mezlocillin	Erythromycin
Azlocillin	Nitrofurantoin	Metronidazole
Cefaclor	Norfloxacin	Penicillin G
Cefazolin	Pipemidic acid	Quinupristin/dalfopristin
Cefoperazone	Piperacillin	Rifampin
Cefotaxime	Rifampicin	Teicoplanin
Ceftriaxone	Streptomycin	Vancomycin
Cephalothin	Tetracycline	
Chloramphenicol	Ticarcillin	
Ciprofloxacin	Tobramycin	
Doxycycline	Trimethoprim/sulfamethoxazole	
Gentamicin		

Adapted from Sonnenborn et al 2009

a Like *E. coli* in general, *E. coli* Nissle shows natural resistance against the listed antibiotics. As an isolate from the pre-antibiotic era, *E. coli* Nissle has no acquired resistance, and no antibiotic resistance plamids are present.