Title Page

Protocol Title:	Phase 1/1b Study of the Safety of TTX-030 as a Single Agent and in Combination with Pembrolizumab or Chemotherapy in Patients with Lymphoma or Solid Tumor Malignancies
Protocol Number:	TTX-030-001
Amendment Number:	Version 8.0, December 15, 2020
Compound:	TTX-030
Study Phase:	1/1b
Sponsor Name:	Trishula Therapeutics, Inc.
Legal Registered Address:	4000 Shoreline Court, Suite 200 South San Francisco, CA 94080
Regulatory Agency Identifier Number(s):	IND Number 138313

Approval Date:	October 1, 2018	Version 1.0
	October 24, 2018	Version 1.1
	December 28, 2018	Version 2.0
	September 12, 2019	Version 3.0
	October 21, 2019	Version 4.0
	November 22, 2019	Version 5.0
	January 2, 2020	Version 6.0
	October 12, 2020	Version 7.0
	December 15, 2020	Version 8.0

CONFIDENTIAL

PROTOCOL APPROVAL PAGE

PROTOCOL TITLE:

Phase 1/1b Study of the Safety of TTX-030 as a Single Agent and in Combination with Pembrolizumab or Chemotherapy in Patients with Lymphoma or Solid Tumor Malignancies

PROTOCOL NUMBER:	TTX-030-001
STUDY TREATMENT:	TTX-030
IND NUMBER:	138313
SPONSOR:	Trishula Therapeutics, Inc. 4000 Shoreline Court, Suite 200 South San Francisco, CA 94080
MEDICAL MONITOR:	[redacted]

DATE FINAL:		
	October 1, 2018	Version 1.0
	October 24, 2018	Version 1.1
	December 28, 2018	Version 2.0
	September 12, 2019	Version 3.0
	October 21, 2019	Version 4.0
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[redacted]

12/15/20

Date

PROTOCOL ACCEPTANCE FORM

PROTOCOL TITLE:

Phase 1/1b Study of the Safety of TTX-030 as a Single Agent and in Combination with Pembrolizumab or Chemotherapy in Patients with Lymphoma or Solid Tumor Malignancies

PROTOCOL NUMBER:	TTX-030-001
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IND NUMBER:	138313
SPONSOR:	Trishula Therapeutics, Inc. 4000 Shoreline Court, Suite 200 South San Francisco, CA 94080
DATE FINAL:	December 15, 2020 Version 8.0

By my signature below, I hereby state that I have read and agree to abide by the instructions, conditions, and restrictions of the protocol, in accordance with Good Clinical Practice guidelines, the Declaration of Helsinki, and all applicable laws and regulations.

_____

Name of investigator (print) Name of investigator (signature)

_____

Date

Protocol Amendment Summary of Changes Table

DOCUMENT HISTORY	
Document	**Date**
Version 8.0 Amendment 8 (Trishula)	15 Dec 2020
Version 7.0 Amendment 7 (Trishula)	12 Oct 2020
Version 6.0 Amendment 6	2 Jan 2020
Version 5.0 Amendment 5	22 Nov 2019
Version 4.0 Amendment 4	21 Oct 2019
Version 3.0 Amendment 3	12 Sep 2019
Version 2.0 Amendment 2	28 Dec 2018
Version 1.1 Amendment 1	24 Oct 2018
Version 1.0 Original Protocol	01 Oct 2018

Amendment 8 (15 December 2020)

Overall Rationale for the Amendment:

Section #	Description of Change	Brief Rationale
Entire document	Minor spelling, grammar, punctuation, capitalization, abbreviations, and other formatting edits were made throughout the document	Consistency
Section 1.3	Schedule of Activities – update footnotes	Clarity
Section 1.1 Section 3.0 Section 4.1.1.2 Section 5.1 Section 9.2	Remove the number of prior lines requirement for RCC population	Population consideration
Section 5.2 Section 5.3	Inclusion Criterion #22 Exclusion Criterion #27 -Update washout requirement from 28 to 14 days	Treatment consideration for advanced cancer population
Section 6.1.13 Table 11	-Update TTX-030 administration guidance to follow the Pharmacy Manual	Clarity

The rationale for prior amendments is included in Section 11.1.

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List of Abbreviations

5-PS	5-point scale
A2AR	adenosine A2A receptor
ACTH	adrenocorticotropic hormone
ADA	anti-drug antibodies
ADP	adenosine diphosphate
AE	adverse event
ALT	alanine aminotransferase
ALP	alkaline phosphatase
AMP	adenosine monophosphate
ANOVA	analysis of variance
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the serum concentration curve
AUC_{0-t}	area under the serum concentration curve (AUC) from time zero to the last time of the last quantifiable concentration
$AUC_{0-\infty}$	area under the serum concentration curve (AUC) from time zero extrapolated to infinity
B-LCL	Epstein Barr virus-transformed lymphoblastoid B cell line
BOR	best overall response
BP	blood pressure
BUN	blood urea nitrogen
C	Cycle
CA19-9	carbohydrate antigen
CBC	complete blood count
CD	cluster of differentiation
$CD39^{-/-}$	cluster of differentiation 39 deficient
CEA	carcinoma embryonic antigen
cIg	isotype control
CL	apparent total body clearance of the drug from serum
C_{max}	maximum serum observed concentration
C_{min}	minimum serum observed concentration
CMV	cytomegalovirus
CNS	central nervous system
CRS	cytokine release syndrome
CT	computed tomography
D	day
DCR	disease control rate
DLT	dose-limiting toxicity
DOR	duration of response
DVT	deep vein thrombosis

eATP	extracellular ATP
EC_{50}	half-maximal effective concentration
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EHNA	erythro-9-(2-hydroxy-3-nonyl)adenine
ELISA	enzyme-linked immunosorbent assay
ENTPDase	ectonucleoside triphosphate diphosphohydrolase
EOI	end of infusion
EOT	end of treatment
FDG	fluorodeoxyglucose
FIH	first-in-human
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GH	growth hormone
GLP	Good Laboratory Practice
Hgb	hemoglobin
HgbA1c	hemoglobin A1c
HED	human equivalent dose
HR	heart rate
IB	Investigator Brochure
IC_{50}	half-maximal inhibition concentration
ICF	informed consent form
IEC	Independent Ethics Committee
IFN	interferon
Ig	immunoglobulin
IL	interleukin
IND	Investigational New Drug
INR	international normalized ratio
IR	immune response
irAE	immune-related adverse event
IRB	Institutional Review Board
iRECIST	immune-related Response Evaluation Criteria in Solid Tumors
IV	intravenous(ly)
LD	longest diameter
LDi	longest transverse diameter of a lesion
LFT	liver function tests
LH	luteinizing hormone
LYRIC	LYmphoma Response to Immunomodulatory Therapy Criteria
mAb	monoclonal antibody

mCRM	modified continual reassessment method
mCRPC	metastatic castration-resistant prostate cancer
MFI	mean fluorescence intensity
MRI	magnetic resonance imaging
MRSD	maximum recommended starting dose
MTD	maximum tolerated dose
N/A	not applicable
NCI CTCAE	National Cancer Institute Common Terminology Criteria
NP	Nab-paclitaxel (Abraxane®)
NHP	non-human primate
NOAEL	no-observed-adverse-effect level
O_2	oxygen
ORR	objective response rate
OS	overall survival
PAD	pharmacologically active dose
PBMC	peripheral blood mononuclear cell
PCWG3	Prostate Cancer Clinical Trials Working Group 3
PD	progressive disease
PD-1	programmed cell death protein-1
PD-L1	programmed cell death protein ligand-1
PET	positron-emission tomography
PFS	progression-free survival
PLT	platelets
PK	pharmacokinetic(s)
PPD	cross product of the LDi and perpendicular diameter
PSA	prostate-specific antigen
PT	prothrombin time
PTT	partial thromboplastin time
Q	every
RCC	renal cell carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	recommended Phase 2 dose
SAE	serious adverse event
SDi	shortest axis perpendicular to the LDi
SPD	sum of the product of the perpendicular diameters for multiple lesions
$t_{1/2}$	terminal elimination half-life
TEAE	treatment-emergent adverse event
TIL	tumor infiltrating leukocytes
Tizona	Tizona Therapeutics, Inc.
TK	toxicokinetics
TKI	tyrosine kinase inhibitor

TLS	tumor lysis syndrome
T_{max}	time at which C_{max} is reached
TME	tumor microenvironment
T_{reg}	regulatory T cells
Trishula	Trishula Therapeutics, Inc.
TSH	thyroid stimulating hormone
TTP	time to progression
TTR	time to tumor response
Tx	treatment
ULN	upper limit of normal
V_{ss}	volume of distribution at steady state
W	week(s)
WT	wild type

1. Protocol Summary

1.1. Synopsis

Protocol Title:

Phase 1/1b Study of the Safety of TTX-030 as a Single Agent and in Combination with Pembrolizumab or Chemotherapy in Patients with Lymphoma or Solid Tumor Malignancies

Rationale:

There are compelling preclinical data to support the hypothesis that cluster of differentiation (CD)39-mediated reduction of adenosine triphosphate (ATP) and accumulation of adenosine both play a central role in establishing and maintaining the immunosuppressive microenvironment within solid tumors. CD39 expression is associated with worse outcomes in several cancer types. The enzymatic inhibition of CD39 maintains high levels of pro-inflammatory extracellular ATP (eATP), leading to immune activation and significant anti-tumor immune responses. The CD39 pathway contributes to T-cell exhaustion and limits the immunogenicity of chemotherapy, which provides the rationale for combining CD39 inhibition with checkpoint blockade and conventional cytotoxic therapies.

Targeting CD39 is a rational strategy for the treatment of subjects with cancer, and blockade of CD39 may result in less potential for autoimmune side effects compared with rates seen with other immunotherapy agents. Although regulatory T cells (T_{reg}) from CD39-deficient ($CD39^{-/-}$) knockout mice have impaired suppressive activity, the mice do not show autoimmunity (Sun et al. 2010). TTX-030, as a single agent or in combination, is expected to have a manageable safety profile, thereby providing additional therapeutic options for subjects whose malignancies are otherwise refractory to or relapsed on current standard of care therapies.

The indications selected were defined based on expression of CD39 on tumor infiltrating leukocytes (TIL), expression of CD39 on tumor cells, and tumor types that are amenable to be combined with chemotherapy or anti-programmed cell death protein-1 (PD-1) therapy.

Objectives and Endpoints

Objectives	Endpoints
Primary	
• To assess the safety profile, to determine the dose-limiting toxicity (DLT), and to determine the maximum tolerated dose (MTD) and/or recommended Phase 2 dose (RP2D) of TTX-030 when administered intravenously (IV) as a single agent or in combination with agents in specified regimens to subjects with advanced solid tumor malignancies or lymphoma	• DLTs, MTD, and/or RP2D of TTX-030 as a single agent • DLTs, MTD, and/or RP2D of TTX-030 in combination with specified regimens
• Arm 1 and Arm 2 Expansion Cohorts: To assess the objective response rate (ORR) per immune-related Response Evaluation Criteria in Solid Tumors (iRECIST) after treatment with TTX-030 as a single agent or TTX-030 in combination with pembrolizumab in subjects with advanced or metastatic renal cell carcinoma (RCC) with clear-cell component	• ORR
Secondary	
• To evaluate anti-tumor activity in subjects treated with TTX-030 as a single agent or in combination with specified regimens	• Best overall response (BOR) • ORR (except for Arm 1 and 2 Expansion Cohorts, where ORR is a primary endpoint) • Duration of response (DOR) • Time to tumor response (TTR) • Disease control rate (DCR) • Time to progression (TTP) • Progression-free survival (PFS) • Overall survival (OS)

Objectives	Endpoints
• To evaluate the pharmacokinetics (PK) of TTX-030	• Serum PK parameters for TTX-030 following single and multiple doses including the maximum serum observed concentration (C_{max}), time at which C_{max} is reached (T_{max}), minimum serum observed concentration (C_{min}), volume of distribution at steady state (V_{SS}), apparent total body clearance of the drug from serum (CL), terminal elimination half-life ($t_{1/2}$), area under the serum concentration curve (AUC) to last quantifiable concentration (AUC_{0-t}), and AUC from time zero extrapolated to infinity ($AUC_{0-\infty}$) for TTX-030
• To assess the effects of TTX-030 on pharmacodynamic biomarkers relating to mechanism of action and immune responses	• Immunogenicity (detection of anti-drug antibodies [ADA]) • Expression of CD39 • Exploratory pharmacodynamic biomarkers

Overall Design

Study Design:

This is a Phase 1/1b, open-label, and multicenter study of TTX-030 in adult subjects with lymphoma or solid tumor malignancies. The study will evaluate the safety and tolerability of:

- TTX-030 as a single agent, and
- TTX-030 in combination with pembrolizumab, a PD-1 inhibitor, and
- TTX-030 in combination with specified chemotherapy regimens

Number of Subjects:

Up to 43 subjects may be enrolled across 1 dose escalation and 2 safety lead-in arms.

TTX-030 will first be evaluated in the monotherapy dose escalation (Arm 1). Once the RP2D of TTX-030 has been determined in the monotherapy dose-escalation arm, the combination-therapy safety lead-in arms (Arms 2 and 4) can be initiated. For the safety lead-in arms, the TTX-030 starting dose will be the RP2D identified during monotherapy dose escalation; de-escalation to

one dose level below the RP2D is permitted. Upon completion of the monotherapy dose-escalation and combination-therapy safety lead-in arms, each of those arms may have the option to enroll additional subjects per expansion cohort at the RP2D.

As of Jan 2020, a RP2D was determined to be 40 mg/kg loading dose 7 days prior to Cycle 1 Day 1, followed by either 30 mg/kg Q3W or 20 mg/kg Q2W.

Arm 1 (single agent), n = up to 21

- TTX-030 single-agent dose escalation (several doses and schedules may be evaluated)

Arm 1 (expansion), n = 25

- TTX-030 single-agent; loading dose 7 days prior to Cycle 1 Day 1, followed by 30 mg/kg Q3W

Arm 2 (anti-PD-1 combination), n = up to 6

- Pembrolizumab 200 mg every (Q) 3 weeks (W) in combination with TTX-030 Q3W safety lead-in

Arm 4 (chemotherapy combination), n = up to 6

- Gemcitabine 1000 mg/m^2 + nab-paclitaxel 125 mg/m^2 Days 1, 8, and 15 every 28 days + TTX-030 Q2W safety lead-in (Note: dose modification allowed for gemcitabine 1000 mg/m^2 + nab-paclitaxel 125 mg/m^2 (e.g., modify to Days 1 and 8 or Days 1 and 15 every 28 days + TTX-030 Q2W)

Expansion cohorts, n = 25 each in Arms 1 and 2 and n = up to 20 in Arm 4. The number of subjects evaluated in the combination-therapy safety lead-in Arm 4 may count toward the total number of subjects in an expansion cohort.

Note: The sponsor may choose not to open one of the safety lead-in arms and/or expansion cohorts.

Reference Therapy and Mode of Administration:

- TTX-030 IV infusion Q3W for Arm 1 Dose Escalation (21-day cycle)
- TTX-030 IV infusion Q3W for Arms 1 and 2 Safety Lead-in and Expansion (21-day cycle + a 7-day loading dose prior to C1D1)
- TTX-030 IV infusion Q2W for Arm 4 (28-day cycle + a 7-day loading dose prior to C1D1)

The investigational formulation of TTX-030 will be supplied as a sterile, single-use, preservative-free liquid containing 200 mg of antibody. The dosage form of TTX-030 is a liquid formulation containing 30 mg/mL of TTX-030 in 10 mM sodium citrate, 280 mM sucrose, 0.02% polysorbate 20, and 1.0 mM methionine, with a pH of 6.5. The liquid TTX-030 will be

filled into a Type 1 glass serum vial with an elastomeric stopper and aluminum over seal with a plastic flip top. The fill volume will be 6.7 mL in a 10-cc glass vial stored at temperatures of 2°C to 8°C. TTX-030 will be administered IV Q3W or Q2W according to the study treatment arm schedule.

Duration of Treatment:

If well tolerated, treatment with TTX-030 may continue until disease progression, drug toxicity, or death. For combination arms, if a subject discontinues the pembrolizumab or chemotherapy regimens, treatment with TTX-030 may continue if it is well tolerated and if both the investigator and sponsor approve.

Criteria for Evaluation:

Safety: Clinical adverse events (AEs) will be graded according to the National Cancer Institute Common Terminology Criteria (NCI CTCAE) Version 5.0. AEs are to be monitored and collected for the duration of study treatment until 90 days after the last dose. A DLT is defined as any clinically significant AE that occurs during Treatment Cycle 1, is considered related to TTX-030 as a single agent or the combination of TTX-030 and other agent(s), and meets the criteria outlined in the protocol. In the dose escalation arm, for the Q3W dose schedule, the cycle length is 3 weeks and the DLT period will be the first cycle (21 days).
In the safety lead-in Arm 2, the DLT evaluation period is 21-day (TTX-030 regimen given every 3 weeks [Q3W]) plus a 7-day of loading dose. In the safety lead-in Arm 4, for the Q2W dose schedule, the cycle length is 4 weeks and the DLT period will be 28-day (TTX-030 regimen given every 2 weeks [Q2W]) plus a 7-day of loading dose.

Pharmacokinetics: PK analyses will be conducted using samples drawn based on the provided schedule of assessments. TTX-030 concentrations will be measured by validated electrochemiluminescence-based method and used to calculate PK parameters including $t_{1/2}$, C_{max}, T_{max}, C_{min}, CL, V_{ss}, AUC_{0-t}, and $AUC_{0-\infty}$.

Pharmacodynamics: Pharmacodynamic analyses will be performed using blood and tissue samples taken at designated time points. Parameters to be assessed include target and mechanism of action-related biomarkers, ADA formation, and pro-inflammatory cytokine release. Additionally, several exploratory analyses are planned, including immunoprofiling, target receptor coverage, and serum metabolomics. Detailed instructions for pharmacodynamic blood sample collection, processing, and shipping will be provided to the study sites in a separate laboratory manual.

Early Signals of Activity: Anti-tumor activity, including BOR, ORR, DOR, TTR, DCR, TTP, PFS, and OS, will be assessed based on iRECIST, LYmphoma Response to Immunomodulatory Therapy Criteria (LYRIC), or Prostate Cancer Clinical Trials Working Group 3 (PCWG3) response criteria when applicable.

Statistical Methods:

A primary objective of the trial is to determine the MTD and/or RP2D for each arm. Dose escalation (Arm 1) will be conducted according to a modified continual reassessment method (mCRM) for doses up to 10 mg/kg and according to a 3 + 3 design for doses > 10 mg/kg, with the aim of estimating the dose-toxicity relationship and determining the MTD. The MTD will be

defined as the dose that has a model-estimated DLT rate closest to 30% and has less than 55% probability of a DLT rate exceeding 30%.

Dose-toxicity modeling will be used to estimate the log-odds of DLT across dose levels and will be conducted using a 2-parameter logistic regression model. A detailed adaptive design and simulation report is included in Section 11.6.

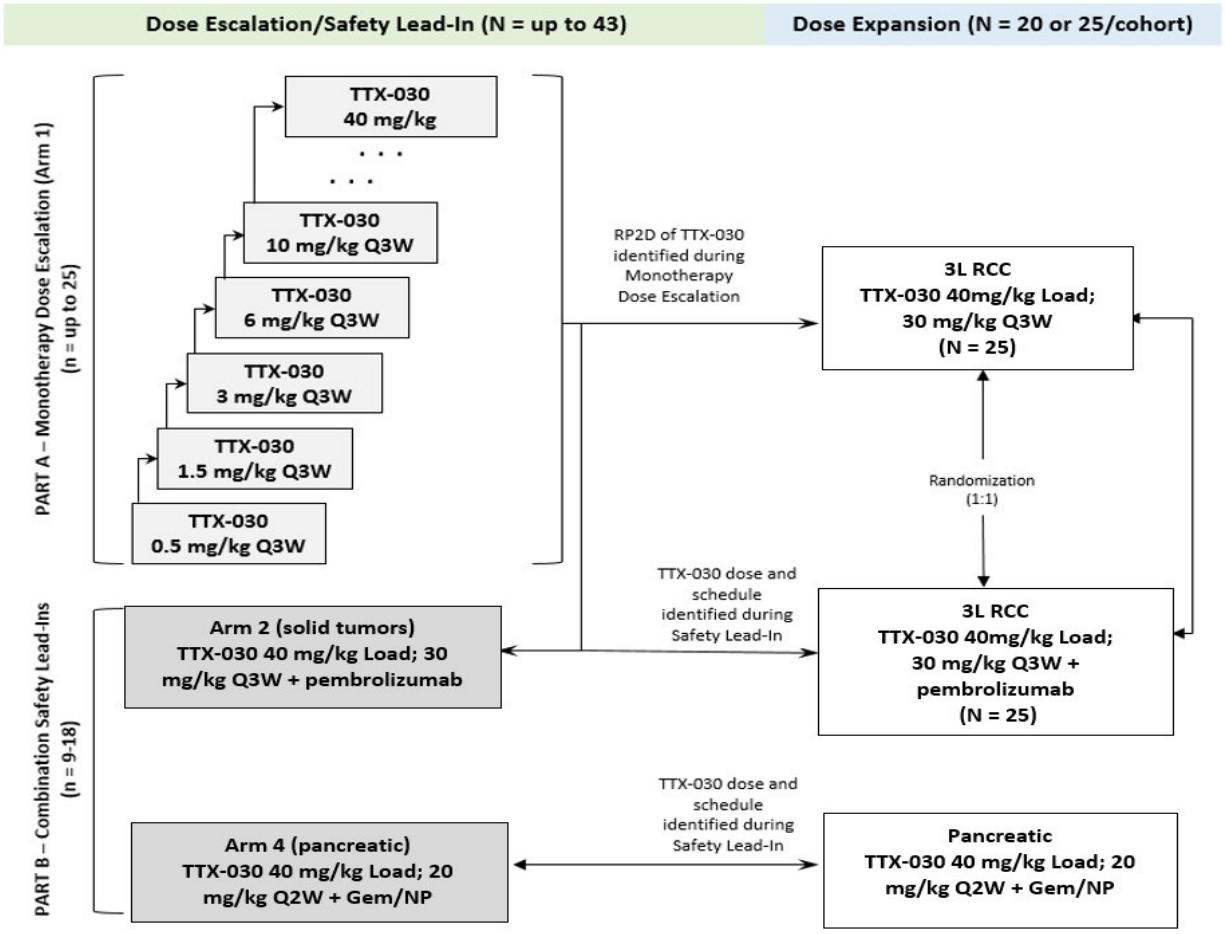
The RP2D will be determined based on the totality of data, including the estimated MTD, plus other endpoints such as observed efficacy, PK, and other AEs.

Descriptive summary statistics will be presented. Longitudinal data will be presented by appropriate time intervals, such as monthly or quarterly, depending on the nature of the data.

1.2. Schema

The study schema is depicted in Figure 1.

Figure 1: Study Schema



Abbreviations: Gem = gemcitabine, NP = nab-paclitaxel, Q = every, RCC = renal cell carcinoma; RP2D = recommended Phase 2 dose, Q3W = every 3 weeks, Q2W = every 2 weeks

Note: Arm 3 is removed as a similar population is being evaluated in another protocol (TTX-030-002).

1.3. Schedule of Activities

The study-specific assessments and procedures are outlined in Table 1/Table 2, and Table 3/Table 4.

Table 1: Arms 1 and 2: Schedule of Assessments (Screening Through Treatment Period Cycle 1 and Cycle 2) – Q3W Dosing Schedule (21-Day Cycle)

	Pretreatment		Loading Dose	Study Treatment Period = 2 cycles (1 cycle = 21 days)						
				Cycle 1					Cycle 2	
				Day 1	Day 2	Day 3	Day 8	Day 15	Day 22 (+ 2 days)	Day 29 (± 2 days)
Assessment	**Screening[a] (up to 28 days prior to loading dose)**	**Baseline (up to 3 days prior to loading dose)**	**Day -7[a] / Pre-C1D1**	**C1D1**	**C1D2**	**C1D3**	**C1D8**	**C1D15**	**C2D1**	**C2D8**
Informed consent	X									
Inclusion/Exclusion	X									
Demographics	X									
Medical history & prior anti-cancer treatments	X									
Physical examination	X	X	X						X	
Symptom-directed examination				X	X		X	X		X
Vital signs (temperature, O_2, HR, BP, weight, height)	X	X	X	X	X		X	X	X	X
12-lead ECG	X									
HgbA1c	X									
ECOG performance status	X			X					X	
Tumor assessment (CT or PET-CT)[b]	X									
Serum tumor-associated markers as applicable (eg, CEA, CA19-9, PSA)	X									

Assessment	Pretreatment		Loading Dose	Study Treatment Period = 2 cycles (1 cycle = 21 days)						
	Screening[a] (up to 28 days prior to loading dose)	Baseline (up to 3 days prior to loading dose)	Day -7[a]	Cycle 1					Cycle 2	
				Day 1	Day 2	Day 3	Day 8	Day 15	Day 22 (+ 2 days)	Day 29 (± 2 days)
			Pre-C1D1	C1D1	C1D2	C1D3	C1D8	C1D15	C2D1	C2D8
Thyroid function tests[c]		X	X							
Clinical laboratory tests (CBC, LFTs, chemistry); LDH at Screening only	X	X	X	X		X	X	X	X	
Measurements of ACTH, FSH, LH, GH, amylase, lipase		X								
Coagulation profile[d] (PT/INR, PTT, fibrinogen)	X	X	X				X	X	X	
Pregnancy test[e]	X	X	X						X	
Arm 1: TTX-030 only infusion			X	X					X	
Arm 2: TTX-030 and pembrolizumab infusion			TTX-030 only	X					X	
PK blood samples[f]		X	pre & post	multiple	X	X	X	X	pre & post	
ADA blood samples		X		X	X				X	
Pharmacodynamic and correlative blood samples[g]		X		X	X		X	X	X	
Tumor biopsy[h] (optional for dose escalation, mandatory for safety lead-in/cohort expansion)	X								X (-7 days)	
Concomitant medications[i]	X	X	X	X	X	X	X	X	X	X

Assessment	Pretreatment		Loading Dose	Study Treatment Period = 2 cycles (1 cycle = 21 days)						
	Screening[a] (up to 28 days prior to loading dose)	Baseline (up to 3 days prior to loading dose)	Day -7[a]	Cycle 1					Cycle 2	
				Day 1	Day 2	Day 3	Day 8	Day 15	Day 22 (+ 2 days)	Day 29 (± 2 days)
			Pre-C1D1	C1D1	C1D2	C1D3	C1D8	C1D15	C2D1	C2D8
AEs[j]	X	X	X	X	X	X	X	X	X	X

Abbreviations: ACTH = adrenocorticotropic hormone, ADA = anti-drug antibodies, AE = adverse event, BP = blood pressure, C = cycle, CA19-9 = carbohydrate antigen, CBC = complete blood count, CEA = carcinoma embryonic antigen, CT = computed tomography, D = day, ECG = electrocardiogram, ECOG = Eastern Cooperative Oncology Group, FSH = follicle-stimulating hormone, GH = growth hormone, HgbA1c = hemoglobin A1c, HR = heart rate, INR = international normalized ratio, LDH = Lactate Dehydrogenase, LFT = liver function test, LH = luteinizing hormone, O_2 = oxygen, PD-L1 = programmed cell death protein ligand-1, PET = positron-emission tomography, PK = pharmacokinetic, PSA = prostate-specific antigen, PT = prothrombin time, PTT = partial thromboplastin time, Q = every, RP2D = recommended Phase 2 dose, TSH = thyroid stimulating hormone, W = weeks.

[a] Screening assessments and enrollment should occur prior to loading dose/first dose of TTX-030. Assessments done up to 3 days prior to each dosing visit do not need to be repeated.

[b] Subjects with solid tumors will require CT scans of the chest, abdomen, and pelvis unless contraindicated. Subjects with prostate cancer will also require bone scan. Brain scans and bone scans will be performed at screening if disease is suspected and on study as appropriate to follow disease. Subjects with lymphoma will require CT or PET-CT; however, the method for each subject's tumor assessments should remain consistent.

[c] Thyroid function tests are to be performed at baseline, at the loading dose/first dose, and then every 3 months: TSH, T3, free T4, and thyroid antibody.

[d] See Section 8.3.5. Fibrinogen is only required for eligibility.

[e] Pregnancy test is for women of childbearing potential only.

[f] Timing of PK draws: See Table 5. If using the same infusion filter line for PK draw, flushing is required.

[g] Pharmacodynamic blood draws should occur at the same time as PK blood draws and tumor biopsy when applicable. When multiple PKs are collected, collect PD with the pre-dose PK.

[h] Subjects enrolled in the safety lead-in and expansion arms will be required to have a site of disease that is safely accessible for biopsy (paired) upon enrollment. If a subject has had a biopsy within 90 days with no intervening treatment prior to the loading dose/first dose of TTX-030, that tissue can be used for this study and the subject does not need to repeat a baseline biopsy. Invasive procedures that require general anesthesia should not be performed to obtain a biopsy specimen. If a surgical procedure is performed for a clinical indication, a sample may be used for research purposes. During the course of treatment, a decision may be made to perform the on-treatment biopsy earlier than C2D1 (-7 days) if there will be no available tumor for biopsy due to a rapid clinical response. Similarly, tumor biopsy may be delayed if there is evidence of a delayed response. Accessible lesions are defined as those that can be biopsied (at screening) and are amenable to repeat biopsy at C2D1 (-7 days), unless clinically contraindicated. It is optimal to have the screening and on-treatment biopsy obtained from the same lesion if possible.

[i] Concomitant medications are to be collected from screening through 30 days after the last dose of study treatment.

[j] Adverse events (e.g., including COVID-19 symptoms) and AESIs are to be collected from the time subject provides written informed consent through 90 days after the last dose of TTX-030 or until initiation of a new systemic anticancer therapy, whichever occurs first. Positive COVID-19 test result will be collected as a medical event and submitted as SAE.

Table 2: Arms 1 and 2: Schedule of Assessments (Treatment Extension Cycles Through Post-Treatment) – Q3W Dosing Schedule (21-Day Cycle)

	21-Day Study Treatment Extension Period (Dosing Q3W)			Post-Treatment	
	Cycle 3	Cycles 4+	EOT Visit	Follow-Up Safety Contact	Follow-up Survival Contact
Assessments	Day 43 (± 2 days) C3D1	Day 64 (± 2 days) and Q21D thereafter C4D1	30 (± 9) Days After Last Dose[a]	60 and 90 (± 7) days After Last Dose	180 (± 15) Days After Last Dose
Physical examination	X	X	X		
Symptom-directed examination					
Vital signs (temperature, O_2, HR, BP, weight, height)	X	X	X		
12-lead ECG					
ECOG performance status	X	X	X		
Clinical laboratory tests (CBC, LFTs, chemistry)	X	X	X		
Coagulation profile (PT/INR, PTT)	X	X	X		
Pregnancy test[b]	X	X	X		
Tumor assessment (CT or PET-CT)		Q9W (- 7D) starting on C4D1			
Serum tumor-associated markers as applicable (eg, CEA, CA19-9, PSA)		Q9W (- 4D) starting on C4D1			
Arm 1: TTX-030 infusion	X	X			
Arm 2: TTX-030 and pembrolizumab infusion	X	X			
PK blood samples[c]	multiple	pre	X		

Assessments	21-Day Study Treatment Extension Period (Dosing Q3W)			Post-Treatment	
	Cycle 3	Cycles 4+	EOT Visit	Follow-Up Safety Contact	Follow-up Survival Contact
	Day 43 (± 2 days) C3D1	Day 64 (± 2 days) and Q21D thereafter C4D1	30 (± 9) Days After Last Dose[a]	60 and 90 (± 7) days After Last Dose	180 (± 15) Days After Last Dose
Pharmacodynamic, ADA, and correlative blood samples[d]	X	X	X		
Tumor biopsy (optional for dose escalation, mandatory for safety lead-in/cohort expansion)					
Concomitant medications[e]	X	X	X		
AEs[f]	X	X	X	X	
Post-treatment anti-cancer therapy			X	X	X
Follow-up contact			X	X	X

Abbreviations: ADA = anti-drug antibodies, AE = adverse event, BP = blood pressure, C = cycle, CA19-9 = carbohydrate antigen, CBC = complete blood count, CEA = carcinoma embryonic antigen, CT = computed tomography, D = day, ECG = electrocardiogram, ECOG = Eastern Cooperative Oncology Group, EOT = End of Treatment, HR = heart rate, INR = international normalized ratio, LFT = liver function test, O_2 = oxygen, PET = positron-emission tomography, PK = pharmacokinetic, PSA = prostate-specific antigen, PT = prothrombin time, PTT = partial thromboplastin time, Q = every, W = weeks.

[a] EOT laboratory assessment do not need to be repeated if laboratory assessments were collected in the study within the past 5 days.

[b] Pregnancy test is for women of childbearing potential only.

[c] Timing of PK draws: See Table 5. If using the same infusion filter line for PK draw, flushing is required.

[d] Pharmacodynamic blood draws should occur at the same time as PK blood draws and tumor biopsy when applicable. When multiple PKs are collected, collect PD with the pre-dose PK.

[e] Concomitant medications are to be collected from screening through 30 days after the last dose of study treatment.

[f] Adverse events (e.g., including COVID-19 symptoms) and AESIs are to be collected from the time subject provides written informed consent through 90 days after the last dose of TTX-030 or until initiation of a new systemic anticancer therapy, whichever occurs first. Positive COVID-19 test result will be collected as a medical event and submitted as SAE.

Table 3: Arm 4: Schedule of Assessments (Screening Through Treatment Period 1 (Cycle 1) – Q2W Dosing Schedule (28-Day Cycle)

	Pretreatment		Loading Dose[a]	28-Day Study Treatment Period (Dosing Every 14 Days)					
				Cycle 1					
	Screening[a] (up to 28 days prior to loading dose)	Baseline (up to 3 days prior to loading dose)	Day -7	Day 1	Day 2	Day 3	Day 8	Day 15	Day 22
Assessment			Pre-C1D1	C1D1	C1D2	C1D3	C1D8	C1D15	C1D22
Informed consent	X								
Inclusion/Exclusion	X								
Demographics	X								
Medical history & prior anti-cancer treatments	X								
Physical examination	X	X	X					X	
Symptom-directed examination				X	X		X		X
Vital signs (temperature, O_2, HR, BP, weight, height)	X	X	X	X	X		X	X	X
12-lead ECG	X								
HgbA1c	X								
ECOG performance status	X			X					
Thyroid function tests[b]		X	X						
Clinical laboratory tests (CBC, LFTs, chemistry); LDH at Screening only	X	X	X	X		X	X	X	X
Measurements of ACTH, FSH, LH, GH, amylase, lipase		X							
Coagulation profile (PT/INR, PTT, fibrinogen[c])	X	X	X				X	X	X
Pregnancy test[d]	X	X	X					X	
Tumor assessment (CT or PET-CT)[e]	X								
Serum tumor-associated markers as applicable (eg, CEA, CA19-9, PSA, CA 125)	X								

Assessment	Pretreatment		Loading Dose[a]	28-Day Study Treatment Period (Dosing Every 14 Days)					
				Cycle 1					
	Screening[a] (up to 28 days prior to loading dose)	Baseline (up to 3 days prior to loading dose)	Day -7	Day 1	Day 2	Day 3	Day 8	Day 15	Day 22
			Pre-C1D1	C1D1	C1D2	C1D3	C1D8	C1D15	C1D22
Arm 4: TTX-030 and gemcitabine + nab-paclitaxel infusion[f]			TTX-030 only	X			Gem/nab only	X	
PK blood samples[g]		X	pre & post	multiple	X	X	X	pre & post	X
Pharmacodynamic, ADA, and correlative blood samples[h]		X	X	X	X		X	X	
Tumor biopsy (optional for dose escalation, mandatory for safety lead-in/cohort expansion)[i]	X								
Concomitant medications[j]	X	X	X	X	X	X	X	X	X
AEs[k]	X	X	X	X	X	X	X	X	X

Abbreviations: ACTH = adrenocorticotropic hormone, ADA = anti-drug antibodies, AE = adverse event, BP = blood pressure, C = cycle, CA19-9 = carbohydrate antigen, CBC = complete blood count, CEA = carcinoma embryonic antigen, CT = computed tomography, D = day, ECG = electrocardiogram, ECOG = Eastern Cooperative Oncology Group, EOT = End of Treatment, FSH = follicle-stimulating hormone, GH = growth hormone, HgbA1c = hemoglobin A1c, HR = heart rate, INR = international normalized ratio, LDH = Lactate Dehydrogenase, LH = luteinizing hormone, LFT = liver function test, O_2 = oxygen, PD-L1 = programmed cell death protein ligand-1, PET = positron-emission tomography, PK = pharmacokinetic, PT = prothrombin time, PTT = partial thromboplastin time, Q = every, RP2D = recommended Phase 2 dose, TSH = thyroid stimulating hormone, W = weeks.

[a] Screening assessments and enrollment should occur prior to loading dose/first dose of TTX-030. Assessments done up to 3 days prior to each dosing visit do not need to be repeated.

[b] Thyroid function tests are to be performed at baseline, at the loading dose, and then every 3 months: TSH, T3, free T4, and thyroid antibody.

[c] See Section 8.3.5. Fibrinogen is only required for eligibility.

[d] Pregnancy test is for women of childbearing potential only.

[e] Subjects with solid tumors will require CT scans of the chest, abdomen, and pelvis unless contraindicated. Subjects with prostate cancer will also require bone scan. Brain scans and bone scans will be performed at screening if disease is suspected and on study as appropriate to follow disease. Subjects with lymphoma will require CT or PET-CT; however, the method for each subject's tumor assessments should remain consistent.

[f] Gemcitabine + nab-paclitaxel are administered on Days 1, 8, and 15 every 28 days. After the loading dose, TTX-030 will be administered every 14 days. (Note: dose modification allowed for gemcitabine 1000 mg/m^2 + nab-paclitaxel 125 mg/m^2 (e.g, Days 1 and 8 or Days 1 and 15 every 28 days) + TTX-030 every 14 days).

[g] Timing of PK draws: See Table 6. If using the same infusion filter line for PK draw, flushing is required.

[h] Pharmacodynamic blood draws should occur at the same time as PK blood draws and tumor biopsy when applicable. When multiple PKs are collected, collect PD with the pre-dose PK.

[i] Subjects enrolled in the safety lead-in and expansion arms will be required to have a site of disease that is safely accessible for biopsy (paired) upon enrollment. If a subject has had a biopsy within 90 days with no intervening lines of therapy prior to the loading dose/first dose of TTX-030, that tissue can be used for this study and the subject does not need to repeat a baseline biopsy. Accessible lesions are defined as those that can be biopsied (at screening) and are amenable to repeat biopsy on C2D1 (-7 days), unless clinically contraindicated. It is optimal to have the screening and on-treatment biopsy obtained from the same lesion if possible. Invasive procedures that require general anesthesia should not be performed to obtain a biopsy specimen. If a surgical procedure is performed for a clinical indication, a sample may be used for research purposes. During the course of treatment, a decision may be made to perform the on-treatment biopsy earlier than C2D1 (-7 days) if there is evidence to suggest a possibility that there will be no available tumor for biopsy due to a rapid clinical response. Similarly, tumor biopsy may be delayed if there is evidence of a delayed response.

[j] Concomitant medications are to be collected from screening through 30 days after the last dose of study treatment.

[k] Adverse events (e.g., including COVID-19 symptoms) and AESIs are to be collected from the time subject provides written informed consent through 90 days after the last dose of TTX-030 or until initiation of a new systemic anticancer therapy, whichever occurs first. Positive COVID-19 test result will be collected as a medical event and submitted as SAE.

Table 4: Arm 4: Schedule of Assessments (Treatment Extension Cycles Through Post-Treatment) – Q2W Dosing Schedule (28-Day Cycle)

	28-Day Treatment Extension Period (Dosing Q2W)						Post-Treatment		
	Cycle 2			Cycle 3+					
Assessment	Day 29 (+ 2 days) C2D1	Day 36 (± 2 days) C2D8	Day 43 (± 2 days) C2D15	Day 57 (± 2 days) and Every Cycle X Day 1 Thereafter C3D1	Day 64 (± 2 days) and Every Cycle X Day 8 Thereafter C3D8	Day 71 (± 2 days) and Every Cycle X Day 15 Thereafter C3D15	EOT Visit 30 (± 7) Days After Last Dose	Follow-Up Safety Contact 60 and 90 (± 7) Days After Last Dose	Follow-up Survival Contact 180 (± 15) Days After Last Dose
Physical examination	X	X	X	X	X	X	X		
Symptom-directed examination									
Vital signs (temperature, O_2, HR, BP, weight, height)	X	X	X	X	X	X	X		
12-lead ECG									
ECOG performance status	X	X	X	X	X	X	X		
Clinical laboratory tests (CBC, LFTs, chemistry)	X	X	X	X	X	X	X		
Coagulation profile (PT/INR, PTT)	X	X	X	X	X	X	X		
Pregnancy test [a]	X		X	X		X	X		
Tumor assessment (CT or PET-CT)[b]				Q8W (-7D) starting on C3D1					
Serum tumor-associated markers as applicable (eg, CEA, CA19-9)				X (Q8W)					

Assessment	28-Day Treatment Extension Period (Dosing Q2W)						Post-Treatment		
	Cycle 2			Cycle 3+					
	Day 29 (+ 2 days)	Day 36 (± 2 days)	Day 43 (± 2 days)	Day 57 (± 2 days) and Every Cycle X Day 1 Thereafter	Day 64 (± 2 days) and Every Cycle X Day 8 Thereafter	Day 71 (± 2 days) and Every Cycle X Day 15 Thereafter	EOT Visit 30 (± 7) Days After Last Dose	Follow-Up Safety Contact 60 and 90 (± 7) Days After Last Dose	Follow-up Survival Contact 180 (± 15) Days After Last Dose
	C2D1	C2D8	C2D15	C3D1	C3D8	C3D15			
Arm 4: TTX-030 and gemcitabine + nab-paclitaxel infusion[c]	X	Gem/nab only	X	X	Gem/nab only	X			
PK blood samples [d]	multiple		pre	pre		pre	X		
ADA blood samples	X			X		X	X		
Pharmacodynamic and correlative blood samples[e]	X			X		X	X		
Tumor biopsy (optional for dose escalation, mandatory for safety lead-in/cohort expansion)[f]	X (-7 days)								
Concomitant medications[g]	X		X	X	X	X	X		
AEs[h]	X		X	X	X	X	X	X	
Post-treatment anti-cancer therapy							X	X	X
Follow-up contact							X	X	X

Abbreviations: ADA = anti-drug antibodies, AE = adverse event, BP = blood pressure, C = cycle, CA19-9 = carbohydrate antigen, CBC = complete blood count, CEA = carcinoma embryonic antigen, CT = computed tomography, D = day, ECG = electrocardiogram, ECOG = Eastern Cooperative Oncology Group, EOT = End of Treatment, HR = heart rate, INR = international normalized ratio, LFT = liver function test, O_2 = oxygen, PET = positron-emission tomography, PK = pharmacokinetic, PSA = prostate-specific antigen, PT = prothrombin time, PTT = partial thromboplastin time, Q = every, W = weeks.

[a] Pregnancy test is for women of childbearing potential only.

[b] Subjects with solid tumors will require CT scans of the chest, abdomen, and pelvis unless contraindicated. Subjects with prostate cancer will also require bone scan. Brain scans and bone scans will be performed at screening if disease is suspected and on study as appropriate to follow disease. Subjects with lymphoma will require CT or PET-CT; however, the method for each subject's tumor assessments should remain consistent.

[c] Gemcitabine + nab-paclitaxel are administered on Days 1, 8, and 15 every 28 days. After the loading dose, TTX-030 will be administered every 14 days. Cycle 4 and beyond also requires a dosing visit at Days 1, 8, and 15 for gemcitabine/nab-paclitaxel and Day 15 for TTX-030. (Note: dose modification allowed for gemcitabine 1000 mg/m^2 + nab-paclitaxel 125 mg/m^2 (e.g., Days 1 and 8 or Days 1 and 15 every 28 days) + TTX-030 every 14 days).

[d] Timing of PK draws: See Table 6. If using the same infusion filter line for PK draw, flushing is required.

[e] Pharmacodynamic blood draws should occur at the same time as PK blood draws and tumor biopsy when applicable. When multiple PKs are collected, collect PD with the pre-dose PK.

[f] Subjects enrolled in the safety lead-in and expansion arms will be required to have a site of disease that is safely accessible for biopsy (paired) upon enrollment. If a subject has had a biopsy within 90 days with no intervening lines of therapy prior to loading dose/first dose of TTX-030, that tissue can be used for this study and the subject does not need to repeat a baseline biopsy. Accessible lesions are defined as those that can be biopsied (at screening) and are amenable to repeat biopsy on C2D1 (-7 days), unless clinically contraindicated. It is optimal to have the screening and on-treatment biopsy obtained from the same lesion if possible. Invasive procedures that require general anesthesia should not be performed to obtain a biopsy specimen. If a surgical procedure is performed for a clinical indication, a sample may be used for research purposes. During the course of treatment, a decision may be made to perform the on-treatment biopsy earlier than C2D1 (-7 days) if there is evidence to suggest a possibility that there will be no available tumor for biopsy due to a rapid clinical response. Similarly, tumor biopsy may be delayed if there is evidence of a delayed response.

[g] Concomitant medications are to be collected from screening through 30 days after the last dose of study treatment.

[h] Adverse events (e.g., including COVID-19 symptoms) and AESIs are to be collected from the time subject provides written informed consent through 90 days after the last dose of TTX-030 or until initiation of a new systemic anticancer therapy, whichever occurs first. Positive COVID-19 test result will be collected as a medical event and submitted as SAE.

Table 5: PK Sampling Intervals During Treatment (21-day treatment schedule Q3W)

Visit Identifier	Pre-C1D1 Loading Dose		Cycle 1								Cycle 2		Cycle 3				Cycles 4+	EOT
Study Day	-7		1				2	3	8	15	22 (+ 2)		43 (± 2)				64 (± 2) and Q21D thereafter	30 days after last Tx
Hours Before/After Dose	0[a]	EOI[b]	0[a]	EOI[b]	4	8	24	48	168	336	0[a]	EOI[b]	0[a]	EOI[b]	4	8	0[a]	0
PK blood serum sampling[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: C = cycle, D = day(s), EOI = end of infusion, EOT = End of Treatment, PK = pharmacokinetics, Q = every, Tx = treatment, W = weeks.

[a] Predose sample collection

[b] Sample to be collected at the EOI; specifically, collect the PK sample 15 ± 5 minutes after infusion of TTX-030 has completed.

[c] Blood will be collected for determining the concentration of TTX-030: Loading Dose (pre-C1D1): predose (if not already drawn with baseline assessments) and at EOI (15 ± 5 minutes after end of TTX-030 infusion); Dose 2 (C1D1): predose, EOI (15 ± 5 minutes after end of TTX-030 infusion), and 4, 8, 24, and 48 hours (± 30 minutes) from start of TTX-030 infusion and on Day 8 and Day 15; Dose 3 (C2D1): predose and at EOI (15 ± 5 minutes after end of TTX-030 infusion); Dose 4 (C3D1): predose and at EOI (15 ± 5 minutes after end of TTX-030 infusion), and 4 and 8 hours (± 30 minutes) from start of TTX-030 infusion; Dose 5 and beyond (Cycles 4+): predose. Detailed instructions for sample collection, processing, and shipment will be provided in a separate laboratory manual.

Table 6: PK Sampling Intervals During Treatment (28-day treatment cycle, Q2W)

Visit Identifier	**Pre-C1D1 Loading Dose**		**Cycle 1**										**Cycle 2**					**Cycles 3+**	**EOT**
Study Day	**-7**		**1**				**2**	**3**	**8**	**15**		**22**	**29 (+ 2)**				**43 (± 2)**	**57 (± 2) and Q28D thereafter**	**30 days after last Tx**
Hours Before/After Dose	**0[a]**	**EOI[b]**	**0[a]**	**EOI[b]**	**4**	**8**	**24**	**48**	**168**	**0[a]**	**EOI[b]**	**0**	**0[a]**	**EOI[b]**	**4**	**8**	**0[a]**	**0[a]**	**0**
PK blood serum sampling[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: C = cycle, D = day(s), EOI = end of infusion, EOT = End of Treatment, PK = pharmacokinetics, Q = every, Tx = treatment, W = weeks.

[a] Predose sample collection

[b] Sample to be collected at the EOI; specifically, collect the PK sample 15 ± 5 minutes after infusion of TTX-030 has completed.

[c] Blood will be collected for determining the concentration of TTX-030: Loading Dose (pre-C1D1): predose (if not already drawn with baseline assessments), and at EOI (15 ± 5 minutes after end of TTX-030 infusion); Dose 2 (C1D1): predose, EOI (15 ± 5 minutes after end of TTX-030 infusion), and 4, 8, 24, and 48 hours (± 30 minutes) from start of TTX-030 infusion and on Day 8; Dose 3 (C1D15): predose and at EOI (15 ± 5 minutes after end of TTX-030 infusion) and on Day 22; Dose 4 (C2D1): predose, EOI (15 ± 5 minutes after end of TTX-030 infusion), and 4 and 8 hours (± 30 minutes) from start of TTX-030 infusion; Dose 5 (C2D15) and beyond (Cycles 3+): predose. Detailed instructions for sample collection, processing, and shipment will be provided in a separate laboratory manual.

2. Introduction

Adenosine Pathway

Purinergic signaling plays a key role in regulation of immune response in the tumor microenvironment (TME). eATP released during cell death and/or apoptosis serves as an immune cell activator through interaction with and downstream signaling of P2 purinergic receptors (Yip et al. 2009; Schenk et al. 2008; Bours et al. 2006). Pro-inflammatory adenosine triphosphate (ATP) can be converted to adenosine via an enzymatically controlled phosphohydrolytic cascade, leading to the accumulation of adenosine in the TME. Adenosine exerts its suppressive function by binding to A2A receptors (A2AR) on multiple immune cells such as phagocytes, dendritic cells, natural killer cells, T cells, and B cells and inhibiting their pro-inflammatory properties (Eppell et al. 1989; Haskó et al. 1996; Haskó et al. 2000; Zhang et al. 2004; Lappas et al. 2005; Minguet et al. 2005; Deaglio et al. 2007; Ohta et al. 2009).

CD39

CD39, the cell surface-located prototypic member of the ectonucleoside triphosphate diphosphohydrolase (ENTPDase) family, is an integral membrane protein that is responsible for phosphohydrolysis of eATP to yield adenosine diphosphate (ADP) and adenosine monophosphate (AMP) (Kaczmarek et al. 1996; Dwyer et al. 2007). AMP is further converted to adenosine via another ecto-enzyme, CD73 (ecto-5, ecto-nucleotidase). CD39 and CD73 have a major impact on the dynamic balance of pro-inflammatory eATP and ADP nucleotides compared with the immunosuppressive adenosine nucleosides (Takenaka et al. 2016). Moreover, evidence supports CD39 and CD73 ectonucleotidases having an involvement in the regulation of other aspects of T-cell biology, such as naïve cell homeostatic survival, memory cell survival, and differentiation (Bono et al. 2015). CD39 is constitutively expressed on immune cell populations, such as B cells, natural killer cells, dendritic cells, Langerhans cells, monocytes, macrophages, mesangial cells, neutrophils, and T_{reg} as well as normal endothelial cells and platelets (Koziak et al. 1999).

In the TME, CD39 expression is most prominent on T_{reg} and myeloid derived suppressor cells (Whiteside 2015). Additionally, exhausted CD8+ T cells upregulate CD39 in the context of the tumor, which is accompanied by reduced functional capacity (Canale et al. 2018). CD39 has been recognized as an activation marker and is increasingly appreciated as a regulatory marker (Zhao et al. 2017).

Increasing or inhibiting CD39 can interfere with the abnormal pathophysiological process of disorders, especially inflammation and tumor (Zhao et al. 2017).

CD39 and Tumor Activity

One mechanism by which immunotherapies modulate an individual's immune system is through the blocking of suppressive pathways, thus making the adenosine pathway a promising therapeutic target in this area of clinical research. Reportedly, both CD39 and CD73 are elevated in blood neoplasias such as leukemia and lymphoma, as well as in multiple solid tumor settings (Bastid et al. 2013). In solid tumors, ATP is abundantly released in the extracellular space, achieving concentrations more than a thousand times higher than in healthy tissues. This is

primarily due to cell death in the tumor core, metabolic or hypoxic stress, and pro-inflammatory signals that stimulate active export of ATP (Pellegatti et al. 2008; Di Virgilio et al. 2016). In addition, immunogenic cell death mediated by chemotherapy is in part mediated by increasing extracellular ATP. Tumors are proficient at converting ATP into adenosine via CD39 and CD73 on malignant cells, regulatory immune cells, and the vasculature. These ectonucleotidases modulate purinergic signaling by scavenging mainly pro-inflammatory ATP and generating immunosuppressive adenosine (Allard et al. 2017).

CD39, the main rate-limiting enzyme of this cascade, has an important role in tumor progression. Blockade of CD39 enzymatic activity may then stimulate anti-tumor immunity across a wide range of tumors by preventing the production of immunosuppressive adenosine and by promoting the accumulation of ATP in the TME. CD39 can be viewed as an immunological switch that shifts ATP-driven pro-inflammatory immune cell activity toward an anti-inflammatory state mediated by adenosine and is, therefore, a unique therapeutic target for oncology indications (Antonioli et al. 2014; Cai et al. 2016).

TTX-030

TTX-030 is a novel, fully human anti-CD39 antibody that inhibits CD39 ATPase enzymatic function allosterically with sub-nanomolar affinity and potency. The constant domain of TTX-030 is a hinge stabilized (S228P) human immunoglobulin (Ig)G4 containing the S228P mutation, which reduces antigen-binding fragment arm exchange by stabilizing the disulfides in the core-hinge of the IgG4 molecule, which has low/no effector function.

TTX-030 is specific to CD39 and does not bind to other ENTPDase family member proteins. This antibody does bind to and inhibit cynomolgus monkey CD39 but is not cross-reactive with murine CD39. Trishula Therapeutics, Inc. (Tizona Therapeutics, Inc.'s spin-out company) (hereafter referred to as Trishula) has conducted studies demonstrating that TTX-030 binds to CD39+ cancer cell lines and primary human and cynomolgus monkey monocytes and T cells with high affinity. TTX-030 specifically and potently inhibits CD39-driven processing of ATP by tumor and immune cells and by CD39+ tumor tissues, as demonstrated by a tumor tissue slice immunohistochemistry assay. In addition to preserving pro-inflammatory ATP by inhibiting CD39 ATPase activity, TTX-030 inhibits downstream adenosine production in SK-MEL-28 melanoma cell line. Functionally, TTX-030 reverses adenosine-driven suppression of proliferation of activated CD4+ and CD8+ human T cells in a dose-dependent manner. Inhibition of CD39 by TTX-030 in CD3/CD28 stimulated peripheral blood mononuclear cells (PBMCs) in the presence of exogenous ATP increased proliferation of both CD4+ and CD8+ T cells from multiple donors. TTX-030 incubation with stimulated PBMCs led to increased secretion of pro-inflammatory cytokines interferon (IFN)-γ, interleukin (IL)-2, and tumor necrosis factor-α. Additionally, TTX-030 reversed adenosine-driven suppression of T-cell IFN-γ responses in cytomegalovirus (CMV) peptide recall assay (see also Section 2.2.1.3.3).

2.1. Study Rationale

The goal of this first-in-human (FIH) Phase 1/1b study is to evaluate the safety, tolerability, and preliminary anti-tumor activity of intravenous (IV) TTX-030 as monotherapy or in combination regimens in adults with unresectable or metastatic solid tumor malignancy, including

relapsed-refractory lymphoma. There are compelling preclinical data to support the hypothesis that CD39-mediated reduction of adenosine triphosphate (ATP) and accumulation of adenosine both play a central role in establishing and maintaining the immunosuppressive microenvironment within solid tumors. CD39 expression is associated with worse outcomes in several cancer types. The enzymatic inhibition of CD39 maintains high levels of pro-inflammatory extracellular ATP (eATP), leading to immune activation and significant anti-tumor immune responses. The CD39 pathway contributes to T-cell exhaustion and limits the immunogenicity of chemotherapy, which provides the rationale for combining CD39 inhibition with checkpoint blockade and conventional cytotoxic therapies. Targeting CD39 is a rational strategy for the treatment of patients with cancer. TTX-030, as a single agent or in combination, is expected to have a manageable safety profile, thereby providing additional therapeutic options for subjects whose malignancies are otherwise refractory to or relapsed on current standard of care therapies. The indications selected were defined based on the following criteria: expression of CD39 on TIL, expression of CD39 on tumor cells, and tumor types that are amenable to be combined with chemotherapy or anti-PD-1 therapy.

While impressive and durable responses are observed in subsets of patients treated with anti-PD-1 agents, response rates are rarely > 50%, and for most patients, a response is short lasting. Thus, a synergistic strategy may be beneficial for patients. Co-expression of PD-1 and CD39 is highly prevalent on TILs and marks exhausted effector T-cell subsets in multiple tumor types (Canale et al. 2018; Trishula data on file Study No. 18-006-TRL). The rationale for combining with an anti-PD-1 antibody is that CD39 inhibition and subsequent ATP accumulation and adenosine reduction in the TME may make the tumor more susceptible to anti-PD-1 therapy. This combination strategy is supported by the in vivo data generated in using an anti-murine CD39 antibody B66 in multiple syngeneic tumor models (Section 2.2.1.4). Combining anti-CD39 therapy with an anti-PD-1 antibody in an MC38 syngeneic colorectal tumor model resulted in a significant decrease in tumor growth compared with the control or either monotherapy. Anti-PD-1 treatment of SM1WT1, which is generally refractory to immunotherapies, including anti-PD-1, shows marked tumor growth inhibition when anti-PD-1 treatment is combined with an antibody capable of inhibiting enzymatic function of CD39, suggesting that the latter treatment sensitizes an otherwise refractory tumor to checkpoint blockade. These findings are consistent with previously reported data in $CD39^{-/-}$ animals that suggested that anti-PD-1 treatment of MCA205 resulted in a more pronounced tumor growth inhibition and increased number of complete responders in $CD39^{-/-}$ mice compared with the same treatment in wild-type mice (Lapierre et al. 2016).

Similarly, treatment with oxaliplatin either alone or in combination with an anti-PD-1 antibody resulted in regression of MCA205 tumors in $CD39^{-/-}$ mice but not in wild-type mice (Boyd-Tressler et al. 2014). A study by Michaud et al. 2012 compared the effects of treatment with the anthracycline mitoxantrone on tumor growth of MCA205 tumor cells versus MCA205 expressing mouse CD39. Tumor cells were pretreated with mitoxantrone prior to injection into wild-type mice – only MCA205 tumor cells expressing mouse CD39 grew (Michaud et al. 2012). Chemotherapy drugs such as oxaliplatin, cisplatin, and mitoxantrone have been shown to induce the release of ATP from dying tumor cells (Boyd-Tressler et al. 2014); CD39 then processes ATP in concert with CD73 resulting in the generation of immune-suppressive adenosine. Inhibition of CD39 prevents phosphohydrolytic processing and maintains high levels of

pro-inflammatory ATP in the TME and can lead to immune activation. These data support the potential synergy of the combination of chemotherapy and TTX-030.

2.2. Background

2.2.1. Nonclinical Data

2.2.1.1. Non-human Primate Pharmacokinetic Study with TTX-030

Multiple non-Good Laboratory Practice (GLP) pharmacokinetic (PK) studies were conducted with TTX-030, including a single-dose study at 10 mg/kg IV and multiple-dose studies at 30 mg/kg and 100 mg/kg. Titers of TTX-030 were measured using an antigen-down enzyme-linked immunosorbent assay (ELISA) method and used for PK parameter calculations. Linear PK was observed for doses starting at 10 mg/kg, 30 mg/kg, and 100 mg/kg. In addition to PK parameters, blood samples were collected for assessment of treatment-related clinical observations and clinical pathology parameters (hematology, clinical chemistry, and coagulation). There were no treatment-related effects noted for clinical observations or for the hematology and clinical chemistry parameters collected in any of the cynomolgus monkeys in either the single- or multiple-dose cohorts.

[redacted]

2.2.1.2. Non-human Primate Good Laboratory Practice Toxicology

Since TTX-030 has equivalent binding and functional potency on both human and cynomolgus monkey CD39, testing TTX-030 in healthy male and female cynomolgus monkeys allowed for the characterization of effects of TTX-030 on immune cells, endothelial cells, and other tissues.

A multiple-dose 28-day GLP toxicology study with 28-day recovery period was performed in cynomolgus monkeys, and no TTX-030-related findings were observed. The animals were IV dosed with 0, 30, or 100 mg/kg of TTX-030 once per week for 4 weeks (5 doses total). The main study groups consisted of 3 males and 3 females with an additional 2 males and 2 females included as part of the control and high-dose recovery groups.

Three animals per sex from each group were sacrificed at the end of the dosing period. The 2 remaining animals of each sex from each group were allowed to recover for 4 additional weeks and then were sacrificed. The 4-week recovery period provides approximately 5 to 6 half-lives for TTX-030 elimination, based on the preliminary PK measured in the pilot tolerability study. The recovery cohorts served to determine whether any toxicities observed at the end of the 30-day dosing period are reversible.

Data collected from this toxicology study are summarized in Table 7. Gross observations on animal health (body weight, food consumption, and behavior) were collected and a battery of serological analyses from blood samples (hematology, blood chemistry, toxicokinetics, ADA, and cytokine analysis) were performed throughout the course of the study. In addition, a set of safety pharmacology assessments were conducted, including evaluation of heart rate and blood pressure, body temperature measurements, quality and rate of respiration, ophthalmology examinations, and neurological/central nervous system behavior evaluations. At sacrifice, a comprehensive examination of all animals (necropsy, urinalysis, and histopathology) was performed. The groups were compared using a parametric dose-response curve or non-parametric analysis of variance (ANOVA) method, as appropriate.

Table 7: Procedures and Parameters for the 28-Day GLP Toxicology Study of the Administration of TTX-030 to Male and Female Cynomolgus Monkeys

Procedure/Parameters	Time Points
Mortality/morbidity observations	All animals: Twice daily, beginning Day -7 and continuing through the End of Study
Cage-side observations	All animals: Twice daily, beginning Day -14 and continuing through the End of Study
Postdose observations	All animals: For 1 continuous hour following dose
Body weight	All animals: Twice pre-study (Weeks -2 and -1) and weekly thereafter starting on Day 7
Food consumption (qualitative)	All animals: Daily beginning Day -14 and continuing through the End of Study
Clinical pathology (hematology)	All animals: Predose on Days 1, 15, and 29
Clinical pathology (serum chemistry, and coagulation [PT/INR, aPTT])	All animals: Predose and postdose on Days 1, 15, and 29 Recovery animals: Day 56
TK	All animals: Day 1 predose and 0.5, 4, 12, 24, 96, and 168 hours postdose; Day 15: predose; Day 29: predose and 0.5, 4, 12, 24, 96, and 168 hours postdose
ADA	All animals: Day -2 and predose on Days 15 and 29 Recovery animals: At the end of recovery period on Day 56
Cytokines	All animals: Predose, and multiple time points in 24 hours following first and last dose

Procedure/Parameters	Time Points
Electrocardiograms	All animals: Once pretreatment (Week -2), on Day 1 (1 to 4, 12, and 24 hours postdose), and at Week 4 (1 to 4, 12, and 24 hours postdose) Recovery animals: At the end of the recovery period prior to necropsy
Blood pressure measurements	All animals: Once during acclimation and at multiple time points within 24 hours after first and last dose
Respiratory examinations	All animals: By visual assessment pretreatment (Week -1), predose, and 2, 6, and 24 hours postdose on Days 1, 8, 15, and 22 Recovery animals: At the end of the recovery period prior to necropsy
Neurological / CNS behavior examinations	All animals: Once pretreatment (Week -1), on Day 1 (1 to 4 hours postdose), and on Day 22 (predose and 1 to 4 hours postdose) Recovery animals: At the end of the recovery period prior to necropsy
Body temperature	All animals: Using infrared monitor, pretreatment (Week -1), and predose and 2, 6, and 24 hours postdose on Days 1, 8, 15, and 22 Recovery animals: At the end of the recovery period prior to necropsy
Ophthalmology examinations	All animals: Assessed pretreatment and during Week 4 Recovery animals: Additionally, at the end of the recovery period prior to necropsy Examinations were performed by a board-certified veterinarian ophthalmologist
Urinalysis	All animals: At necropsy
Necropsy	Full necropsy of 12 main study animals on Day 30 and 8 recovery animals on Day 56
Organ weights	Standard organs measured
Histopathology	Full histopathologic evaluation on all animals

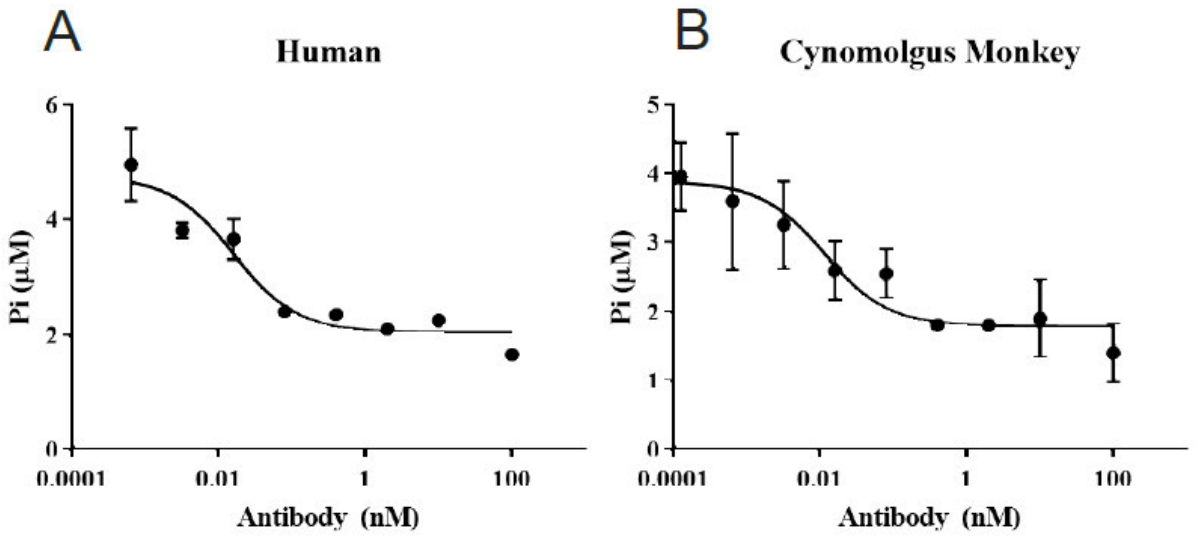
Abbreviations: ADA = anti-drug antibodies, aPTT = activated partial thromboplastin time, CNS = central nervous system, INR = international normalized ratio, PT = prothrombin time, TK = toxicokinetics.
Trishula data on file Study No. 18-002-TOX.

2.2.1.3. Modeling and In Vitro Studies

2.2.1.3.1. Enzymatic Inhibition of CD39 by TTX-030

TTX-030 was evaluated for its ability to inhibit enzymatic activity of endogenous CD39 expressed on human and cynomolgus monkey primary cells and multiple tumor cell lines. CD39 expressing cells were pretreated with TTX-030 before administering ATP; phosphate release resulting from enzymatic phosphohydrolysis was subsequently measured. TTX-030 inhibited CD39 activity in multiple cell types with sub-nanomolar half-maximal inhibition concentration (IC_{50}) values, ranging from 51.4% inhibition in primary human artery endothelial cells to 85.3% in the SK-MEL-28 cell line. TTX-030 also inhibited activity of CD39 in human monocytes and in cynomolgus monkey monocytes with similar IC_{50} values (Figure 2).

Figure 2: Short-Term Inhibition of CD39 by TTX-030 on Human and Cynomolgus Monocytes



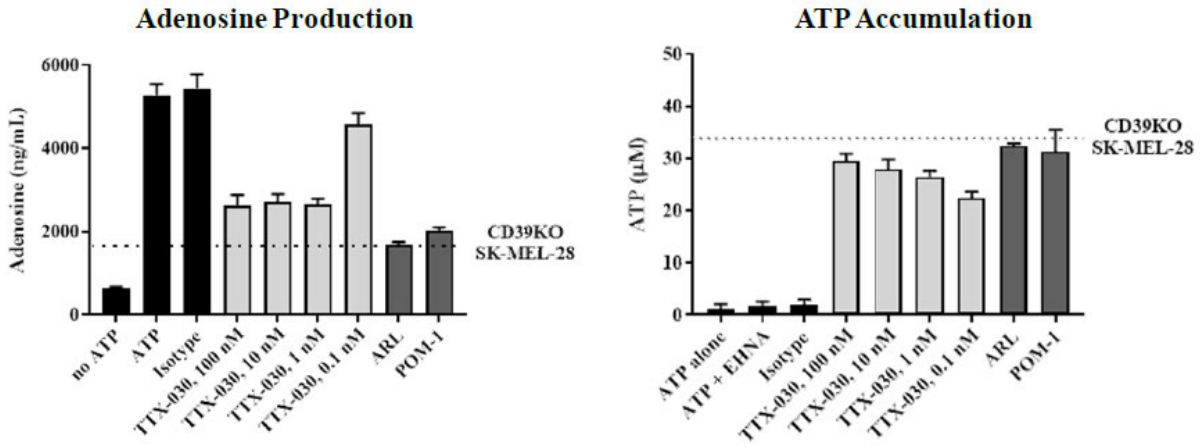
Abbreviation: IC_{50} = half-maximal inhibition concentration.

Trishula data on file Study No. 17-001-IVT: TTX-030 inhibits CD39 enzymatic activity on human and cynomolgus monkey monocytes. Inhibition curves of ATPase activity by TTX-030 on human (A) and cynomolgus monkey (B) monocytes (average and standard deviation are shown, n = 2). Enzymatic inhibition on human monocytes was calculated at 72.0 ± 1.1% with an IC_{50} of 0.02 nM. For cynomolgus monocytes, enzymatic inhibition was 98.7 ± 8.8%, with IC_{50} of 0.01 nM.

2.2.1.3.2. TTX-030 Reduces Adenosine and Preserves ATP in SK-MEL-28 Cancer Cell Line

To assess functional effect of CD39 inhibition by TTX-030, the antibody was evaluated for its ability to inhibit CD39-mediated adenosine production on tumor cell lines. The melanoma cell line SK-MEL-28 (CD39 and CD73 positive) was pretreated with TTX-030 and adenosine production following ATP addition was measured by liquid chromatography-tandem mass spectrometry. TTX-030 (100 nM) inhibited 73.0 ± 7% of CD39-mediated adenosine production by SK-MEL-28 cells compared with cells treated with ATP only. TTX-030 also preserved ATP levels in the supernatant of SK-MEL-28 cells by preventing ATP processing by CD39 (Figure 3). ATP was not hydrolyzed by SK-MEL-28 cells that had CD39 expression ablated by Clustered Regularly Interspaced Short Palindromic Repeats (designated as CD39KO). Additionally, adenosine levels were lower in supernatants of CD39KO cells, indicating that CD39 is critical for ATP processing to adenosine by SK-MEL-28. These data demonstrate that inhibition of CD39 enzymatic activity by TTX-030 is sufficient to impact downstream immunosuppressive adenosine production while simultaneously preserving pro-inflammatory ATP.

Figure 3: TTX-030 Reduces CD39-Mediated Adenosine Production and Preserves ATP in SK-MEL-28 Cells

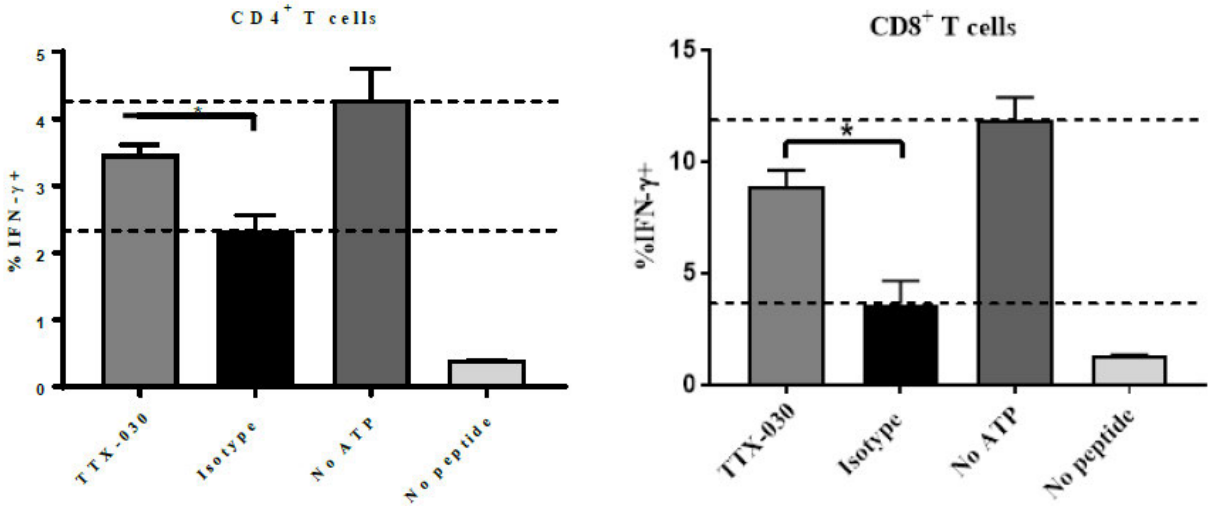


Abbreviations: ATP = adenosine triphosphate, EHNA =41rythrono-9-(2-hydroxy-3-nonyl)adenine.
Trishula data on file Study No. 17-012-IVT: Average and standard deviation of adenosine production from SK-MEL-28 (n = 3) and average and standard deviation of ATP accumulation in SK-MEL-28 (n = 6). Dotted line denotes the adenosine production and ATP accumulation from CD39KO SK-MEL-28 cells. Adenosine was measured by liquid chromatography-tandem mass spectrometry. ATP was measured by EnzyLight Kit. The nonspecific small molecule inhibitors ARL67156 and POM-1 were used as positive controls. EHNA is an adenosine deaminase inhibitor.

2.2.1.3.3. TTX-030 Induces IFN-γ in CMV Recall Assay

Human PBMCs express both CD39 and CD73 and stimulation of T-cell proliferation in the presence of ATP results in generation of immunosuppressive adenosine. Adenosine can lead to decrease in pro-inflammatory cytokine release by activated T cells. The effect of TTX-030 on T-cell response was measured using human PBMCs that were stimulated with a mixture of immunogenic CMV peptides. Following an initial recall stimulation period of 5 days using PBMCs, T cells were purified and rested overnight, prior to treatment with TTX-030. T cells were then re-stimulated with CMV peptides in the presence of autologous CD39+ Epstein Barr virus-transformed lymphoblastoid B cell lines (B-LCLs) and ATP (Figure 4). Addition of ATP in this system led to suppression of IFN-γ responses by CD4+ and CD8+ T cells, which was nearly completely reversed by TTX-030 treatment (25 µg/mL).

Figure 4: TTX-030 Reverses Suppression of IFN-γ Response in a CMV Recall Assay



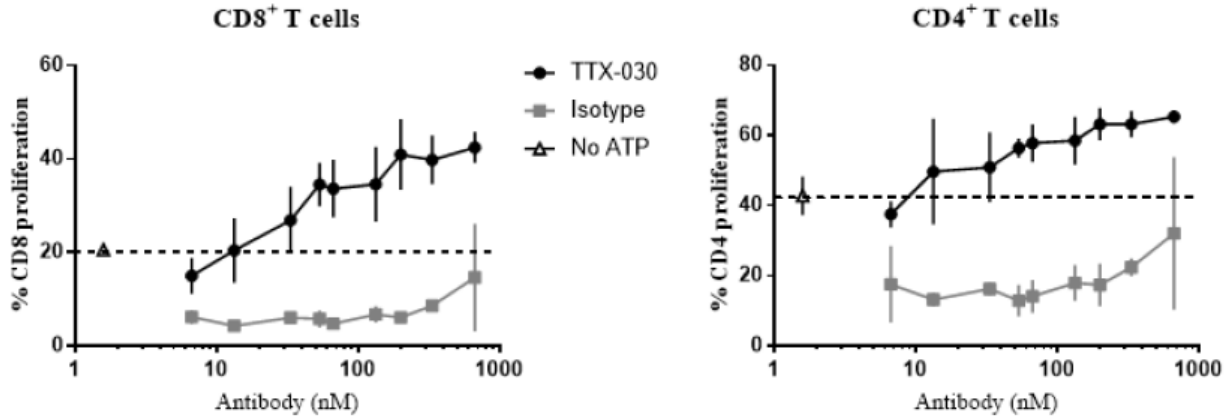
Abbreviations: ATP = adenosine triphosphate, B-LCL = Epstein Barr virus-transformed lymphoblastoid B cell line, CMV = cytomegalovirus, IFN = interferon, PBMC = peripheral blood mononuclear cell.

Trishula data on file Study No. 17-011-IVT: TTX-030 reverses adenosine-driven suppression of IFN-γ response in T-cell CMV recall assay. Human PBMCs were stimulated with CMV peptides and rested prior to re-stimulation with the same peptides in the presence of ATP, autologous B-LCL, and TTX-030. Levels of IFN-γ produced by CD4+ and CD8+ T cells were analyzed by intracellular cytokine staining (* $P < 0.05$ by t test).

2.2.1.3.4. TTX-030 Induces T Cell Proliferation and Cytokine Response

The impact of TTX-030 treatment on CD39-mediated adenosine production in PBMCs was measured using functional readouts of CD4+ and CD8+ T-cell proliferation and cytokine expression. Addition of ATP to activated PBMCs decreased proliferation, which was reversed through addition of TTX-030 in a dose-dependent manner compared with isotype control and untreated T cells (Figure 5). Similar results were obtained with TTX-030 without the addition of exogenous ATP (data are not presented).

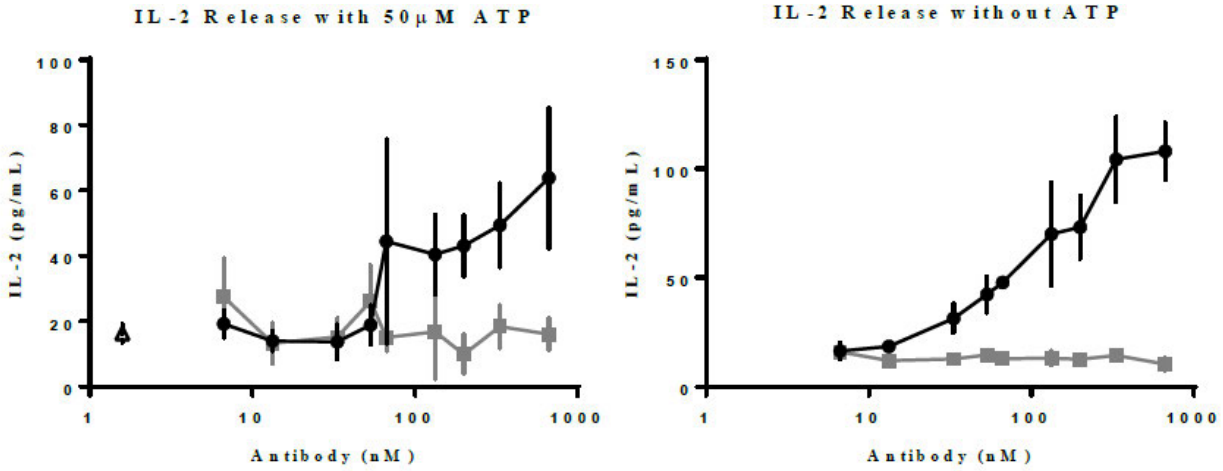
Figure 5: TTX-030 Increases the Proliferation of Stimulated CD8+ and CD4+ T Cells in the Presence of ATP



Abbreviations: ATP = adenosine triphosphate, PBMC = peripheral blood mononuclear cell.
Trishula data on file Study No. 17-014-IVT: PBMCs isolated from human donor were fluorescently labeled, stimulated with anti-CD3/anti-CD28 antibody cocktail, and incubated with TTX-030 and ATP for 96 hours. Proliferation of CD4+ and CD8+ T cells was assessed by flow cytometry gating on the specific T-cell populations. Dotted line depicts baseline level of proliferation without the addition of exogenous ATP or antibody.

The effects of TTX-030 treatment on pro-inflammatory cytokines secretion by anti-CD3/anti-CD28 activated PBMC was measured using a multi-cytokine electrochemiluminescent assay. TTX-030 increased IL-2 secretion by stimulated PBMCs compared with isotype control in a dose-dependent manner with a half-maximal effective concentration (EC_{50}) of 129.10 ± 43.99 nM (19.37 µg/mL) when 50 µM ATP was added to the culture and an EC_{50} of 155.20 ± 24.47 nM (23.30 µg/mL) without the addition of exogenous ATP (Figure 6).

Figure 6: TTX-030 Induces IL-2 Release by Stimulated PBMCs in Dose-Dependent Manner



Abbreviations: ATP = adenosine triphosphate, IL-2 = interleukin-2, PBMC = peripheral blood mononuclear cell.
Trishula data on file Study No. 17-014-IVT: TTX-030 induces dose-dependent IL-2 production by anti-CD3/anti-CD28 stimulated PBMC. Assay performed with addition of 50 µM ATP (left) and without exogenous ATP (right) is shown. TTX-030 indicated by black squares while gray squares indicate isotype control. Data are representative of 3 independent PBMC donors.

2.2.1.4. In Vivo Studies: Mouse

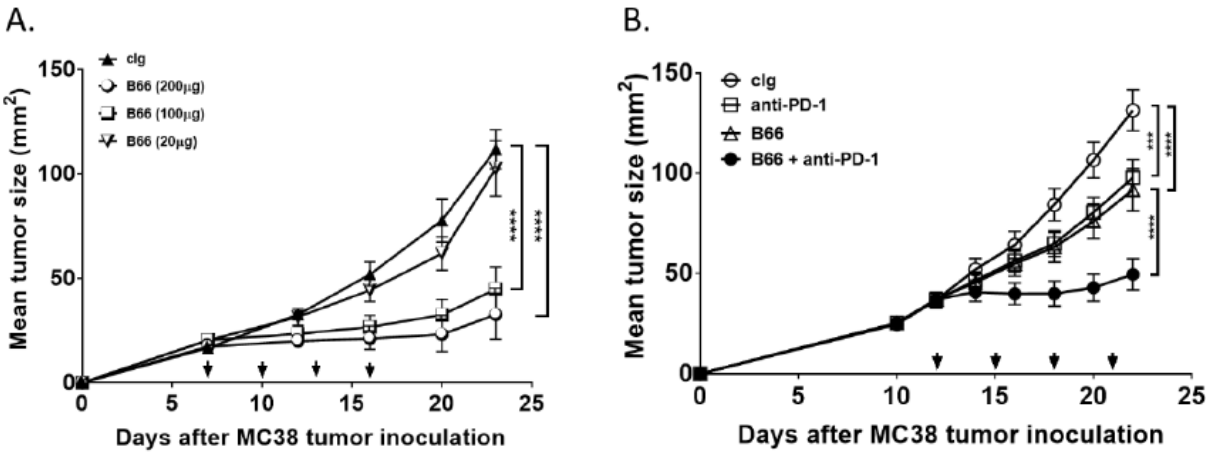
The impact of CD39 on tumor growth and anti-tumor immunity has previously been studied using CD39$^{-/-}$ knockout mice. Published data suggest that growth of syngeneic tumors, including MC38 and B16F10 (Sun et al. 2010; Feng et al. 2011), is reduced in these mice. These effects are more pronounced when combined with therapeutic intervention via anti-programmed cell death ligand-1 (PD-L1) (Lapierre et al. 2016) or chemotherapeutic agents (Michaud et al. 2012; Lapierre et al. 2016). In both cases, combinatorial efficacy was observed in tumor-bearing CD39$^{-/-}$ mice but not in matched wild-type C57BL/6 animals.

To test the effect of therapeutic inhibition of CD39 enzymatic activity on tumor growth and to complement findings from the CD39$^{-/-}$ mouse studies, Trishula has generated antibody clone B66, an antibody specific for murine CD39 with the ability to inhibit CD39-mediated ATP hydrolysis (Trishula data on file Study No. 18-001-IVT). This antibody was used to characterize the role of pharmacological inhibition of CD39 activity on tumor growth. B66 was used both as a single agent and in combination with anti-PD-1 in the syngeneic MC38 mouse tumor model (Figure 7). In single-agent studies, treatment with B66 significantly decreased tumor growth of a subcutaneous murine colorectal tumor cell line in a dose-dependent fashion (Figure 7A). Therefore, inhibition of CD39 enzymatic activity in the MC38 syngeneic mouse tumor model by B66 monoclonal antibody (mAb) is sufficient to decrease tumor growth as a single agent. These data agree with the previous findings from MC38 tumors grown in the CD39$^{-/-}$ mice.

Given the single-agent efficacy observed with B66, a suboptimal dosing strategy with a delayed treatment schedule (100 µg/dose on Days 12, 15, 18, and 21) was employed to maximize the window for seeing therapeutic combination effects (Figure 7B). Employing this treatment

regimen, efficacy was reduced but was still significant versus isotype control for B66 monotherapy. Additionally, the anti-tumor effect was similar to that seen for anti-PD-1 treatment alone. In combination, anti-PD-1 and B66 resulted in a highly significant increase in anti-tumor efficacy compared with either monotherapy or isotype control.

Figure 7: Anti-Mouse CD39 Antibody B66 Inhibits MC38 Tumor Growth as Single Agent and in Combination with Anti-PD-1



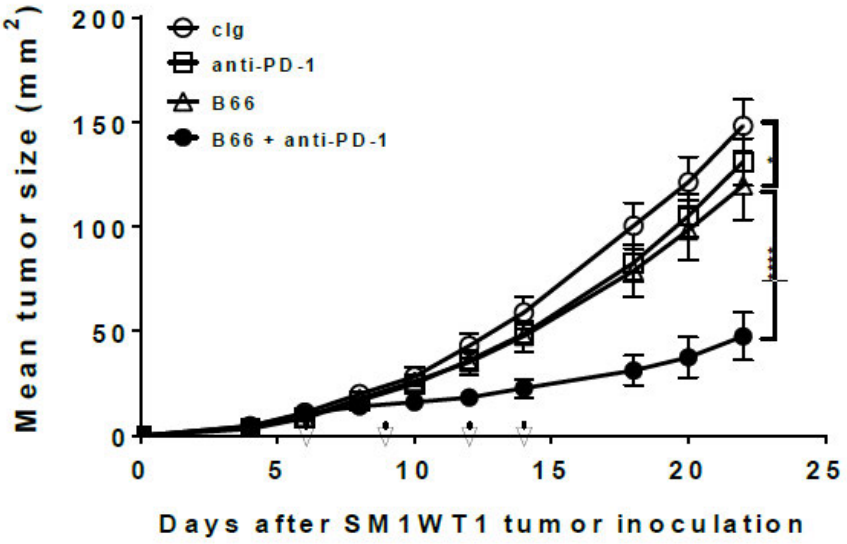
Abbreviations: ANOVA = analysis of variance, cIg = isotype control, PD-1 = programmed cell death protein-1.
Trishula data on file Study No.17-015-IVO: MC38 cells were injected subcutaneously on Day 0.

A) B66 was administered intraperitoneally at 20, 100, or 200 µg, and cIg was administered at 20 µg/dose on Days 7, 10, 13, and 16.

B) B66 was administered intraperitoneally 100 µg/dose and anti-PD-1 at 250 µg/dose on Days 12, 15, 18, and 21 (*** $P < 0.001$, **** $P < 0.0001$ by 2-way ANOVA). Arrows indicate dosing days.

Similar responses were observed in an immunotherapy-resistant melanoma tumor model SM1WT1 (Figure 8). Treatment with B66 demonstrated a slight, but statistically significant effect on subcutaneous SM1WT1 tumor growth alone, while anti-PD-1 treatment demonstrated no single-agent efficacy. In combination, B66 and anti-PD1 led to significant tumor growth reduction compared with either agent alone. Therefore, combination treatment of anti-PD1 and B66 in both anti-PD1-sensitive MC38 and anti-PD1-resistant SM1WT1 tumors resulted in an additive and potentially synergistic impact on tumor growth. These findings suggest that PD-1/PD-L1 and CD39/adenosine represent non-overlapping immune resistance mechanisms.

Figure 8: Combinatorial Anti-Tumor Efficacy of Anti-PD-1 and Anti-Mouse CD39 Antibody B66 in an Immunotherapy-Resistant SM1WT1 Tumor Cell Line

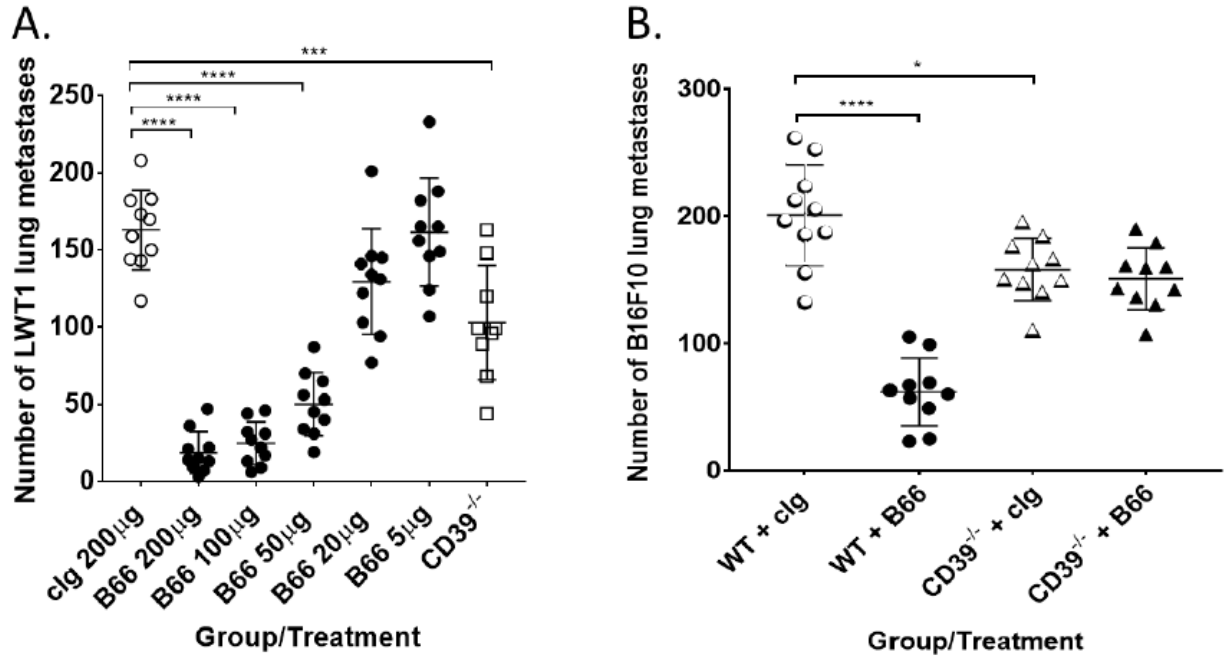


Abbreviations: ANOVA = analysis of variance, cIg = isotype control, PD-1 = programmed cell death protein.
Trishula data on file Study No.17-015-IVO: SM1WT1 cells were injected subcutaneously on Day 0. B66 and cIg were administered intraperitoneally at 200 µg/dose and anti-PD1 was administered at 250 µg/dose on Days 6, 9, 12, and 14. (* $P < 0.05$, **** $P < 0.0001$ by 2-way ANOVA). Arrows indicate dosing days.

To assess the impact of inhibition of CD39 enzymatic function on models of disseminated disease, the anti-mouse CD39 inhibitory antibody B66 was tested in lung metastasis seeding models of 2 melanoma-derived tumors, LWT1 and B16F10. Treatment with B66 significantly inhibited lung metastasis of LWT1 in wild-type mice in a dose-dependent fashion – the 50 µg dose of B66 was sufficient to mediate significant reduction in lung metastatic burden; maximal inhibition of metastasis formation was observed at the 200 µg dose of antibody in this model (Figure 9A). Therapeutic inhibition of CD39 activity by B66 antibody in wild-type mice had a more pronounced effect on metastasis compared with metastasis observed in $CD39^{-/-}$ mice. This effect of B66 was not limited to LWT1 as similar results were observed in B16F10 metastasis model (Figure 9B). Anti-CD39 inhibitory antibody treatment in wild-type mice in the B16F10 model was more efficacious in reducing metastasis compared with $CD39^{-/-}$ animals. However, there was no benefit observed between B66 treatment and the control treatment groups in the $CD39^{-/-}$ background, suggesting that the observed reduction of lung metastatic burden is dependent on CD39 inhibition.

Inhibition of CD39 enzymatic activity in mouse metastasis models of cancer is sufficient to reduce number of lung metastasis in vivo. Reduction in number of lung metastasis by B66 antibody in both LWT1 and B16F10 models resulted in a more pronounced effect compared with decreases in metastasis observed in $CD39^{-/-}$.

Figure 9: Anti-CD39 Antibody Inhibits Lung Metastasis Formation in Models of Disseminated Disease



Abbreviations: ANOVA = analysis of variance, cIg = isotype control, IV = intravenously, WT = wild type.
Trishula data on file Study No. 17-016-IVO: Anti-tumor efficacy of B66 in disseminated models of disease.

A) LWT1 cells were injected IV on Day 0. Mice were injected intraperitoneally with B66 or cIg at indicated doses (all doses are in µg) on Day 0 and on Day 3. Lungs were collected on Day 14 and perfused with India ink and analyzed for number of metastasis.

B) B16F10 cells were injected IV on Day 0. Mice were injected intraperitoneally with B66 or cIg at 200 µg/dose on Days 0 and 3. Lungs were collected on Day 14 and perfused with India ink and analyzed for number of metastasis. (*$P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$ by one-way ANOVA).

2.2.1.5. In Vivo Studies: Cynomolgus Monkey

TTX-030 has been tested in vivo in cynomolgus monkeys in single-dose (10 mg/kg) and multiple-dose non-GLP and GLP (30 and 100 mg/kg) studies. No adverse effects have been observed; all clinical chemistry, hematology, and coagulation results were within the normal range for cynomolgus monkeys.

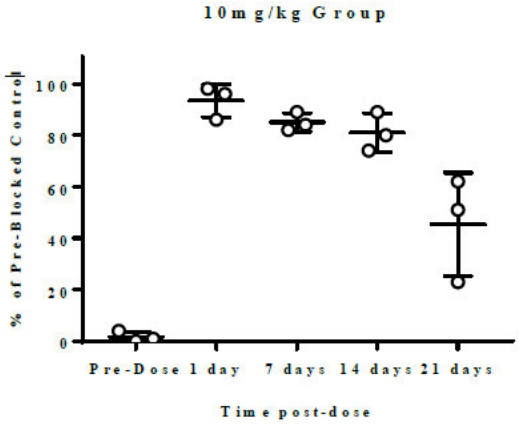
CD39 receptor occupancy by TTX-030 in cynomolgus monkeys was analyzed as part of a non-GLP study. Target coverage was assessed by flow cytometry using fluorescently labeled TTX-030 on whole blood samples and gated on monocyte population. Free CD39 receptor was calculated relative to baseline (mean fluorescence intensity [MFI] of an unblocked sample at

predose). The percent of free receptor at each time point was calculated using the following formula:

% of Free Receptor =	(MFI of Unblocked Sample MFI of Pre-Blocked Sample) ÷ (MFI of Unblocked Sample at Predose—- MFI of Pre-Blocked Sample)

Receptor occupancy exceeding 90% was achieved following TTX-030 dosing at 10 mg/kg. Occupancy was 91.1% 24 hours postdose and remained high (83% ± 3%) through Day 14 (Figure 10) with C_{max} for that group calculated as 19.8 µg/mL.

Figure 10: Receptor Occupancy Analysis for 10 mg/kg Group in Cynomolgus Monkeys



Trishula data on file Study No. 17-019-PK: TTX-030 covers CD39 cynomolgus monkey peripheral blood monocytes for > 2 weeks at 10 mg/kg. Cynomolgus monkey whole blood was incubated in fluorescently labeled TTX-030 for 30 minutes and analyzed by flow cytometry.

2.2.2. Clinical Data

As of the data cutoff date of 10 January 2020, 21 subjects in Study TTX-030-001 have received TTX-030 monotherapy every 3 weeks (Q3W) at the following escalating doses: 0.5 mg/kg (n = 1), 1.5 mg/kg (n = 2), 3.0 mg/kg (n = 2), 6.0 mg/kg (n = 4), 10 mg/kg (n = 3), 20 mg/kg (n = 3), or 40 mg/kg (n = 6).

As of 10 January 2020, 17 of 21 subjects in Study TTX-030-001 experienced treatment-emergent adverse events (TEAEs). A majority of the TEAEs were of Grade 1 or 2 severity. TEAEs of ≥ Grade 3 severity were reported in 6 subjects: Grade 3 lipase increase (1.5 mg/kg cohort), Grade 3 hyperbilirubinemia (3.0 mg/kg cohort), Grade 3 gamma-glutamyltransferase (GGT) increase (10 mg/kg cohort), Grade 3 bilateral lower extremity muscle weakness (10 mg/kg cohort), Grade 3 dyspnea, ascites, increased alkaline phosphate, esophageal hemorrhage, upper gastrointestinal (GI) bleed, and decreased lymphocyte count (40 mg/kg cohort), and Grade 5 sepsis (6.0 mg/kg cohort). There were no Grade 4 events. Treatment-related TEAEs were reported in 9 subjects and included arthralgia in 2 subjects and malaise, myalgia, worsening back pain, intermittent rib pain, dizziness, worsening diarrhea, foot maculo-papular rash, bruising left hand, vaginal discharge, nausea, headache, creatine phosphokinase (CPK) increased, and fatigue in 1 subject

each; all of these events were of Grade 1 or 2 severity. Five subjects experienced a total of 6 serious adverse events (SAEs): Grade 2 bee sting reaction (1.5 mg/kg cohort), Grade 3 hyperbilirubinemia (3.0 mg/kg cohort), Grade 3 bilateral lower extremity muscle weakness (10 mg/kg cohort), Grade 3 dyspnea and esophageal hemorrhage (40 mg/kg cohort), and Grade 5 sepsis (6.0 mg/kg cohort). None of the SAEs were considered to be treatment-related by the Investigator. No dose-limiting toxicities (DLTs) were observed, and the maximum tolerated dose has not been determined.

As of 08 January 2020, PK data are available for 21 subjects treated at TTX-030 doses through 40 mg/kg. Overall, the 2-compartment model was consistent with the individual subject data. Clearance appears to be independent of dose at doses ≥ 6 mg/kg suggesting linear or dose-proportional PK. For doses from 6 to 40 mg/kg, there was reasonable consistency among doses and subjects with respect to the PK parameters. The elimination half-life over that dose range averaged 18.9 days.

Refer to the TTX-030 Investigator Brochure (IB) for more information.

2.2.3. Rationale for TTX-030 Dose and Schedule

Based on the current PK, receptor occupancy data and safety from the ongoing first-in-human Phase 1 study (TTX-030-001) that is evaluating TTX-030 as monotherapy and in combination with other agents in subjects with advanced solid tumor malignancies or lymphoma, a RP2D was determined to be 40 mg/kg loading dose 7 days prior to Cycle 1 Day 1 and then either 30 mg/kg Q3W or 20 mg/kg Q2W.

As of the clinical data cut-off date of 10 January 2020, doses of TTX-030 monotherapy up to 40 mg/kg Q3W have been evaluated in Study TTX-030-001 and demonstrated to be well tolerated. No DLTs have been reported (Section 2.2.2).

PK demonstrated that a dose of 40 mg/kg Q3W achieved a minimal functional trough level based on activity of TTX-030 in preclinical human functional assays (i.e., enhancement of IL-2 from PBMCs and inflammasome activation). Clearance appears to be independent of dose at doses ≥ 6 mg/kg, suggesting linear or dose-proportional PK. The half-life was 18.9 days. After the completion of the dose escalation portion of the study, the totality of the PK data was used for PK modeling for the RP2D. Based on the simulations, it was determined that a loading dose of 40 mg/kg one week prior to Cycle 1, Day 1 dosing of 30 mg/kg Q3W or 20 mg/kg Q2W will provide a minimal functional trough level in greater than 80% of patients before Cycle 1, Day 1 and in cycles thereafter.

In the 0.5, 1.5, 3.0, 6.0, 10.0 and 20.0 mg/kg Q3W dose groups, receptor occupancy data demonstrated that doses greater than or equal to 6 mg/kg achieved and maintained higher levels of peripheral blood receptor occupancy through 21 days post-Cycle 1 on $CD39^{+}$ monocytes, B cells, and regulatory T cells. At doses less than or equal to 3 mg/kg, receptor occupancy levels were lower and decreased 7-14 days post-Cycle 1.

Refer to the TTX-030 Investigator's Brochure for more information.

2.3. Benefit/Risk Assessment

Blockade of CD39 may result in less potential for autoimmune side effects compared with rates seen with other immunotherapy agents. Although T_{reg} from $CD39^{-/-}$ knockout mice have impaired suppressive activity, the mice do not show autoimmunity (Sun et al. 2010).

TTX-030 has a different mechanism of action from each of the regimens in the combination arms (Arms 2 and 4, presented in Table 8), and toxicities are anticipated to be manageable and nonoverlapping. Conversely, it is also possible that TTX-030 could potentiate some of the existing toxicities of the combination regimens.

The clinical risks and benefits of TTX-030 are unknown at this time; however, several investigational agents targeting A2AR and CD73 in the adenosine pathway have reportedly entered Phase 1 trials and may offer insights on potential risks and benefits of targeting the adenosine pathway. Clinical data from an ongoing Phase 1 trial with CPI-444 (NCT02655822), an oral small molecule inhibitor of the A2A receptor, suggest that inhibiting adenosine-mediated suppression can be achieved with a favorable safety profile (Hotson et al. 2017). Similarly, no SAEs were reported in a Phase 1b/II study in Parkinson's Disease with the same compound (Pinna 2014). A similar safety profile was observed with NIR178 (previously known as PBF-509), also a small molecule inhibitor of A2AR, which has been evaluated in both healthy volunteers (NCT01691924 and NCT02111330) and subjects with advanced non-small-cell lung cancer (NCT02403193). NIR178 was reportedly well-tolerated. Adverse events (AEs) were manageable, and there were no Grade 4 drug-related AEs; immune-related AEs (irAEs) may indicate immune stimulation (Chiappori et al. 2018).

Antibodies targeting CD73 have also recently entered clinical development. Data from NZV930/SFF3737 (NCT03549000) and CPI-006 (NCT03454451) have not been reported. The anti-CD73 antibody MEDI9447 (oleclumab) was evaluated for safety, efficacy, and PK alone or in combination with the anti-PD-L1 antibody (IMFINZI®, 2018) in advanced pancreatic cancer or colorectal cancer (NCT02503774). No treatment-related deaths or DLTs were reported, and no SAEs were reported in any of the oleclumab monotherapy dose-escalation cohorts. Overall, treatment with oleclumab alone or with durvalumab demonstrated a manageable safety profile as measured by low incidence of treatment-related discontinuation and SAEs (Overman et al. 2018).

Overall, clinical experience with other investigational agents targeting A2AR and CD73 in the adenosine pathway were shown to be well tolerated. Publicly disclosed safety data have been summarized in more detail in the IB.

3. Objectives and Endpoints

Objectives	Endpoints
Primary	
• To assess the safety profile, to determine the DLT, and to determine the MTD and/or RP2D of TTX-030 when administered IV as a single agent or in combination with agents in specified regimens to subjects with advanced solid tumor malignancies or lymphoma.	• DLTs, MTD, and/or RP2D of TTX-030 as a single agent • DLTs, MTD, and/or RP2D of TTX-030 in combination with specified regimens
• Arm 1 and Arm 2 Expansion Cohorts: To assess the ORR per iRECIST after treatment with TTX-030 as a single agent or TTX-030 in combination with pembrolizumab in subjects with advanced or metastatic RCC with clear-cell component	• ORR
Secondary	
• To evaluate anti-tumor activity in subjects treated with TTX-030 as a single agent or in combination with specified regimens	• BOR • ORR (except for Arm 1 and 2 Expansion Cohorts, where ORR is a primary endpoint) • DOR • TTR • DCR • TTP • PFS • OS
• To evaluate the PK of TTX-030	• Serum PK parameters for TTX-030 following single and multiple doses including the C_{max}, T_{max}, C_{min}, V_{SS}, CL, $t_{1/2}$, AUC_{0-t}, and $AUC_{0-\infty}$ for TTX-030
• To assess the effects of TTX-030 on pharmacodynamic biomarkers relating	• Immunogenicity (detection of ADA) • Expression of CD39

to mechanism of action and immune responses	• Exploratory pharmacodynamic biomarkers

4. Study Design

4.1. Overall Design

4.1.1. Overview

This is a Phase 1/1b, FIH, open-label, and multicenter study to investigate the safety, tolerability, PK, pharmacodynamic, and preliminary clinical activity of TTX-030 in adult subjects with advanced solid tumor malignancies or lymphoma. The study will evaluate the safety and tolerability of:

- TTX-030 as a single agent, and
- TTX-030 in combination with pembrolizumab, a PD-1 inhibitor, and
- TTX-030 in combination with specified chemotherapy regimens.

The study consists of 2 stages: dose escalation/safety lead-in and dose expansion Figure 1. Dose escalation/safety lead-in comprises Part A (1 dose-escalation arm of TTX-030 monotherapy [Arm 1]) and Part B (2 safety lead-in arms of combination therapy [TTX-030 with other agents; Arms 2 and 4]). Arm 3 is removed from study design as similar population is being evaluated in another Trishula protocol, TTX-030-002.

Up to 43 subjects may be enrolled across the dose-escalation and safety lead-in arms. Cohort expansions of n=25 subjects each in Arms 1 and 2 and n=up to 20 in Arm 4. The number of subjects evaluated in the combination-therapy safety lead-in Arm 4 may count toward the total number of subjects in an expansion cohort. Note: The sponsor may choose not to open one of the safety lead-in arms and/or expansion cohorts.

The study includes pretreatment (screening and baseline), study treatment, treatment extension, and post-treatment (end-of-treatment and follow-up) periods.

4.1.1.1. Dose Escalation and Safety Lead-in

Dose escalation (Arm 1) will be conducted according to a mCRM for doses up to 10 mg/kg (see Section 4.4 for details) and according to a traditional 3 + 3 design for doses > 10 mg/kg (see Section 4.7 for details) to estimate the dose-toxicity relationship and determine the MTD (defined as the dose that has a model-estimated DLT rate closest to 30% and also has less than 55% probability that the DLT rate exceeds 30%). The study will be overseen by a Cohort Review Committee.

A DLT is defined as any clinically significant AE that occurs during Treatment Cycle 1, is considered related to TTX-030 as a single agent or the combination of TTX-030 and other agent(s), and meets the criteria outlined in Section 4.12. In Arm 1 dose escalation, for the Q3W dose schedule, the DLT period will be the first cycle (21 days).

In the safety lead-in Arm 2, the DLT evaluation period is 21-day (TTX-030 regimen given every 3 weeks [Q3W]) plus a 7-day of loading dose. In the safety lead-in Arm 4, for the Q2W dose schedule, the cycle length is 4 weeks and the DLT period will be 28-day (TTX-030 regimen given every 2 weeks [Q2W]) plus a 7-day of loading dose.

Safety and tolerability will be assessed by physical examination (including symptom-directed examinations), vital signs, and clinical laboratory tests and evaluated as the incidence, severity, duration, seriousness, type, and relationship of AEs that occur during the treatment and follow-up periods. Subjects will be followed for determination of AE incidence through through 90 days after the last dose of TTX-030 or until initiation of a new systemic anticancer therapy, whichever occurs first. Clinical AEs will be graded according to the NCI CTCAE Version 5.0.

TTX-030 will first be evaluated in monotherapy dose escalation (Arm 1). Once the RP2D of TTX-030 has been determined in the monotherapy dose-escalation arm, the combination-therapy safety lead-in arms (Arms 2 and 4) can be initiated. For the safety lead-in arms, the TTX-030 starting dose will be the RP2D identified during monotherapy dose escalation; de-escalation to 1 dose level below the RP2D is permitted. Upon completion of the dose-escalation and safety lead-in arms, each of those arms may have the option to enroll up to 20 to 25 additional subjects per expansion cohort. The number of subjects evaluated in the combination-therapy safety lead-in Arm 4 may count toward the total number of subjects in an expansion cohort.

Dose escalation and safety lead-in arms are presented in Table 8, and dosing regimens are presented in Table 11. Subjects in Arms 1 and 2, are to be dosed Q3W on a 21-day schedule (Table 1/Table 2). Subjects in Arm 4 may be dosed Q2W on a 28-day schedule (Table 3/Table 4).

As of Jan 2020, a RP2D was determined to be 40 mg/kg loading dose 7 days prior to Cycle 1 Day 1, followed by either 30 mg/kg Q3W or 20 mg/kg Q2W.

Enrollment will continue in the monotherapy dose-escalation arm (Arm 1) until the MTD is determined or the highest dose level has been reached and deemed safe by the Cohort Review Committee. The Cohort Review Committee may select interim dose levels as recommended by the mCRM for doses up to 10 mg/kg and by the 3 + 3 design for doses > 10 mg/kg and deemed appropriate.

4.1.1.2. Expansion Phase

Once a dose level in the dose-escalation arm has been declared safe, that particular dose level may be expanded to further explore the PK/pharmacodynamics of TTX-030 in the tumor cohorts listed below (see Section 5 for disease-specific eligibility criteria for each cohort). A total of up to 90 subjects (20 to 25 subjects per cohort) may be enrolled for PK/pharmacodynamic exploration.

The expansion phase includes 3 arms, as described below. Multiple expansion cohorts may enroll in parallel.

Arm 1 and 2 Expansion Cohorts

In Arm 1 and 2 Expansion Cohorts, 50 subjects (25 subjects in each arm) with advanced or metastatic renal cell carcinoma with a clear-cell component and previously treated with anti-PD-(L)1 therapy and tyrosine kinase inhibitor (TKI) therapy, will be randomized 1:1 to TTX-030 or TTX-030 and pembrolizumab. These agents will be administered at the doses and schedules identified in the safety lead-in.

- Arm 1 Expansion: TTX-030 (n = 25)
- Arm 2 Expansion: TTX-030 and pembrolizumab (n = 25)

Expansion Arm 4

In Expansion Arm 4, up to 20 subjects will receive TTX-030 40 mg/kg 7-day prior to C1D1, followed by 20 mg/kg Q2W in combination with gemcitabine + nab-paclitaxel at a dosing schedule equivalent to that evaluated in the safety lead-in.

- Expansion Arm 4: Histologically or cytologically confirmed diagnosis of locally advanced, unresectable, or metastatic pancreatic adenocarcinoma, naïve to any prior treatment for metastatic disease. Prior adjuvant therapy (including chemotherapy and/or radiotherapy) is permitted if neoadjuvant or adjuvant therapy was completed at least 6 months prior to study enrollment.

4.1.2. Cohort Review Committee

The Cohort Review Committee is composed of study investigators, the sponsor's medical monitor (or qualified delegate), and a statistician. Treatment cohorts will be dosed in escalating order as determined by the mCRM (for doses up to 10 mg/kg) or 3 + 3 design (for doses > 10 mg/kg) and with approval from the Cohort Review Committee, only after the safety of each dose level has been established, or until the MTD has been determined. Subsequent cohorts will not be dosed until safety data from the Study Treatment Period are obtained from subjects in the current cohort.

A review of the safety data for each cohort will be conducted by the Cohort Review Committee. The Cohort Review Committee will be fully aware of clinical and laboratory data, the mCRM recommendation (where applicable), and will determine whether dose escalation to the next cohort is appropriate. Safety will be evaluated based on AEs, clinical laboratory test results, and other relevant clinical findings observed during the Study Treatment Period, as well as data from the Treatment Extension Period for prior cohorts. PK and pharmacodynamic data that are available will also be reviewed but are not required to make a dose-escalation decision.

No dose escalation will take place until the Cohort Review Committee has reviewed the safety data and approved escalation. Cohort Review Committee evaluations will determine whether a DLT has occurred. Cohort Review Committee evaluations are also required to open combination treatment in Arms 2 through 4. The mCRM for doses up to 10 mg/kg and 3 + 3 design for doses > 10 mg/kg will be applied to each treatment arm independently; however, the Cohort Review Committee will be made aware of all safety data across all arms and cohorts. The Cohort Review Committee may choose to apply safety knowledge gained from one arm to more conservatively manage other arms.

Any toxicity occurring within an earlier dose level after formal Cohort Review Committee review will be reviewed by the Cohort Review Committee along with safety data from the current dose level and used to make decisions regarding further dose escalation.

4.2. Justification for Dose in FIH study

4.2.1. Rationale for TTX-030 Starting Dose and Dosing Schedule

To inform selection of the starting dose for a FIH study, Trishula will primarily employ the no-observed-adverse-effect level (NOAEL) method from the Good Laboratory Practice (GLP) toxicology studies in non-human primates (NHPs) (FDA, 2005). Additionally, the pharmacologically active dose (PAD) method was used to independently calculate a starting dose value, with both methodologies arriving at similar dose levels.

No adverse effects were observed in the non-GLP and GLP NHP multi-dose studies performed with TTX-030. As such, Trishula proposes to calculate the maximum recommended starting dose (MRSD) for TTX-030 based on a 10-fold safety factor over the NOAEL. A multiple-dose GLP NHP study found no observed toxicities at a maximum dose level of 100 mg/kg, administered weekly for a total of 5 doses. The human equivalent dose (HED) based on the body size mg/kg scaling factor is calculated at 32.36 mg/kg. Coupled with a 10-fold safety factor, the resulting MRSD for TTX-030 is 3.23 mg/kg.

A PAD level was calculated based on in vitro functional data to derive the FIH starting dose. In vitro human PBMC bioactivity assays (IL-2 release from stimulated PBMC in the presence of TTX-030) predict that a minimum exposure concentration of 10 µg/mL of TTX-030 is required to maintain efficacy (Section 2.2.1.3.4). In this assay, the half maximal dose of TTX-030 needed for increased cytokine release was calculated at 19.37 µg/mL when 50 µM ATP was added to the culture and 23.3 µg/mL without the addition of exogenous ATP; cytokine release dropped off when TTX-030 concentration was below 10 µg/mL (see Figure 6). By combining these in vitro results with exposure levels in NHP studies and allometric scaling from cynomolgus monkey to human, a 1.8 mg/kg Q3W HED dose was calculated as the PAD, which is almost 2-fold lower than MRSD.

A dose of 0.5 mg/kg given Q2W or Q3W is 2 to 3 dose levels below the predicted PAD dose and below the 10-fold safety margin based on NOAEL. The FIH starting dose of 0.5 mg/kg Q3W initiated in the single-agent arm.

4.2.2. Selection of Agents in Combination with TTX-030

TTX-030 is postulated to potentiate the effectiveness of each of these combination-therapy regimens.

The backbone regimens chosen for each combination arm are approved and/or standard of care for several indications, and the regimens all have a well-characterized safety profile. TTX-030 has a different mechanism of action from each of the regimens in the combination arms, and toxicities are anticipated to be manageable and nonoverlapping.

Treatment with pembrolizumab 200 mg every 21 days has been approved for multiple oncology indications, including melanoma, non-small-cell lung cancer, small-cell lung cancer, head and neck squamous cancer, classical Hodgkin lymphoma, primary mediastinal large B-cell lymphoma, urothelial carcinoma, MSI-H/MMR deficient malignancies, gastric cancer, esophageal cancer, cervical cancer, hepatocellular carcinoma, Merkel cell carcinoma, RCC, and endometrial carcinoma (KEYTRUDA®, 2019).

Treatment with docetaxel 75 mg/m^2 every 21 days is widely used in lung, breast, prostate, bladder, and other cancers (TAXOTERE®, 2015).

Treatment with gemcitabine 1000 mg/m^2 + nab-paclitaxel 125 mg/m^2 is widely used in treatment of metastatic pancreatic cancer (GEMZAR®, 2017 and ABRAXANE®, 2018).

4.3. Treatment Assignment

This is an open-label study with multiple arms. At times, multiple arms may be enrolling in parallel. Investigators are responsible for contacting the sponsor, or delegate, for treatment assignment before treating subjects with TTX-030. The sponsor will have records of the number of subjects treated within a specific cohort and will determine which treatment cohort to assign the newly enrolled subjects.

Subject's treatment assignment/randomization may be done in EDC or alternative randomization system upon enrollment confirmation by the sponsor or designee. Subjects in Arm 1 and 2 Expansion Cohorts will be randomized (as described in Section 4.1.1.2). A separate instruction manual will be provided to each site. A subject is considered entered into the study once the investigator (or designee) confirms eligibility and notifies the sponsor that the subject is enrolled in the study.

4.4. Dose Levels

The doses for this trial will include a set of primary doses (0.5, 1.5, 3, 6, 10, 20, and 40 mg/kg) as well as several intermediate doses. As mentioned above, dose escalation (Arm 1) will be conducted according to a mCRM for doses up to 10 mg/kg and according to a 3 + 3 design for doses > 10 mg/kg. Not all dose levels may be explored as dose levels may be skipped during escalation based on mCRM recommendation. A detailed adaptive design and simulation report is included in Section 11.6.

TTX-030 will first be evaluated as a single agent (Arm 1). Subjects will be enrolled into the study sequentially beginning with a single-subject cohort at 0.5 mg/kg. Beginning with 1.5 mg/kg, subjects will be enrolled in dosing cohorts of 2 subjects each. If no Grade 2 toxicities (related to TTX-030) and no DLTs are observed, the trial may enroll 2 subjects each to the primary dose levels (1.5, 3, 6, 10, 20, and 40 mg/kg). Upon the first instance of a DLT or a Grade 2 or above toxicity that is related to TTX-030, that cohort and subsequent cohorts receiving higher doses will enroll at least 3 subjects each. Each dosing cohort must complete its DLT observation period (see Section 4.12 for discussion of DLT) of 1 cycle before enrollment of the next dosing cohort.

Based on clinical data as of 10 Jan 2020, a loading dose of 40 mg/kg one week prior to Cycle 1, Day 1 followed by dosing of 30 mg/kg Q3W or 20 mg/kg Q2W has been established. See Section 2.2.3 for RP2D dose selection details.

Subjects who discontinue from the study for reasons other than a DLT (eg, noncompliance, subject request, or disease progression), and are not evaluable for at least a single complete cycle of TTX-030 as single agent or in combination, will be replaced. Subjects who discontinue after Cycle 1 will not be replaced. For the cohort expansion, subjects will not be replaced.

4.5. Dose Escalation and Safety Lead-In Arms

Up to 43 subjects across 1 dose escalation and 2 safety lead-in arms are planned. The 3 arms are summarized in Table 8.

Refer to Section 5 for details on subject eligibility.

Table 8: Dose-Escalation and Safety Lead-In Arms

Arm	Monotherapy or Combination Therapy	Examples of Eligible Indications	Treatment	Number of Subjects
Dose-Escalation Arm				
1	Monotherapy	All tumors	TTX-030	Up to 21
Safety Lead-In Arm				
2	Anti-PD-1 Combination	Indicated tumors	TTX-030 with pembrolizumab	Up to 6
4	Chemotherapy Combination	Pancreas	TTX-030 with gemcitabine + nab-paclitaxel	Up to 6

Abbreviation: PD-1 = programmed cell death protein-1

4.6. Dose Escalation Using Modified Continual Reassessment Method

Dose escalation will be conducted according to the mCRM, with the aim of estimating the dose-toxicity relationship and determining the MTD. The MTD will be defined as the dose that has a model-estimated DLT rate closest to 30% and has less than 55% probability of a DLT rate higher than 30%.

The starting dose for TTX-030 will be 0.5 mg/kg. Ascending IV doses of TTX-030 as a single agent will be administered to subjects on the first day of each 21-day treatment cycle. See Section 4.4 for dose levels.

Subjects in Arm 1 will be enrolled sequentially beginning with a single subject at 0.5 mg/kg and followed by dosing cohorts of 2 subjects each. Upon the first instance of a DLT or a Grade 2 or above toxicity that is related to TTX-030, that cohort and subsequent cohorts receiving higher doses will be expanded to a minimum of 3 subjects each.

Each dosing cohort must be completed through the DLT observation window before escalation can be allowed within its arm. After 1.5 mg/kg, untried dose levels may be skipped if they are deemed safe by the dose-toxicity model and are no more than a 100% increase (a doubling) from the current dose. If the dose-toxicity model (described in Section 4.8) deems the current dose to be unsafe and de-escalation is required, the dose will be decreased to the highest safe dose level. Thus, if no DLTs are observed in Arm 1, the trial would enroll 1 subject to 0.5 mg/kg followed by 2 subjects each to 1.5 mg/kg, 3 mg/kg, 6 mg/kg, 10 mg/kg, 20 mg/kg, and then proceed to the highest dose, 40 mg/kg.

If the dose-toxicity model deems all doses up to 40 mg/kg to be safe, the Cohort Review Committee will have the option to dose escalate above 40 mg/kg, or a comparable flat dose, in

appropriate increments (no more than a 100% increase) if it is felt a higher dose would be safe and potentially beneficial to study subjects.

4.7. Dose Escalation Using 3 + 3 Design

Escalation of doses > 10 mg/kg will progress according to a traditional 3 + 3 design. This design proceeds with cohorts of 3 subjects; the first cohort is treated at the starting dose, and subsequent cohorts are treated at increasing dose levels.

- If none of the 3 subjects in a cohort experiences a DLT, dose escalation may proceed to the next higher dose level (ie, another 3 subjects will be treated at the next higher dose level).
- If 1 of the first 3 subjects in a cohort experiences a DLT, 3 more subjects will be treated at the same dose level.
- Dose escalation continues until ≥ 2 subjects in a cohort of 3 to 6 subjects experience a DLT (ie, ≥ 33% of subjects with a DLT at that dose level). The MTD is defined as the dose level just below this toxic dose level.

The recommended RP2D of TTX-030 was selected based on the overall safety/tolerability and available PK/pharmacodynamic data. The RP2D may or may not be the same as the MTD identified during dose escalation. For example, if the MTD is not reached despite increasing drug dose, or if exposure at the MTD is much higher than the level believed to be required for efficacy, or if subsequent cycles of treatment provide additional insight regarding the safety profile, then the RP2D may be a different dose.

4.8. Dose-Toxicity Modeling

Dose-toxicity modeling will be used to estimate the log-odds of a DLT across the dose levels and to guide dose recommendation. The model is a 2-parameter logistic regression model. Full details of the statistical model are provided in Section 11.6.

4.9. Early Stopping

The trial may be stopped early if all doses are considered unsafe (that is, if all doses have more than 55% probability that the DLT rate exceeds 30%). Conversely, the study may be stopped early for having sufficiently characterized the MTD if at least 6 subjects have been enrolled to the current highest safe dose level, have completed the DLT observation period, and additional dose escalation is not recommended. After the highest safe dose level is identified, the Cohort Review Committee will have the option to explore a comparable flat dose.

4.10. Initiation of Safety Lead-In Combination Therapies (Arms 2 & 4)

Enrollment into combination Arms 2 and 4 will be initiated once the RP2D of TTX-030 has been determined in monotherapy dose escalation in Arm 1. For each of Arms 2 and 4, the TTX-030 starting dose will be the RP2D identified in Arm 1.

In Arm 2, eligible pembrolizumab-naive subjects and other eligible subjects with pembrolizumab experience may be entered into the study. Subjects who tolerated prior PD-(L)1-containing regimens, and now want to try a new PD-(L)1 regimen again, can enter.

Treatment in Arm 4 is planned to be TTX-030 in combination with separate chemotherapy regimens used for selected malignancies. The chemotherapy regimens suggested for combination with TTX-030 will be considered standard of care for the planned subject populations in the respective arms. Pembrolizumab will not be included in Arm 4.

In Arm 4, TTX-030 is planned to be administered with gemcitabine + nab-paclitaxel.

In each safety lead-in cohort, all subjects will be treated and closely monitored for the occurrence of DLTs. If ≥ 2 of up to 6 subjects experience a DLT during the DLT period, the Cohort Review Committee will discuss whether dose de-escalation to a lower dose of TTX-030 will be evaluated as appropriate and if further addition of subjects is needed to re-assess the RP2D.

4.11. Maximum Tolerated Dose and Recommended Phase 2 Dose

Dose escalation (Arm 1) will be conducted according to a mCRM for doses up to 10 mg/kg and according to a 3 + 3 design for doses > 10 mg/kg, with the aim of estimating the dose-toxicity relationship and determining the MTD. The MTD will be defined as the dose that has a model-estimated DLT rate closest to 30% and has less than 55% probability of a DLT rate higher than 30%. Escalation will continue until the MTD has been determined for TTX-030 as a single agent or the highest dose level has been reached and deemed safe by the Cohort Review Committee using either the 3 + 3 design or mCRM.

The RP2D may be defined as any dose level that is either explored using the mCRM or 3 + 3 design or determined using PK modeling equal to or below the determined MTD. The RP2D determination may also depend on PK, pharmacodynamic, efficacy, and other safety data as well as the MTD.

4.12. Definition of Dose-limiting Toxicity

A DLT is defined as any clinically significant AE that occurs during Treatment Cycle 1 that the investigator or sponsor considers as possibly or likely related to TTX-030 as a single agent, or the combination of TTX-030 and other agent(s), and meets the following criteria: NCI CTCAE Version 5.0 Grade 5 event, Grade 4 hematological or ≥ Grade 3 non-hematological toxicities, or ≥ Grade 3 irAEs. Laboratory abnormalities that are asymptomatic and deemed not clinically significant will not be regarded as a DLT, unless specified below.

DLTs include the following:

- Grade ≥ 3 neutropenia lasting ≥ 5 days
- Any febrile neutropenia
- Grade ≥ 3 thrombocytopenia with clinically significant hemorrhage
- Grade 4 thrombocytopenia
- Grade ≥ 3 non-hematologic adverse events, except the following

- Grade ≥ 3 nausea, vomiting, or diarrhea lasting < 72 hours in the absence of maximal medical therapy.
- Grade ≥ 3 abnormal laboratory values that are not clinically significant and corrected within 72 hours
- Grade ≥ 3 fatigue lasting < 5 days
- Grade 3 aspartate aminotransferase (AST) or alanine aminotransferase (ALT) elevations lasting < 7 days
- Grade 3 non-hepatic-related increases in alkaline phosphatase will not be considered a DLT

- AST or ALT > 3 x upper limit of normal (ULN) (> 2 x baseline AND > 3 x ULN in subjects with baseline elevation) AND total bilirubin > 2 x ULN (> 2 x baseline AND > 2 x ULN in subjects with baseline elevation) or clinical jaundice, without initial findings of cholestasis AND no other immediately apparent identifiable possible causes of elevated liver enzymes and hyperbilirubinemia

Safety data will be evaluated by the Cohort Review Committee (described in Section 4.1.2).

Delayed DLTs will be evaluated on a case-by-case basis. If 2 or more delayed DLTs are noted within a dose-escalation/safety lead-in arms, further accrual will be held pending safety analysis of the AEs and will be restarted only with Cohort Review Committee review and approval. For the Q3W dose escalation dosing schedule, the cycle length is 3 weeks and the DLT period will be the first cycle (21 days). For the safety lead-in Q2W dosing schedule, the cycle length is 4 weeks and the DLT period will be the first cycle (28 days) plus a 7-day of loading dose.

For the safety lead-in Q3W dosing schedule, the cycle length is 3 weeks and the DLT period will be the first cycle (21 days) plus a 7-day of loading dose.

The DLT must either return to the baseline level at which the subject was enrolled or to a Grade 1 or less prior to subsequent administration of TTX-030 or a TTX-030 combination.

If an AE does not meet the DLT criteria but is deemed clinically significant and one that can require a change in the dosing interval, then the investigator is asked to contact the medical monitor to develop a subject management plan. With mutual agreement, such a case may lead to the dosing interval being prolonged for an additional 21 days without the AE being deemed a DLT. If the AE leads to the dosing interval being extended beyond the additional 21 days, then the event will be deemed a DLT.

4.13. Dosing Delays

During the dose-escalation/safety lead-in period, prolonging infusion times and treatment delays are allowed in response to treatment toxicity. Delays of more than one month necessitate medical monitor discussion and approval for continuation of therapy.

Any necessary interim visits will be captured as unscheduled visits on the electronic case report form (eCRF).

If toxicity is attributed by the investigator as likely related to TTX-030, the administration of TTX-030 should be managed per Table 9. The dose adjustment criteria apply whether TTX-030 is administered as a single agent or in combination therapy. Please refer to Section 8.3.6.5 for details on adverse events of special interest, including immune-related adverse event (irAE) management.

4.14. Safety Criteria for Adjustment or Stopping Doses

If toxicity is attributed by the investigator as likely related to TTX-030, the administration of TTX-030 should be managed per Table 9. The dose adjustment criteria apply whether TTX-030 is administered as a single agent or in combination therapy. Please refer to Section 8.3.6.5 for details on toxicity management, including irAE management.

If toxicity is attributed by the investigator as likely related to chemotherapy in the combination arms, and unlikely related to TTX-030, the subject should be medically managed per standard of care, and the dose and schedule of chemotherapy should be managed per institutional guidelines. Administration of TTX-030 may continue as long as the investigator feels TTX-030 is unlikely contributing to toxicity. If the subject continues to experience significant toxicities despite medical management and after dose/schedule adjustment of chemotherapy, then TTX-030 must be managed per Table 9.

If an irAE is observed in Arm 2, the AE should be attributed to the combination of pembrolizumab and TTX-030, and not pembrolizumab alone. Management of pembrolizumab dosing should follow institutional guidelines. Management of TTX-030 should follow Table 9.

Table 9: Criteria for Adjusting or Stopping Doses of TTX-030

	Management/Next Dose for TTX-030
≤ Grade 1	No change in dose; medical management per standard of care.
Grade 2	If Grade 2 AE despite medical management, hold TTX-030 until ≤ Grade 1. Resume at same dose level.
Grade 3	If Grade 3 AE despite medical management, hold TTX-030. Discontinuation or dose modification to be determined by investigator and Cohort Review Committee on a case-by-case basis.
Grade 4	Discontinue TTX-030.

4.15. Pharmacokinetic Criteria for Adjustment or Stopping Doses

Toxicity-based dose escalation/safety lead-in decisions may depend on PK parameters like C_{max}, C_{min}, and AUC that correlate with desired target stimulation or suppression. For example, C_{max} may correlate with toxicity and $t_{1/2}$ may predict recovery from toxicity. Available PK data will be reviewed by the Cohort Review Committee but is not required for dose escalation/safety lead-in decisions.

4.16. Intra-subject Dose Escalation

Subjects in their Treatment Extension Period may be eligible to receive study treatment at a higher dose level that has been deemed safe. If there are multiple successful dose escalations

throughout the study, subjects who started with lower doses may be eligible for multiple intra-subject dose escalations up to 40 mg/kg. Each case of intra-subject dose escalation must be approved by the Cohort Review Committee (Section 4.1.2) and may not exceed the current highest dose level deemed safe. The first subject treated on the study at 0.5 mg/kg is the exception; in the absence of toxicity during their first cycle, the subject may initiate doses of 1.5 mg/kg Q3W without prior approval from the Cohort Review Committee.

Safety data from intra-subject dose escalation will not be assessed by the mCRM or 3 + 3 method but will be reviewed and assessed by the Cohort Review Committee.

4.17. Expansion Arms

Upon completion of dose escalation (Arm 1) and safety lead-in (Arms 2 and 4), each arm may have the option to enroll additional subjects per expansion cohort at the RP2D. Arm 1 and Arm 2 Expansion Cohorts will enroll an additional 50 total subjects (25 per randomized treatment arm), and Expansion Arm 4 will enroll up to 20 subjects. The number of subjects evaluated in the safety lead-in of Arm 4 may count toward the total number of subjects in an expansion arm. The sponsor will determine the eligible patient population for each expansion cohort after discussion with the Cohort Review Committee. Each dose-escalation and safety lead-in arm may lead to more than one expansion cohort if different patient populations will be studied.

Monitoring of all safety and toxicity data are performed by the investigator and the Cohort Review Committee. The Cohort Review Committee may choose to apply safety knowledge gained from any arm or cohort to more conservatively manage other arms or cohorts.

4.18. Retreatment Criteria

After safely completing the Study Treatment Period, each subject may continue to receive dosing during the Treatment Extension Period until discontinuation for any reason. Upon discontinuation, the subject has a Post-treatment Period that includes an End of Treatment (EOT) Visit, and post-treatment evaluations, including both follow-up safety and survival contact (Table 2 and Table 4).

Arm 1 dose escalation has a 21-day cycle.

Arm 1 Expansion and Arm 2 have a 21-day cycle plus a 7-day of loading dose.

Arm 4 has a 28-day cycle plus a 7-day of loading dose.

4.19. Follow-Up Safety and Survival Contact

Subjects will be followed for a minimum of 30 days from the last dose of TTX-030 (EOT Visit). Scheduled follow-up safety contact will be scheduled for 60 and 90 days after the last dose and a follow-up survival contact will be scheduled for 180 days after the last dose.

Subjects removed from study for unacceptable AE(s) will be followed until resolution or stabilization of the AE.

4.20. Study Completion

A subject is considered to have completed the study if he or she has completed the protocol-defined activities for the EOT Visit shown in the Schedule of Activities (Table 2 and Table 4).

5. Study Population

5.1. Target Population and Arm-Specific Eligibility Criteria

Arm 1 (Escalation): Single-Agent TTX-030 Q3W

1. Histologically confirmed diagnosis of unresectable or metastatic solid tumor malignancy, or relapsed/refractory lymphoma, for which all standard therapies have been previously given.
2. Meets all Common Inclusion and Exclusion Criteria below.

Arm 2 (Safety Lead-In): Pembrolizumab 200 mg Q3W + TTX-030 Q3W

3. Histologically confirmed diagnosis of unresectable or metastatic solid tumor malignancy, or relapsed/refractory lymphoma.
4. Subjects must fulfill **one** of the following criteria:
   a) Eligible to receive single-agent pembrolizumab as standard of care.
   b) Received prior anti-PD-(L)1 containing regimen and had refractory or relapsed disease, for which all standard therapies have been previously given.
5. Subjects who experienced immune-related or other AEs on prior anti-PD-(L)1 therapy that required discontinuation or modification are not eligible for this study.
6. Meets all Common Inclusion and Exclusion Criteria listed below.

Arm 1 and 2 Expansion Cohorts
Randomized 1:1 to TTX-030 Q3W or TTX-030 Q3W + Pembrolizumab 200 mg Q3W

7. Histologically or cytologically confirmed diagnosis of advanced (not amenable to curative surgery or radiation therapy) or metastatic (AJCC stage IV) RCC with a clear-cell component.
8. Failed any anti-PD-(L)1 and TKI agents. Subjects refractory/relapsed to an anti-PD-(L)1-containing regimen must have confirmed radiographic disease progression no earlier than 4 weeks after initial disease progression.

Arm 4 (Safety Lead-In and Expansion): Gemcitabine 1000 mg/m^2 + nab-paclitaxel 125 mg/m^2 Days 1, 8, and 15 every 28 days + TTX-030 Q2W

9. Histologically or cytologically confirmed diagnosis of locally advanced, unresectable, or metastatic pancreatic adenocarcinoma.
10. Naïve to any prior treatment for metastatic disease. Prior adjuvant therapy (including chemotherapy and/or radiotherapy) is permitted if neoadjuvant or adjuvant therapy was completed at least 6 months prior to study enrollment.
11. Eligible to receive gemcitabine + nab-paclitaxel as standard of care.
12. Meets all Common Inclusion and Exclusion Criteria listed below.

All Expansion Cohorts

13. At least one tumor site (primary or metastasis) that is amenable to biopsy, and subject must be willing to consent for both pretreatment (screening) and on-treatment biopsy.
14. Meets eligibility criteria for subject population(s) specified by sponsor.
15. Meets all Common Inclusion and Exclusion Criteria listed below.

5.2. Common Inclusion Criteria

16. Age 18 years or older and is willing and able to provide informed consent.
17. Fresh and/or archival tumor tissue is optional for dose-escalation; fresh and/or archival tumor biopsies at screening and on-treatment are mandatory for safety lead-in arms and expansion cohorts.
18. Evidence of measurable disease by CT, CT-positron-emission tomography (PET-CT) for dose escalation only, or MRI. (NOTE: Subjects with prostate cancer may instead have evaluable disease using bone scan per PCWG3 criteria and are not required to have Response Evaluation Criteria in Solid Tumors [RECIST] measurable disease.)
19. Life expectancy > 12 weeks.
20. Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1 (see Appendix 2).
21. Demonstrated adequate organ function, including:
    a. Oxygen saturation ≥ 92% on room air.
    b. Absolute neutrophil count ≥ 1.5 k/µL, platelets (PLT) ≥ 75 k/µL (PLT ≥ 100 k/µL required for chemotherapy arms), and hemoglobin (Hgb) ≥ 8 g/dL (Hgb ≥ 9 g/dL for chemotherapy arms). NOTE: Prior red blood cell transfusion is allowed.
    c. Serum creatinine ≤ 1.5 x ULN or creatinine clearance ≥ 40 mL/min.
    d. AST/ALT ≤ 3 x ULN (or ≤ 5 x ULN with liver metastases).
    e. Total bilirubin ≤ 1.5 x ULN (or ≤ 3 x ULN with Gilbert's syndrome).
    f. Prothrombin time (PT)/international normalized ratio (INR) and activated partial thromboplastin time (aPTT) ≤ 1.2 x ULN; fibrinogen ≥ 150 mg/dL.
22. At least 14 days since last dose of chemotherapy or biological therapy, or tyrosine kinase inhibitor or high-dose (eg, > 10 mg prednisone or equivalent) steroid therapy prior to first dose of study treatment.
23. Resolution of adverse effects from any prior chemotherapy, immunotherapy, or prior systemic anti-cancer therapy, radiotherapy, or surgery to Grade 1 or baseline (except Grade 2 alopecia and Grade 2 sensory neuropathy).
24. Women of childbearing potential and all men must agree to use highly effective methods of birth control through 120 days after the last dose of study treatment.

    NOTE: Highly effective contraception methods include:

- Total abstinence
- Female sterilization (tubal ligation, bilateral oophorectomy, and/or hysterectomy)
- Male sterilization, at least 6 months prior to screening
- Intrauterine device
- Oral, injected, or implanted hormonal contraception AND barrier methods of contraception

25. Willing to comply with and able to tolerate study procedures.

5.3. Common Exclusion Criteria

26. History of allergy or hypersensitivity to study treatment components. Subjects with a history of severe hypersensitivity reaction to any mAb should also be excluded.
27. Use of investigational agent within 14 days prior to the first dose of study treatment and throughout the study.
28. Prior surgery or radiotherapy within 14 days of study treatment.
29. Receiving high-dose (eg, > 10 mg prednisone or equivalent) systemic steroid therapy or any other form of immunosuppressive therapy within 14 days prior to the first dose of study treatment. NOTE: inhaled, intranasal, intraocular, topical, and intraarticular steroids are allowed. Transient steroid administration as anti-emetic or chemotherapy preconditioning (eg, for paclitaxel) is allowed per institutional guidelines.
30. Receiving therapeutic anticoagulation. NOTE: Low molecular weight heparin, Factor Xa inhibitors, and low-dose aspirin are allowed for deep vein thrombosis (DVT) prophylaxis.
31. History of autoimmune disease requiring systemic treatment or transplant that requires systemic steroids or immunosuppressive agents. NOTE: History of vitiligo, autoimmune thyroiditis, or mild psoriasis is allowed.
32. Subjects who are on steroids for any irAE that has not resolved, Grade 3 or higher irAE, and ocular or neurologic toxicity of any grade.
33. Known history of human immunodeficiency virus or other chronic immunodeficiency.
34. Uncontrolled intercurrent illness including, but not limited to:
    a. Uncontrolled diabetes with hemoglobin A1c (HgbA1c) $\geq$ 8.0%
    b. Ongoing or active bacterial, viral, or fungal infection requiring systemic treatment
    c. Clinically significant congestive heart failure defined by New York Heart Association Class 3 or Class 4
    d. Unstable angina, arrhythmia, or myocardial infarction within 6 months prior to screening
35. Known brain metastases or central nervous system (CNS) involvement. NOTE: Subjects with asymptomatic and stable brain metastases following prior treatment may be eligible.
36. Other active malignancy requiring ongoing treatment.

37. Women who are pregnant or breastfeeding.
38. For Arm 1 and 2 Expansion Cohorts only (Renal Cell Cancer):
    a. Subjects with Lactate Dehydrogenase (LDH) ≥ 1.5x ULN

5.4. Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently entered/enrolled in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes date of screening, informed consent, reason for screen failure (eg, eligibility criteria), and any SAEs.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened.

5.5. Subject Adherence to Protocol Schedule

General study instructions to be discussed or provided by the study staff:

- Subjects must use highly effective birth control.
    - Women of childbearing potential and all men should be counseled and must agree to use highly effective birth control during treatment through 120 days after the last dose of study treatment.
- Subjects may not receive other investigational or approved anti-cancer therapy while participating in this trial.
- Subjects must come to the clinic on days defined in Table 1/Table 2, and Table 3/Table 4 for study test procedures and post-study follow-up contact information.
- From the first dose of TTX-030 until all procedures are completed on Cycle 2 Day 1, subjects should be instructed not to change their concomitant medications to the extent possible.
- On dosing days, subjects should be discharged from the clinic with directions on self-administration of medications as needed to ameliorate potential delayed reactions (which should be described) to study treatment infusion.
- Subjects should report any concerns of pregnancy immediately.

6. Investigational Product

6.1. Investigational Product Description and Administration

6.1.1. Investigational Product Description

The investigational formulation of TTX-030 will be supplied as a sterile, single-use, preservative-free liquid containing 200 mg of antibody (Table 10). The dosage form of TTX-030 is a clear, colorless liquid formulation containing 30 mg/mL of TTX-030 in 10 mM sodium citrate, 280 mM sucrose, 0.02% polysorbate 20, and 1.0 mM methionine, with a pH of 6.5. The liquid TTX-030 will be filled into a Type 1 glass serum vial with an elastomeric stopper and aluminum over seal with a plastic flip top. The fill volume will be 6.7 mL in a 10-cc glass vial stored at temperatures of 2°C to 8°C. TTX-030 will be administered IV Q3W, Q2W, or Q4W depending on the study arm.

Table 10: Investigational Product

Product name:	TTX-030
Dosage form:	injection, solution in a vial
Unit dose:	200 mg/15 mL (30 mg/mL)
Route of administration:	intravenous infusion
Physical description:	clear liquid in a serum vial with an elastomeric stopper
Manufacturer:	ProBioGen AG

6.1.2. Other Study Treatments

TTX-030 will be administered in combination with standard of care regimens for selected malignancies (Table 8). Sites will obtain approved agents listed in Table 11 for combination therapy with TTX-030. Pembrolizumab will be provided by Trishula.

6.1.3. Investigational Product Administration

TTX-030 can be administered in an outpatient setting. The infusion should be administered under the supervision of a physician. Doses will be administered through an IV line controlled by a volumetric pump. TTX-030 should not be co-administered with other medications through the same IV line during the TTX-030 infusion. TTX-030 doses of ≤ 20 mg/kg should be infused over 30 to 60 minutes, and TTX-030 doses of > 20 mg/kg may be infused over 60 minutes (see Pharmacy Manual for details), unless otherwise revised by the study's Cohort Review Committee. Subjects should be monitored for any infusion-related AEs for 8 hours after the first dose and for 1 hour after subsequent doses. If an infusion-related reaction (IRR) of Grade 3 or above occurs, the medical monitor should be contacted prior to any further subject dosing. It is recommended to have vital signs check every 1-hour for 4 hours and then every 2-hour based on clinical conditions. Vital signs monitoring at subsequent dosing visits may occur as per Investigator's discretion.

Monitoring of the subject should include blood pressure, pulse, temperature, respirations, and pulse oximetry. Additionally, subjects should be monitored for infusion-related reactions such as

rash, acute allergic reaction, bronchospasm, respiratory distress, and acute vascular leak syndrome.

Treatment will be administered as presented in Table 11. Subjects in any combination treatment arm may receive premedication according to institutional practice.

Table 11: Treatment Regimen Description

Arm	Treatment	Dose	Route	Schedule
1[a]	TTX-030	Loading dose 40 mg/kg	IV[b]	Day -7
1	TTX-030	30 mg/kg	IV[b]	Q3W
2[a]	TTX-030	Loading dose 40 mg/kg	IV[b]	Day -7
2	TTX-030	30 mg/kg	IV[b]	Q3W
2	Pembrolizumab	200 mg	IV[b], 60 minutes after TTX-030	Q3W
4[a]	TTX-030	Loading dose 40 mg/kg	IV[b]	Day -7
4	TTX-030	20 mg/kg	IV[b], prior to nab-paclitaxel	Q2W Days 1 and 15 of each 28-day cycle
4	Nab-paclitaxel	125 mg/m^2	IV over 30-40 minutes, 60 minutes after TTX-030 and prior to gemcitabine	Days 1, 8, and 15 of each 28-day cycle
4	Gemcitabine	1000 mg/m^2	IV over 30-40 minutes, immediately after nab-paclitaxel	Days 1, 8, and 15 of each 28-day cycle

Abbreviations: IV = intravenous, Q = every, RP2D = recommended Phase 2 dose, W = week(s).

[a] A loading dose of TTX-030 40 mg/kg is to administer on Day -7 prior to Cycle 1 Day 1.

[b] Refer to the Pharmacy Manual for dosing instruction. Infusion duration is dependent on subject's weight and IV bag size and may be longer than 60 minutes.

6.2. Preparation/Handling/Storage/Accountability

The study treatment will be administered by the study personnel; the day and time of study treatment administration will be recorded by the study personnel.

6.2.1. Study Treatment Packaging and Labeling

The study treatment will be packaged and labeled according to current Good Manufacturing Practice. Details of the packaging and labeling of clinical supplies may be found in the pharmacy manual.

6.2.2. Study Treatment Storage

Cartons will be used for secondary packaging. Each carton will be labeled per regulations with the product name, product lot number, recommended storage condition, manufacturer, and the Federal Caution Statement "Caution: New Drug – Limited by Federal (US) Law to Investigational Use Only." The sponsor's name and address will also be included.

TTX-030 vials must be stored at a temperature of 2°C to 8°C and should be protected from light and freezing. If stored in a glass-front refrigerator, vials should be stored in the carton. Recommended safety measures for preparation and handling of TTX-030 include laboratory coats and gloves. After TTX-030 has been prepared for administration, the total storage time (combination of refrigeration and room temperature) is not to exceed 4 hours.

The product storage manager should ensure that the study treatment is stored in accordance with the environmental conditions (temperature, light, and humidity) as determined by the sponsor. If concerns regarding the quality or appearance of the study treatment arise, do not dispense the study treatment and contact the sponsor immediately.

6.2.3. Study Treatment Preparation

Steps for preparing the study treatment are provided in the pharmacy manual.

6.2.4. Study Treatment Accountability

The study treatment is to be used only as directed by this protocol. Clinical supplies are not to be dispensed to any individual who is not enrolled in the study. An accurate and timely record of the receipt of all clinical supplies and dispensing of study treatment to the subject will be maintained. This may include (a) documentation of receipt of clinical supplies, (b) study treatment dispensing/return reconciliation log, (c) study treatment accountability log, and (d) all shipping service receipts.

6.2.5. Study Treatment Handling and Disposal

If study treatments (those supplied by the sponsor or sourced by the investigator) are to be destroyed on site, it is the investigator's responsibility to ensure that arrangements have been made for the disposal; procedures for proper disposal have been established according to applicable regulations, guidelines, and institutional procedures; and appropriate records of the disposal have been documented. The unused study treatment may be destroyed only after being inspected and reconciled by the responsible study monitor.

6.3. Measures to Minimize Bias: Randomization and Blinding

Only subjects in Arm 1 and Arm 2 Expansion Cohorts will be randomized (as described in Section 4.1.1.2) and treatment assignment will occur as described in Section 4.3.

This is an open-label study.

6.4. Treatment Compliance

The study treatment will be administered by the study personnel; the day and time of study treatment administration will be recorded by the study personnel.

6.5. Concomitant Therapy

6.5.1. Concomitant Medications Allowed During the Study

On dosing days, subjects should be discharged from the clinic with directions on self-administration of medications as needed to ameliorate potential delayed reactions to study treatment infusion, such as fever, chills, and myalgia. Medications may include antipyretics and antihistamines. All concomitant medications, including self-administered medications, are to be recorded. Concomitant medications are to be collected from screening through 30 days after the last dose of study treatment.

Inhaled, intranasal, intraocular, topical, and intraarticular steroids are allowed. Transient steroid administration as anti-emetic or chemotherapy preconditioning (eg, for paclitaxel) is allowed per institutional guidelines. Supportive care may be given as indicated after a subject experiences nausea, vomiting, fever, or diarrhea while on study treatment. Use of supportive care agents must be recorded. Prednisone doses ≤ 10 mg (physiological doses) are permitted.

Supportive treatments that are indicated for treatment of AEs should be given as medically required. Standard supportive medications may be used such as hematopoietic growth factors to treat neutropenia or thrombocytopenia in accordance with American Society for Clinical Oncology guidelines.

Concomitant medications may be necessary in the event of an acute infusion-related reaction, cytokine release syndrome, or another AE as detailed in Section 8.3.6.5.4.

Low molecular weight heparin, Factor Xa inhibitors, and low-dose aspirin are allowed for DVT prophylaxis.

6.5.2. Concomitant Medications Prohibited During the Study

Concomitant treatments or procedures with any of the following are not allowed while the subject is receiving study treatment, unless approved by the sponsor, or as otherwise described in the protocol:

- Any alternative drug treatments or procedures directed toward the treatment of solid tumors, including immunotherapy, chemotherapy, and radiation therapy. NOTE: Palliative radiation may be allowed during trial on a case-by-case basis – please discuss with the medical monitor.
- Any investigational product, including investigational symptomatic treatment or procedures for solid tumors and investigational treatment or procedures for non-cancer indications.

Any concomitant treatment or procedure required for the subject's welfare may be given by the investigator. However, it is the responsibility of the investigator to ensure that details regarding the treatment or procedure are to be recorded.

7. Discontinuation of Study Treatment and Subject Discontinuation

7.1. Discontinuation of Study Treatment

Treatment with TTX-030 may continue until precluded by toxicity, progression, or death.

For combination arms, if a subject discontinues the pembrolizumab or chemotherapy regimens, treatment with TTX-030 may continue if it remains well tolerated and if approved by the investigator and sponsor.

For all arms, in the absence of treatment delays due to AE(s), treatment may continue until 1 of the following criteria applies:

- Disease progression (includes radiological or clinical progression).
- Intercurrent illness that prevents further administration of treatment
- Unacceptable AE
- Subject decides to withdraw from the study
- General or specific changes in the subject's condition that render the subject unacceptable for further treatment in the judgment of the investigator
- Subject non-compliance
- Pregnancy
    - All women of childbearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (eg, missed or late menstrual period) at any time during study participation (see Section 8.3.6.4 for reporting requirements)
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study treatment

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in the eCRF.

7.2. Subject Discontinuation/Withdrawal from the Study

Any subject can withdraw from the trial at any time.

Reasons why a subject may discontinue or be withdrawn from the study include lack of efficacy, AE, DLT, death, withdrawal by subject, physician decision, study terminated by sponsor, protocol deviation, pregnancy, lost to follow-up, or other reasons not otherwise specified within this list.

When a subject discontinues or is withdrawn from the study after receiving ≥ 1 dose of study treatment, the investigator will, when possible, perform the procedures indicated for the EOT Visit (Table 2 and Table 4).

The Sponsor may terminate enrollment into Arm 1 of the Expansion Cohort (i.e. TTX-030 monotherapy, see Section 4.1.1.2) should there be evidence of a lack of efficacy. The totality of

available data will be used to make this determination, however, should zero responders be seen out of the first 16 subjects the 90% one-sided confidence interval (using exact methods) excludes a responder rate of 13.4% or more.

Subjects who have been consented or randomized into Arm 1 before the termination will continue to receive study treatment as per protocol.

7.3. Lost to Follow-up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, he/she will be considered to have withdrawn from the study.

8. Study Assessments and Procedures

All screening and baseline assessments must be performed prior to loading dose/first dose of TTX-030.

Assessments done up to 3 days prior to the loading dose do not need to be repeated. Assessment results must be deemed acceptable before the subject is cleared to receive the first dose of study treatment.

Unless otherwise indicated, laboratory test collections are to be performed before the start of study treatment infusion on infusion days.

COVID-19 guidance from applicable local regulatory authorities will be followed as necessary during the study in order to ensure subject safety. Variance from the study assessments schedule may be permitted at the Sponsor's discretion in consultation with the Investigator and will be documented accordingly.

After safely completing the Study Treatment Period, each subject may continue to receive dosing during the Treatment Extension Period until discontinuation for any reason. Upon discontinuation, the subject has a Post-treatment Period that includes an EOT Visit, and post-treatment evaluations including both follow-up safety and survival contact (Table 1/Table 2, and Table 3/Table 4).

- Arm 1 (dose escalation) has a 21-day cycle (Q3W).
- Arms 1 and 2 expansion have a 21-day cycle (Q3W) plus a 7-day of loading dose.
- Arm 4 has a 28-day cycle (Q2W) plus a 7-day of loading dose.

8.1. Study Visits

8.1.1. Study Visits for Arms 1 and 2 Safety Lead-in and Expansion

- Arm 1 treatment is TTX-030 as a single agent.
- Arm 2 treatment is TTX-030 and pembrolizumab.

Overview of Study Periods for Arms 1 and 2 (21-Day Cycle)

Pretreatment Period

Pretreatment consists of screening (up to 28 days prior to loading dose/first dose) and baseline (up to 3 days prior to loading dose/first dose).

Study Treatment and Treatment Extension Period

- A loading dose of TTX-030 40 mg/kg is to administer on Day -7 prior to Cycle 1 Day 1.
- After the loading dose, TTX-030 30 mg/kg will be administered every 21 days, on Day 1 of each cycle.
- Cycle 1 includes assessments on Day 1, Cycle 1 Day 1 (C1D1) (dosing), Day 2, C1D2; Day 3, C1D3; Day 8, C1D8; and Day 15, C1D15.

- Cycle 2 includes assessments on Day 22 + 2 days, C2D1 (dosing) and Day 29 ± 2 days, C2D8.
- Treatment Extension Period begins with Cycle 3, with treatment on Day 43 ± 2 days, C3D1 (dosing), followed by Cycles 4+, Day 64 ± 2 days, C4D1 (dosing), and every 21 days thereafter, with dosing on Day 1 of each subsequent cycle.

Post-treatment Period

- The Post-treatment Period includes the EOT Visit at 30 ± 7 days after the last dose, follow-up safety contact at 60 and 90 ± 7 days and a follow-up survival contact at 180 ± 15 days after last dose.

8.1.2. Study Visits for Arm 4 (TTX-030 Q2W, 28-Day Cycle)

Arm 4 treatment is TTX-030 and nab-paclitaxel + gemcitabine.

Overview of Study Periods for Arm 4 (28-Day Cycle)

Pretreatment Period

Pretreatment consists of screening (up to 28 days prior to loading dose/first dose) and baseline (up to 3 days prior to loading dose/first dose).

Study Treatment and Treatment Extension Period

- A loading dose of TTX-030 40 mg/kg is to administer on Day -7 prior to Cycle 1 Day 1.
- After the loading dose, TTX-030 20 mg/kg will be administered every 14 days, on Day 1 and Day 15 of each cycle.
- Cycle 1 includes assessments on Day 1, C1D1 (dosing); Day 2, C1D2; Day 3, C1D3; Day 8, C1D8 (Gem/NP dosing only); Day 15, C1D15 (dosing); and Day 22, C1D22.
- Treatment Extension begins with Cycle 2, with treatment on Day 29 + 2 days, C2D1 (dosing), Day 36 ± 2 days, C2D8 (Gem/NP dosing only) and Day 43 ± 2 days, C2D15 (dosing), and every 14 days thereafter, with TTX-030 dosing on Day 1 and Day 15 of each subsequent cycle.
- Treatment Extension continues with Cycle 3, with treatment on Day 57 ± 2 days, C3D1 (dosing), Day 64 ± 2 days, C3D8 (Gem/NP dosing only) and Day 71 ± 2 days C3D15 (dosing), followed by Cycles 4+, with treatment on Days 1, 8 (Gem/NP dosing only) and 15 thereafter.

Post-treatment Period

- The Post-treatment Period includes the EOT Visit at 30 ± 7 days after the last dose, follow-up safety contact at 60 and 90 ± 7 days, and a follow-up survival contact at 180 ± 15 days after last dose.

8.2. Efficacy Assessments

8.2.1. Tumor Assessment/Tumor Response Assessment

Tumor response assessments will be performed as scheduled in Table 1/Table 2, and Table 3/Table 4.

Radiological assessments for subjects with solid tumors include CT scans of the chest, abdomen, and pelvis unless contraindicated. Subjects with prostate cancer will also require bone scans. Subjects with lymphoma may be assessed with CT or PET-CT; however, the method for each subject's radiology assessments should remain consistent.

8.2.1.1. Criteria for Response Assessment for Solid Tumors

To evaluate early signs of activity, iRECIST will be used for solid tumors. Response criteria to be determined by the investigator are found in Appendix 3 (Section 11.3).

Additional response criteria for subjects with prostate cancer are found in Appendix 4 (Section 11.4).

Lymphoma will be assessed per LYRIC (see Section 8.2.1.2).

For immunotherapeutics, response assessment may be confounded by a delayed onset of clinical effect of these agents allowing for early tumor growth or even the appearance of new lesions known as pseudo-progression. Efforts have been made to modify standard response criteria to account for the impact of immunotherapeutics on anti-tumor activity with the utilization of iRECIST for solid tumor and LYRIC for lymphoma to evaluate tumor response.

In an attempt to apply response criteria and optimize response assessments with the use of immunotherapeutics, the RECIST Version 1.1 criteria have been modified to include an immune-related progressive disease response category in iRECIST. The addition of the immune response (IR) category to the more standard RECIST for solid tumors may provide information to the immune response as well as distinguish delayed responses and capturing pseudo progression (flare reaction). A comparison of RECIST and iRECIST response criteria for solid tumor is provided in Section 11.3 (Appendix 3).

8.2.1.2. Criteria for Response Assessment for Lymphoma

Subjects with lymphoma should have their response assessments conducted per LYRIC. Response criteria to be determined by the investigator are found in Section 11.5 (Appendix 5).

The current Lugano criteria have been used for more traditional therapies in lymphoma. The proposed provisional modification, LYRIC, for immunomodulatory agents has introduced the term "IR". The term "IR" does not make a direct reference to the underlying mechanism, acknowledging that both a delayed response and an immune-mediated flare ("pseudo progression") can occur in the early treatment period and may be challenging to differentiate from progression by physical examination or imaging alone. The term provides the flexibility to allow subjects to continue treatment past IR in some circumstances with a mandatory subsequent evaluation within 12 weeks to confirm or refute true progressive disease.

A comparison of response based on PET-CT and CT evaluations is provided in Section 11.5 (Appendix 5). Exceptions to Lugano for the LYRIC response assessments accounting for a development of a flare reaction ("pseudo-progression") and a true progressive disease are provided in Section 11.5 (Appendix 5).

For details on the response criteria and evaluations for lymphomas, see Cheson et al. 2014 and Cheson et al. 2016.

8.3. Safety Assessments

8.3.1. Demographic/Medical History

Subject demographics and baseline characteristics (including age, sex, race, ethnicity, weight and height, disease information, and medical conditions) are to be summarized at screening.

8.3.2. Physical Examinations (Including Symptom-focused Examination)

A physical examination requires assessment of all major body systems to evaluate any new clinically significant abnormalities within the following: general appearance, skin, head/eyes/ears/nose/throat, neck, heart, lungs, abdomen, extremities, back/spinal, and lymph nodes. This includes worsening of baseline conditions.

For a symptom-focused examination, only the relevant or affected body system(s) must be examined and a full physical examination is not required.

8.3.3. Vital Signs

Sitting blood pressure, pulse rate, respiratory rate, and oral temperature will be measured after the subject has been resting for at least 5 minutes. Arterial oxygen saturation will be measured by pulse oximetry.

8.3.4. Electrocardiograms

Electrocardiograms (ECGs) will be obtained via 12-lead ECGs at screening with the subject in semi-recumbent position after 5 minutes of rest. The investigator may perform additional unscheduled ECGs to manage or evaluate a suspected AE as clinically necessary. Unscheduled ECGs will be documented.

8.3.5. Laboratory Assessments

Blood samples will be collected for clinical laboratory testing, including complete blood count (CBC), chemistry, coagulation, and other tests. Clinical laboratory tests are summarized in Table 12.

Additional or repeated clinical laboratory testing may be performed while on treatment as clinically indicated by the investigator. Laboratory assessments may be performed up to 3 days prior to the dosing visit specified in Table 1/Table 2, and Table 3/Table 4. Visits conducted outside the windows are to be discussed with the medical monitor (or designee). On treatment days, samples for clinical laboratory testing should be drawn prior to the infusion.

Table 12: Clinical Laboratory Tests

Hematology Tests	Coagulation Tests
RBC count	PT/INR
Hgb	PTT
Hematocrit	Fibrinogen (within 14 days of dosing)
WBC count	**Other**
Neutrophils	Serum pregnancy
Lymphocytes	HgbA1c (at screening only)
Monocytes	Creatinine clearance (at screening)
Basophils	Thyroid function tests: TSH, T3, free T4, thyroid antibody (at baseline, at the loading dose/first dose, and every 3 months thereafter)
Eosinophils	ACTH (at baseline only)
PLT	FSH (at baseline only)
Chemistry Tests	LH (at baseline only)
Sodium	GH (at baseline only)
Potassium	Amylase (at baseline only)
Chloride	Lipase (at baseline only)
Bicarbonate	LDH (at screening only) – for RCC
Glucose	
Creatinine	
ALT	
AST	
ALP	
GGT	
Lactate dehydrogenase	
Total bilirubin	
Total protein	
Albumin	
BUN	
Calcium	
Phosphorous	
Magnesium	
Creatine kinase	
Uric acid	

Abbreviations: ACTH = adrenocorticotropic hormone, ALT = alanine aminotransferase, ALP = alkaline phosphatase, AST = aspartate aminotransferase, BUN = blood urea nitrogen, FSH = follicle-stimulating hormone, GGT = gamma-glutamyl transferase, GH = growth hormone, Hgb = hemoglobin, HgbA1c = hemoglobin A1c, INR = international normalized ratio, LH = luteinizing hormone, PLT = platelets, PT = prothrombin time, PTT = partial thromboplastin time, RBC = red blood cell, TSH = thyroid stimulating hormone, WBC = white blood cell, Lactate dehydrogenase (LDH).

8.3.5.1. Hematology

Hematology assessments are included in the clinical laboratory assessments in Table 1/Table 2, and Table 3/Table 4.

8.3.5.2. Blood Chemistry

Chemistry is included in the clinical laboratory assessments in the Table 1/Table 2, and Table 3/Table 4.

8.3.5.3. Urinalysis

Urinalysis is not required for this study but allowed for pregnancy testing.

8.3.5.4. Virus Serology

Virus serology is not required.

8.3.5.5. Drug Screen

Drug screen is not required.

8.3.5.6. Pregnancy Screen

For women of childbearing potential, pregnancy test (serum or urine, per institutional practice) will be administered at screening, baseline, and on C2D1 and the first day of each subsequent cycle.

8.3.6. Adverse Events and Serious Adverse Events

Any subject who receives at least 1 dose of study treatment will be included in the evaluation for safety. Safety will be assessed by physical examinations, vital signs, and laboratory assessments. Safety will also be evaluated using the proportion of subjects requiring modifications of the infusion schedule.

Safety and tolerability will be evaluated by the incidence, severity, seriousness, type, and causality of AEs that occur during the treatment and follow-up periods.

The incidence of AEs and SAEs will be evaluated for each dose level and for all subjects combined. Subjects are to be followed for AEs that begin or worsen after start of study treatment through EOT or 90 days (± 7 days) after the last dose of study treatment, whichever is later.

“Lack of efficacy” or “failure of expected pharmacological action” per se will not be reported as an AE or SAE; nor will progressive disease. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Clinical AEs will be graded according to the NCI CTCAE Version 5.0. Adverse events are to be monitored and collected for the duration of study treatment until 90 days after the last dose.

8.3.6.1. Definition of Adverse Events

8.3.6.1.1. Adverse Event

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (eg, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a treatment and does not imply any judgment about causality. An AE can arise with any use of the treatment (eg, off-label use, use in combination with another treatment) and with any route of administration, formulation, or dose, including an overdose.

All AEs that occur after any subject has been enrolled, before treatment, during treatment, or within 90 days following the cessation of treatment, whether or not the events are related to the study, must be recorded on the eCRF provided by the sponsor.

8.3.6.1.2. Suspected Adverse Reaction

Suspected adverse reactions are a subset of all AEs for which there is reasonable possibility that the treatment caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the treatment and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a treatment.

The principal investigator is responsible for determining whether there is a reasonable possibility that the treatment caused the AE and will capture the causality assessment in the eCRF.

8.3.6.1.3. Adverse Reaction

An adverse reaction is any AE caused by a treatment. Adverse reactions are a subset of all suspected adverse reactions where there is reason to conclude the treatment caused the event.

8.3.6.1.4. Unexpected

An AE or suspected adverse reaction is considered “unexpected” if it is not listed in the IB or is not listed at the specificity or severity that has been observed. The sponsor is responsible for determining whether an event meets the definition of “unexpected,” based on adverse reactions listed in the sponsor’s IB.

8.3.6.1.5. Serious Adverse Event

An SAE is an AE occurring during any study period (baseline, treatment, washout, or follow-up), and at any dose of the study treatment, comparator, or placebo, that fulfils 1 or more of the following:

- It results in death
- It is immediately life-threatening
- It requires in-patient hospitalization or prolongation of existing hospitalization
- It results in persistent or significant disability or incapacity
- It results in a congenital abnormality or birth defect

- It is an important medical event that may jeopardize the subject or may require medical intervention to prevent 1 of the outcomes listed above

All SAEs that occur after any subject has been enrolled, before treatment, during treatment, or within 90 days following the cessation of treatment, whether or not they are related to the study, must be recorded on eCRF provided by the sponsor.

8.3.6.1.6. Further AE and SAE Definitions

Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the investigator, it should be recorded as a separate AE. Laboratory data are to be collected as stipulated in this protocol. Clinical syndromes associated with laboratory abnormalities are to be recorded as appropriate (eg, diabetes mellitus rather than hyperglycemia).

Scheduled hospitalizations or elective surgical procedures will not be considered as AEs or SAEs. Complications associated with scheduled procedures are considered AEs or SAEs.

An abnormal test finding will be classified as an AE if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms.
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant treatment or other therapy. NOTE: Simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an AE.
- The test finding leads to a change in study treatment dosing or discontinuation of subject participation in the clinical research study.
- The test finding is considered an AE by the investigator.

8.3.6.1.7. Other Adverse Event

Other AEs will be identified by the sponsor and, if applicable, also by the Clinical Study Team Physician during the evaluation of safety data for the clinical study report. Significant AEs of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from the study, will be classified as other AEs. For each other AE, a narrative may be written and included in the clinical study report.

8.3.6.2. Relationship to Study Treatment

An investigator who is qualified in medicine must make the determination of relationship to the investigational product for each AE (unrelated, possibly related, or probably related). The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the study treatment. If no valid reason exists for suggesting a relationship, then the AE should be classified as “unrelated.” If there is any valid reason, even if undetermined, for suspecting a possible cause-and-effect relationship between the study treatment and the occurrence of the AE, then the AE should be considered “related.”

If the relationship between the AE/SAE and the study treatment is determined to be "possible" or "probable," the event will be considered to be related to the study treatment for the purposes of expedited regulatory reporting.

8.3.6.3. Recording Adverse Events

Adverse events spontaneously reported by the subject and/or in response to an open question from the study personnel or revealed by observation will be recorded during the study at the investigational site. Clinically significant changes in laboratory values, blood pressure, and pulse need not be reported as AEs. However, abnormal values that constitute an SAE or lead to discontinuation of administration of study treatment must be reported and recorded as an AE. Information about AEs will be collected from the signing of consent form until the end of the study. Serious adverse event information will be collected from signing of consent form until 90 days following the last dose of study treatment. The AE term should be reported in standard medical terminology when possible. For each AE, the investigator will evaluate and report the onset (date and time), resolution (date and time), intensity, causality, action taken, serious outcome (if applicable), and whether or not it caused the subject to discontinue the study.

Adverse event grading will be defined by the NCI CTCAE Version 5.0. In the event the NCI CTCAE Version 5.0 criterion does not apply, the general severity descriptions of mild, moderate, severe, life-threatening and death will be used as listed below by NCI CTCAE:

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.[a]
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.[b]
Grade 4 Life-threatening consequences: urgent intervention indicated.
Grade 5 Death related to AE.

[a] Instrumental activities of daily living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

[b] Self-care activities of daily living refer to bathing, dressing, and undressing; feeding self; using the toilet; taking medications; and not bedridden.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity, whereas seriousness is defined by the criteria under Section 8.3.6.1.5. An AE of severe intensity may not be considered serious.

8.3.6.3.1. Pregnancy

Should a pregnancy occur, it must be reported to and recorded by the sponsor. Pregnancy in itself is not regarded as an AE unless there is a suspicion that a study treatment may have interfered with the effectiveness of a contraceptive medication.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed up and documented even if the subject was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

8.3.6.4. Reporting Adverse Events

All AEs (e.g., including COVID-19 symptoms) and SAEs will be collected from the time the subject signs informed consent through 90 days after the last dose of TTX-030 or until initiation of a new systemic anticancer therapy, whichever occurs first. Positive COVID-19 test result will be collected as a medical event and submitted as SAE.

Any SAEs considered possibly or probably related to the study treatment and discovered by the investigator at any time after the study should be reported. All SAEs must be reported to the sponsor within 1 business day of the first awareness of the event. The investigator must complete, sign, and date the SAE pages; verify the accuracy of the information recorded on the SAE pages with the corresponding source documents; and send a copy to the contact information listed on the SAE forms.

Additional follow-up information, if required or available, should all be faxed to the sponsor within 1 business day of receipt, and this should be completed on a follow-up SAE form and placed with the original SAE information and kept with the appropriate section of the eCRF and/or study file.

The sponsor is responsible for notifying the relevant regulatory authorities of certain events. It is the investigator's responsibility to notify the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) of all SAEs that occur at his or her site. Investigators will also be notified of all unexpected, serious, treatment-related events (7/15 Day Safety Reports) that occur during the clinical trial. Each site is responsible for notifying its IRB or IEC of these additional SAEs.

8.3.6.4.1. SAE Reporting

The investigator is obligated to report immediately to the sponsor or designee each SAE that occurs during this study, within 24 hours from knowledge of the event, whether or not it is considered study treatment related. All requested supplementary documents (eg, discharge summary and autopsy report) and relevant data (eg, ECGs, laboratory tests, discharge summaries, and postmortem results) must be faxed to the number on the SAE form within 24 hours after available to the sponsor or sponsor's designee. If any questions or considerations regarding SAE arise, the sponsor's medical monitor or designee should be consulted.

The information provided in a SAE report should be as complete as possible, but contain a minimum of the following:

- A short description of the AE (diagnosis) and the reason why the AE was categorized as serious
- Subject identification and treatment
- Investigator's name and phone number (if applicable)
- Name of the suspect medicinal product and dates of administration
- Assessment of causality

If all information about the SAE is not yet known, the investigator will be required to report any additional information within 24 hours as it becomes available.

All SAEs will be evaluated by the sponsor's medical monitor or designee.

The investigators must notify their governing IRB of any SAEs.

8.3.6.4.1.1.Serious and Unexpected Suspected Adverse Reaction

For any suspected adverse reaction that is both serious and unexpected (not documented in the IB or package insert), an Investigational New Drug (IND) safety report or revision to the IB may be issued to inform all investigators involved in any study with the same study treatment.

8.3.6.4.2. Post-treatment Safety Follow-up

In this study, all AEs occurring after the informed consent is signed will be captured on the eCRF. All subjects should be instructed to report AEs or SAEs occurring up to 30 (± 7) days after the last dose of study treatment. Unresolved study treatment-related AEs and SAEs at the time of treatment discontinuation, or new study treatment-related AEs and SAEs that occur during the 30-day postdose timeframe, will be followed until they have, in opinion of the investigator, resolved to baseline, have stabilized, or are deemed to be irreversible.

8.3.6.5. Adverse Events of Special Interest

8.3.6.5.1. Adverse Events from Commercial Study Agents

For AEs related to any of the commercially available agents used in combination treatment in this study, please refer to the discussion in Section 4.2.2 and the individual package inserts referenced there.

8.3.6.5.2. Infections

Follow National Comprehensive Cancer Network guidelines for the prevention and treatment of cancer-related infections (Baden et al. 2012).

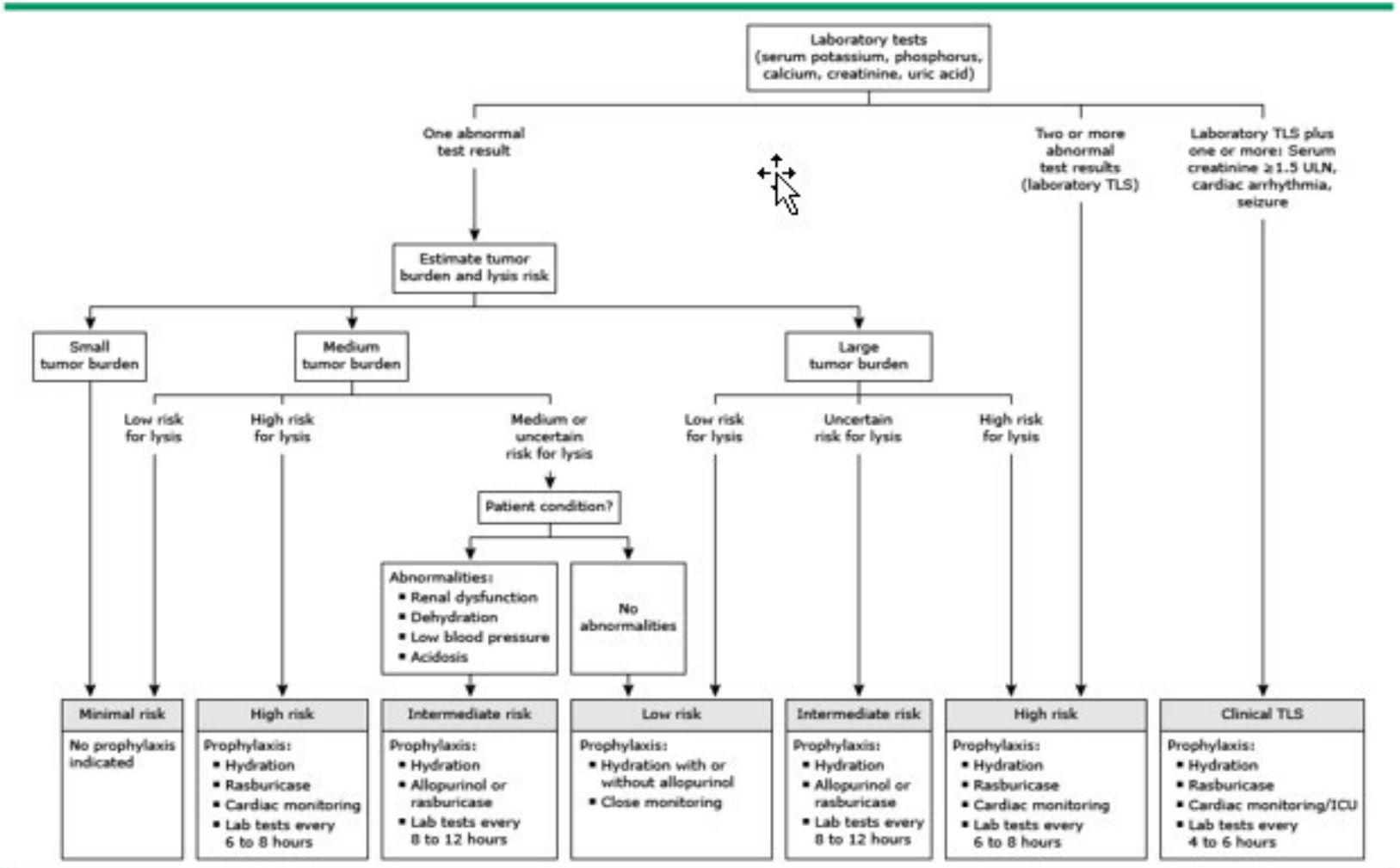
8.3.6.5.3. Tumor Lysis Syndrome

Tumor lysis syndrome (TLS) is defined by both laboratory criteria and by clinical features. It most commonly occurs after initiation of cytotoxic therapy in individuals with aggressive lymphomas and acute lymphoblastic leukemia but can occur spontaneously and with other tumor types that have high proliferate rate or large tumor burden.

For individuals who are at risk for TLS, please monitor and select prophylaxis methods based on institutional guidelines. Guidelines commonly include IV hydration, hypouricemic agents (eg allopurinol, rasburicase), frequent monitoring of urine output, and serial assays of electrolytes and serum uric acid.

The following algorithmic approach to risk assessment and monitoring of tumor lysis syndrome should also be used as guidance (Figure 11):

Figure 11: Algorithmic Approach to Risk Assessment of Tumor Lysis Syndrome



TLS: tumor lysis syndrome; ULN: upper limit of normal; ICU: intensive care unit.

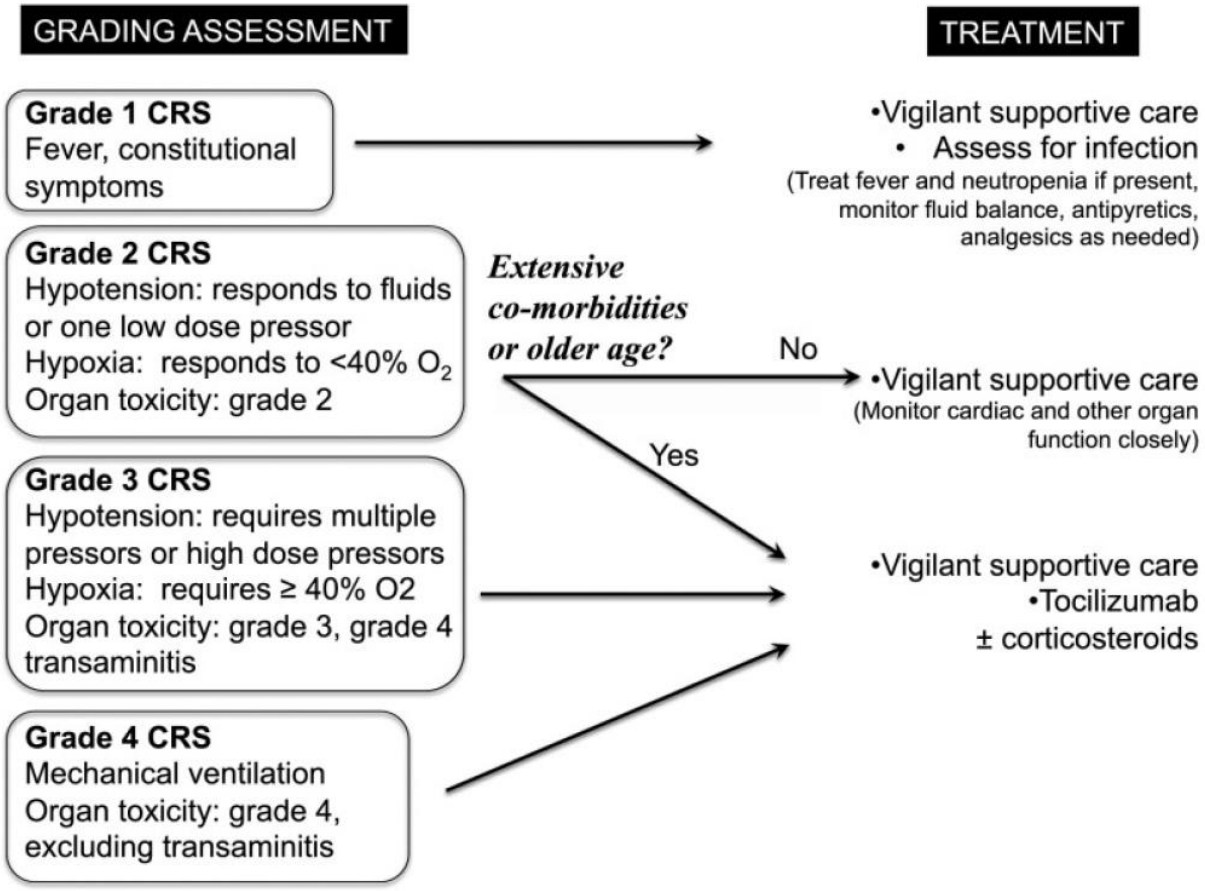
Modified from Howard SC, Jones DP, Pui CH. The Tumor Lysis Syndrome. N Engl J Med 2011; 364:1844.

8.3.6.5.4. Cytokine Release Syndrome

Cytokine release syndrome (CRS) is a potentially severe immune reaction that may occur in response to immunotherapies. The largest risk factor is high tumor load. Symptoms may include high fevers, rigors, myalgia, headache, nausea, vomiting, malaise, hypotension, rash, dyspnea, hypoxia, and tachycardia. Elevations in serum aminotransferases and bilirubin can be seen, and, in some cases, disseminated intravascular coagulation, capillary leak syndrome, and hemophagocytic lymphohistiocytosis-like syndrome have been reported.

Management of CRS depends on the severity and may include interruption of the infusion, discontinuation of therapy, symptomatic treatment, IV fluids, and ventilator and/or pressor support, as well as corticosteroids and anti-cytokine therapy (Lee et al. 2014). Vigilant supportive care is recommended including empiric treatment of concurrent bacterial infections and maintenance of adequate hydration and blood pressure for every subject regardless of CRS grade.

The following treatment algorithm for management of CRS is recommended by Lee et al. 2014:



In the event of an acute infusion-related reaction/CRS, the infusion of study treatment will be stopped, and the subject will be closely monitored until resolution of the reaction. Drugs that may be used to facilitate resolution and permit resumption of study treatment administration

include epinephrine and other vasopressors, acetaminophen, antihistamines (eg, diphenhydramine), opiates for pain (eg, short-term meperidine), supplemental oxygen, IV fluids, and corticosteroids. For acute infusion-related reactions less than Grade 3, resumption of study treatment administration at a slower infusion rate may be considered at the investigator's discretion.

Cytokine release syndrome may have a delayed onset following the completion of the infusion. Study subjects must be instructed to contact their study site immediately if they develop a fever.

The lack of any clinical symptoms related to CRS in Trishula cynomolgus monkey PK studies may indicate a low potential for this effect. However, serum cytokine levels were monitored after the first and last administration of TTX-030 in the GLP cynomolgus monkey safety study.

8.3.6.5.5. Immune-related Adverse Events

(Content for this section has been adapted from UpToDate, Postow M;Wolchok J, 2018.)

Checkpoint inhibition is associated with irAEs, including dermatologic, gastrointestinal, hepatic, endocrine, and other inflammatory events. These irAEs are typically transient but occasionally can be severe or life-threatening. In general, treatment of moderate or severe irAEs requires interruption of the checkpoint inhibitor and the use of corticosteroid immunosuppression. Treatment is based upon the severity of the observed toxicity.

- For subjects with Grade 2 (moderate) immune-mediated toxicities, treatment with the checkpoint inhibitor should be withheld and should not be resumed until symptoms or toxicity is Grade 1 or less. Corticosteroids (prednisone 0.5 mg/kg/day or equivalent) should be started if symptoms do not resolve within a week.
- For subjects experiencing Grade 3 or 4 (severe or life-threatening) immune-mediated toxicities, treatment with the checkpoint inhibitor should be permanently discontinued. High doses of corticosteroids (prednisone 1-2 mg/kg/day or equivalent) should be given. When symptoms subside to Grade 1 or less, steroids can be gradually tapered over at least 1 month.

Subjects who will benefit from corticosteroids generally do so within days. If symptoms do not clearly improve, particularly after approximately 3 days with IV steroids, administering infliximab (5 mg/kg) rather than continuing with a prolonged course of high-dose IV corticosteroids is suggested. If symptoms persist after the first infliximab dose, a second dose of infliximab (5 mg/kg) can be repeated 2 weeks after the initial dose.

Fatigue

- Fatigue is among the most common side effect seen and is generally mild. Severe fatigue is rare as a side effect of checkpoint inhibitors. When fatigue is present, it is important to exclude thyroid, pituitary, and other endocrine disorders, such as primary adrenal insufficiency.

Infusion-related Reactions

- Mild infusion-related side effects have been reported in up to 25% of individuals treated with anti-PD-1 or anti-PD-L1 agents. The reported incidence of severe or life-threatening infusion-related reactions has been less than 2%. Premedication with acetaminophen and an antihistamine may be considered as needed.

Dermatologic and Mucosal Toxicity

- Dermatologic toxicity is the most common irAE associated with checkpoint inhibitors. Approximately 30% to 50% of individuals will have dermatologic complications. Alopecia has been reported in approximately 1% to 2% of cases. For most individuals, dermatologic toxicity is the earliest irAE experienced, with onset an average of 3.6 weeks after treatment initiation.
- Oral mucositis and/or complaints of dry mouth may also occur. Oral candidiasis remains an important consideration in the differential diagnosis, particularly if an individual has been on oral corticosteroids for management of other irAEs. In some individuals, oral corticosteroid rinses and lidocaine have been effective.
- Most checkpoint inhibitor rashes can be treated with topical corticosteroid creams. If pruritus is a prominent symptom, oral antipruritics (eg, hydroxyzine or diphenhydramine) can be helpful. Severe rashes (Grade 3/4) should be managed with oral corticosteroids, and treatment with checkpoint blockade should be held as per established management algorithms. Any rash that does not promptly resolve with corticosteroid creams or shows signs of blistering should be evaluated promptly by a dermatologist and biopsy considered.

Diarrhea/Colitis

- Diarrhea is a common clinical complaint in individuals undergoing treatment with checkpoint-blocking antibodies and most commonly presents approximately 6 weeks into treatment. Subjects being treated with a checkpoint inhibitor should be counseled on the importance of maintaining oral hydration if diarrhea develops. If symptoms persist for more than 3 days or increase and no infectious causes are identified, prompt assessment and use of oral or IV corticosteroids are required.
- Mild (Grade 1) symptoms (fewer than 4 stools per day over baseline) can be managed symptomatically. Some clinicians feel that the American Dietary Association colitis diet and anti-motility agents (loperamide or oral diphenoxylate atropine sulfate) can be helpful for mild symptoms. Oral or rectal budesonide may be helpful in the early treatment of mild noninfectious diarrhea symptoms that persist but do not escalate after 2 to 3 days of dietary modification and anti-motility agents.

- Colonoscopy may be helpful if Grade 2 symptoms (increase of 4 to 6 stools per day over baseline) or greater occur or in situations where the diagnosis is unclear. Treatment should be initiated if colitis is observed.
- For individuals with severe or life-threatening enterocolitis (Grade 3 or 4, increase of 7 or more stools per day over baseline or other complications), treatment with checkpoint inhibitor should be permanently discontinued. High doses of corticosteroids (prednisone 1-2 mg/kg/day or equivalent) should be given.
- If individuals do not improve with IV corticosteroids after approximately 3 days on IV steroids, infliximab at a dose of 5 mg/kg Q2W is typically recommended. The dose and schedule of infliximab in this setting is based upon experience treating individuals with inflammatory bowel disease. In cases refractory to infliximab, mycophenolate may be needed.
- In very rare cases, colitis can result in bowel perforation, potentially requiring colostomy.
- Prophylactic treatment with the matrix-release corticosteroid budesonide is not recommended for the prevention of diarrhea/colitis. This approach was studied as a way to reduce the incidence and/or severity of diarrhea in a double-blind, placebo-controlled Phase 2 study and the rates of diarrhea were similar in both study arms.

Hepatotoxicity

- Elevations in serum levels of the hepatic enzymes, AST and ALT, may be observed. Most episodes involve asymptomatic laboratory abnormalities, but occasionally individuals have an associated fever. Rarely, elevations in total bilirubin are seen as well, usually in association with a prolonged period of AST and ALT increase. Among individuals that develop hepatic-related toxicities, the most common time of onset is 8 to 12 weeks after initiation of treatment, although early or delayed events may also be seen.
- Hepatic function (transaminases and bilirubin) should be monitored prior to each dose of checkpoint inhibitor. When an individual has an elevated AST and/or ALT, exclusion of viral or other drug-induced causes of hepatitis is important. If no other etiology is obvious, then prompt treatment with corticosteroids following an established algorithm is the next step. Hepatitis may persist for quite some time and may require prolonged or repeated corticosteroid tapers (a minimum of 3 weeks of treatment is suggested) and/or additional immunosuppression.
- Grade 2 hepatic toxicity: AST or ALT > 2.5 times the ULN but ≤ 5 times the ULN, or total bilirubin > 1.5 times the ULN but ≤ 3 times the ULN. Treatment with the checkpoint inhibitor should be withheld.

- Grade 3 or greater hepatic toxicity: AST or ALT > 5 times the ULN, or total bilirubin > 3 times the ULN. Treatment should be permanently discontinued.
- In rare cases, elevations in AST and ALT are refractory to corticosteroid therapy, and mycophenolate mofetil (500 mg every 12 hours) may be administered concurrently with corticosteroids. The use of antithymocyte globulin therapy has also been described.
- Infliximab should not be given to individuals with elevated AST/ALT since infliximab itself carries a risk of hepatotoxicity.

Pneumonitis

- Pneumonitis is an uncommon but potentially severe or fatal complication of treatment with checkpoint inhibitor immunotherapy. Drug-induced pneumonitis is a diagnosis of exclusion, and alternative diagnoses, including infection and malignancy, need to be excluded.
- An empiric approach to treatment includes the following:
    - For asymptomatic, Grade 1 pneumonitis, clinicians generally withhold drug for 2 to 4 weeks with close follow-up. If symptoms arise or there is radiographic progression, corticosteroids are appropriate.
    - Subjects with Grade 2 or higher pneumonitis should have their drug withheld and be treated using corticosteroids with close follow-up. Additional immunosuppression may be used in individuals with worsening of pneumonitis, although the benefit of this approach is uncertain.

Endocrinopathies

- Inflammation of the pituitary, thyroid, or adrenal glands as a result of checkpoint blockade often presents with nonspecific symptoms such as nausea, headache, fatigue, and vision changes. The most common endocrinopathies are hypothyroidism, hyperthyroidism, and hypophysitis.
- Thyroid function should be monitored prior to each dose of a checkpoint inhibitor. Hypothyroidism and hyperthyroidism should be managed per standard clinical guidelines and an endocrinologist should be consulted.
- Typically, hypophysitis is manifested by clinical symptoms of fatigue and headache. The diagnosis is established by low levels of the hormones produced by the pituitary, including adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH), and prolactin. When hypophysitis is suspected, a course of high-dose corticosteroids (1 mg/kg of prednisone daily) given during the acute phase may result in reversal of the inflammatory process in some cases and prevent the need for longer term hormone replacement. In most individuals, however, long-term supplementation

of the affected hormones is necessary due to secondary hypothyroidism (treated with levothyroxine) or secondary hypoadrenalism (treated with replacement doses of hydrocortisone, typically 20 mg each morning and 10 mg each evening). In some cases, individuals can be successfully weaned from replacement steroids over time.

Adrenal Insufficiency

- The most critical endocrinopathy is adrenal insufficiency, which can cause dehydration, hypotension, and electrolyte imbalances (hyperkalemia, hyponatremia) and constitutes an emergency. Adrenal insufficiency is rare and has been reported in 0.7% of individuals treated in randomized clinical trials. When an adrenal crisis is suspected, IV corticosteroids and immediate hospitalization is warranted. Consultation with an endocrinologist, aggressive hydration, and evaluation for sepsis are also critical.

Type 1 Diabetes Mellitus

- Treatment with checkpoint inhibitors has been associated with acute onset of type 1 diabetes mellitus in approximately 0.2% of cases. It is important to monitor glucose with each dose of immunotherapy.

Renal

- Acute renal injury is a rare complication of checkpoint inhibitor immunotherapy. Discontinuation of checkpoint inhibitor immunotherapy and treatment with corticosteroids are indicated for individuals with severe renal injury. The most common reported underlying pathology is acute tubulointerstitial necrosis, but acute interstitial nephritis, immune complex glomerulonephritis, and thrombotic microangiopathy have also been observed. Acute renal injury due to checkpoint inhibitor therapy has been managed by discontinuing immunotherapy and administering a course of corticosteroids, typically 1 mg/kg tapering over 1-2 months. Rechallenging with checkpoint inhibitor after resolution of the renal injury has been successful in some cases (Wanchoo et al. 2017).

Exocrine Pancreas

- Monitoring serum amylase and lipase in asymptomatic individuals is not recommended unless pancreatitis is suspected clinically. Corticosteroid treatment is not indicated in individuals with modest asymptomatic elevations in serum amylase and lipase, as long as there are no other signs or symptoms of pancreatic inflammation.

Neurologic

- A wide range of neurologic syndromes have been associated with checkpoint blockade neurotoxicity, which is estimated to occur in approximately 1-3% of individuals. Serious neurologic irAEs should be treated with corticosteroids. Consultation with neurology is indicated to consider additional treatment such as plasmapheresis and IV Ig.

Cardiotoxicity

- Cardiotoxicity (eg, myocarditis) may develop in the absence of a history of significant cardiac risk factors and may be associated with a more general myositis as well as other irAEs. High-dose steroids have been used to treat cardiac complications, but symptoms may progress in some cases despite aggressive therapy. Immediate transfer to a coronary care unit or, if available, cardiac transplant unit should be considered for individuals with elevated troponin or conduction abnormalities. The early institution of cardiac transplant rejection doses of steroids (methylprednisolone 1 g every day) and the addition of mycophenolate, infliximab, or antithymocyte globulin should be considered in individuals without an immediate response to high-dose steroids. Mechanical circulatory support may also be considered in severe cases when deemed clinically appropriate.

Hematologic

- Red cell aplasia, neutropenia, thrombocytopenia, acquired hemophilia A, and cryoglobulinemia have been described in individuals treated with checkpoint inhibitors. As with other irAEs, the standard approach is initial corticosteroid treatment with addition of other immune-suppressing agents if symptoms are steroid-refractory.

Ophthalmologic

- Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) blockade with ipilimumab has been associated with eye inflammation, which can be manifested by episcleritis, conjunctivitis, uveitis, or orbital inflammation. An ophthalmology consultation is recommended, and treatment with topical corticosteroids (eg, 1% prednisolone acetate suspension) may be helpful. Oral corticosteroids can be used for severe (Grade 3 or 4) for refractory cases.

Rheumatologic and Musculoskeletal

- A wide range of rheumatologic toxicities has been observed with checkpoint inhibition immunotherapy. These include inflammatory arthritis, salivary gland

dysfunction (sicca syndrome), and inflammatory myositis, among others. The incidence of these side effects has not been clearly determined.

8.4. Pharmacokinetics

PK assessments are for TTX-030, whether administered as a single agent or in combination with other agents.

8.4.1. Pharmacokinetic Blood Sample Collection

The serial sampling scheme for TTX-030 concentration assessments scheduled in Table 1/Table 2, Table 3/Table 4, and Table 5/Table 6 will contribute to the characterization of the TTX-030 PK profile. Blood will be collected in the vacutainers specified in the Laboratory Manual. Serum will be harvested from these collections and stored for subsequent analysis. Samples from each collection period will be shipped to the analytical laboratory selected by the sponsor and analyzed for concentrations of TTX-030 by validated methods. Instructions for processing and shipping samples will be provided in a separate Laboratory Manual. All sampling should be collected as close as possible to the sample collection times specified. The actual time and date of each blood sample will be recorded. If a subject has a dosing interruption or delay for any reason, PK sampling may be delayed or skipped after discussion with the sponsor. If the infusion was interrupted or a sample was not drawn, the reason will be documented on the eCRF.

Post-infusion samples should be obtained from a site other than the infusion site (ie, contralateral arm) on infusion days.

The number of samples taken, volume required for each analysis, and timing of the PK sampling may change during the course of the study as additional data emerge.

8.4.2. Pharmacokinetic Sample Analysis

Serum concentrations of TTX-030 will be determined by a validated electrochemiluminescence-based method at the time points listed in Table 1/Table 2, Table 3/Table 4, and Table 5/Table 6.

TTX-030 concentration data will be tabulated and summarized for each cycle at which PK are to be measured. Descriptive statistics will include means, medians, ranges, and standard deviations, as appropriate. Dose-exposure relationships will be characterized. Additional analyses may be conducted if they further address the PK objectives.

PK parameters for TTX-030 will be estimated by fitting a 2-compartment IV infusion model to the serum concentration-time data using Phoenix 64 WinNonlin. The serum PK parameters are listed in Table 13.

Table 13: Serum Pharmacokinetic Parameters to be Computed

Parameter	Definition
$t_{1/2}$	terminal elimination half-life
C_{max}	maximum serum observed concentration
V_{ss}	volume of distribution at steady state
CL	apparent total body clearance of the drug from serum
AUC	Area under the curve (AUC)

Concentrations below the lower limit of quantitation will be treated as 0.00 for descriptive statistics and noncompartmental PK analysis. Values for missing PK samples will not be imputed unless supported by PK principles (eg, concentration[s]) prior to dosing or in the elimination phase following decline below the assay lower limit of quantitation and will be identified as "missing" in the data listings.

8.5. Pharmacodynamics

8.5.1. Pharmacodynamic Blood Samples

Pharmacodynamic blood samples should occur at the same time as PK blood draws or tumor biopsy when applicable as shown in Table 1/Table 2, Table 3/Table 4, and Table 5/Table 6. Detailed instructions for pharmacodynamic blood sample collection, processing, and shipping will be provided to the study sites in a separate Laboratory Manual.

Peripheral blood mononuclear cells, whole blood, and serum and/or plasma will be analyzed for pharmacodynamic readouts, including TTX-030 relevant target- and mechanism-of-action-related biomarkers.

Pro-inflammatory cytokine release will be measured as a marker of immune activation. Additionally, several exploratory analyses are planned. Whole blood will be collected to enable immunoprofiling, measurements of lymphocyte activation status, and analysis of CD39 receptor occupancy by the test article. Additionally, recent reports (Giannakis et al. 2017) have suggested that elevated presence of adenosine and its metabolites in serum may correlate with intratumoral CD39 activity. Therefore, potential exploratory biomarkers may include the detection of adenosine and inosine using liquid chromatography-tandem mass spectrometry techniques or similar alternatives.

8.5.2. Pharmacodynamic Tissue Samples

Subjects may consent to undergo pretreatment biopsy or may provide archival tumor tissue (either a paraffin embedded tissue block or ≥ 10 unstained slides) obtained from a procedure that was performed any time prior to first dose.

Fresh and/or archival tumor tissue is optional for dose escalation; fresh tumor biopsy at screening and on-treatment is mandatory for safety lead-in arms and expansion cohorts. If a subject is clinically contraindicated for tumor biopsy, discuss with Medical Monitor. If a subject has had a biopsy within 90 days with no intervening treatment prior to the loading dose/first dose of TTX-

030, that tissue can be used for this study and the subject does not need to repeat a baseline biopsy.

Follow-up tissue biopsies will occur on C2D1 (-7 days) [Table 1/Table 2 and Table 3/Table 4 Invasive procedures that require general anesthesia should not be performed to obtain a biopsy specimen. If a surgical procedure is performed for a clinical indication, a sample may be used for research purposes. During the course of treatment, a decision may be made to perform the on-treatment biopsy earlier than Cycle 2 Day 1 (-7 days) if there is evidence to suggest a possibility that there will be no available tumor for biopsy due to a rapid clinical response. Similarly, tumor biopsy may be delayed if there is evidence of a delayed response.

When PK and pharmacodynamic sampling are scheduled on days when tissue samples (tumor biopsy) are to be obtained, every effort will be made to schedule the assessments together.

Archival/pretreatment and on-treatment tumor biopsies will be collected and embedded in paraffin. Expression of CD39 will be assessed by immunohistochemistry. Additional analysis may include immunohistochemistry staining for markers of TIL populations (eg, CD3, CD8, CD73, FoxP3, or CD68), functional markers (Ki67), or immune checkpoints (eg, PD-L1). Additionally, expression analysis of tumor tissue may be performed using Nanostring™ or Illumina RNA-seq.

Other TTX-030-relevant targets and mechanism-of-action-related biomarkers may be examined using available assays. Tissue blocks or slides from fresh biopsies will be retained to research disease pathways. Any future use of samples following study completion will be in compliance with the Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable Guidance for Sponsors, Institutional Review Boards, and Food and Drug Administration Staff (FDA, 2006). Tissue blocks from archival biopsies will be returned to the archival facility from which it was originally received. Other TTX-030-relevant targets and mechanism-of-action-related biomarkers may be examined using available assays.

Detailed instructions for tissue sample preparation and shipping will be provided to the study sites as needed in a separate laboratory manual.

8.6. Immunogenicity Assessments

8.6.1. Anti-drug Antibody Assessments

Formation of ADA against TTX-030 will be evaluated in serum samples collected from all subjects, whether administered as a single agent or in combination with other agents.

8.6.2. Blood Sample Collection

Serum samples will be collected for the purpose of ADA analysis from all subjects as scheduled in Table 1/Table 2, Table 3/Table 4, and Table 5/Table 6. Blood will be collected in the vacutainers specified in the laboratory manual and serum extracted as specified. Actual time and date of each blood draw are to be recorded. Samples from each scheduled collection period will be shipped to the analytical laboratory selected by the sponsor and analyzed for ADA reactivity to TTX-030.

Additionally, serum samples should also be collected at the final visit from subjects who discontinued study treatment or were withdrawn from the study. These samples will be tested by the sponsor or sponsor's designee.

8.6.3. Analysis of ADA Formation

Immunogenicity results will be analyzed descriptively by summarizing the number and percentage of subjects who develop detectable anti-TTX-030 antibodies (population defined as per Table 16). The immunogenicity titer will be reported for samples confirmed positive for the presence of anti-TTX-030 antibodies.

9. Statistical Considerations

9.1. Statistical Hypotheses

Details of the statistical model are included in Section 11.6 (Appendix 6).

A primary objective of the trial is to determine the MTD and/or RP2D for each arm. Dose escalation (Arm 1) will be conducted according to a mCRM for doses up to 10 mg/kg and according to a 3 + 3 design for doses > 10 mg/kg, with the aim of estimating the dose-toxicity relationship and determining the MTD. The MTD will be defined as the dose that has a model-estimated DLT rate closest to 30% and also has less than 55% probability of a DLT rate exceeding 30%. The log-odds of a DLT will be modeled across the dose levels with a 2-parameter logistic regression model.

The model will be fit to the available data following each dosing cohort to support escalation decisions. The observed DLT data will be summarized by dose (within each dose-escalation/safety lead-in arm) and reported along with the model fit. Summaries from the model will include the posterior means and 95% confidence intervals (CI) for each dose along with the posterior probability that the DLT rate exceeds 30%.

The RP2D was determined based on the totality of data, including the estimated MTD, plus other endpoints such as observed efficacy, PK, and other AEs.

Descriptive summary statistics will be presented. Longitudinal data will be presented by appropriate time intervals, such as monthly or quarterly depending on the nature of the data.

The efficacy in subjects treated with TTX-030 as a single agent or in combination with specified regimens will be evaluated and analyzed using the endpoints listed in Table 14.

Table 14: Efficacy Endpoints

Efficacy Endpoint	Definition
Best Overall Response (BOR)	Best response recorded after the start of the study treatment/randomization
Objective Response Rate (ORR)	Confirmed partial response or confirmed complete response
Duration of Response (DOR)	Time from documentation of tumor response to disease progression
Time to Progression (TTP)	Time from the start of study treatment/randomization until objective tumor progression; TTP does not include deaths
Time to Tumor Response (TTR)	Time from the start of study treatment/randomization to the first documentation of an objective response
Progression-Free Survival (PFS)	Time from the start of study treatment/randomization until objective tumor progression or death
Disease Control Rate (DCR)	Percentage of subjects who have achieved complete response, partial response, or stable disease for at least 6 weeks
Overall Survival (OS)	Time from the start of study treatment/randomization until death from any cause and is measured in the Efficacy Evaluable Population

9.2. Statistical Considerations for Arm 1 and 2 Expansion Cohorts

In Arm 1 and 2 Expansion Cohorts (n = 25 subjects each) subjects with advanced or metastatic RCC with a clear-cell component and previously treated with anti-PD-(L)1 therapy and TKI therapy, will be randomized 1:1 to TTX-030 or TTX-030 and pembrolizumab. These agents will be administered at the doses and schedules identified in the safety lead-in.

- Arm 1: TTX-030 (n = 25)
- Arm 2: TTX-030 and pembrolizumab (n = 25)

The primary objective of this randomization procedure is to obtain unbiased estimates of safety and efficacy parameters of TTX-030 plus pembrolizumab compared with TTX-030. For example, a 25% improvement in ORR (ie, ORR Δ) in Arm 2 relative to Arm 1 (assuming a 15% ORR in Arm 1) will have an 80% CI of (9%, 41%) and a power of 76%. See Table 15.

Demographic and baseline characteristics will be summarized by treatment arm for all randomized subjects. Descriptive summaries of continuous data will be presented by the group mean, standard deviation, median, minimum, and maximum. Descriptive summaries of discrete data will be presented by the category counts as frequencies and percentages.

An estimate of the difference between the ORR in the two arms will be computed along with its 90% CI and 80% CI. The Mantel-Haenszel test will be used to compare the ORR between the two treatment arms at the two-sided significance level of 5% and 10%.

PFS and OS will be analyzed when 65% of the 50 subjects have experienced a PFS event and an OS event, respectively. Data for a subject without disease progression or death as of the clinical data cutoff date will be censored at the time of the last tumor assessment (or at the date of randomization plus 1 day if no tumor assessment was performed after the baseline visit). Data from a subject who is lost to follow-up will be included in the analysis and censored on the last date of tumor assessment that the subject was known to be progression free or death. A stratified Cox proportional-hazards model will be used to estimate the hazard ratio and its 90% CI and 80% CI. The two-sided stratified log-rank test will be used to compare PFS and OS between the two treatment arms. Kaplan-Meier methodology will be used to estimate the PFS curve and OS curve as well as the median PFS and OS for each treatment arm.

Safety analyses will be conducted in all randomized subjects who received at least one dose of TTX-030 or pembrolizumab. Safety analyses will be performed by treatment arm and will be based on actual treatment received. Specifically, a subject will be included in Arm B in the safety analyses if the subject receives any amount of pembrolizumab, regardless of the initial treatment assignment at randomization. Safety endpoints will include incidence, nature, and severity of adverse events (using NCI CTCAE v5.0), including SAEs and AEs of special interest.

Table 15: Power for Study Design for Several Possible True Underlying Delta ORR Values

	True Underlying ORRΔ			
	13%	**17%**	**21%**	**25%**
Power to detect ORRΔ[a]	44%	55%	66%	76%
80% CI	(-2%, 28%)	(2%, 32%)	(6%, 36%)	(9%, 41%)

Abbreviations: CI = confidence interval, ORR = objective response rate.

[a]Two-sided $\alpha = 0.20$.

Notes: Arm A is assumed to have an ORR of 15%. Results are based on 25 subjects in each arm.

9.3. Populations for Analyses

The following populations are defined for statistical analyses (Table 16).

Table 16: Analysis Populations

Population	Population Definition
Enrolled Population	All subjects who sign an ICF.
Safety Population	All subjects who receive at least 1 dose or any partial dose of study treatment.
Efficacy Evaluable Population	All treated subjects with measurable disease at baseline and 1 of the following: 1) at least 1 on-treatment tumor assessment, 2) clinical progression, or 3) death.
MTD Analysis Population	All subjects dosed at the MTD (or the highest dose tested if the MTD is not determined) in the Safety Population who have the valid baseline tumor assessment showing measurable disease, and at least 1 valid post-baseline tumor assessment. Any subject who has a major inclusion/exclusion deviation, major dosing deviation, or major protocol conduct deviation that impacts MTD assessment will be excluded from the MTD Analysis Population. A subject who withdraws from the study during Cycle 1 for reasons other than a DLT will be replaced and will be excluded from the MTD Analysis Population.
PK Population	All subjects who receive at least 1 dose of study treatment and have, in the opinion of the pharmacokineticist, sufficient data for PK analysis.
Biomarker/Pharmacodynamic Population	All subjects who receive at least 1 dose of study treatment and have available biomarker data.
Immunogenicity Population	All subjects who receive at least 1 dose of study treatment and have available ADA data.
DLT Evaluable Population	All subjects who receive 1 infusion during the treatment cycle, completed the DLT evaluation period, and are DLT evaluable.

Abbreviations: ADA = anti-drug antibodies, DLT = dose-limiting toxicity, ICF = informed consent form, MTD = maximum tolerated dose, PK = pharmacokinetic.

9.4. Interim Analyses

No formal interim analysis is planned.

An informal interim analysis will be performed when 16 subjects treated in Arm 1 of the Expansion Cohort (i.e. TTX-030 monotherapy, see Section 4.1.1.2) have had 2 response evaluations completed or experienced progression. The Sponsor may terminate enrollment into Arm 1 Expansion should there be evidence of a lack of efficacy. The totality of available data will be used to make this determination, however, should zero responders be seen out of the first 16 subjects the 90% one-sided confidence interval (using exact methods) excludes a responder rate of 13.4% or more.

10. Regulatory, Ethical, and Study Oversight Considerations

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation/Good Clinical Practice (GCP), applicable regulatory requirements and the sponsor's policy on bioethics.

10.1. Criteria for Study Termination

The sponsor or designee may suspend or terminate the study or part of the study at any time for any reason.

If the investigator suspends or terminates the study, the investigator will promptly inform the sponsor or designee and the IRB/IEC and provide them with a detailed written explanation. The investigator will destroy all unused (after final study treatment accountability has been performed) and partially used investigational product per site standard operating procedures. Upon study completion, the investigator will provide the sponsor or designee, IRB/IEC, and regulatory agency with final reports and summaries as required by regulations. For IND studies, or when the data will be used in support of an IND, the investigator must submit a written report to the sponsor or designee and the IRB/IEC within 3 months after the completion or termination of the study.

Study enrollment may be paused at any time for safety reasons by the sponsor and/or Cohort Review Committee. In the event enrollment is suspended, the decision as to whether subjects who have already undergone dosing may be made on a case-by-case decision in consultation with the Cohort Review Committee. Subjects who have already been treated with TTX-030 will be followed throughout the study.

Enrollment and treatment with the study treatment may be temporarily suspended for death that is not related to disease progression (timeframe) or underlying disease. Re-initiation of enrollment will be considered depending on the determination of the cause of death.

10.2. Protocol Adherence and Amendment

The treating physician or other involved health care professionals will apply due diligence to avoid protocol deviations. The protocol should be amended and updated as needed throughout the course of the study. Any change or addition to the protocol requires a written protocol amendment that must be approved by the sponsor, the treating physician, and the relevant IEC/IRB before implementation, unless the amendment is safety related. Safety-related amendments will be implemented immediately and documented with a Note to File. Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IEC/IRB approval, but the IEC/IRB must be kept informed of such administrative changes.

10.3. Investigator Compliance

The protocol and the proposed informed consent form (ICF) must be reviewed and approved by a properly constituted IRB/IEC or equivalent board before the study start. A signed and dated

statement that the protocol and informed consent have been approved by the IEC/IRB must be given to the sponsor before study initiation. Prior to study start, the treating physician is required to sign a protocol signature page confirming agreement to conduct the study in accordance with these documents and all the instructions and procedures found in this protocol and to give access to all relevant date and records to the sponsor's representatives or designees, IECs/IRBs, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulator authority, the investigator must inform the sponsor immediately that this request has been made.

10.4. Institutional Review Board or Independent Ethics Committee

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC as appropriate. The investigator must submit written approval to the sponsor before he or she can enroll any subject into the study.

The investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

The investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the study treatment. The sponsor will provide this information to the investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or IEC according to local regulations and guidelines.

10.5. Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.6. Informed Consent

The investigator(s) at each center will ensure that the subject is given full and adequate oral and written information about the nature, purpose, and possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any study procedures.

The investigator(s) must maintain the original, signed ICF. A copy of the signed ICF must be given to the subject.

10.7. Data Protection

The sponsor maintains confidentiality standards by assigning a unique study-specific number to each subject enrolled in the study. This means that subject names are not included in data sets that are transmitted to any sponsor location.

Subject medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the ICF (or separate authorization for use and disclosure of personal health information) signed by the subject or as permitted or required by law.

Medical information may be given to a subject's personal physician or other appropriate medical personnel responsible for the subject's welfare, for treatment purposes.

Data generated by this study must be available for inspection upon request by representatives of national and local health authorities, the sponsor or its designee, and the IRB/IEC for each study site, as appropriate.

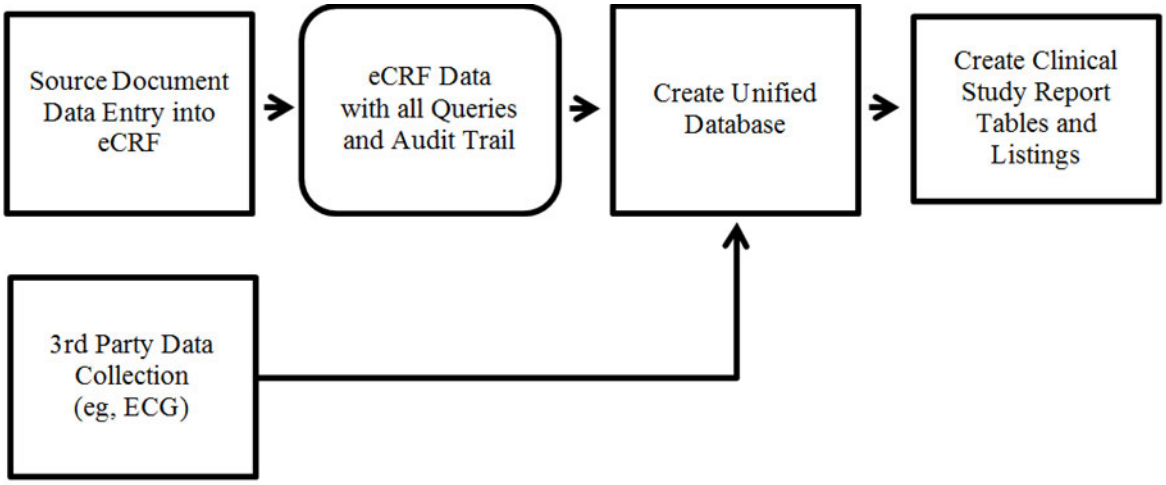
10.8. Committee Structure

The investigator must obtain IRB approval for the investigation. Initial IRB approval and all materials approved by the IRB for this study including the consent form and recruitment materials must be maintained by the investigator and made available for inspection.

10.9. Dissemination of Clinical Study Data

The standard flow of the clinical study data from collection to clinical study report is illustrated in Figure 12.

Figure 12: Transmission of Electronic Data



Abbreviations: eCRF = electronic case report form.

Data will be transcribed from source into a validated 21 CFR Part 11 compliant, electronic data capture system using single data entry. External data will also be imported into the system. PK data will be merged into the database using SAS or Excel transfers. Data originators, as well as system administrators, will be trained, authorized, and each assigned an individual identifier

(username and password). Electronic tools (prompts), as well as data review and query, will be used to alert users to and correct for missing data, data inconsistencies, inadmissible values, entries out of range, and to request additional data as applicable. An audit trail will be maintained to record data entries and edits. Data originators will have access to their data during study conduct and will be provided a read-only copy of the final data, audit trail, and queries in a human readable file suitable for long term storage.

The investigator and any vendors will permit study-related monitoring, audits, IRB review, and regulatory inspection(s) by providing direct access to source data/documents. Data entered into the eCRF will be available to the parties responsible for study data management (sponsor, contract research organization) as it is entered and can be exported into files compatible with SAS and Excel, among other data formats.

Subject data necessary for analysis and reporting of this study will be transferred via a validated procedure into a validated database or data system (eg, SAS), to enable the data to be restructured into industry standards. Clinical data management will be performed in accordance with applicable sponsor or designee's standards and data cleaning procedures. This is applicable for data recorded on eCRF as well as for data from other sources. Examples of other data would be the medical glossary coding (eg, AEs, medication) that will be performed with internationally recognized and accepted dictionaries. Additional details are recorded in the monitoring and data management documentation.

10.10. Data Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, the sponsor may conduct a quality assurance audit. Please see Section 10.11 for more details regarding the audit process.

10.11. Inspection of Records

Authorized representatives of the sponsor, a regulatory authority, an IEC, or an IRB may visit the site to perform audits or inspections, including source data verification. The purpose of a sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the International Conference on Harmonisation, and any applicable regulatory requirements. The investigator should contact the sponsor immediately if contacted by a regulatory agency about an inspection.

The sponsor will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the study treatment storage area, study treatment stocks, treatment accountability records, subject charts and study source documents, and other records relative to study conduct.

10.12. Retention of Records

The investigator must maintain all documentation relating to the study for a period of 2 years after the last marketing application approval, or if not approved, for 2 years following the discontinuance of the test article for investigation. If it becomes necessary for the sponsor or the

regulatory authority to review any documentation relating to the study, the investigator must permit access to such records.

10.13. Study and Site Start and Closure

Before an investigational site can enter a subject into the study, a representative of the sponsor will visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the investigator(s) and other personnel their responsibilities with regard to protocol adherence and the responsibilities of the sponsor or its representatives. This will be documented in a Clinical Study Agreement between the sponsor and the investigator.

During the study, a monitor from the sponsor or representative will have regular contacts with the investigational site, for the following:

- Provide information and support to the investigator(s).
- Confirm that facilities remain acceptable.
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the case report forms, and that study treatment accountability checks are being performed.
- Perform source data verification. This includes a comparison of the data in the eCRF with the subject's medical records at the hospital or practice and other records relevant to the study. This will require direct access to all original records for each subject (eg, clinic charts).
- Record and report any protocol deviations not previously sent to the sponsor.
- Confirm AEs and SAEs have been properly documented on eCRFs and confirm any SAEs have been forwarded to the sponsor and those SAEs that met criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the investigator(s) or other staff needs information or advice.

10.14. Publication Policy

The sponsor's policy is to publish or otherwise communicate the results of its hypothesis testing clinical studies, regardless of outcome, for compound(s) being investigated that are later approved for marketing. Hypothesis-testing clinical studies are those studies intended to provide meaningful results by examining prestated questions using predefined statistically valid plans for data analysis, thereby providing firm evidence of safety and/or efficacy to support product claims.

Exploratory studies, in contrast, serve to set direction for possible future studies. They have significant statistical limitations, provide only preliminary information about a disease,

condition, or product, and are not designed to provide final conclusions on product claims. The sponsor does not commit to publish or otherwise communicate the results of every exploratory study because this information is of an exploratory nature and often highly proprietary. However, if information from an exploratory study is of significant medical importance, the sponsor will publish or otherwise communicate the results.

The sponsor's decision to publish or otherwise publicly communicate the results of this study will be made in accordance with all applicable laws, regulations, and sponsor policies regarding publication and communication of clinical study results.

11. Appendices

11.1. Appendix 1: Protocol Amendment History

Version Number	Section Number or Title	Description of Change	Reason for Change
7.0	Entire document	Minor spelling, grammar, punctuation, capitalization, abbreviations, and other formatting edits were made throughout the document	Consistency
7.0	Entire document	Tizona Therapeutics, Inc. to Trishula Therapeutics, Inc.	Change company name to Trishula (Tizona's spin-out company)
7.0	Sponsor Approval Page	Update Medical Monitor	Personnel change
7.0	Section 1.1	Synopsis: Revised to align with protocol body	Consistency
7.0	Section 1.2	Modified study schema to reflect updated study design	Consistency
7.0	Section 1.3	Schedule of assessments: - update Tables 1-4 - remove Tables 5-6 - add missing D8 gem/nab dosing visits to Arm 4 - add COVID-19 AE collection guidanace - removed optional sampling for PK and PD correlative blood sample at C1D3	 - for consistency with text in protocol - to align with RP2D dosing schedules -for clarity and consistency - additional safety monitoring
7.0	Section 2.2.2 Section 2.2.3 Section 2.3	Update clinical data and rationale for RP2D dose and schedule	RP2D dose selection
7.0	Section 4.1 Section 4.1.1.2 Section 4.5 Section 4.10 Section 4.17 Section 4.18	-Remove Arm 3 -Update "n" for expansion arms	-Update study design as a similar population is being evaluated in another Trishula protocol (TTX-030-002) -Clarity
7.0	Section 4.1.1.1 Section 4	Update DLT evaluation period and RP2D dose and schedule	To reflect RP2D dose and schedule
7.0	Section 4.4	Remove dose schedule scenarios	To reflect established RP2D dose and schedule
7.0	Section 4.13	Update dose delays guidance	Safety monitoring
7.0	Section 5.1	Inclusion Criteria: -remove Arm 3 criteria	Consistency due to removal of Arm 3

7.0	Section 6.1.3	Add vital signs checks for infusion-related-reaction (IRR) management	Safety monitoring
7.0	Section 6.5.2	Remove mCRPC requirement	Consistency due to removal of Arm 3
7.0	Section 7.2 Section 9.4	Add Arm 1 Expansion rule for termination	Efficacy analysis
7.0	Table 11 Section 8 Section 8.1	Remove Arm 3 and update dose and schedules	To reflect established RP2D dose and schedule
7.0	Section 8 Section 8.3.6.4	Add COVID-19 guidance for AEs/SAEs management	Safety monitoring
7.0	Section 8.4.2	Update Table 13 for PK sample analysis	Clarity
7.0	Section 8.5.2 Section 1.3	Update timing for archival tumor biopsy collection from 45 to 90 days	Recruitment flexibility
7.0	Section 6.3	Update ADA analysis	Program consistency
7.0	Section 11.1	Update protocol amendment history	Consistency
6.0	Entire document	Updated protocol to a new template	Update
6.0	Entire document	Minor spelling, grammar, punctuation, capitalization, and other formatting edits were made throughout the document	Consistency
6.0	Section 1.1	Synopsis: Revised to align with protocol body	Consistency
6.0	Section 1.2	Modified study schema to reflect updated study design	Consistency
6.0	Section 1.3	Schedule of assessments: - clarified CT/CT-PET scans - update Table 2 for tumor assessments and serum tumor-associated markers to be collected Q2M to Q9W (21-day cycle) - add clinical laboratory test to be collected at C1D3 - removed optional sampling for PK and PD correlative blood sample at C1D3 - add PD-L1 to serum tumor-associated markers at screening - Included dose modification for Arm 4 for gemcitabine/nab-paclitaxel	- for consistency with text in protocol - to align with 21-day cycle visits - additional safety monitoring - optional samples removed - to assess for this serum tumor-associated marker - to allow dose modification in case of toxicities
6.0	Section 1.3 Section 8.3.5	Clarify that fibrinogen is only required for eligibility (within 14 days of dosing)	To align with inclusion criterion
6.0	Section 2.1.3	Updated indications of pembrolizumab	New indications have been approved since the original version of the protocol
6.0	Section 3	Updated objectives and endpoints for Arm 1 and Arm 2 Expansion Cohorts	To align with randomized expansion cohorts
6.0	Section 4.4	Modified loading dose language to remove reference to Q2W and Q1W (loading dose on Day -14 or Day -7 only).	Clarification

6.0	Section 4.1.1.2 Section 4.3 Section 4.17	Modified study design for Arm 1 and Arm 2 expansion from single arm cohort expansion to randomized 1:1 to TTX-030 or TTX-030 and pembrolizumab in subjects with advanced or metastatic RCC with a clear-cell component and previously treated with up to 3 lines of prior therapies, including anti-PD-L1 therapy and TKI therapy.	To allow for clearer decision making than single arm expansion
6.0	Section 5.1	Modified the disease-specific inclusion criteria for Arm 1 and Arm 2 Expansion Cohorts	Defining of subject populations
6.0	Section 5.1	Modified inclusion criteria for Arm 3 to clarify the type of prior therapy and to clarify that subjects had not previously received docetaxel	Clarify population
6.0	Section 5.3	Updated exclusion criteria to exclude: Subjects who are on steroids for any irAE that has not resolved, and Grade 3 or higher irAE, and ocular or neurologic toxicity of any grade.	For subject safety
6.0	Section 6.1.2	Clarified that pembrolizumab would be provided by Tizona	Clarity
6.0	Section 6.3	Updated randomization section to refer to treatment assignment and randomization for Arm 1 and Arm 2 Expansion Cohorts	To align with randomized expansion cohorts
6.0	Section 6.5.1	Clarified allowed prednisone dose	Clarity
6.0	Section 6.13	Updated treatment regimen description table to include optional loading dose	Consistency
6.0	Section 7.3	Added section entitled Lost to Follow Up	To define and address lost to follow-up subjects
6.0	Section 8.2.1	Clarified that CT scans are to be performed unless contraindicated	Subject safety
6.0	Section 8.3.6	Clarified that AEs/SAEs are to be monitored/followed until EOT or 90 days (rather than 30 days) after the last dose	Consistency with safety follow-up contact at 60 and 90 days after the last dose
6.0	Section 8.4.2	Updated description of PK parameter estimation	Clarity
6.0	Section 9.1	Updated definitions of efficacy endpoints to from start of study treatment rather than from time of randomization	Modified since the study is not randomized (except for Arm 1 and 2 Expansion Cohorts)
6.0	Section 9.2	Added statistical considerations for Arm 1 and Arm 2 Expansion Cohorts	Added due to modified study design
6.0	Section 9.3	Changed “Response Evaluable Population” to “Efficacy Evaluable Population” and “Per-protocol Population” to “MTD Analysis Population”. In addition, revised the definition of the DLT Evaluable Population	Clarity
6.0	Section 11.3	Replaced the comparison of RECIST and iRECIST table with the version from Seymour et al. 2017	Clarity
5.0	Section 2.2.2	Summarized clinical safety data up to first 3 subjects in the 40 mg/kg from Study TTX-030-001	To support 40 mg/kg dose level

5.0	Synopsis, Section 1.3, Section 5.1, Section 5.4, Section 5.11, Section 6.6, Table 4, Sections 9.1-9.3.	Added text to allow for a TTX-030 loading dose to be explored Added Schedule of Assessments for loading dose visit (Tables 7-12) Added PK time points to reflect the loading dose intervals (Tables 13-15)	Based on PK and reaching functional trough concentration
4.0	Synopsis	Revised to align with protocol body	Consistency
4.0	List of Abbreviations, Section 2.1.5, Section 4.2, Section 10.7.2	Replaced plasma with serum for PK-related items	PK parameters are/were measured in serum
4.0	Section 2.2	Summarized clinical safety data up to 20 mg/kg from Study TTX-030-001	To support the 20 mg/kg dose
4.0	Section 2.1.2, Section 5.1, Section 5.2, Section 5.4, Section 5.5, Section 5.6, Section 5.10, Section 5.11, Section 5.12, Section 5.13, Section 5.15, Section 5.17, Section 6.2, Section 13	Added 2 higher dose levels (20 and 40 mg/kg) for monotherapy dose escalation (Arm 1) and allowed for several doses and schedules to be tested (added Q2W and Q4W). Changed the combination-therapy dose-escalation arms (Arms 2-4) to safety lead-in arms. These arms are to start at the RP2D to be identified in the monotherapy dose-escalation arm (Arm 1). Modified the text throughout the protocol to reflect this change. Added a study design schema (Figure 10).	Based on PK and safety higher doses and varying schedules are being explored
4.0	Section 6.1	Modified the disease-specific inclusion criteria for Arm 3 and Arm 4.	Defining of subject populations
4.0	Section 6.6, Section 6.7, Section 9, Section 10.5, Section 10.5.1, Section 10.5.2, Section 10.6.2, Section 10.6.3, Section 10.7.1, Section 10.7.2, Section 10.8.1, Section 10.8.2, Section 10.9, Section 10.10	Added Schedule of Assessments for the Q4W schedule – Arm 1 (Table 11/Table 12) and included Arm 1 in the existing Schedule of Assessments for Q2W schedule (Table 9/Table 10). Added PK sampling interval tables for Q3W schedule (Table 13), Q2W schedule (Table 14), and Q4W schedule (Table 15).	Added to support the varying schedules being explored
3.0	Protocol Approval Page	Replaced Angela Shen with Joyson Karakunnel as medical monitor	New Chief Medical Officer
3.0	Synopsis	Revised to align with protocol body	Consistency
3.0	Section 5.1, Section 5.2, Section 5.7, Section 5.11,	Added text to indicate that a traditional 3 + 3 dose-escalation design will be implemented for doses > 10 mg/kg	Due to evaluation of higher doses than anticipated

	Section 5.16, Section 13		
2.0	Section 5.11	Clarification of DLT definition	Request from FDA
2.0	Section 6.1, 6.2	Clarification of eligibility criteria #1, 4, and 25	Request from FDA
2.0	Section 8.3, 8.4	Guidance provided on management of TLS and CRS	Request from FDA
2.0	Section 5.18 Section 9 Table 8, 10	Added follow-up safety contact at 60 and 90 days (± 7 days) after last dose of study drug	Request from FDA
2.0	Table 7	Corrected footnotes d and e	Correction of typo
1.1	Section 2.1	Updated data from non-human primate PK study	Final report completed

DOCUMENT HISTORY	
Document	**Date**
Version 8.0 Amendment 8 (Trishula)	15 Dec 2020
Version 7.0 Amendment 7 (Trishula)	12 Oct 2020
Version 6.0 Amendment 6	2 Jan 2020
Version 5.0 Amendment 5	22 Nov 2019
Version 4.0 Amendment 4	21 Oct 2019
Version 3.0 Amendment 3	12 Sep 2019
Version 2.0 Amendment 2	28 Dec 2018
Version 1.1 Amendment 1	24 Oct 2018
Original Protocol	01 Oct 2018

11.2. Appendix 2: Eastern Cooperative Oncology Group Performance Status

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, eg, light housework, office work
2	Ambulatory and capable of all self-care; but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Source: Oken et al. 1982.

11.3. Appendix 3: RECIST and iRECIST

Comparison of RECIST and iRECIST

	RECIST 1.1	**iRECIST**
Definitions of measurable and nonmeasurable disease; numbers and site of target disease	Measurable lesions are ≥ 10 mm in diameter (≥ 15 mm for nodal lesions); maximum of 5 lesions (2 per organ); all other disease is considered non-target (must be ≥ 10 mm in short axis for nodal disease)	No change from RECIST 1.1; however, new lesions are assessed as per RECIST 1.1 but recorded separately on the case report form (but not included in the sum of lesions for target lesions identified at baseline)
CR, PR, or SD	Cannot have met criteria for progression before CR, PR, or SD	Can have had iUPD (1 or more instances), but not iCPD, before iCR, iPR, or iSD
Confirmation of CR or PR	Only required for non-randomized trials	As per RECIST 1.1
Confirmation of SD	Not required	As per RECIST 1.1
New lesions	Result in progression; recorded but not measured	Results in iUPD but iCPD are only assigned on the basis of this category if at next assessment additional new lesions appear or an increase in size of new lesions is seen (≥ 5 mm for sum of new lesion target or any increase in new lesion non-target); the appearance of new lesions when none have previously been recorded can also confirm iCPD
Independent blinded review and central collection of scans	Recommended in some circumstances (eg, in some trials with progression-based endpoints planned for marketing approval)	Collection of scans (but not independent review) recommended for all trials
Confirmation of progression	Not required (unless equivocal)	Required
Consideration of clinical status	Not included in assessment	Clinical stability is considered when deciding whether treatment is continued after iUPD

Abbreviations: CPD = confirmed progressive disease; CR = complete response; iRECIST = immune-related Response Evaluation Criteria in Solid Tumors; PR = partial response; RECIST = Response Evaluation Criteria in Solid Tumors; SD = stable disease; UPD = unconfirmed progressive disease. NOTE: "i" indicates immune responses assigned using iRECIST.
Source: Seymour et al. 2017.

11.4. Appendix 4: Response Criteria for Metastatic Prostate Cancer (Based on Prostate Cancer Clinical Trials Working Group 3 Criteria)

Prostate-specific Antigen

As long as subject safety is the primary concern, in the absence of other indicators of disease progression, therapy should not be discontinued solely on the basis of a rise in PSA.

PSA progression is defined as the date that a 25% or greater increase and an absolute increase of 2 ng/mL or more from the nadir are documented and confirmed by a second value obtained 3 or more weeks later. Where no decline from baseline is documented, PSA progression is defined as a 25% increase from the baseline value along with an increase in absolute value of 2 ng/mL or more after 12 weeks of treatment.

Bone

Record post-treatment changes as either “no new lesions” or “new lesions.”

Progressing disease on bone scan is considered when at least 2 new lesions are observed. If these 2 or more new lesions are identified at the first post-treatment bone scans, progression remains unconfirmed unless at least 2 additional new lesions appear at a subsequent time point at least 6 or more weeks later (“2 + 2” rule). This is intended to account for bone scan flare response that is generally seen at earlier time points. If progression (defined as at least 2 new lesions on bone scan) is first identified at a time point beyond the first post-treatment bone scan (ie, beyond the typical flare response window), then these 2 (or more) lesions should be confirmed on a subsequent bone scan, performed at least 6 weeks later. When further progression is documented on the confirmatory scan, the date of progression recorded for the trial is the date of the first scan that shows the change.

Prostate Cancer Clinical Trials Working Group Outcome Measures

Variable	Prevent/Delay Endpoints
PSA	**Decline from baseline:** record time from start of therapy to first PSA increase that is ≥ 25% and ≥ 2 ng/mL above the nadir and that is confirmed by a second value 3 or more weeks later (ie, a confirmed rising trend)[a] **No decline from baseline:** PSA progression ≥ 25% from baseline and ≥ 2 ng/mL increase from baseline after 12 weeks
Soft-tissue lesions	Use iRECIST criteria
Bone	The appearance of approximately 2 new lesions, and, for the first reassessment only, a confirmatory scan performed 6 or more weeks later that shows at least 2 or more additional new lesions ("2 + 2" rule). If progression (2 or more new lesions) is identified at a time point beyond the first post-treatment scan, then these lesions must be confirmed on a subsequent scan. The date of progression is the date of the first scan that shows the change

Abbreviations: iRECIST = immune-related Response Evaluation Criteria in Solid Tumors, PSA = prostate-specific antigen.

[a] Particularly important when anticipated effect on PSA is delayed or for biologic therapies.

Source: Scher et al. 2016

11.5. Appendix 5: Response Criteria for Lymphoma

Comparison of Response Based on PET-CT and CT

Response and Site	**PET-CT-Based Response**	**CT-Based Response**
Complete	**Complete metabolic response**	**Complete radiologic response (all of the following)**
Lymph nodes and extralymphatic sites	Score 1, 2, or 3[a] with or without a residual mass on 5-PS[b] It is recognized that in Waldeyer's ring or extranodal sites with high physiological uptake or with activation within spleen or marrow, eg, with chemotherapy or myeloid colony stimulating factors, uptake may be greater than normal mediastinum and/or liver. In this circumstance, complete metabolic response may be inferred if uptake at sites of initial involvement is no greater than surrounding normal tissue even if the tissue has high physiological uptake	Target nodes/nodal masses must regress to ≤ 1.5 cm in LDi No extralymphatic sites of disease
Non-measured lesion	N/A	Absent
Organ enlargement	N/A	Regress to normal
New lesions	None	None
Bone marrow	No evidence of FDG-avid disease in marrow	Normal by morphology; if indeterminate, immunohistochemistry negative

Response and Site	PET-CT-Based Response	CT-Based Response
Partial	**Partial metabolic response**	**Partial remission (all of the following)**
Lymph nodes and extralymphatic sites	Score 4 or 5[b] with reduced uptake compared with baseline and residual mass(es) of any size At interim these findings suggest responding disease At End of Treatment these findings indicate residual disease	≥ 50% decrease in SPD of up to 6 target measurable nodes and extranodal sites When a lesion is too small to measure on CT, assign 5 mm × 5 mm as the default value When no longer visible, 0 mm × 0 mm For a node > 5 mm × 5 mm, but smaller than normal, use actual measurement for calculation
Non-measured lesions	N/A	Absent/normal, regressed, but no increase
Organ enlargement	N/A	Spleen must have regressed by > 50% in length beyond normal
New lesions	None	None
Bone marrow	Residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy, or an interval scan	N/A

Response and Site	PET-CT-Based Response	CT-Based Response
No response or stable disease (SD)	**No metabolic response**	**Stable disease**
Target nodes/nodal masses, extranodal lesions	No response: score 4 or 5 with no significant change in FDG uptake from baseline, at interim or End of Treatment	Stable disease: < 50% decrease from baseline in SPD of up to 6 dominant, measurable nodes and extranodal sites; no criteria for PD are met
Non-measured lesions	N/A	No increase consistent with progression
Organ enlargement	N/A	No increase consistent with progression
New lesions	None	None
Bone marrow	No change from baseline	N/A

Response and Site	PET-CT-Based Response	CT-Based Response
Progressive disease	**Progressive metabolic disease**	**Progressive disease requires at least 1 of the following**
Individual target nodes/nodal masses, extranodal lesions	Score 4, 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	PPD progression: An individual node must be abnormal with: • LDi >1.5 cm • Increase by ≥50% from PPD nadir 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 (eg, a 15 cm spleen must increase to > 16 cm). If no prior splenomegaly, must increase by at least 2 cm from baseline New or recurrent splenomegaly
Non-measured lesions	None	New or clear progression of pre-existing non-measured lesions

Response and Site	PET-CT-Based Response	CT-Based Response
New lesions	New FDG-avid foci consistent with lymphoma rather than another etiology, eg, infection, inflammation. If uncertain regarding etiology of new lesions, biopsy or interval scan may be considered	Regrowth of previously resolved lesions A new node > 1.5 cm in any axis A new extranodal site > 1.0 cm in any axis if less than 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
Bone marrow	New or recurrent FDG avid foci	New or recurrent involvement

Abbreviations: 5-PS = 5-point scale, CT = computed tomography, FDG = fluorodeoxyglucose, LDi = longest transverse diameter of a lesion, MRI = magnetic resonance imaging, N/A = not applicable, PD = progressive disease, PET = positron emission tomography, PPD = cross product of the LDi and perpendicular diameter, SDi = shortest axis perpendicular to the LDi, SPD = sum of the product of the perpendicular diameters for multiple lesions.

[a] Score 3 in many individuals 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 score 3 as inadequate response (to avoid under-treatment).
Measured dominant lesions: Up to 6 of the largest dominant nodes, nodal masses, and extranodal lesions selected to be clearly measurable in 2 diameters. Nodes should preferably be from disparate regions of the body and should include, where applicable, mediastinal and retroperitoneal areas. Non-nodal lesions include those in solid organs, eg, liver, spleen, kidneys, lungs, etc., gastrointestinal involvement, cutaneous lesions of those noted on palpation. Non-measured lesions: any disease not selected as measured, dominant disease and truly assessable disease should be considered not measured. These sites include any nodes, nodal masses, and extranodal sites not selected as dominant, measurable, or which do not meet the requirements for measurability but are still considered abnormal. In addition, they include truly assessable disease, which is any site of suspected disease that would be difficult to follow quantitatively with measurement, including pleural effusions, ascites, bone lesions, leptomeningeal disease, abdominal masses, and other lesions that cannot be confirmed and followed by imaging. In Waldeyer's ring or in extranodal sites, eg, gastrointestinal tract, liver, and bone marrow, FDG uptake may be greater than mediastinum with complete metabolic response but should be no higher than surrounding normal physiological uptake, eg, with marrow activation due to chemotherapy or myeloid growth factors.

[b] PET 5-PS: 1, no uptake above background; 2, uptake ≤ mediastinum; 3, uptake > mediastinum, but ≤ liver; 4, uptake moderately > liver; 5, uptake markedly higher than liver and/or new lesions; X, new areas of uptake unlikely to be related to lymphoma.

Features Distinguishing Between Lugano and LYRIC

Criteria	Complete Response	Partial Response	Progressive Disease
LYRIC	Same as Lugano	Same as Lugano	As with Lugano with the following exceptions: IR IR(1): > 50% increase in SPD in first 12 weeks IR(2): < 50% increase in SPD with New lesion(s), or > 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 meeting criteria for PD.

Abbreviations: FDG = fluorodeoxyglucose, IR = immune response; LYRIC = LYmphoma Response to Immunomodulatory Therapy Criteria, PD = progressive disease; PPD = cross product of the longest transverse diameter of a lesion and perpendicular diameter; SPD = sum of the product of the diameters.
Source: Cheson et al. 2016

11.6. Appendix 6: Adaptive Design Report for the Phase I Dose Escalation Study of TTX-030 in Patients with Lymphoma or Solid Tumor Malignancies

Note: This appendix only applies to the monotherapy dose-escalation portion of the study.

Submitted to Trishula
By Berry Consultants

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1.0 Introduction

This is a Phase I dose-escalation study of TTX-030 in patients with lymphoma or solid tumor malignancies. The primary objective of this trial is to characterize the dose-toxicity relationship and to estimate the maximum tolerated dose (MTD) that will help determine the RP2D for each arm. Dose escalation to determine the MTD will be conducted according to a modified continual reassessment method (mCRM). The single-agent investigation may enroll up to 21 subjects.

Safety lead-in combination investigations may enroll up to 12 subjects. The single-agent and the combination investigations will be conducted independently and identically with the exception of the starting dose.

2.0 Dose Escalation

2.1 Overview

The primary objective of the dose escalation portion of the study is to estimate an MTD to help determine the RP2D. We target a dose-limiting toxicity (DLT) rate of 30% and define the MTD as the highest safe dose where the DLT rate is closest to 30%.

There are a total of 11 possible dose levels between 0.5 mg/kg and 10 mg/kg. Dose levels are shown in Table 1. These include a set of primary dose levels (indicated by asterisks) as well as several intermediate dose levels.

Table 1: Dose Levels

Dose in mg/kg	Dose Level Modeled as (v_d)
0.5*	1
1.5*	2
2	3
3*	4
4	5
5	6
6*	7
7	8
8	9
9	10
10*	11

Dose escalation will be conducted according to a mCRM. Dose escalation is also conducted according to a set of rules that govern entry into the study and assignment of dose level. These rules allow skipping untried dose levels provided they are estimated to be safe. The trial is continuously monitored for safety and for early stopping for successfully identifying the MTD.

2.2 DLT Observation Period

The DLT observation period, for the purpose of dose-escalation, is one cycle. One cycle is 3 weeks in length for the single agent. Once a subject has completed this first cycle DLT observation period, the subject will be considered to have complete DLT information for the purposes of making dose escalation decisions for the next subject enrolling into the trial.

2.3 Dose-Toxicity Model

For each dose we model the log-odds of a DLT, π, at each dose level with a 2-parameter model,

$$\log\left(\frac{\pi_d}{1-\pi_d}\right) = \alpha + \beta(v_d).$$

where v_d is the modeled dose level as show in Table 1.

We place the following prior distributions on the model parameters:

$$\begin{bmatrix} \alpha \\ \ln(\beta) \end{bmatrix} \sim N\left[\begin{bmatrix} \mu_\alpha \\ \mu_\beta \end{bmatrix}, \begin{bmatrix} \sigma_\alpha^2 & \rho\sigma_\alpha\sigma_\beta \\ \rho\sigma_\alpha\sigma_\beta & \sigma_\beta^2 \end{bmatrix}\right]$$

where

$\mu_\alpha = -4$ and $\sigma_\alpha = 2.5$

$\mu_\beta = -1.6$ and $\sigma_\beta = 0.9$

$\rho = 0$.

Figure 1 shows 100 draws from the prior dose-toxicity curve. The solid blue line corresponds to the mean probability of a DLT at each dose. The dotted blue lines correspond to 95% confidence interval (CI) for the probability of a DLT at each dose. The MTD based on the prior distribution is 9 mg/kg, but with large uncertainty.

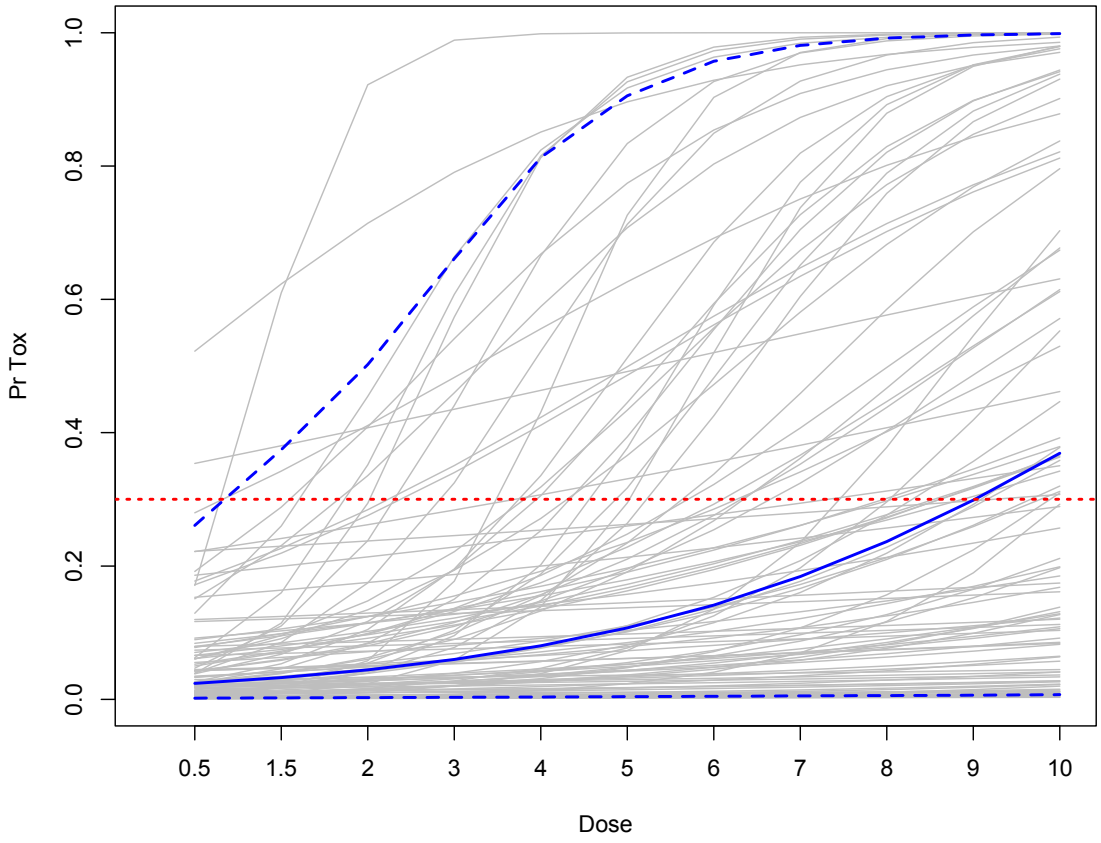

Figure 1: *Prior dose-toxicity curve. The solid blue line represents the mean probability of a DLT at each dose. The gray lines represent draws from the prior distribution. The dotted blue lines show the 95% confidence interval around the probability of DLT at each dose level. The dotted red line is at 30% for reference.*

The model will be updated as frequently as necessary. In particular, the model may be updated as each dosing cohort is treated and assessed for DLT. When the model is updated, the distributions of all parameters are updated using Markov chain Monte Carlo methods and the MTD is estimated based on the posterior probability of DLT at each dose.

Additionally, we define a dose as safe if there is at least a 45% probability that the DLT rate is less than 30%,

$$\Pr(\pi_{vd} < 30\%) > 45\%.$$

If the DLT rate is estimated to be exactly 30%, the $\Pr(\pi d < 30\%)$ will be approximately 50%. Therefore, only doses with a mean estimated DLT rate close to 30% will be considered safe by this definition.

2.4 Dose Escalation Rules

2.4.1 Entry into the Study

Dose escalation for the single agent begins with a run-in of a single subject on the 0.5 mg/kg dose. Beginning with the next subject enrolled, this trial will enroll in dosing cohorts. Dosing cohorts will be 2 subjects until the first Grade 2 toxicity related to study treatment or a DLT is observed. After that, dosing cohorts will be 3 subjects.

Dose escalation for the combinations may begin at a higher dose level depending on which dose levels have already been determined to be safe in the single-agent investigation. For the combination investigations, enrollment will begin with dosing cohorts.

2.4.2 Assignment of Dose Levels

The dose-toxicity model will be used to determine which doses are safe and to assign subjects to doses, ie, to assign subjects to the highest safe dose. However, assignment of dose levels is also governed by rules concerning the speed of dose escalation and rules that determine what untried dose levels may be skipped.

When the dose is escalated, it may only escalate to the highest safe dose that is no more than a 100% increase over the current dose level. The only exception to this is the escalation from the 0.5 mg/kg dose to the 1.5 mg/kg dose. Therefore, the fastest dose escalation for the single agent that could occur would be 0.5 mg/kg to 1.5 mg/kg, then 3 mg/kg, 6 mg/kg, and then finally to 10 mg/kg (skipping all intermediate dose levels). If the dose must be de-escalated, the dose will de-escalate to the highest safe dose regardless of how large a decrease in dose level.

As long as no Grade 2 toxicity related to study drug or DLT has yet been observed, there must be complete DLT information on at least 2 subjects at the current dose level in order to escalate (with the exception of the single subject required at the 0.5 mg/kg dose for the single agent). Once the first Grade 2 toxicity related to study drug or DLT is observed in the trial, there must be complete DLT information on at least 3 subjects at the current dose level in order to escalate.

2.5 Interim Monitoring

Dose escalation will be continuously monitored for safety and for success in identifying the MTD. In this section, we describe the prespecified rules to stop the dose-escalation early. If the dose-escalation is not stopped early, dose escalation will stop when the maximum number of subjects has been enrolled. This is 25 subjects for the single agent and 12 subjects for each combination. At that time, the MTD will be the highest safe dose as estimated by the dose toxicity model.

2.5.1 Safety Monitoring

If no doses are safe, dose escalation will stop and no MTD will be declared. Formally if all

$$\Pr(\pi_d < 30\%) < 45\% \text{ for all } v_d = 1,\ldots 11$$

then the trial will stop early for safety.

2.5.2 Success in Identifying the MTD

Dose escalation may be stopped early when we are sufficiently confident the MTD has been identified. We define this as having 6 subjects with complete DLT information at the current estimate of the MTD.

3.0 Example Trials

In this section we show a single simulated trial in order to illustrate the dose-toxicity modeling and dose escalation decisions of the mCRM.

Figure 2 shows the allocation of the first 3 subjects in the trial. The trial begins with the single agent with a single subject run-in on the 0.5 mg/kg dose level. This subject experiences no toxicity and the dose is escalated. Dosing cohorts begin after this single subject run-in. The first dosing cohort of size 2 is enrolled at the 1.5 mg/kg dose. Neither of these subjects experiences a toxicity event. The highest safe dose is estimated to be 10 mg/kg; however, the largest increase allowed is a 100% increase.

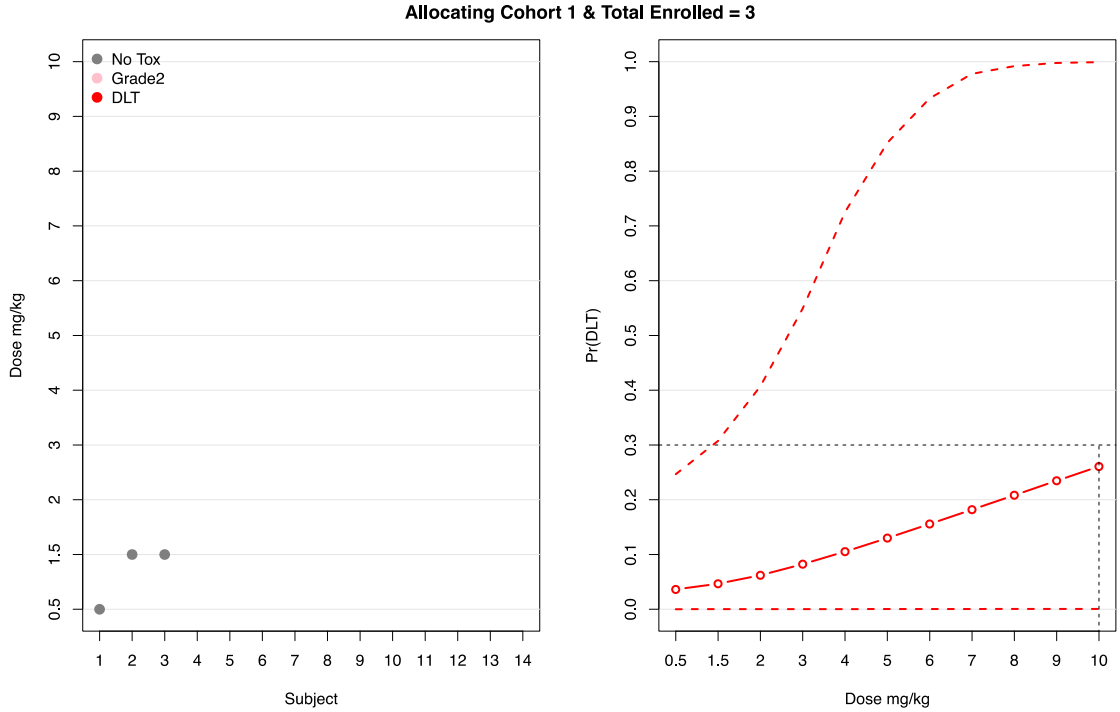

Figure 2: *Example trial with 3 total subjects enrolled. The left panel shows subject allocation where each dot represents one subject. The right panel shows the dose-toxicity model estimates. The solid red line is the mean and the dotted red lines show the 95% confidence interval. The black horizontal reference line is at 30% for reference and the black vertical reference line indicates the highest safe dose.*

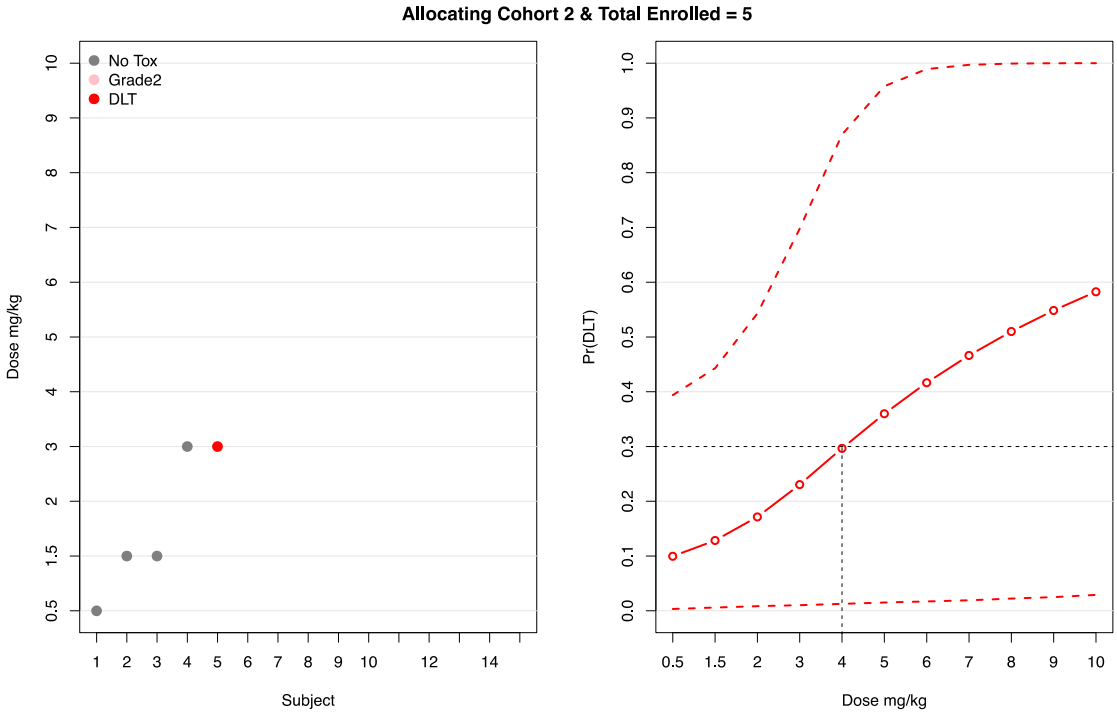

Figure 3: *Example trial with 5 total subjects enrolled. The left panel shows subject allocation where each dot represents one subject. The right panel shows the dose-toxicity model estimates. The solid red line is the mean and the dotted red lines show the 95% confidence interval. The black horizontal reference line is at 30% for reference and the black vertical reference line indicates the highest safe dose.*

The next dosing cohort is enrolled at 3 mg/kg (Figure 3). One of these subjects experiences a DLT. The highest safe dose level is now estimated to be 4 mg/kg. When either a Grade 2 toxicity related to study drug or a DLT is observed, the dosing cohort size must expand to 3 subjects. Because 3 mg/kg is still considered safe, a third subject is added to the 3 mg/kg dose (Figure 4).

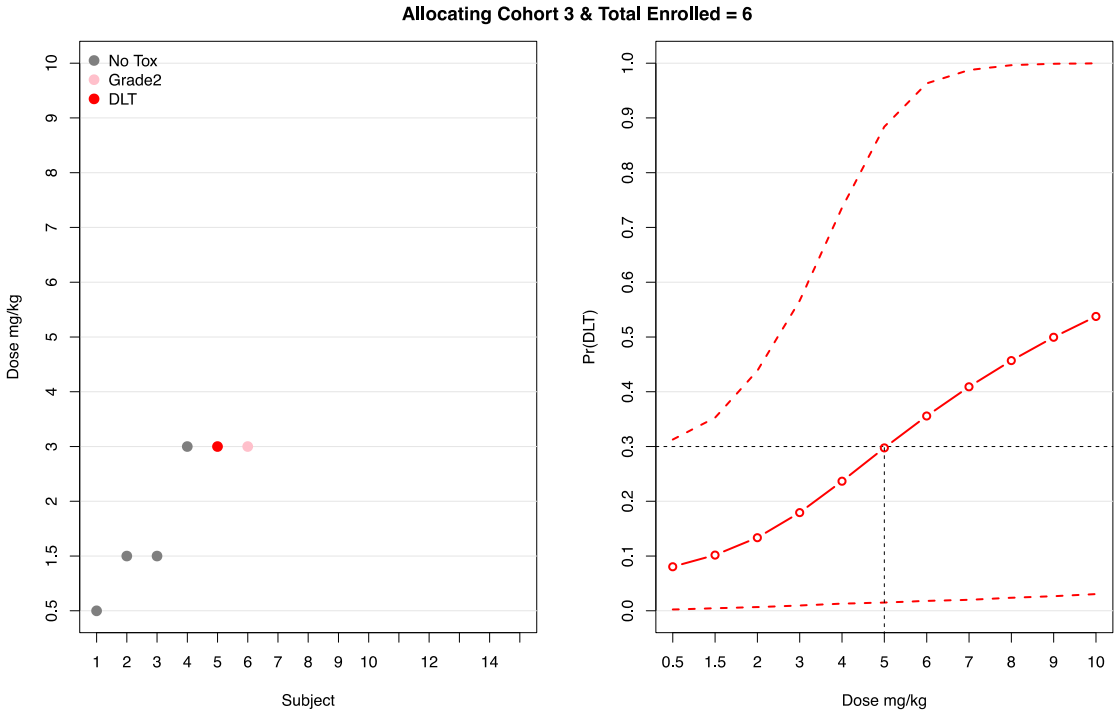

Figure 4: *Example trial with 6 total subjects enrolled. The left panel shows subject allocation where each dot represents one subject. The right panel shows the dose-toxicity model estimates. The solid red line is the mean and the dotted red lines show the 95% confidence interval. The black horizontal reference line is at 30% for reference and the black vertical reference line indicates the highest safe dose.*

This third subject does not experience a DLT, and the highest safe dose is now 5 mg/kg (Figure 4), one of the intermediate doses. The observed toxicity results in the model slowing down the escalation so that the next dose is a 67% increase from the current dose. A dosing cohort of 3 subjects is enrolled at the 5 mg/kg dose (Figure 5).

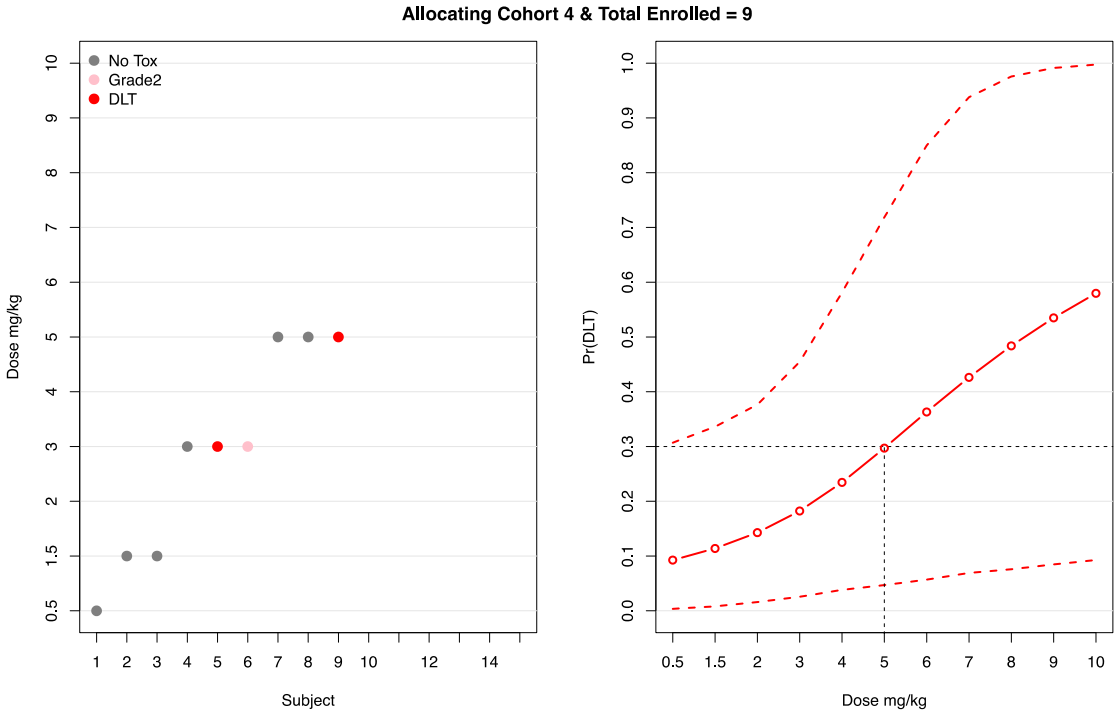

Figure 5: *Example trial with 9 total subjects enrolled. The left panel shows subject allocation where each dot represents one subject. The right panel shows the dose-toxicity model estimates. The solid red line is the mean and the dotted red lines show the 95% confidence interval. The black horizontal reference line is at 30% for reference and the black vertical reference line indicates the highest safe dose.*

One of the subjects at 5 mg/kg experiences a DLT (Figure 5). The 5 mg/kg remains the highest safe dose, and a second dosing cohort of 3 subjects is added to the 5 mg/kg dose (Figure 6).

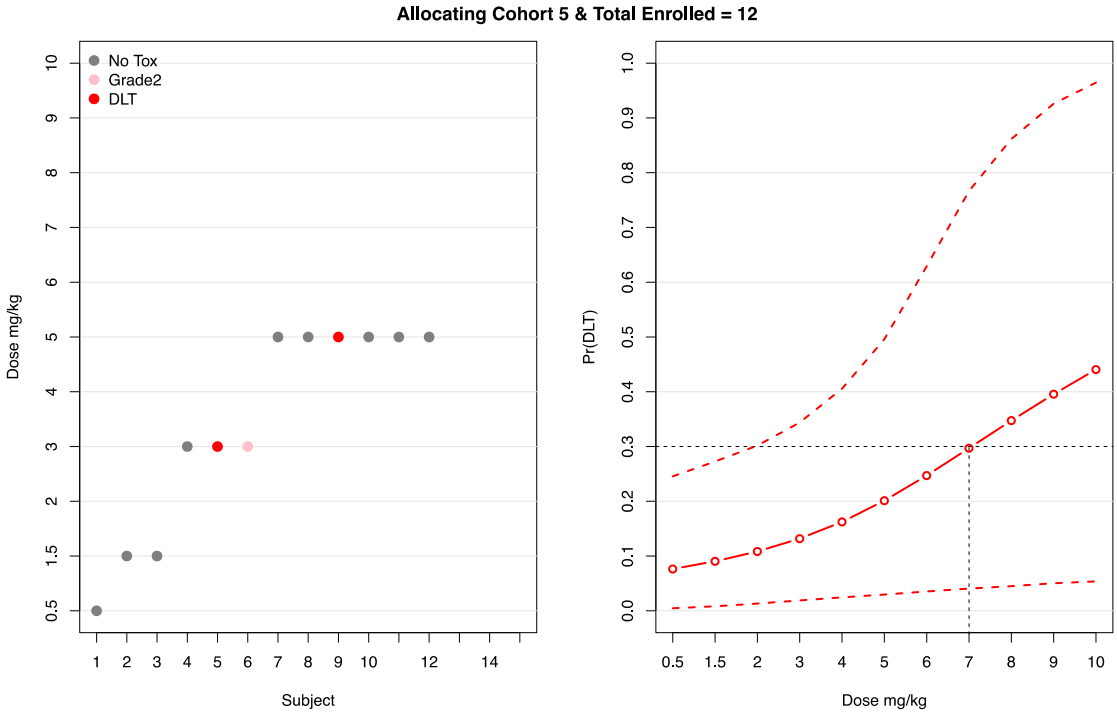

Figure 6: *Example trial with 12 total subjects enrolled. The left panel shows subject allocation where each dot represents one subject. The right panel shows the dose-toxicity model estimates. The solid red line is the mean and the dotted red lines show the 95% confidence interval. The black horizontal reference line is at 30% for reference and the black vertical reference line indicates the highest safe dose.*

We have now observed 1 DLT out of 6 subjects on the 5 mg/kg dose (Figure 6). The highest safe dose is 7 mg/kg. A dosing cohort of 3 subjects is added to 7 mg/kg, and 2 of these subjects experience a DLT (Figure 7).

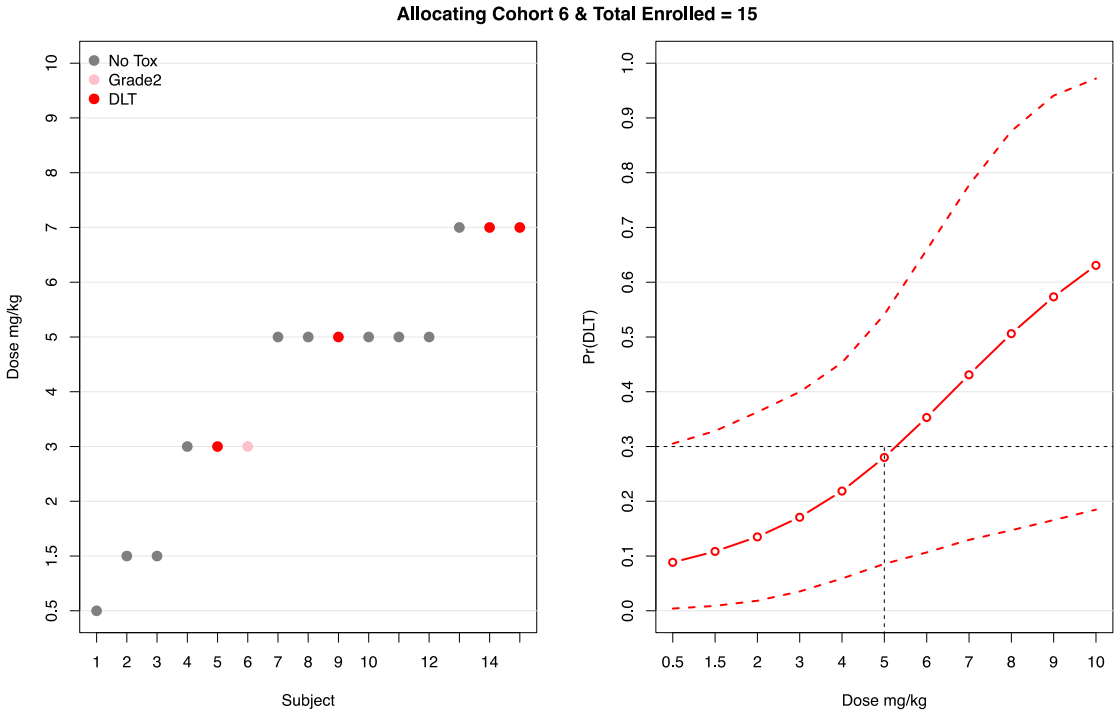

Figure 7: *Example trial with 15 total subjects enrolled. The left panel shows subject allocation where each dot represents one subject. The right panel shows the dose-toxicity model estimates. The solid red line is the mean and the dotted red lines show the 95% confidence interval. The black horizontal reference line is at 30% for reference and the black vertical reference line indicates the highest safe dose.*

The dose is de-escalated, and the highest safe dose is 5 mg/kg (Figure 7). There are already 6 subjects with complete DLT information at 5 mg/kg and this trial stops early for having identified the MTD.

4.0 Single-Agent Operating Characteristics

We evaluate the design by clinical trial simulation. We hypothesize several possible underlying truths of the dose-toxicity curve and determine how the trial will behave in those scenarios. For example, if all the doses were toxic, we would want the trial to recognize that all doses were unsafe and stop as soon as possible. If the smaller doses are safe and a higher dose is in truth the MTD, we would like the algorithm to identify a dose near the MTD as much as possible. The same design can ideally handle different scenarios. Note that this ideal is difficult to achieve in practice with any Phase 1 design due to the small sample sizes.

4.1 Simulation Assumptions

We consider 7 scenarios for the dose-toxicity relationship to characterize the mCRM for different possible underlying truths for the probability of DLT at each dose level. Each scenario assumes the probability of Grade 2 toxicities related to study drug are a constant increase from

the probability of DLT on the log-odds scale. For technical reasons, simulations always assume that the run-in subject assigned to the 0.5 mg/kg dose level experiences no Grade 2 toxicity or DLT. This subject is included in the simulations as a "pseudo-subject" and as such is not reflected in the mean sample size or mean number of DLT operating characteristics presented below.

4.2 Simulation Scenarios

Figure 8 shows the different simulation scenarios used to characterize the performance of the mCRM. We consider a scenario in which all doses are considered safe, a scenario in which no doses are considered safe, and then explore other dose-toxicity curves where the true MTD is in various locations across the dose range.

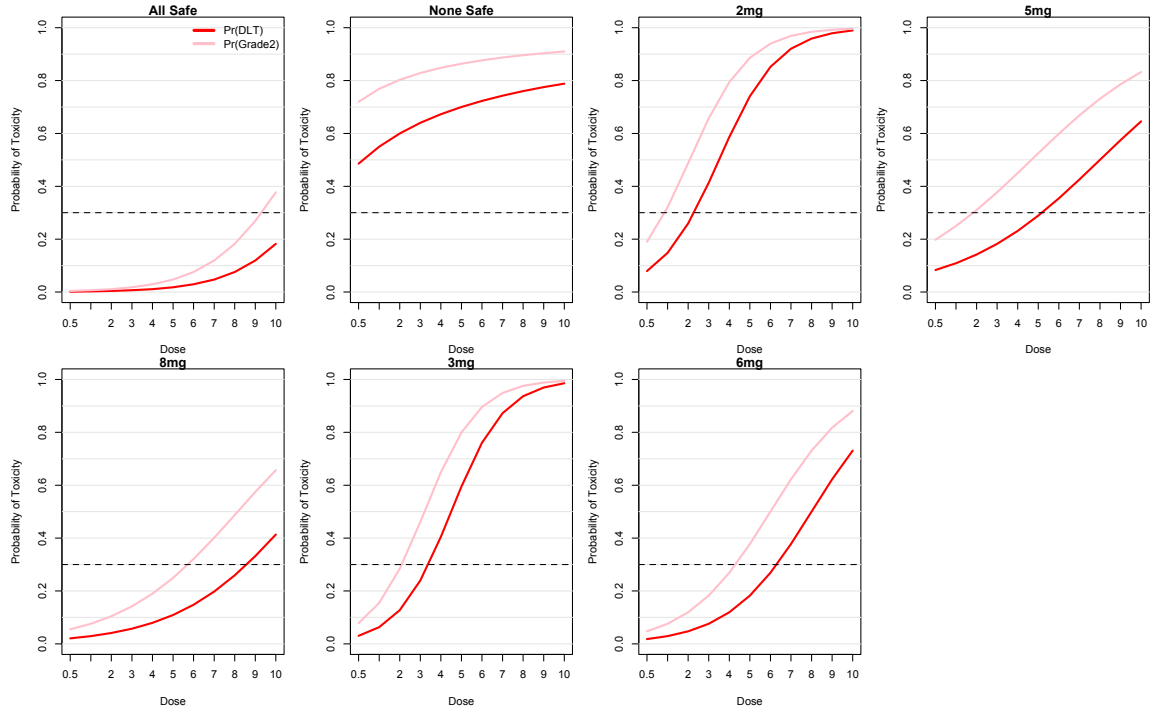

Figure 8: *Dose-toxicity scenarios. The red line indicates the probability of DLT, and the pink line indicates the probability of a Grade 2 or higher toxicity. The horizontal line is at 30% for reference.*

4.1.1 All Doses Safe

In this scenario all doses are considered safe. The true MTD is 10 mg/kg with a DLT rate of 18%. The 10 mg/kg dose is the dose most likely to be selected as the MTD. There is a 97% probability of selecting 10 mg/kg dose as the MTD. The total mean number of subjects in this scenario is 13.7. The mean samples sizes per dose reflect the skipping rules that allow untried doses to be skipped. On average, most subjects are allocated to the primary dose levels, with fewer subjects allocated to the intermediate dose levels.

Scenario 1: All Doses Safe											
	0.5	1.5	2	3	4	5	6	7	8	9	***10***
True Pr(DLT)	0.00	0.00	0.00	0.01	0.01	0.02	0.03	0.05	0.08	0.12	***0.18***
Mean N	0	2.0	0.0	2.0	0.0	0.1	2.2	0.0	0.5	0.3	***6.5***
Mean DLTs	0.00	0.00	0.00	0.01	0.00	0.00	0.07	0.00	0.04	0.04	***1.16***
Pr(MTD)	0	0	0	0	0	0	0	0.003	0.02	0.02	***0.97***

4.1.2 No Doses Safe

In this scenario, none of the dose levels would be considered safe. The lowest dose level, 0.5 mg/kg has a DLT rate of 49%. This lowest dose is selected as the MTD with 12% probability. There is a 69% probability of stopping for toxicity and selecting no dose as the MTD. The total mean number of subjects in this scenario is 9. The operating characteristics of this scenario may be slightly more aggressive than in truth due to the simulation assumption that the first run-in subject on the 0.5 mg/kg dose level does not experience a toxicity.

Scenario 2: No Doses Safe											
	0.5	1.5	2	3	4	5	6	7	8	9	10
True Pr(DLT)	0.49	0.55	0.60	0.64	0.67	0.70	0.72	0.74	0.76	0.78	0.79
Mean N	2.7	4.0	0.5	1.6	0.1	0.1	0.1	0.0	0.0	0.0	0.0
Mean DLTs	1.3	2.2	0.3	1	0.0	0.1	0.0	0.0	0.0	0	0.0
Pr(MTD)	0.12	0.14	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00

4.1.3 True MTD = 2 mg/kg

In this scenario the true MTD is 2 mg/kg with a DLT rate of 26%. This dose is declared as the MTD with 31% probability. The 3 mg/kg dose is slightly more likely to be selected as the MTD. The 3 mg/kg dose has a true DLT rate of 41% is selected as the MTD with 41% probability. The probability of selecting a dose within 1 dose level of the true MTD is 22% + 31% + 41% = 94%. A mean of 5 subjects are enrolled at the 3 mg/kg dose and a mean of 2 DLTs are observed. The total mean number of subjects in this scenario is 14.5.

Scenario 3: 2 mg/kg											
	0.5	1.5	***2***	3	4	5	6	7	8	9	10
True Pr(DLT)	0.08	0.15	***0.26***	0.41	0.59	0.74	0.85	0.92	0.96	0.98	0.99
Mean N	0.5	3.9	***2.8***	4.8	0.5	1.3	0.6	0.0	0.0	0.0	0.0
Mean DLTs	0.0	0.6	***0.7***	2.0	0.3	0.9	0.5	0.0	0.0	0.0	0.0
Pr(MTD)	0.02	0.22	***0.31***	0.41	0.01	0.01	0.00	0.00	0.00	0.00	0.00

4.1.4 True MTD = 5 mg/kg

The true MTD in this scenario is 5 mg/kg with a true DLT rate of 29%. This dose is the mostly likely to be selected as the MTD and is selected with 25% probability. There is 57% probability of selecting a dose within 1 dose level of the true MTD. The total mean number of subjects is 17.6.

Scenario 4: 5 mg/kg											
	0.5	1.5	2	3	4	***5***	6	7	8	9	10
True Pr(DLT)	0.08	0.11	0.14	0.18	0.23	***0.29***	0.35	0.43	0.50	0.57	0.65
Mean N	0.1	2.7	0.5	3.6	1.0	***2.8***	2.9	1.2	1.3	0.5	1.0
Mean DLTs	0.0	0.3	0.1	0.7	0.2	***0.8***	1.0	0.5	0.7	0.3	0.6
Pr(MTD)	0.00	0.02	0.03	0.16	0.10	***0.25***	0.22	0.09	0.07	0.02	0.02

4.1.5 True MTD = 8 mg/kg

The true MTD in this scenario is 8 mg/kg with a true DLT rate of 26%. This dose is selected as the MTD with 22% probability. The 10 mg/kg dose, which has a true DLT rate of 41% is selected as the MTD with 46% probably. A mean of 4.2 subjects are enrolled at the 10 mg/kg dose and a mean of 1.7 DLTs are observed. The total mean sample size in this scenario is 17.6.

Scenario 5: 8 mg/kg											
	0.5	1.5	2	3	4	5	6	7	8	9	10
True Pr(DLT)	0.02	0.03	0.04	0.06	0.08	0.11	0.15	0.20	***0.26***	0.33	0.41
Mean N	0.0	2.2	0.1	2.5	0.1	0.8	3.0	1.1	***2.6***	1.1	4.2
Mean DLTs	0.0	0.1	0.0	0.2	0.0	0.1	0.5	0.2	***0.7***	0.4	1.7
Pr(MTD)	0.00	0.00	0.00	0.01	0.00	0.03	0.09	0.10	***0.22***	0.08	0.46

4.1.6 True MTD = 3 mg/kg

The true MTD in this scenario is 3 mg/kg with a DLT rate of 24%. This dose is the most likely to be selected as the MTD and is selected with 56% probability. The total mean sample size in this scenario is 16.

Scenario 6: 3 mg/kg

	0.5	1.5	2	***3***	4	5	6	7	8	9	10
True Pr(DLT)	0.03	0.06	0.13	***0.24***	0.40	0.60	0.76	0.87	0.94	0.97	0.99
Mean N	0.1	2.6	1.6	***5.4***	2.0	2.5	1.6	0.0	0.2	0.0	0.0
Mean DLTs	0.0	0.2	0.2	***1.3***	0.8	1.5	1.2	0.0	0.2	0.0	0.0
Pr(MTD)	0.00	0.03	0.15	***0.56***	0.18	0.08	0.01	0.00	0.00	0.00	0.00

4.1.7 True MTD = 6 mg/kg

The true MTD in this scenario is 6 mg/kg with a true DLT rate of 27%. This dose is the most likely to be selected as the MTD and is selected with 38% probability. The total mean sample size in this scenario is 18.6.

Scenario 7: 6 mg/kg											
	0.5	1.5	2	3	4	5	***6***	7	8	9	10
True Pr(DLT)	0.02	0.03	0.05	0.08	0.12	0.18	***0.27***	0.38	0.50	0.62	0.73
Mean N	0.0	2.2	0.1	2.7	0.6	2.4	***4.4***	1.8	2.4	0.5	1.6
Mean DLTs	0	0.1	0.0	0.2	0.1	0.4	***1.2***	0.7	1.2	0.3	1.2
Pr(MTD)	0.00	0.00	0.00	0.02	0.05	0.23	***0.38***	0.16	0.13	0.02	0.02

5.0 Combination Operating Characteristics

In the section we describe the behavior of the mCRM for the combination in the case that the starting dose is higher. If the starting dose is a low dose, the behavior will be very similar to that described for the single agent. The highest starting dose for the combination is likely to be 5 mg/kg dose. Therefore, in this section we describe the behavior of the mCRM assuming this higher starting dose.

5.1 First Dosing Cohort Behavior

At the 5 mg/kg starting dose, the dosing cohort will be size 2. If no DLTs are observed, the dose may escalate to the 10 mg/kg dose. If 1 DLT is observed, the dosing cohort will add 1 subject to

be size 3. If out of a dosing cohort of 3 subjects, only 1 DLT is observed the dose will escalate to 7 mg/kg. If 2 DLTs are observed out of a dosing cohort of 3 subjects, the dose will de-escalate to 1 mg/kg and if 3 DLTs are observed the dose will de-escalate to 0.5 mg/kg.

5.2 Operating Characteristics

We simulate the behavior of the design where the starting dose is 5 mg/kg assuming the same true dose-toxicity scenarios as described for the single agent. Generally, we see similar trends in behavior to the single-agent escalation in terms of correctly identifying the MTD. Several scenarios show that should 5 mg/kg be an unsafe dose, the mCRM is able to de-escalate to safe doses.

Scenario 1: All Doses Safe											
	0.5	1.5	2	3	4	5	6	7	8	9	***10***
True Pr(DLT)	0.00	0.00	0.00	0.01	0.01	0.02	0.03	0.05	0.08	0.12	***0.18***
Mean N	0.0	0.0	0.0	0.0	0.0	2.1	0.2	0.3	0.1	0.3	***6.4***
Mean DLTs	0.00	0.00	0.00	0.00	0.00	0.04	0.01	0.01	0.01	0.04	***1.18***
Pr(MTD)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.05	***0.92***

Scenario 2: No Doses Safe											
	0.5	1.5	2	3	4	5	6	7	8	9	10
True Pr(DLT)	0.49	0.55	0.60	0.64	0.67	0.70	0.72	0.74	0.76	0.78	0.79
Mean N	1.2	2.1	0.3	0.5	0.1	3.1	0.6	0.1	0.0	0.0	0.2
Mean DLTs	0.54	1.16	0.19	0.31	0.06	2.16	0.46	0.04	0.01	0.00	0.12
Pr(MTD)	0.04	0.09	0.04	0.03	0.02	0.01	0.01	0.00	0.00	0.00	0.00

Scenario 3: 2 mg/kg											
	0.5	1.5	***2***	3	4	5	6	7	8	9	10

True Pr(DLT)	0.08	0.15	***0.26***	0.41	0.59	0.74	0.85	0.92	0.96	0.98	0.99
Mean N	1.3	3.1	***1.2***	1.8	0.2	3.1	0.4	0.0	0.0	0.0	0.1
Mean DLTs	0.10	0.45	***0.32***	0.75	0.09	2.30	0.37	0.01	0.00	0.00	0.09
Pr(MTD)	0.04	0.26	***0.20***	0.29	0.08	0.01	0.00	0.00	0.00	0.00	0.00

Scenario 4: 5 mg/kg											
	0.5	1.5	2	3	4	***5***	6	7	8	9	10
True Pr(DLT)	0.08	0.11	0.14	0.18	0.23	***0.29***	0.35	0.43	0.50	0.57	0.65
Mean N	0.1	0.7	0.2	1.0	0.4	***3.6***	2.0	1.0	0.3	0.2	2.0
Mean DLTs	0.01	0.06	0.02	0.20	0.09	***1.03***	0.69	0.44	0.13	0.12	1.31
Pr(MTD)	0.01	0.02	0.03	0.09	0.19	***0.18***	0.20	0.12	0.08	0.02	0.06

Scenario 5: 8 mg/kg											
	0.5	1.5	2	3	4	5	6	7	***8***	9	10
True Pr(DLT)	0.02	0.03	0.04	0.06	0.08	0.11	0.15	0.20	***0.26***	0.33	0.41
Mean N	0.0	0.1	0.0	0.1	0.0	2.8	1.1	1.2	***0.6***	0.5	4.4
Mean DLTs	0.00	0.00	0.00	0.01	0.00	0.28	0.16	0.25	***0.14***	0.20	1.84
Pr(MTD)	0.00	0.00	0.00	0.01	0.02	0.05	0.09	0.14	***0.16***	0.11	0.43

Scenario 6: 3 mg/kg											
	0.5	1.5	2	***3***	4	5	6	7	8	9	10

True Pr(DLT)	0.03	0.06	0.13	***0.24***	0.40	0.60	0.76	0.87	0.94	0.97	0.99
Mean N	0.6	2.3	1.1	***2.7***	0.6	3.4	0.8	0.0	0.0	0.0	0.3
Mean DLTs	0.02	0.12	0.13	***0.66***	0.22	2.04	0.65	0.00	0.00	0.00	0.29
Pr(MTD)	0.01	0.07	0.12	***0.40***	0.31	0.07	0.00	0.00	0.00	0.00	0.00

Scenario 7: 6 mg/kg											
	0.5	1.5	2	3	4	5	***6***	7	8	9	10
True Pr(DLT)	0.02	0.03	0.05	0.08	0.12	0.18	***0.27***	0.38	0.50	0.62	0.73
Mean N	0.0	0.2	0.0	0.5	0.3	3.9	***2.0***	1.5	0.3	0.2	2.6
Mean DLTs	0.00	0.01	0.00	0.04	0.03	0.70	***0.54***	0.57	0.16	0.15	1.86
Pr(MTD)	0.00	0.00	0.01	0.02	0.11	0.22	***0.27***	0.21	0.09	0.03	0.04

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