A Phase 1b Safety and Feasibility Study of Personalized Immunotherapy in Adults with Advanced Cancers

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A Phase 1b Safety and Feasibility Study of Personalized Immunotherapy in Adults with Advanced Cancers

PROTOCOL

Study Chair (Principal Investigator)
Ezra Cohen, M.D.
Professor of Medicine
UC San Diego Moores Cancer Center
3855 Health Sciences Drive
La Jolla, CA 92093-0658
Phone: 858-534-6161
Fax: 858-822-5754
Email: ecohen@ucsd.edu

Study Site
UCSD Moores Cancer Center
3855 Health Sciences Drive #0698
La Jolla, CA 92093-0698

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IND Holder Name: Ezra Cohen, MD

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Confidentiality Statement

LIST OF INVESTIGATORS

PRINCIPAL INVESTIGATOR

Ezra Cohen, M.D.
Professor of Medicine
UC San Diego Moores Cancer Center
3855 Health Sciences Drive
La Jolla, CA 92093-0658
Phone: 858-534-6161
Fax: 858-822-5754
Email: ecohen@ucsd.edu

CO-INVESTIGATOR(S)

Stephen Schoenberger, Ph.D.
Professor
Division of Developmental Immunology
La Jolla Institute for Immunology
9420 Athena Circle
La Jolla, CA 92037
Email: sps@lji.org

Sandip Patel, M.D.
Associate Professor of Medicine
UC San Diego Moores Cancer Center
3855 Health Sciences Drive
La Jolla, CA 92093-0820
Email: sandippatel@ucsd.edu

Aaron Miller, M.D.
Associate Professor of Medicine
UC San Diego Moores Cancer Center
3855 Health Sciences Drive
La Jolla, CA 92093-0658
Email: ammiller@health.ucsd.edu

Rebecca Shatsky, M.D.
Associate Professor of Medicine
UC San Diego Moores Cancer Center
3855 Health Sciences Drive
La Jolla, CA 92093-0658
Email: rshatsky@ucsd.edu

Rana McKay, M.D.
Assistant Professor of Medicine
UC San Diego Moores Cancer Center
3855 Health Sciences Drive
La Jolla, CA 92093-0987
Email: rmckay@ucsd.edu

Shumei Kato, M.D.
Associate Professor of Medicine
UC San Diego Moores Cancer Center
3855 Health Sciences Drive
La Jolla, CA 92093-0987
Email: smkato@ucsd.edu

BIOSTATISTICIAN

Karen Messer, Ph.D.
Professor of Family Medicine & Public Health
UC San Diego Moores Cancer Center
3855 Health Sciences Drive #0901
La Jolla CA, 92093-0901
Phone: 858-822-6823
E-mail: kmesser@ucsd.edu

Xinlian Zhang, PhD
Assistant Professor of Family Medicine & Public Health
UC San Diego Moores Cancer Center
3855 Health Sciences Drive #0901
La Jolla CA, 92093-0901
Phone: (858) 822-4838E-mail: xizhang@ucsd.edu

Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator / Study Chair

_____
Printed Name

_____ _____
Signature Date

Participating Site Principal Investigator

_____ _____
Printed Name Institution

_____ _____
Signature Date

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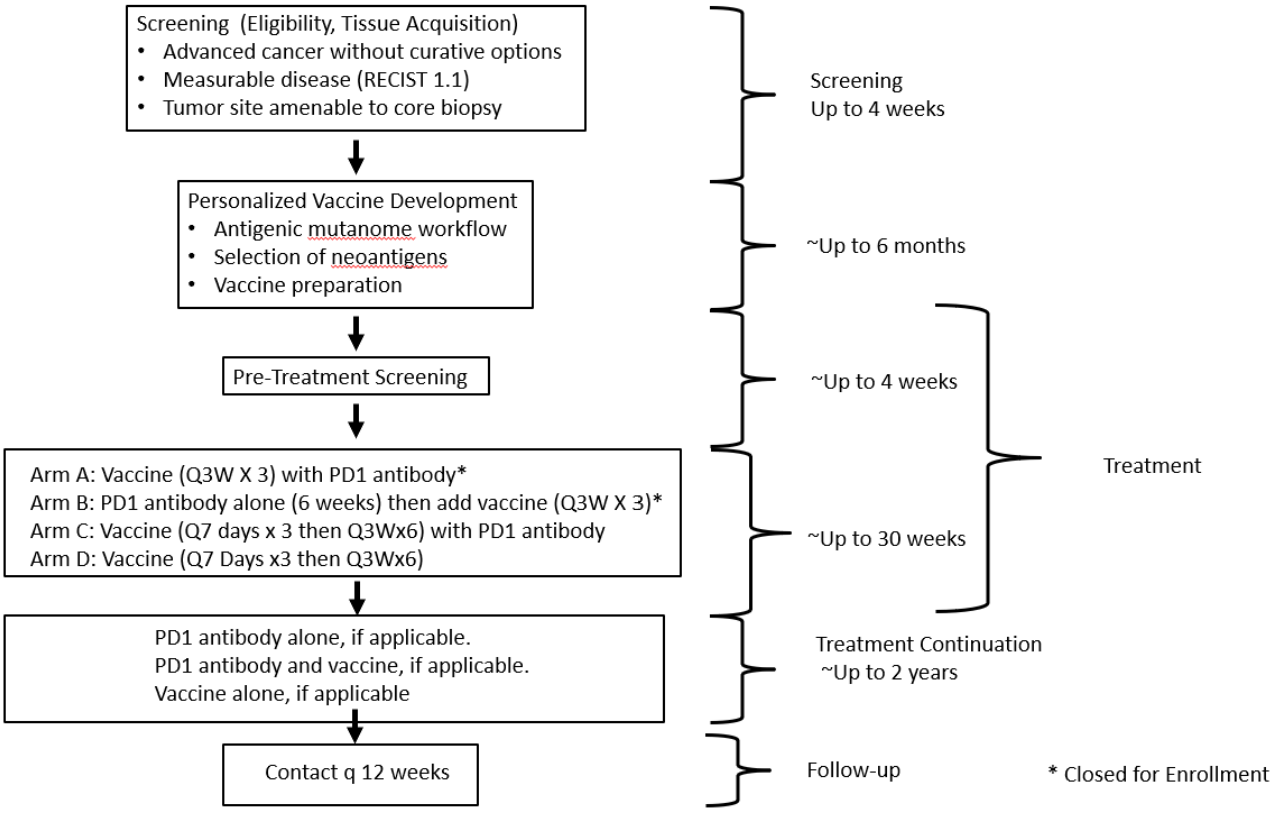
LIST OF ABBREVIATIONS

AE	Adverse Event
ALC	Absolute Lymphocyte Count
ALT	Alanine Aminotransferase
aPTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CNS	Central Nervous System
CR	Complete Response
CRP	C-reactive Protein
CSF	Cerebrospinal Fluid
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DC	Dendritic Cell
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HIV	Human Immunodeficiency Virus
HIPAA	Health Insurance Portability and Accountability Act
HNSCC	Head and Neck Squamous Cell Carcinoma
HPV	Human Papillomavirus
HRPP	Human Research Protections Program
ICH	International Conference on Harmonization
IHC	Immunohistochemistry
IRB	Institutional Review Board
IUD	Intrauterine Device
I.V.	Intravenous
LDH	Lactate Dehydrogenase
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
ORR	Overall Response Rate
OS	Overall Survival
PD	Progressive Disease
PET	Positron Emission Tomography
PBMCs	Peripheral Blood Mononuclear Cells
p.o.	per os/by mouth/orally
PR	Partial Response

PT	Prothrombin Time
RCC	Renal Cell Carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase
TNF	Tumor Necrosis Factor
ULN	Upper Limit of Normal
UPR	Unanticipated Problems involving Risk to subjects or others

STUDY SCHEMA



STUDY SUMMARY

Title	**A Phase 1b Safety and Feasibility Study of Personalized Immunotherapy in Adults with Advanced Cancers**
Short Title	Personalized Vaccine in Advanced Cancer Patients
Phase	1b
Methodology	Open label, single arm, unblinded
Study Duration	Approximately 4 years
Study Center(s)	Single-center
Objectives	Demonstrate induction or increase in T cell neoantigen reactivity
Number of Subjects	n=30
Diagnosis and Main Inclusion Criteria	• Advanced Solid Tumor patients who are not candidates for curative therapy • Measurable disease • At least one site of disease amenable to core needle biopsy
Study Product(s), Dose, Route, Regimen	Personalized vaccine (encoding tumor-specific neoantigens) at a dose of 100 µg per peptide. Arm A: Personalized vaccine and anti- PD-1 administered concurrently at the start of study therapy. Personalized vaccine will be administered by subcutaneous injection. Vaccine will be administered every three weeks for a total of three doses. Vaccine may continue to be administered at 3 week intervals for an additional 9 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit. Arm B: Anti-PD-1 antibody for 6 weeks followed by personalized vaccine therapy. Personalized vaccine will be administered by subcutaneous injection. Vaccine will be administered every three weeks for a total of three doses. Vaccine may continue to be administered at 3 week intervals for an additional 9 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit. Arm C: Personalized vaccine and anti- PD-1 administered concurrently in a boosted schedule at the start of study therapy. Personalized vaccine will be administered by intramuscular injection. Vaccine will be administered three weekly priming vaccine doses, followed by six vaccine doses administered every three weeks. Vaccine may continue to be administered at 3 week intervals for an additional 18 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit. Arm D: Personalized vaccine alone, in a boosted schedule at the start of study therapy. Personalized vaccine will be administered by intramuscular injection. Vaccine will be administered three weekly priming vaccine doses, followed by six vaccine doses administered every three weeks. Vaccine may continue to be administered at 3 week intervals for an additional 18 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit.

TABLE 1a: SCHEDULE OF EVENTS: ARM A (CLOSED TO ENROLLMENT)

Assessments[r]	Screening		Treatment Period [a]								Treatment Continuation	End of Treatment[b]	Follow-up
	Eligibility	Pre-vaccine	Week 0		Week 3		Week 6		Week 9		Q 3 Wks	4 wks post final treatment	Q 12 Wks[c]
Day		Within 14 days of Week 0 Day1	D1	D8	D1 [a]	D8	D1 [a]	D8	D1 [a]	D8	D1	D1	
Visit Window (days)				±1	±3	±1	±3	±1	±3	±3	±3	±7	±7
Informed consent	X	X											
Inclusion/exclusion	X	X											
Medical history, height[d]	X	X											
Virology screen[e]	X	X											
Coagulation panel[n]		X							X				
CT or MRI[p]		X							X		Q 9 wks ±7 days[p]	X	
Genome sequencing (blood)	X												
Tumor biopsy	X[f]								X[f,l]			X[f,l]	
Physical exam[g]	X	X	X		X		X		X		X	X	
ECOG status	X	X	X		X		X		X		X	X	
Vital signs[h]	X	X	X		X		X		X		X	X	
Pregnancy test[i]		X											
Clinical labs[j]	X[j]	X[j]	X[j]		X[j]		X[j]		X[j]		X[j]	X[j]	
TSH		X			X[q]				X[q]		X[q]	X	
Con. Meds		X	X	X	X	X	X	X	X	X	X	X	
AEs			X	X	X	X	X	X	X	X	X	X	
Blood for Correlative Studies[k]	X[k]	X[k]						X[k]	X[k]		X[k]		
Vaccine administration[o]			X		X		X				X[o]		
Pembrolizumab[m]			X		X		X		X		X		
Status, cancer treatment													X

FOOTNOTES

a. At visits where dosing is indicated, all assessments should be completed prior to dosing unless stated otherwise. If any doses are delayed, adjust subsequent dosing schedule accordingly. The Week 3, Day 1 visit must be ≥ 21 days post the Week 0, Day 1 visit.
b. EOT Visit will occur within 4 weeks after the last dose of study drug or prior to commencing the new therapy. If the EOT visit occurs earlier than 4 weeks, a safety follow-up telephone call on Day 28 (-3 days) is required; document contact in the study records.
c. Follow-up begins 12 weeks after final anti-PD1 dose and every 12 weeks thereafter.
d. Medical history, medication history, and demographics will be recorded, including cancer-related treatments and procedures.
e. Virology screen only if clinically indicated and includes HIV antibody, hepatitis B surface antigen, and hepatitis C antibody. Subjects who are hepatitis C antibody positive with confirmed negative viral load are eligible.
f. A core needle biopsy may be obtained at any accessible tumor site (primary or metastatic); CT scan-may be needed to assist during the biopsy procedure.
g. Complete physical examinations conducted at screening and EOT; symptom-directed physical examinations conducted at all other indicated visits (up to 3 days prior to dosing).
h. Vital signs include blood pressure, pulse, respiratory rate, temperature, pulse oximetry, and weight.
i. Urine or serum pregnancy tests administered to WOCBP only. In case of delayed menstrual period (> 1 month) confirm absence of pregnancy prior to dosing. Pregnancy test for screening must be completed within 28 days prior to dosing.
j. Clinical hematology includes complete blood count with differential ANC and platelet count; serum chemistry includes sodium, potassium, chloride, bicarbonate, glucose, blood urea nitrogen, creatinine, lactate dehydrogenase, ALT, AST, alkaline phosphatase, bilirubin (total, direct, indirect), total protein, albumin, calcium, magnesium, uric acid, and phosphate. Urinalysis includes blood, glucose, ketones, leukocytes, nitrite, pH, protein, and specific gravity; perform using test strip (dipstick) unless full urinalysis with microbiology is clinically indicated. All prior to dosing; testing may be done up to 3 days prior to dosing.
k. Arm A patients will have blood for correlative studies collected at the following time points: 1) Screening, eligibility 2) Screening, Pre-Vaccine (within 14 days prior to Week 0 Day 1 vaccine administration); 3) Week 6 (within 7 days post-vaccine administration); 4) Within Week 9-12 5) Week 18 (within 12 weeks of last vaccine dose (+/- 3 days) and anti-PD1 antibody administration). Please see section 5.5 for details. If additional vaccine doses are administered, additional samples may be collected per investigator, a sample should collected within 12 weeks of last vaccine dose (+/-3 days).
l. A tumor biopsy within Weeks 9-12 and at disease progression is not required, but strongly encouraged.
m. Pembrolizumab will be administered for a maximum of 35 cycles (approximately 2 years) if there is no evidence of disease progression.
n. Coagulation panel includes PT, INR, and APTT.
o. Vaccine may be administered for an additional 12 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit.
p. CT or MRI scans will be performed every 9 weeks (+/- 7 days) for the first 6 months of therapy and then can be performed as clinically indicated.
q. Starting Week 3, TSH will be drawn every other pembrolizumab administration (i.e., Week 9, Week 15, Week 21, etc.)
r. Alternative methods for informed consent and safety assessments (e.g., phone contact, virtual visit, alternative location for assessment, including local labs or imaging centers) may be implemented when necessary (e.g. COVID-19 pandemic) and feasible.
s. Survival contacts via medical record review, telephone call, or review of the Social Security Index.

ABBREVIATIONS FOR TABLE

ALT=alanine aminotransferase; ANC = absolute neutrophil count; APTT = activated partial thromboplastin time; AST=aspartate aminotransferase; CT = computed tomography; ECOG = Eastern Cooperative Oncology Group; EOT = End of Treatment; HIV = human immunodeficiency virus; INR = international normalized ratio of prothrombin time; MRI = magnetic resonance imaging; PBMC = peripheral blood mononuclear cells; PT = prothrombin time; WOCBP = woman of childbearing potential.

TABLE 1b: SCHEDULE OF EVENTS: ARM B (CLOSED TO ENROLLMENT)

Assessments[s]	Screening		Treatment Period [a]								Treatment Continuation	End of Treatment[b]	Follow-up
	Eligibility	Pre-vaccine	Week 0		Week 3		Week 6		Week 9		Q 3 Wks	4 wks post final treatment	Q 12 Wks[c]
Day		Within 14 days of Week 0 Day1	D1	D8	D1 [a]	D8	D1 [a]	D8	D1 [a]	D8	D1	D1	
Visit Window (days)				±1	±3	±1	±3	±1	±3	±1	±3	±7	±7
Informed consent	X	X											
Inclusion/exclusion	X	X											
Medical history, height[d]	X	X											
Virology screen[e]	X	X											
Coagulation panel[o]		X							X				
CT or MRI[q]		X							X		Q 9 wks ±7 days[q]	X	
Genome sequencing (blood)	X												
Tumor biopsy	X[f]										X[f,l,p]	X[f,l]	
Physical exam[g]	X	X	X		X		X		X		X	X	
ECOG status	X	X	X		X		X		X		X	X	
Vital signs[h]	X	X	X		X		X		X		X	X	
Pregnancy test[i]		X											
Clinical labs[j]	X[j]	X[j]	X[j]		X[j]		X[j]		X[j]		X[j]	X[j]	
TSH		X			X[r]				X[r]		X[r]	X	
Con. Meds		X	X	X	X	X	X	X	X	X	X	X	
AEs			X	X	X	X	X	X	X	X	X	X	
Blood for Correlative Studies[k]	X[k]	X[k]					X[k]		X[k]		X[k]		
Vaccine administration[m]							X		X		X[m]		
Pembrolizumab[n]			X		X		X		X		X		
Status, cancer treatment													X

FOOTNOTES

a. At visits where dosing is indicated, all assessments should be completed prior to dosing unless stated otherwise. If any doses are delayed, adjust subsequent dosing schedule accordingly. The Week 3, Day 1 visit must be ≥ 21 days post the Week 0, Day 1 visit.
b. EOT Visit will occur within 4 weeks after the last dose of study drug or prior to commencing the new therapy. If the EOT visit occurs earlier than 4 weeks, a safety follow-up telephone call on Day 28 (-3 days) is required; document contact in the study records.
c. Follow-up begins 12 weeks after final anti-PD1 dose and every 12 weeks thereafter.
d. Medical history, medication history, and demographics will be recorded, including cancer-related treatments and procedures.
e. Virology screen only if clinically indicated and includes HIV antibody, hepatitis B surface antigen, and hepatitis C antibody. Subjects who are hepatitis C antibody positive with confirmed negative viral load are eligible.
f. A core needle biopsy may be obtained at any accessible tumor site (primary or metastatic); CT scan-may be needed to assist during the biopsy procedure.
g. Complete physical examinations conducted at screening and EOT; symptom-directed physical examinations conducted at all other indicated visits (up to 3 days prior to dosing).
h. Vital signs include blood pressure, pulse, respiratory rate, temperature, pulse oximetry, and weight.
i. Urine or serum pregnancy tests administered to WOCBP only. In case of delayed menstrual period (> 1 month) confirm absence of pregnancy prior to dosing. Pregnancy test for screening must be completed within 28 days prior to dosing.

j. Clinical hematology includes complete blood count with differential ANC and platelet count; serum chemistry includes sodium, potassium, chloride, bicarbonate, glucose, blood urea nitrogen, creatinine, lactate dehydrogenase, ALT, AST, alkaline phosphatase, bilirubin (total, direct, indirect), total protein, albumin, calcium, magnesium, uric acid, and phosphate. Urinalysis includes blood, glucose, ketones, leukocytes, nitrite, pH, protein, and specific gravity; perform using test strip (dipstick) unless full urinalysis with microbiology is clinically indicated. All prior to dosing; testing may be done up to 3 days prior to dosing.
k. Arm B patients will have PBMC Research samples collected at the following time points: 1) Screening, eligibility 2) Screening, Pre-Vaccine (within 14 days prior to Week 0 Day 1); 3) Week 6 (within 7 days prior to vaccine administration); 4) Week 12 (within 7 days post-3rd vaccine administration); 5) Week 24 (within 12 weeks of last vaccine dose (+/- 3 days) and anti-PD1 antibody administration) If additional vaccine doses are administered, additional samples may be collected per investigator, a sample should collected within 12 weeks of last vaccine dose (+/-3 days).
l. A tumor biopsy within Week 12-15 and at disease progression is not required, but strongly encouraged.
m. Vaccine administered on Week 12. Vaccine may continue to be administered at 3 week intervals for an additional 12 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit.
n. Pembrolizumab will be administered for a maximum of 35 cycles (approximately 2 years) if there is no evidence of disease progression.
o. Coagulation panel includes PT, INR, and APTT.
p. 2nd tumor biopsy on arm B should be performed after the 3rd dose of vaccine is administered and is optional
q. CT or MRI scans will be performed every 9 weeks (+/- 7 days) for the first 6 months of therapy and then can be performed as clinically indicated
r. Starting Week 3, TSH will be drawn every other pembrolizumab administration (i.e., Week 9, Week 15, Week 21, etc.)
s. Alternative methods for informed consent and safety assessments (e.g., phone contact, virtual visit, alternative location for assessment, including local labs or imaging centers) may be implemented when necessary (e.g. COVID-19 pandemic) and feasible.
t. Survival contacts via medical record review, telephone call, or review of the Social Security Index.

ABBREVIATIONS FOR TABLE

ALT=alanine aminotransferase; ANC = absolute neutrophil count; APTT = activated partial thromboplastin time; AST=aspartate aminotransferase; CT = computed tomography; ECOG = Eastern Cooperative Oncology Group; EOT = End of Treatment; HIV = human immunodeficiency virus; INR = international normalized ratio of prothrombin time MRI = magnetic resonance imaging; PBMC = peripheral blood mononuclear cells; PT = prothrombin time WOCBP = woman of childbearing potential.

TABLE 1c: SCHEDULE OF EVENTS: ARM C

Assessments[s]	Screening		Treatment Period [a]											Treatment Continuation	End of Treatment[b]	Follow-up
	Eligibility	Pre-vaccine	Week -1	Week 0	Week 1	Week 2 Week 5 Week 8		Week 9	Week 11 Week 14 Week 17		Week 18	Week 20		Q 3 Wks	4 wks post final treatment	Q 12 Wks[c]
Day		Within 28 days of Week -1 Day 1	D1	D1	D1	D1 [a]	D8	D1 [a]	D1 [a]	D8	D1 [a]	D1 [a]	D8	D1	D1	
Visit Window (days)						±3	±1	±7	±3	±1	±7	±3	±1	±3	±7	±7
Informed consent	X[t]	X[t]														
Inclusion/ Exclusion	X	X														
Medical history, height[d]	X	X														
Virology screen[e]	X	X														
Coagulation panel[o]		X						X			X					
CT or MRI[p]		X						X			X			Q 9 wks ±7 days[p]	X	
Genome sequencing (blood)	X															
Tumor biopsy	X[f]							X[f,l]							X[f,l]	
Physical exam[g]	X	X	X[g]	X	X	X			X			X		X	X	
ECOG status	X	X	X[r]	X	X	X			X			X		X	X	
Vital signs[h]	X	X	X[r]	X	X	X			X			X		X	X	
Pregnancy test[i]		X														
Clinical labs[j]	X[j]	X[j]	X[j]	X[j]	X[j]	X[j]			X[j]			X[j]		X[j]	X[j]	
TSH		X				X[q]			X[q]					X[q]	X	
Con. Meds		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
AEs			X	X	X	X	X	X	X	X	X	X	X	X	X	
Blood for Correlative Studies[k]	X[k]	X[k]				X[k]								X[k]		

Vaccine administration[m]				X	X	X			X			X		X[m]		
Pembrolizumab[n]			X[n]			X			X			X		X		
Status, cancer treatment																X

FOOTNOTES

a. At visits where dosing is indicated, all assessments should be completed prior to dosing unless stated otherwise. If any doses are delayed, adjust subsequent dosing schedule accordingly. The Week 5, Day 1 visit must be ≥ 21 days post the Week 2, Day 1 visit.
b. EOT Visit will occur within 4 weeks after the last dose of study drug or prior to commencing the new therapy. If the EOT visit occurs earlier than 4 weeks, a safety follow-up telephone call on Day 28 (-3 days) is required; document contact in the study records.
c. Follow-up begins 12 weeks after final anti-PD1 dose and every 12 weeks thereafter.
d. Medical history, medication history, and demographics will be recorded, including cancer-related treatments and procedures.
e. Virology screen only if clinically indicated and includes HIV antibody, hepatitis B surface antigen, and hepatitis C antibody. Subjects who are hepatitis C antibody positive with confirmed negative viral load are eligible.
f. A core needle biopsy may be obtained at any accessible tumor site (primary or metastatic); CT scan-may be needed to assist during the biopsy procedure.
g. Complete physical examinations conducted at screening and EOT; symptom-directed physical examinations conducted at all other indicated visits (up to 3 days prior to dosing; for Week -1, up to 7 days prior to dosing).
h. Vital signs include blood pressure, pulse, respiratory rate, temperature, pulse oximetry, and weight.
i. Urine or serum pregnancy tests administered to WOCBP only. In case of delayed menstrual period (> 1 month) confirm absence of pregnancy prior to dosing. Pregnancy test for screening must be completed within 28 days prior to dosing.

j. Clinical hematology includes complete blood count with differential ANC and platelet count; serum chemistry includes sodium, potassium, chloride, bicarbonate, glucose, blood urea nitrogen, creatinine, lactate dehydrogenase, ALT, AST, alkaline phosphatase, bilirubin (total, direct, indirect), total protein, albumin, calcium, magnesium, uric acid, and phosphate. Urinalysis includes blood, glucose, ketones, leukocytes, nitrite, pH, protein, and specific gravity; perform using test strip (dipstick) unless full urinalysis with microbiology is clinically indicated. All tests must be performed prior to dosing. For Week 0 Day 1, pre-vaccine screening labs may be used up to 28 days prior to dosing. For subsequent treatment days on which laboratory assessments are required, labs must be performed up to 3 days prior to dosing.
k. Arm C patients should have Blood Collection for Correlative Studies samples collected at the following time points: 1) Screening, eligibility 2) Screening, Pre-Vaccine (within 28 days prior to Week -1 Day 1); 3) Week 8 (within 7 days after vaccine administration); 4) Week 32 (within 12 weeks of last vaccine dose (+/- 3 days) and anti-PD1 antibody administration). If additional vaccine doses are administered, additional samples may be collected per investigator, a sample should collected within 12 weeks of last vaccine dose (+/-3 days). For neoantigen identification, an additional 75-100 mL whole blood (eight to ten 10mL heparin tubes) may be collected every 4 weeks.
l. A tumor biopsy within Weeks 9-12 and at disease progression is not required, but strongly encouraged.
m. Vaccine administered on Week 20. Vaccine may continue to be administered at 3 week intervals for an additional 18 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit.
n. Pembrolizumab will be initiated 1 week prior to the first vaccine dose (week -1). Pembrolizumab will be administered for a maximum of 35 cycles (approximately 2 years) if there is no evidence of disease progression.
o. Coagulation panel includes PT, INR, and APTT
p. CT scans will be performed every 9 weeks (+/- 7 days) for the first 6 months of therapy and then can be performed as clinically indicated
q. Starting Week 2, TSH will be drawn every other pembrolizumab administration following (i.e., Week 8, Week 14, Week 20, etc.)
r. ECOG and Vital signs for Week -1 may be performed up to 7 days prior to dosing.

s. Alternative methods for informed consent and safety assessments (e.g., phone contact, virtual visit, alternative location for assessment, including local labs or imaging centers) may be implemented when necessary (e.g. COVID-19 pandemic) and feasible.
t. Subjects may sign an initial consent allowing the vaccine to be manufactured. Subsequently, the subject would sign a second consent for treatment when they and the IP are ready to proceed.
u. Survival contacts via medical record review, telephone call, or review of the Social Security Index.

ABBREVIATIONS FOR TABLE

ALT=alanine aminotransferase; ANC = absolute neutrophil count; APTT = activated partial thromboplastin time; AST=aspartate aminotransferase; CT = computed tomography; ECOG = Eastern Cooperative Oncology Group; EOT = End of Treatment; HIV = human immunodeficiency virus; INR = international normalized ratio of prothrombin time MRI = magnetic resonance imaging; PBMC = peripheral blood mononuclear cells; PT = prothrombin time WOCBP = woman of childbearing potential.

TABLE 1d: SCHEDULE OF EVENTS: ARM D

Assessments[q]	Screening		Treatment Period [a]										Treatment Continuation	End of Treatment[b]	Follow-up
	Eligibility	Pre-vaccine	Week 0	Week 1	Week 2 Week 5 Week 8		Week 9	Week 11 Week 14 Week 17		Week 18	Week 20		Q 3 Wks	4 wks post final treatment	Q 12 Wks[c]
Day		Within 28 days of Week 0 Day1	D1	D1	D1 [a]	D8	D1 [a]	D1 [a]	D8	D1 [a]	D1 [a]	D8	D1	D1	
Visit Window (days)					±3	±1	±7	±3	±1	±7	±3	±1	±3	±7	±7
Informed consent	X[r]	X[r]													
Inclusion/ Exclusion	X	X													
Medical history, height[d]	X	X													
Virology screen[e]	X	X													
Coagulation panel[n]		X					X			X					
CT or MRI[o]		X					X			X			Q 9 wks ±7 days[o]	X	
Genome sequencing (blood)	X														
Tumor biopsy	X[f]						X[f,l]							X[f,l]	
Physical exam[g]	X	X	X	X	X			X			X		X	X	
ECOG status	X	X	X	X	X			X			X		X	X	
Vital signs[h]	X	X	X	X	X			X			X		X	X	
Pregnancy test[i]		X													
Clinical labs[j]	X[j]	X[j]	X[j]	X[j]	X[j]			X[j]			X[j]		X[j]	X[j]	
TSH		X[p]													

Con. Meds		X	X	X	X	X	X	X	X	X	X	X	X	X	
AEs			X	X	X	X	X	X	X	X	X	X	X	X	
Blood for Correlative Studies[k]	X[k]	X[k]			X[k]								X[k]		
Vaccine administration			X	X	X			X			X		X[m]		
Status, cancer treatment															X

FOOTNOTES

a. At visits where dosing is indicated, all assessments should be completed prior to dosing unless stated otherwise. If any doses are delayed, adjust subsequent dosing schedule accordingly. The Week 5, Day 1 visit must be ≥ 21 days post the Week 2, Day 1 visit.
b. EOT Visit will occur within 4 weeks after the last dose of study drug or prior to commencing the new therapy. If the EOT visit occurs earlier than 4 weeks, a safety follow-up telephone call on Day 28 (-3 days) is required; document contact in the study records.
c. Follow-up begins 12 weeks after final vaccine dose and every 12 weeks thereafter.
d. Medical history, medication history, and demographics will be recorded, including cancer-related treatments and procedures.
e. Virology screen only if clinically indicated and includes HIV antibody, hepatitis B surface antigen, and hepatitis C antibody. Subjects who are hepatitis C antibody positive with confirmed negative viral load are eligible.
f. A core needle biopsy may be obtained at any accessible tumor site (primary or metastatic); CT scan-may be needed to assist during the biopsy procedure.
g. Complete physical examinations conducted at screening and EOT; symptom-directed physical examinations conducted at all other indicated visits (up to 3 days prior to dosing).
h. Vital signs include blood pressure, pulse, respiratory rate, temperature, pulse oximetry, and weight.
i. Urine or serum pregnancy tests administered to WOCBP only. In case of delayed menstrual period (> 1 month) confirm absence of pregnancy prior to dosing. Pregnancy test for screening must be completed within 28 days prior to dosing.
j. Clinical hematology includes complete blood count with differential ANC and platelet count; serum chemistry includes sodium, potassium, chloride, bicarbonate, glucose, blood urea nitrogen, creatinine, lactate dehydrogenase, ALT, AST, alkaline phosphatase, bilirubin (total, direct, indirect), total protein, albumin, calcium, magnesium, uric acid, and phosphate. Urinalysis includes blood, glucose, ketones, leukocytes, nitrite, pH, protein, and specific gravity; perform using test strip (dipstick) unless full urinalysis with microbiology is clinically indicated. For Week 0 Day 1, pre-vaccine screening labs may be used up to 28 days prior to dosing. For subsequent treatment days on which laboratory assessments are required, labs must be performed up to 3 days prior to dosing.
k. Arm D patients may have Blood Collection for Correlative Studies samples collected at the following time points: 1) Screening, eligibility 2) Screening, Pre-Vaccine (within 28 days prior to Week 0 Day 1); 3) Week 8 (within 7 days after vaccine administration); 4) Week 32 (or within 12 weeks of last vaccine dose (+/- 3 days). If additional vaccine doses are administered, additional samples may be collected per investigator, a sample should collected within 12 weeks of last vaccine dose (+/-3 days). For neoantigen identification, an additional 75-100 mL whole blood (eight to ten 10mL heparin tubes) may be collected every 4 weeks.
l. A tumor biopsy within Weeks 9-12 and at disease progression is not required, but strongly encouraged.
m. Vaccine administered on Week 20. Vaccine may continue to be administered at 3 week intervals for an additional 18 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit.
n. Coagulation panel includes PT, INR, and APTT.
o. CT scans will be performed every 9 weeks (+/- 7 days) for the first 6 months of therapy and then can be performed as clinically indicated
p. TSH performed as clinically indicated,

q. Alternative methods for informed consent and safety assessments (e.g., phone contact, virtual visit, alternative location for assessment, including local labs or imaging centers) may be implemented when necessary (e.g. COVID-19 pandemic) and feasible.
r. Subjects may sign an initial consent allowing the vaccine to be manufactured. Subsequently, the subject would sign a second consent for treatment when they and the IP are ready to proceed.
s. Survival contacts via medical record review, telephone call, or review of the Social Security Index.

ABBREVIATIONS FOR TABLE

ALT=alanine aminotransferase; ANC = absolute neutrophil count; APTT = activated partial thromboplastin time; AST=aspartate aminotransferase; CT = computed tomography; ECOG = Eastern Cooperative Oncology Group; EOT = End of Treatment; HIV = human immunodeficiency virus; INR = international normalized ratio of prothrombin time MRI = magnetic resonance imaging; PBMC = peripheral blood mononuclear cells; PT = prothrombin time WOCBP = woman of childbearing potential.

1.0 BACKGROUND AND RATIONALE

1.1 Immunotherapy in Advanced Cancer Patients

Incurable advanced cancers will eventually develop resistance to standard systemic therapies. Recent data have demonstrated that immunotherapy can be an effective modality in several malignancies with regulatory approvals for checkpoint blocking agents in many settings. Moreover, the responses observed to these agents in many patients can be deep and durable emphasizing the role for immunotherapy in cancer. It is now appreciated that the critical determinant of a response to checkpoint blockade and other immunotherapies is the formation of a T cell response to cancer neoantigens. In fact, an expansion of specific T cell clones is the hallmark of response to checkpoint blockade.

Cancer neoantigens can encompass a variety of different molecules but among the most attractive candidates for therapy are ones derived from genomic alterations. Mutation derived neoantigens are recognized by the immune system as non-self and can induce powerful T cell responses. In theory, a neoantigen cancer vaccine should induce both CD4 and CD8 responses and subsequently produce tumor responses. However, several challenges exist in producing an effective neoantigen vaccine for cancer patients including determining which neoantigens are most relevant to the immune system, finding an effective vaccine platform that induces the appropriate immune response, and incorporating the neoantigen sequence into the chosen platform.

1.2 Summary of Clinical Studies with personalized vaccine-based Immunotherapy

Recent studies have shown that neoantigen vaccine approaches are able to induce robust antitumor responses in mice [1-3]. These findings led to phase 1 studies in melanoma patients using different vaccine platforms and neoantigen candidates derived from *in silico* prediction models [4-6]. The first report employed neoantigen-pulsed dendritic cells and demonstrated neoantigen-specific T-cell responses in three patients. However, clinical responses were not reported [6].

Two additional clinical trials have administered neoantigen vaccines in melanoma patients both relying on similar strategies to identify neoantigens based on computational algorithms to predict neoantigen to binding MHC class I molecules and prioritize candidates. Both studies vaccinated patients without evidence of active disease so clinical responses to the vaccine was not available. Ott et al. [4] vaccinated six patients with synthetic long peptides (SLP; up to 20 total peptides in 4 pools for each patient) after surgical resection. Sahin et al. [5] used synthetic RNA vaccines, each encoding five 27-mer neoantigens and up to 10 mutations targeted in each patient's tumor (two RNA vaccines). Importantly, the studies to date have demonstrated only mild treatment-related adverse events consisting of mild flu-like symptoms, injection site reactions, rash, and fatigue – all grade 1.

Immune monitoring analyses of patients' PBMCs (IFN-γ ELISPOT, intracellular cytokine staining, multimer staining) in both studies [4, 5] revealed that SLP and RNA vaccines can (i) enhance pre-existing but weak neoantigen-specific T-cell responses and (ii) generate *de novo* neoantigen-specific T-cell responses. As noted above, the majority of the *ex vivo* IFN-γ responses were generated by CD4+ T cells. Both studies also found that vaccination resulted in an expansion of the neoantigen-specific T-cell repertoire. Taken together, these studies provide strong rationale for further clinical development and testing of neoantigen vaccines.

1.3 Rationale for Personalized Immunotherapy

For many patients with advanced solid tumors, no standard therapy is recognized or patients have failed or are intolerant to standard-of-care (SOC) treatment. For these patients, there is significant unmet medical need in these life-threatening indications. Nonclinical and clinical evidence suggests vaccine-based immunotherapy has the potential to induce potent T cell immune responses and clinical benefit in multiple advanced tumor types.

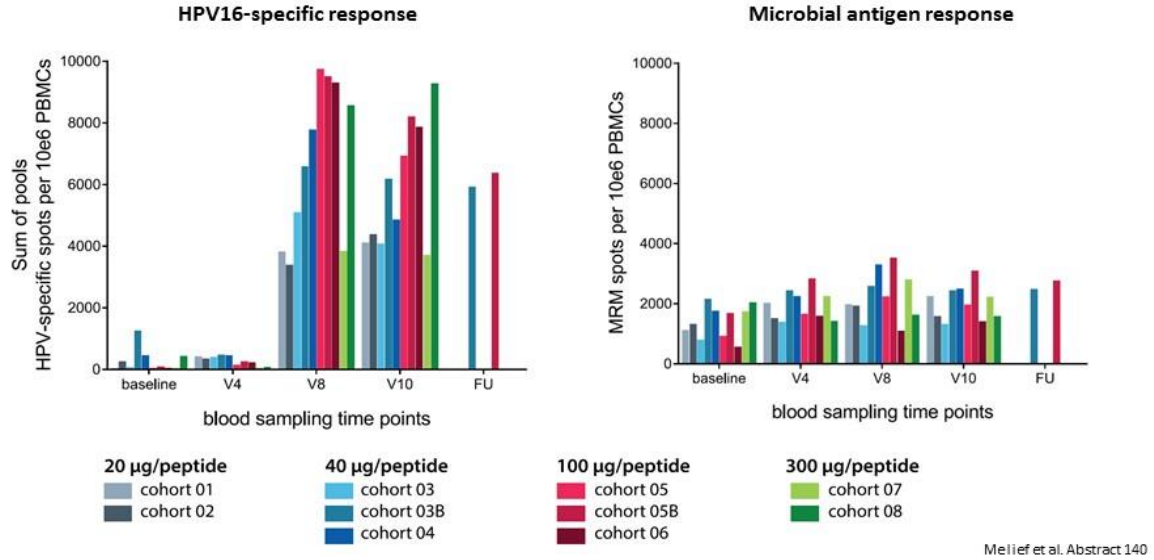
Advances in genome sequencing technology have enabled identification of a new class of tumor-specific antigens derived from mutated proteins that are present only in the tumor (i.e. neoantigens) [7, 8]. Mounting evidence highlights the critical role of these patient-specific neoantigens for the induction of highly specific antitumor immunity. Tumor-infiltrating lymphocyte populations in patients benefiting from immunotherapy represent dominant neoantigen-specific populations sufficient to induce tumor regression in mice and humans [9-12]. In addition to widespread detection of neoantigen-specific T cells in the tumor, biochemical tools have been used to demonstrate that processing and presentation of multiple neoantigens occurs at the tumor site [13, 14]. Moreover, checkpoint blockade and dendritic cell immunotherapies have revealed new and amplified neoantigen-specific responses to be central to disease control [6, 15].

The principal objective for inducing immunity to tumor-specific neoantigens is to generate a systemic population of potent T lymphocytes that traffic to advanced, metastatic disease sites resulting in a T cell inflamed tumor phenotype, reduction of disease burden, and clinical benefit. In both nonclinical and clinical settings, priming of neoantigen-specific $CD8^+$ T cells has been shown to result in significant tumor regression, providing proof of concept for this approach. The strength of neoantigen-based immunotherapy is therefore dependent on stimulation of a potent neoantigen-specific immune response.

1.4 Dose Selection Rationale

Studies involving patients with cervical cancer administering a vaccine of synthetic long peptides covering E6 and E7 of HPV16, ISA101, have demonstrated that an optimal T-cell immune response is reached at 100 ug per peptide and that increasing to 300 ug does not further improve immunogenicity. However, 300 ug/peptide does increase local side effects at the injection site which can be dose limiting. The CervISA trial administered increasing doses of vaccine in combination with chemotherapy to patients with recurrent or metastatic cervical cancer. It was clear from measuring HPV-specific peripheral T cell responses that maximal immune responses were generated at 100 ug/peptide while HPV-specific T cell responses were

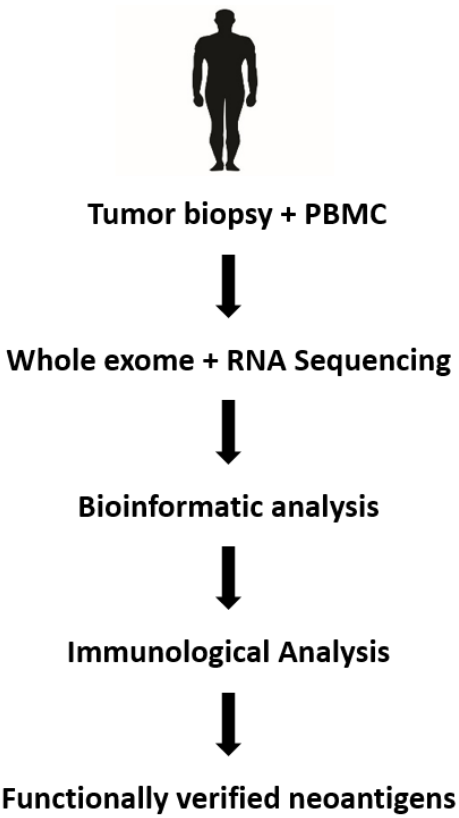
associated with significantly greater overall survival [ASCO-SITC 2017, San Francisco, Abstract 140, Melief et al].



1.5 Selection and Validation of Neoantigens

Neoantigens (NeoAg) are those that are entirely absent from the germline genome. As applied to tumors, NeoAg comprise the set of expressed somatic mutations by which a tumor can be distinguished from the normal self by host $CD4^+$ and $CD8^+$ T cells [10]. As such, tumor NeoAg constitute unique targets of opportunity for directing a patient's immune responses exclusively to the tumor, and thereby sparing normal tissues from the 'collateral damage' normally attendant to most systemic cancer therapies [16]. Accordingly, significant efforts have gone towards the identification of tumor NeoAg in individual patients for a range of therapeutic purposes, including personalized vaccines or adoptive immunotherapy, as for purposes of diagnosis and immune monitoring [17]. The screening strategy to identify immunogenic neo-epitopes in this application (Figure 1) is based on over a decade of experience in the Peters lab of designing and carrying out human T cell epitope mapping

studies in a number of systems, including infectious diseases [18-20], diabetes [21, 22], and allergies [23, 24]. Peptide candidate selection in these studies commonly relied on bioinformatic prediction tools for MHC binding, antigen processing and presentation, and TCR recognition which the Peters lab has a long-standing interest in [19, 25] as part of leading the bioinformatic component of the Immune Epitope Database and Analysis Resource [26, 27]. The approach used in current proposal, and the commitment to the functional verification of NeoAg in cancer patients at the level of a T cell response has emerged from a long-standing collaborative effort between the Schoenberger and Peters labs [28, 29] that has been recently been informed by findings involving the Schoenberger lab that expression data that accompanies genomic sequencing can be used to guide discovery of MHC class II-restricted NeoAg [2].



Neoantigen discovery and workflow

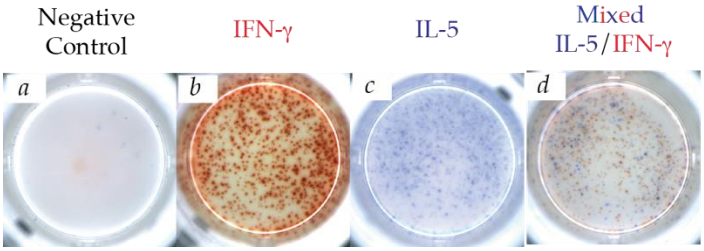
Biospecimens, briefly a small amount (approximately 2 mg – 50 mg) of fresh tumor tissue is obtained via fine-needle aspirate, core biopsy, surgical resection, or as needed, from FFPE material, along with 1 ml PBMC to provide DNA for a patient-specific reference genome.

Coincident to tissue collection or at a later date 75-100 ml whole blood is obtained, yielding 3-5x10^6 PBMC that can be cryopreserved for use in functional testing of T cell response.

Sequencing: Paired tumor/control genomic DNA and tumor RNA is isolated and next-generation sequencing is performed on the Illumina platform. Specifically, whole-exome sequencing (WES) is performed to a depth of at least 200x coverage on both tumor and reference (PBMC) exome and RNA seq on tumor RNA, obtaining at least 30 million reads per sample.

Bioinformatic analysis of the combined exome and RBA sequence is performed using empirically developed algorithm. Selection is primarily based on the confidence that a mutation in DNA is specific to the tumor but not healthy tissue in the patient, and the degree of expression of the mutation in the tumor at the RNA level.

Immunological analysis. Synthesis peptides are used to restimulate patient PBMC and TIL missense mutations selected for testing are represented as pairs of 20-mer peptides with the altered residue at position +6 or +15 in the linear sequence. NeoORFs created by indel (frameshift) mutations are represented as overlapping 15-mer peptides with an increment of 5, to cover all possible peptides and binding frames. Patient PBMC are stimulated with pools of peptides representing 5-10 mutations in 24-well plates for a 14-day expansion culture with autologous patient PBMC. Following the expansion, viable cells are collected and tested for functional reactivity against individual peptides comparing the stimulating pool using 2-color ELISPOT assay that simultaneously detects IFN-gamma and IL-5. Expansion cultures shown to contain significant T cell responses by ELISPOT against specific mutant peptides are immediately retested 1 day later by cytokine-capture assay to allow phenotyping of the responding cells as CD4+ or CD8+ and to allow fluorescence-activated cell sorting of a representative population of 100-500 cytokine-positive cells per neoantigen for T cell receptor analysis by single-cell transcriptomics and bioinformatics analysis [30, 31]. Our studies to-date have indicated that the endogenous response to a given somatic mutation is dominated by 1-5 clonal TCRs. We therefore expect that sampling 100 cells per antigen on the basis of activation-induced cytokine production will allow us to identify the clonotype of the responding cells with a high degree of confidence. The output of these studies will a) identify which somatic mutations are recognized by a patient's T cells, b) the nature of the responding T cell subset (CD4+ or CD8+) and the cytokine polarity (IFN-γ versus IL-5), and c) the TCR clonotypes expressed in the responding cells. This information will be utilized to select which mutated peptides to use in each patient's vaccine formulation, as well as in the post-vaccination immune monitoring.



Two-color ELISPOT assay. 4 representative wells from the ELISPOT used to simultaneously detect T cell secreting IFN-γ, IL-5, or both, are depicted to demonstrate, from left to right, a) Negative Control (irrelevant peptide pool), b) dominant IFN-γ response, c) dominant IL-5 response, and d) mixed IFN-γ/IL-5 response. Double IFN-γ/IL-5 producing cells appear as purple.

1.6 Rationale for Addition of anti-PD1 Antibody

Recently, drug discovery efforts to target the tumor microenvironment and reactivate the

antitumor immunity have been demonstrating great potential. One of the most promising approaches has been to inhibit immune checkpoints that block anti-tumor immune responses. These checkpoint inhibitors can act by blocking interactions between ligands and receptors on tumor cells and T cells, such as PD-L1 and PD-1, respectively, and lead to enhanced anti-tumor immunity. Five anti-PD-1/PD-L1 antibodies have been approved by the Food and Drug Administration (FDA), with others currently in clinical trials. We believe that anti-PD1 therapy will augment vaccine stimulation of CD8+ T cells against recognized neoantigens. Hence, we will accrue this study in four cohorts: A) vaccine administered concurrently with anti-PD1 and B) anti-PD1 antibody alone followed by vaccine and C) vaccine administered concurrently with anti-PD1 and D) vaccine administered alone. In all arms the strength of the T cell response to predicted and validated neo-epitopes will be measured. This design will allow evaluation of the T cell response to anti-PD1 alone versus anti-PD1 with vaccine. In addition, we will measure any change in T cell response in Arm B after addition of the vaccine. We hypothesize that the combination of anti-PD1 and vaccine will demonstrate the greatest T cell response against the desired neo-epitopes.

1.7 Study Rationale

This phase 1b study will evaluate the immune response of patients with advanced cancers to personalized vaccine with an anti-PD1 antibody in sequential cohorts. The primary endpoint of the study will be formation of neoantigen specific T cell responses after 3 doses of personalized vaccine administered every 3 weeks. The primary hypothesis of this study is that administration of the vaccine, based on expressed and functionally validated neoantigens, will result in a quantifiable increase in T cell specific responses. In addition, we hypothesize that the addition of anti-PD1 antibody, either prior to 3 doses of or concurrent with personalized vaccine, will be feasible and safe. Furthermore, we hypothesize that the concurrent administration of anti-PD1 antibody with vaccine therapy will result in a greater neoantigen specific T cell response. Ultimately, the demonstration of a neoantigen specific T cell response will provide the rationale to conduct larger studies of this approach in patients with advanced cancers.

Cohorts A and B will enroll 5 subjects to receive either: Arm A) personalized vaccine and anti-PD-1 administered concurrently at the start of study therapy or Arm B) anti-PD-1 antibody for 6 weeks followed by personalized vaccine therapy. Arm C will enroll 10 subjects to receive personalized vaccine and anti- PD-1 administered concurrently in a boosted schedule at the start of study therapy. An additional 5 subjects may be enrolled on to Arm C. Arm D will enroll 10 subjects to receive personalized vaccine alone, in a boosted schedule at the start of study therapy.

Eligible subjects for this study will include all patients with advanced solid tumors who meet the eligibility criteria. Patients with hematologic malignancies will not be included in the current study due to the relatively low antigen load in many of these cancers, likelihood of bone marrow toxicity from prior therapy, and potential difficulty in extracting tumor infiltrating lymphocytes. Patients who have been previously treated with an anti-PD1 or anti-PDL1 antibody will be eligible to participate if they did not experience severe toxicity to prior immunotherapy and any toxicity has resolved.

2.0 STUDY OBJECTIVES

The purpose of this study is to measure neoantigen specific T cell responses to vaccination with a neo-epitope vaccine with anti-PD1 antibody in patients with advanced cancers.

2.1 Primary Objectives

1. To determine the increase in neoantigen specific T cell response to vaccine with an anti-PD1 antibody compared to anti-PD1 antibody alone using a TCR clonotype assay.
2. To determine the safety and feasibility of generating a personalized, tumor neoantigen-specific therapeutic vaccine and the safety of combining it with checkpoint blockade immunotherapy.

Primary Safety Endpoint
Adverse events, reported overall and by grade, body system, attribution, and dose received.

Primary Pharmacodynamic Endpoint:
Quantitative frequency of neoantigen specific TCR clonotypes – see Section 5.5.2, Assay 3.

2.2 Secondary Objectives (defined in Section 9.0)

1. To describe the efficacy of vaccine with anti-PD1 antibody in patients with advanced cancers that includes
    a. Objective response rate using RECIST 1.1
    b. Time to response
    c. Duration of response
    d. Progression free survival
    e. Overall Survival
2. To determine the safety and feasibility of administering vaccine with anti-PD1 antibody in patients with advanced cancers

2.3 Exploratory Objectives

1. To characterize immune infiltrates in the tumor microenvironment including lymphoid and myeloid cell subsets, immune checkpoint expression, and other immunomodulatory molecules
2. Evaluate genomic changes in the tumor in response to personalized vaccine
3. Evaluate circulating cellular, protein, and genomic changes in response to personalized vaccine

3.0 PATIENT ELIGIBILITY

3.1 Initial Screening Inclusion Criteria

Subjects must meet all of the following inclusion criteria at initial screening eligibility review to participate in this study.

1. Patient has the ability to understand and the willingness to sign a written informed consent.
2. Histologically or cytologically documented incurable solid tumor including lymphoma.
3. Patients can be enrolled in two categories:

a. Measurable disease as defined by RECIST 1.1 that has progressed on or be intolerant to therapies that are known to provide clinical benefit Lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions. These patients must have at least one tumor site accessible for biopsy. Tumor lesions used for biopsy should not be lesions used as RECIST target lesions, unless there are no other lesions suitable for biopsy. If a RECIST target lesion is used for biopsy the lesion must be > 2 cm in its longest diameter (Arms A, B, and C).

b. Non-measurable disease by RECIST 1.1 and high-risk (> 50% over 5 years) of mortality (Arm D).

4. Patient is ≥ 18 years of age.
5. ECOG Performance Status ≤ 1.
6. Patient has adequate organ function as defined below:

- Absolute Neutrophil Count ≥ 1.5 x 10^9/L
- Platelet count ≥ 90 x 10^9/L
- Hemoglobin ≥ 9.0 g/dL
- AST/SGOT and ALT/SPGT ≤ 2.5 X institutional upper limit of normal
- Total Bilirubin ≤ 1.5 x ULN or ≤3× ULN if due to Gilbert's disease
- Serum creatinine ≤ 2 x institution's ULN

3.2 Initial Screening Exclusion Criteria

Subjects meeting any of the exclusion criteria at baseline will be excluded from study participation at the initial screening eligibility review.

1. Patient has severe hypersensitivity (≥Grade 3) to pembrolizumab and/or any of its excipients.
2. Known or suspected allergy or hypersensitivity to any component of vaccine.
3. Has a known history of Human Immunodeficiency Virus (HIV).
4. Has a known history of Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] reactive) or known active Hepatitis C virus (defined as HCV RNA [qualitative] is detected) infection. Note: no testing for Hepatitis B and Hepatitis C is required unless mandated by local health authority.

(Individuals who are hepatitis C antibody positive may be enrolled if negative viral load confirmed).

5. History of autoimmune disease including: inflammatory bowel disease (including ulcerative colitis and Crohn's Disease), rheumatoid arthritis, systemic progressive sclerosis (scleroderma), systemic lupus erythematosus, autoimmune vasculitis (e.g. Wegener's granulomatosis); central nervous system or motor neuropathy considered of autoimmune origin (e.g. Guillain-Barré syndrome, myasthenia gravis, multiple sclerosis). Individuals with vitiligo, Sjogren's Syndrome, interstitial cystitis, Graves' or Hashimoto's Disease, celiac disease, DM1, hypothyroidism stable on hormone replacement, or any autoimmune disease without symptoms and not requiring active therapy for at least 2 years will be allowed with Study Medical Monitor's approval.

6. Has a known history of active TB (Bacillus Tuberculosis).

7. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

8. History of receiving a solid organ transplant or allogeneic bone marrow transplant.

9. Unable or unwilling to withhold or discontinue any prohibited or restricted medications/procedures for the specified windows during the study.

3.3 Pre-Treatment Inclusion Criteria

Subjects must meet all of the inclusion criteria to continue participation in this study.

1. Patient has the ability to understand and the willingness to sign a written informed consent.

2. Histologically or cytologically documented incurable solid tumor including lymphoma.

3. Patients can be enrolled in two categories:

   a. Measurable disease as defined by RECIST 1.1 that has progressed on or be intolerant to therapies that are known to provide clinical benefit Lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions. These patients must have at least one tumor site accessible for biopsy. Tumor lesions used for biopsy should not be lesions used as RECIST target lesions, unless there are no other lesions suitable for biopsy. If a RECIST target lesion is used for biopsy the lesion must be $\geq$ 2 cm in its longest diameter (Arms A, B, and C).

   b. Non-measurable disease by RECIST 1.1 and high-risk (> 50% over 5 years) of mortality (Arm D).

4. Patient is ≥ 18 years of age.

5. ECOG Performance Status ≤ 1.

6. Patient has adequate organ function as defined below:

• Absolute Neutrophil Count	≥ 1.5 x 10^9/L
• Platelet count	≥ 90 x 10^9/L
• PT and PTT	< 1.5 × ULN
• Hemoglobin	≥ 9.0 g/dL
• AST/SGOT and ALT/SPGT	≤ 2.5 X institutional upper limit of normal
• Total Bilirubin	≤ 1.5 x ULN or ≤3× ULN if due to Gilbert's disease
• Serum creatinine	≤ 2 x institution's ULN

7. Women of child-bearing potential and men with partners of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) as detailed in Appendix C prior to study entry, for the duration of study participation, and for 180 days following completion of therapy. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.
    - A woman of child-bearing potential is any female (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:
        - Has not undergone a hysterectomy or bilateral oophorectomy; or
        - Has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months)

8. Women of child-bearing potential has negative pregnancy test prior to initiating vaccine dosing (See Table 1 for pregnancy testing requirements).

3.4 Exclusion Criteria

Subjects meeting any of the exclusion criteria at baseline will be excluded from study participation.

1. Patient is currently receiving or has received another systemic anti-cancer therapy within 4 weeks prior to first dose of study treatment.
    - Note: Participants must have recovered from all AEs due to previous therapies to ≤Grade 1 or baseline. Participants with ≤Grade 2 neuropathy may be eligible.

    - Note: If participant received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting study treatment.

- Note: Participants who have received prior radiotherapy must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis prior to starting study treatment.

- Note: Participants may continue maintenance anti-cancer therapy (e.g. hormone ablation or anti-HER) if clinically indicated.

2. Patient is currently receiving or has received PD1/PDL1 inhibitor immunotherapy within 4 weeks prior to first dose of study treatment.
3. Patient is currently receiving or has received anti-PD1 or anti-CTLA4 treatment during the vaccine preparation period.
4. Has received a live vaccine within 30 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.
5. Has severe hypersensitivity (≥Grade 3) to pembrolizumab and/or any of its excipients.
6. Patient has not recovered (to CTCAE ≤ Grade 1) from all clinically significant toxicities related to prior therapy.
7. Receiving TNF pathway inhibitors, PI3 kinase inhibitors, systemic steroid therapy or any other form of immunosuppressive therapy within 14 days prior to the first dose of study medication.
8. Received an investigational agent within 28 days prior to the first dose of study drug.
9. Untreated brain metastases; individuals with treated and stable metastases are eligible. Eligible subjects should have recovered from the acute effects of radiation therapy or surgery prior to study entry, have discontinued corticosteroid treatment for brain metastases for at least 4 weeks and are neurologically stable for 8 weeks (confirmed by MRI) prior to administration of experimental therapy
10. Known or suspected allergy or hypersensitivity to any component of vaccine.
11. Has a known history of Human Immunodeficiency Virus (HIV).
12. Has a known history of Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] reactive) or known active Hepatitis C virus (defined as HCV RNA [qualitative] is detected) infection. Note: no testing for Hepatitis B and Hepatitis C is required unless mandated by local health authority. (Individuals who are hepatitis C antibody positive may be enrolled if negative viral load confirmed).
13. History of autoimmune disease including: inflammatory bowel disease (including ulcerative colitis and Crohn's Disease), rheumatoid arthritis, systemic progressive sclerosis (scleroderma), systemic lupus erythematosus, autoimmune vasculitis (e.g. Wegener's granulomatosis); central nervous system or motor neuropathy considered of autoimmune origin (e.g. Guillain-Barré syndrome, myasthenia gravis, multiple sclerosis). Individuals with vitiligo, Sjogren's Syndrome, interstitial cystitis, Graves' or Hashimoto's Disease, celiac disease, DM1, hypothyroidism stable on hormone replacement, or any autoimmune disease without symptoms **and** not requiring active therapy for at least 2 years will be allowed with Study Medical Monitor's approval.

14. Has a history of (non-infectious) pneumonitis that required steroids or has current pneumonitis.
15. Has an active infection requiring systemic therapy.
16. Has a known history of active TB (Bacillus Tuberculosis).
17. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
18. History of receiving a solid organ transplant or allogeneic bone marrow transplant.
19. Major surgical procedure within 28 days prior to the first dose of study drug.
20. Unable or unwilling to withhold or discontinue any prohibited or restricted medications/procedures for the specified windows during the study.
21. If female, pregnant or breastfeeding.

4.0 TREATMENT PLAN

4.1 Study Design

This is an open-label Phase 1b, sequential cohort, clinical study in adults with incurable solid tumors that will enroll subject in four arms.

Arm A) Subjects on this arm will receive personalized vaccine and anti-PD-1 administered concurrently at the start of study therapy. In Arm A, the first vaccine dose will be initiated with the anti-PD1 antibody. Vaccine will be administered starting on week 0, day 1 Q3W for 3 doses (9 weeks) at a dose 100 µg per peptide. Pembrolizumab will be administered starting on week 0, day 1 Q3W at a dose of 200 mg IV. Subjects may receive additional doses administered at 3 week intervals for an additional 12 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit. See Table 1a for further details. **(CLOSED TO ENROLLMENT)**

Arm B) Subjects on this arm will receive anti-PD-1 antibody alone for 6 weeks followed by the addition of personalized vaccine therapy. In Arm B, anti-PD1 antibody will be initiated for 6 weeks prior to administration of vaccine. Pembrolizumab will be administered starting on week 0, day 1 Q3W at a dose of 200 mg IV. Vaccine will be administered starting on week 6, day 1 Q3W for 3 doses (9 weeks) at a dose 100 µg per peptide. Subjects may receive additional doses administered at 3 week intervals for an additional 12 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit. See Table 1b for further details**. (CLOSED TO ENROLLMENT)**

Arm C) Subjects on this arm will receive personalized vaccine therapy (PSLP) and anti-PD1 administered concurrently. In Arm C, a prime-boost vaccine schedule will be used with subjects receiving three weekly (Q7day) priming vaccine doses of PSLP followed by 6 boost vaccine doses of PSLP administered every 3 weeks. Vaccine will be administered starting on week 0, at a dose 100 µg per peptide. Subjects will receive 9 doses including priming. Subjects may receive additional doses administered at 3 week intervals for an additional 18 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit. Pembrolizumab (anti-PD1) will be

initiated one week prior to the first priming dose of PSLP (week -1) and continue Q3W at a dose of 200 mg IV. See Table 1c for further details.

Arm D) Subjects on this arm will receive personalized vaccine therapy (PSLP). In Arm D, a prime-boost vaccine schedule will be used with subjects receiving three weekly (Q7day) priming vaccine doses of PSLP followed by 6 boost vaccine doses of PSLP administered every 3 weeks. Vaccine will be administered starting on week 0, at a dose 100 µg per peptide. Subjects will receive 9 doses including priming. Subjects may receive additional doses administered at 3 week intervals for an additional 18 doses if there is no evidence of disease progression. Treatment for up to 9 doses may continue past progression if the treating physician feels there is continued benefit. See Table 1d for further details.

In arm A, B, and C pembrolizumab will be administered for a maximum of 35 cycles (approximately 2 years) if there is no evidence of disease progression.

Toxicities related to the personalized vaccine immunotherapy are expected to be consistent with prior clinical and non-clinical experience, based on the platform and unrelated to the expressed antigens. However, for additional safety monitoring, subjects on each arm will be staggered to enroll a maximum of two subjects at a time and allow at least one week between subject pairs, i.e. enrollment of subject 1 and 2, followed by a one week interval, followed by enrollment of subjects 3 and 4, followed by a one week interval, etc.

Potential subjects will initially be screened for eligibility. If eligible, subjects will provide a blood sample and undergo tumor biopsy. The tumor specimen will be analyzed by next-generation sequencing, and RNAseq to determine expressed neoantigens. *Ex-vivo* T cell reactivity will be determined against prioritized neoantigens in a functional assay. Personalized vaccines will be constructed that stably express multiple prioritized candidate neoantigen-derived epitopes. The personalized vaccine development and manufacture process is targeted to be optimized to 10 weeks or less duration. During the development period, subjects may initiate or continue other anti-cancer therapies as directed by their treating physician.

Subjects will return to the clinic within 28 days prior to the first dose to confirm eligibility and undergo pre-treatment screening procedures. Subjects will begin the Treatment Period when personalized vaccine is available; vaccine will be administered at a dose 100 µg per peptide.

4.2 Definition of Dose Limiting Toxicities (DLTs)

A DLT is defined as the following treatment-related adverse events or laboratory abnormalities, graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0:

- ≥ Grade 3 hematologic adverse events attributable to either agent.
- ≥ Grade 3 non-hematologic adverse events attributable to either agent.
- ≥ Grade 2 allergic reaction attributable to Personalized Synthetic Long Peptide (PSLP)

DLT exceptions

The following Grade 3 immune adverse events experienced may be acceptable as DLT exceptions depending on the duration of the events and treatment response.

- Grade 3 hypothyroidism and hypophysitis
- Grade 3 toxicities such as transient arthralgia or myalgia of 72 hours or less, transient (≤72h) flu-like syndrome
- Grade 3 fever of up to 48h duration
- Grade 3 injection site reaction that resolves to Grade 2 (or lower) level within 72 hours.

Please refer to pembrolizumab-related toxicities outlined on table 4 of the protocol for additional information

In order to be evaluable for DLT assessment, subjects must receive all injections within 1 dose of PSLP (a complete dose of PSLP may contain up to 3 injections) or at least 1 dose of pembrolizumab (pembrolizumab is only administered on Arms A, B, and C). Subjects who experience a DLT within the first 3 weeks of treatment and drop out of the study will be considered evaluable for DLT and will not be replaced. Subjects who drop out of the study for reasons other than DLT will be considered not evaluable and will be replaced.

If a subject experiences a DLT attributable to the investigational product, no additional doses of the investigational product will be given.

If ≥ 6 DLTs are observed, further accrual will be held pending safety analysis of the events, and the study will be restarted only with Principal Investigator approval. DLTs will be evaluated by a Safety Review Team that includes the Investigators who enrolled subjects in the study, the Principal Investigator, and the UCSD DSMC.

If ≥ 2 serious adverse events (e.g. death or grade 4 toxicities) related to study treatment are observed, further accrual will be held pending safety analysis of the events, and the study will be restarted only with Principal Investigator approval.

Safety and tolerability will be assessed by monitoring for AEs. Tumor response assessments will be performed by radiologic evaluation (CT or magnetic resonance imaging [MRI]) at baseline and every 9 weeks thereafter through the EOT visit. A series of blood samples (serum, plasma, and peripheral blood mononuclear cells [PBMC]) and an additional tumor biopsy (if clinically feasible) will be obtained during the study to characterize the immune response to the vaccine and other prognostic tumor markers.

All subjects will complete an End of Treatment (EOT) visit no more than four weeks following the final dose of study medication or prior to receipt of other cancer-related treatment.

4.3 Pembrolizumab

Pembrolizumab (trade name Keytruda®) is a potent and highly selective humanized monoclonal antibody (mAb) of the immunoglobin G4 (IgG4) / kappa isotype designed to directly block the interaction between Programmed Cell Death 1 (PD-1) receptor and its ligands, Programmed Death Ligand 1 (PD-L1) and Programmed Death Ligand 2 (PD-L2). This blockade enhances functional activity of the target lymphocytes to facilitate tumor regression and ultimately immune rejection. Details of preclinical and clinical studies are provided in the Investigator's Brochure. Clinical studies with pembrolizumab have demonstrated efficacy in participants with advanced melanoma, non-small cell lung cancer, head and neck cancer (HNC), bladder cancer, Hodgkin's lymphoma, and microsatellite instability (MSI) high cancers. Pembrolizumab is currently in clinical development for a number of advanced malignancies. For more detail on specific indications, please refer to the Pembrolizumab US Package Insert.

4.4 Treatment Dosage and Administration

The investigational product (study treatment) administered in this study is vaccine. The PSLP vaccine will be constructed for each subject that stably express multiple candidate tumor-derived neoantigens. Thirty peptide sequences will be the maximum per site of injection. Additional medications will be administered prior to, and following each vaccine dose as needed to manage injection -related reactions.

Arm A and Arm B: A dose of 100 µg per peptide PSLP vaccine will be administered by subcutaneous injection in any limb (left upper arm, right upper arm, left upper leg/thigh, or right upper leg/thigh) every 3 weeks. The appropriate volume of product to achieve the target dose is drawn with a syringe for subcutaneous injection. Additional details for storage and preparation of vaccine are provided in the study Pharmacy Manual.

Arm C and Arm D: A dose of 100 µg per peptide PSLP vaccine will be administered by intramuscular injection (left upper arm, right upper arm, left upper leg/thigh, or right upper leg/thigh) using a prime-boost vaccination schedule (see scheme). The appropriate volume of product to achieve the target dose is drawn with a syringe for intramuscular injection. Additional details for storage and preparation of vaccine are provided in the study Pharmacy Manual.

Arm A, B, and C: Pembrolizumab is administered on an outpatient basis. 200 mg pembrolizumab will be administered as a 30 minute (-5 min/+10 min) intravenous (IV) infusion every 3 weeks. Specific instructions for the preparation infusion fluid and administration of infusion solution are provided in the Pharmacy Manual.

4.5 Dosing Eligibility and Delayed Dosing

Prior to the first dose and for each subsequent dose of both the vaccine and pembrolizumab, the subject must have adequate organ function as defined by the laboratory values in the Table 2; laboratory tests may be performed up to 28 days prior to treatment start; laboratory tests may be performed up to 3 days prior to dosing for subsequent visits.

If vaccine dosing is delayed due to dosing eligibility, pembrolizumab toxicity, manufacturing issues (e.g. sterility), medical / surgical events or logistical reasons not related to study therapy (e.g., COVID-19 pandemic, elective surgery, unrelated medical events, patient vacation, and/or holidays) the subsequent dosing schedule will be adjusted according to the delayed dose(s).

If possible, the imaging scans and tumor measurement/response assessment should remain according to the original schedule of every 9 weeks (+/-7 days) for the first 6 months throughout the Treatment and Treatment Continuation Periods. After 6 months of therapy, CT scans will be performed when clinically indicated.

Table 2. Dosing-Eligibility Requirements

During peptide monotherapy and combination therapy

Hematologic	Renal	Hepatic
ANC ≥ 1000/μL Platelets ≥ 90,000/μL Hemoglobin ≥ 8 g/dL PT and PTT < 1.5 × ULN (when drawn)	Creatinine ≤ 2 × ULN	AST/ALT ≤ 2.5 × ULN Bilirubin ≤ 1.5× ULN or ≤3× ULN if due to Gilbert's disease
ALT = alanine aminotransferase; ANC = absolute neutrophil count; AST = aspartate aminotransferase; PT = prothrombin time; ULN = upper limit of normal		

During pembrolizumab monotherapy

Hematologic
Platelets ≥ 90,000/μL Hemoglobin ≥ 8 g/dL

Please refer to package insert and institutional guidelines regarding non-hematologic dosing parameters for pembrolizumab monotherapy.

4.6 Concomitant therapy and procedures

Subjects may receive concomitant medications as required, unless specifically restricted or prohibited in this study. Subjects are anticipated to continue the use of prescribed medications identified during the screening procedures, consistent with study inclusion and exclusion criteria.

All treatments that the investigator considers necessary for a participant's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF. All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs

4.7 Prohibited concomitant therapy

A subject may be discontinued from study treatment for use of prohibited medications. Approval must be obtained from the Principal Investigator for a subject to continue dosing if a prohibited medication is administered within the specified timeframes. The following therapies are not permitted or are restricted following initiation of vaccine:

- Non-study chemotherapy or immunotherapy (approved or investigational)
- TNF pathway inhibitors or PI3 kinase inhibitors
- Any major surgery or surgical procedure; if required must be discussed with the Principal Investigator to determine if it is appropriate for the subject to continue study treatment
- Any other investigational product

In addition, the following medications are only restricted prior to starting treatment as indicated:

- Systemically active steroids for more than 3 days or use of any systemic steroids within 14 days before pembrolizumab or vaccine administration, with the exception of inhaled steroids. **NOTE: Steroids treating immune AEs are allowed. please refer to section 4.8 for additional information)**

In addition, the following medications are only restricted prior to and after vaccine administration as indicated:

- Filgrastim (granulocyte colony stimulating factor; G-CSF) or sargramostim (GM-CSF) within 14 days prior to or 14 days after vaccine administration
- Prophylactic vaccines (e.g. pneumococcal vaccine, influenza vaccine, COVID-19 vaccine) within 14 days prior to or after vaccine administration
- Any investigational agent within 28 days of vaccine administration

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The final decision on any supportive therapy or vaccination rests with the investigator and/or the participants' primary physician.

4.8 Rescue Medications & Supportive Care

Participants should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Section 4.9. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids, as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the Investigator determines the events to be related to pembrolizumab or PSLP injection

Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab or PSLP injection, the Investigator does not need to follow the treatment guidance

4.9 Toxicities and Dosing Delays/Dose Modifications

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed for the development of toxicity according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE), version 5.0. Dose adjustments should be made according to the system showing the greatest degree of toxicity.

4.9.1 Dose modification and toxicity management for immune-related AEs associated with vaccine.

Since the vaccine product administered in this study is unique for each subject, safety data are unavailable for the specific vaccine. However, a similar SLP vaccine directed against HPV, ISA101, has been administered in clinical trials and safety data are available. Please see Section 8.1 for additional information.

Vaccine dose reductions are not permitted. Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Dosing interruptions are also permitted if corticosteroids are administered due to pembrolizumab toxicity. Patients should be placed back on study therapy as soon as possible after the scheduled interruption. The reason for interruption should be documented in the patient's study record. Patients should receive all doses of vaccine whenever possible.

Table 3: PSLP Injection Reaction Dose modification and Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy interruption but responds promptly to symptomatic treatment (eg, antihistamines, NSAIDs, narcotics, corticosteroids, IV fluids); prophylactic medications indicated for ≤24 hrs	Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	No subsequent dosing
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (eg, renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	Additional appropriate medical therapy may include but is not limited to: • Epinephrine** • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further study drug treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov		

4.9.2 Dose modification and toxicity management for immune-related AEs associated with pembrolizumab

Pembrolizumab dose reductions are not permitted. Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Patients should be placed back on study therapy within 3 weeks of the scheduled interruption. The reason for interruption should be documented in the patient's study record.

Adverse events associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than on body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 4.

Table 4 Dose Modification Guidelines for Pembrolizumab Drug-Related immune related Adverse Events

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	Toxicity grade or conditions (CTCAEv5.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2	• Monitor participants for signs and symptoms of

	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue	mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (i.e., diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (i.e., peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4 or recurrent Grade 3	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable

	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		

All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Guillain-Barré Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

NOTE:

For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

4.9.3 Dose modification and toxicity management of infusion-reactions related to pembrolizumab

Pembrolizumab may cause severe or life threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 5.

Table 5 Pembrolizumab Infusion Reaction Dose modification and Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None

Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (eg, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (eg, from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 25-50 mg PO (or equivalent dose of antihistamine). Acetaminophen 500 to 1000 mg PO (or equivalent dose of analgesic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (eg, renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: • Epinephrine** • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further study drug treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov		

4.10 Duration of Study Treatment

In the absence of treatment delays due to adverse events, treatment may continue until:

- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study,
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- Pembrolizumab will be administered for a maximum of 35 cycles (approximately 2 years) if there is no evidence of disease progression.
- Progressive disease; however, treatment for up to 9 doses of vaccine may continue past progression if the treating physician feels there is continued benefit.

4.11 Discontinuation from Study Participation

Subjects will be encouraged to complete the treatment course and study; however, they may voluntarily withdraw from treatment or the study at any time. In the case of a subject who is lost to follow-up, attempts to contact the subject must be made and documented in the subject's study records. Sites will attempt to obtain vital status data from public records or other external sources where possible if a subject withdraws consent from study (i.e. refuse follow-up for vital status) or is lost to follow up.

Participants may discontinue study treatment at any time for any reason or be dropped from the study treatment at the discretion of the investigator should any untoward effect occur. In addition, a participant may be discontinued from study treatment by the investigator or the Sponsor if study treatment is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons:

- The participant or participant's legally acceptable representative requests to discontinue study treatment
- The patient exhibits confirmed radiographic disease progression, unless the patient is clinically stable or clinically improved or the investigator believes continued study treatment is in the best interest of the patient.
- Any progression or recurrence of any malignancy, or any occurrence of another malignancy that requires active treatment
- Unacceptable adverse experiences which indicates to the Investigator that continued participation is not in the best interest of the subject.
- The participant has a medical condition or personal circumstance which, in the opinion of the investigator and/or sponsor, placed the participant at unnecessary risk from

continued administration of study treatment.

- The participant has a confirmed positive serum pregnancy test
- Noncompliance with study treatment or procedure requirements
- Recurrent Grade 2 pneumonitis
- Discontinuation of treatment may be considered for participants who have attained a confirmed complete response (CR) and have been treated for at least 8 cycles (at least 24 weeks), receiving beyond the date when the initial CR was declared.
- The participant is lost to follow-up
- Completion of 35 treatments (approximately 2 years) with pembrolizumab

Note: The number of treatments is calculated starting with the first dose.

- Administrative reasons
- Termination of the study by the Sponsor, clinical site, or the regulatory authority

In addition, a subject will be discontinued from the study if vaccine development is not feasible due to a lack of candidate neoantigens or technical constraints.

Subjects who discontinue or withdraw during the screening period will be replaced.
Subjects in whom the necessary blood samples to measure the primary endpoint are not obtained, will also be replaced.

5.0 STUDY PROCEDURES

Refer to the study Schedule of Events for procedures.

The Screening Period consists of 2 visits. The first should occur within 6 months of vaccine administration and is designed to assess eligibility and collect tissues necessary to prepare the product. Potential subjects will be evaluated for entry into the study according to the stated inclusion and exclusion criteria. For subjects who meet all inclusion/exclusion criteria, enrollment will be completed and reviewed by the Principal Investigator or designee for approval. The second screening visit should occur within 28 days of vaccine administration and is designed to assess suitability for trial participation.

Individuals deemed ineligible for study enrollment do not need to complete all screening procedures. The reason for ineligible status will be documented. A subject who fails screening may repeat the screening process once if the Investigator believes there has been a change in eligibility status.

The Treatment Period consists of multiple clinic visits for dosing and additional visits/phone calls are indicated to assess safety, tumor response, and immune monitoring. During the Treatment Continuation Period, dosing and visits occur every 3 weeks, with tumor responses evaluated at 9-week intervals (+/-7 days). An EOT visit will be conducted approximately 4 weeks following the final anti-PD1 antibody infusion.

The Follow-up Period begins when subject has discontinued/completed treatment. The subject will be contacted by phone every 12 weeks during the Follow-up Period to obtain information on any additional cancer treatments and/or survival.

All study visits (and visit windows), assessments, and procedures will be performed as indicated in the Schedule of Procedures.

5.1 Definitions of Study Assessments

5.1.1 Medical history

A complete medical, surgical and oncology history as well as history of infections are obtained at screening. Any changes from Screening (e.g. worsening severity or abnormal findings) are considered to be adverse events (AEs).

5.1.2 Demographics

Demographic profile will include date of birth, gender, race, and ethnicity.

5.1.3 Review subject eligibility criteria

Review of eligibility criteria as described in Section 3 to ensure subject qualification for study entry.

5.1.4 Concomitant medications

All concomitant therapy, including anesthetic agents, vitamins, homeopathic/herbal remedies, nutritional supplements, received by patients from five days prior to the first day of study treatment until 28 days after the last study dose (or until the start of a new treatment, whichever comes first) will be recorded in the patient's medical record. If a reportable adverse event deemed related to study intervention (see Section 7) occurs within 28 days after last study dose and the patient has not started a new treatment, recording of concomitant medications related to the treatment of that adverse event should continue until resolution of the adverse event.

5.1.5 Physical exam

A complete physical examination should include the evaluation of general appearance; evaluation of head, eyes, ears, nose, and throat (HEENT); and cardiovascular, pulmonary, abdominal, musculoskeletal, skin, lymph nodes, neurological, and genitourinary systems. Subsequent exams may be targeted as appropriate.

Changes from baseline abnormalities should be recorded at each subsequent physical examination. New or worsened abnormalities should be recorded as adverse events if clinically significant.

5.1.6 Vital signs and height

Vital signs should include blood pressure, pulse, respiratory rate, temperature, and pulse oximetry and weight.

5.1.7 Performance status

Performance status is evaluated using the Eastern Cooperative Oncology Group scale.

5.1.8 Adverse event assessment

Baseline assessment of subject status for determining adverse events. See Section 7 for Adverse Event monitoring and reporting.

5.1.9 Hematology

Hematology includes complete blood count with differential ANC and platelet count.

5.1.10 Serum chemistries

Serum chemistry includes sodium, potassium, chloride, bicarbonate, glucose, blood urea nitrogen, creatinine, lactate dehydrogenase, ALT, AST, alkaline phosphatase, bilirubin (total, direct, indirect), total protein, albumin, calcium, magnesium, uric acid, and phosphate.

5.1.11 Blood draw for correlative studies

See Section 5.5 for details.

5.1.12 Pregnancy test (for females of child bearing potential)

See section 3.1 for definition of WOCBP. For study eligibility, WOCBP must have negative urine or serum pregnancy test within 28 days of first study drug administration. If a urine pregnancy test is positive, the results should be confirmed with a serum pregnancy test. Pregnancy of a subject or partner must be reported and followed. Additional pregnancy tests may be performed if clinically indicated.

5.1.13 Urine Analysis

Standard urinalysis dipstick assessment (pH, protein, glucose, blood, ketones, and leukocytes) should be performed. This must be supplemented with laboratory quantification of any potentially relevant abnormalities. In addition, levels for protein and creatinine must be obtained, as a protein: creatinine ratio will be measured.

5.1.14 Tumor assessment

Radiographic tumor evaluation consists of a CT scan of all affected sites performed at screening (pre-treatment baseline assessment) and at 9-week intervals (+/-7 days) for the first 6 months throughout the Treatment and Treatment Continuation Periods. After 6 months of therapy, CT scans will be performed when clinically indicated. If CT scan is contraindicated (e.g. allergy to contrast dye), an MRI should be performed. If there are delays in study drug administration, the subject should continue to follow the schedule for CT scan/tumor assessments as feasible.

For each scan, tumor measurements should be obtained using RECIST 1.1; assessment of response will be determined by the local Investigator. The method of assessment and

technique should be consistent throughout the study to enable characterization of each identified and reported lesion.

5.1.15 Tumor tissue collection (See Section 5.5 for details)

If eligibility criteria are met, the subject will return to provide a blood sample and undergo tumor biopsy if necessary. The tumor specimen will be analyzed by next-generation sequencing; candidate neoantigens will be identified based on nucleotide sequence differences from the individual's non-malignant cells (blood sample). A core needle biopsy using standard techniques will be obtained from accessible tumor site(s) (primary and/or metastatic) outside any previous field of radiation. See section 5.5 for details.

Post-vaccine treated biopsies (if available) will be sequenced to evaluate changes in neoantigens and other genomic changes. Both pre- and post-vaccine biopsies will undergo immune cell subset analysis (e.g. CD4, CD8, T_{reg}, myeloid) by immunohistochemistry, and gene and protein expression profiling.

5.2 Screening/Baseline Procedures

Subjects may sign an initial consent allowing the vaccine to be manufactured. Subsequently, the subject would sign a second consent for treatment when they and the IP are ready to proceed.

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

The Screening Period consists of 2 visits. The first visit is to assess eligibility and collect tissues necessary to prepare the product. Potential subjects will be evaluated for entry into the study according to the stated inclusion and exclusion criteria. For subjects who meet all inclusion/exclusion criteria, enrollment will be completed and reviewed by the Principal Investigator or designee for approval. The second screening visit should occur within 28 days of vaccine administration and is designed to assess suitability for trial participation.

The screening procedures include:

- Written informed consent.
- Review of inclusion and exclusion criteria.
- Complete medical/oncology history.
- Demographics.
- Documentation of concomitant medications.
- Complete physical examination, including vital signs and height.
- Performance status assessment.
- Laboratory tests *(*within 28 days of initial treatment. For subsequent treatment days, labs within 3 days of dosing); pregnancy test within 28 days).

- Blood collection for correlative studies.
- Documentation of tumor staging.
- Tumor biopsy tissue collection for correlative studies (if available)

5.3 Procedures During Treatment

Refer to Schedule of Events for procedures and windows during the Treatment Period and Treatment Continuation.

5.3.1 Prior to Each Treatment

- Documentation of concomitant medications and adverse events
- Physical exam, vital signs
- Hematology
- Serum chemistries

5.3.2 Tumor Assessments

- CT scan or MRI of all affected sites are performed at 9-week intervals (+/- 7 days) for the first 6 months throughout the Treatment and Treatment Continuation Periods. After 6 months of therapy, CT scans will be performed when clinically indicated.

5.3.3 End of Treatment Visit

- Documentation of concomitant medications and adverse events
- Physical exam, vital signs
- Hematology
- Serum chemistries

5.4 Follow-up Procedures

Following the Treatment and Evaluation Period, subjects will be contacted at 12 week (3 month) intervals to assess survival and subsequent cancer-related therapies. Follow-up will continue until death of the subject, loss to follow-up, or close of study by the Sponsor. For a subject who withdraws from the study prior to completion of the Follow-up Period or is documented as lost to follow up, sites will attempt to obtain vital status data from public records or other external sources where possible. All deaths must be reported on the CRF.

5.5 Correlative Studies

Research assessments will be conducted on blood and tumor biopsy samples and will characterize the immune responses mediated by vaccine with or without anti-PD1 antibody. Blood samples required for exploratory laboratory evaluations will be collected, processed, stored, and shipped as outlined below.

5.5.1 Sample Collection Guidelines

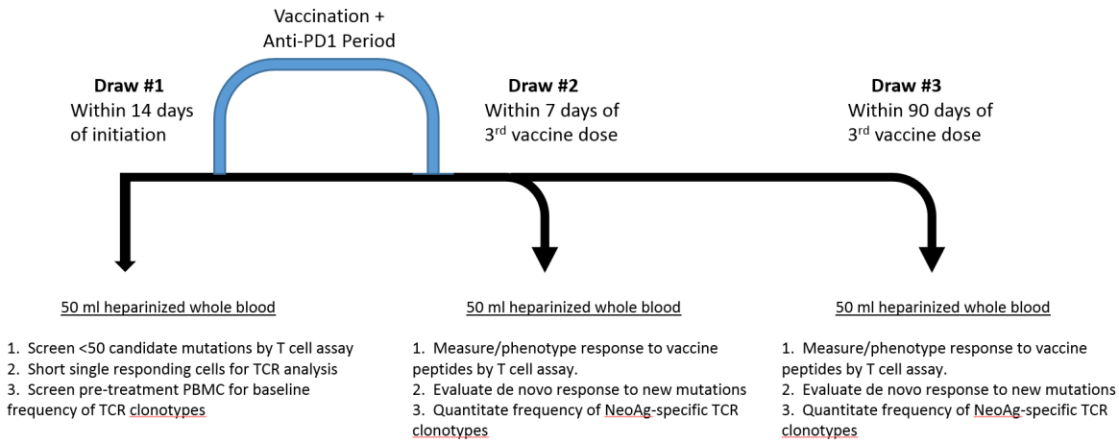
A. Tumor tissue: Approximately 2 mg – 50 mg of fresh tumor tissue is obtained via fine-needle aspirate, core biopsy, surgical resection, or as needed, from FFPE material. Three tumor collections are desired: 1) before, during, or after the screening period to manufacture the personalized vaccine (this collection is absolutely necessary for treatment); 2) within 7 days of 3rd vaccine dose on Arms A and B and within 7 days of 6^{th} vaccine dose in Arm C and D; and 3) at disease progression.

B. Peripheral blood mononuclear cells (PBMC):

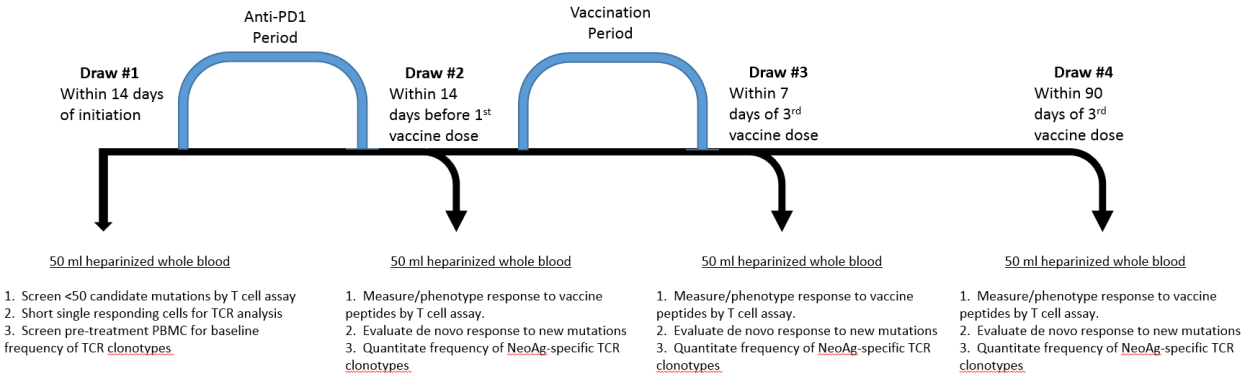
- One 10mL heparin tube will be collected at or before screening to provide DNA for a patient-specific reference genome. The blood will be given to Dr. Schoenberger's group as fresh whole blood.
- 75-100 ml whole blood (eight to ten 10mL heparin tubes) to be collected during screening period to determine neoantigens. The blood will be given to Dr. Schoenberger's group as fresh whole blood.
- For neoantigen identification, an additional 75-100 mL whole blood (eight to ten 10mL heparin tubes) may be collected every 4 weeks.
- 50-75 ml whole blood (5-8 10 mL heparin tubes) to determine T cell specific response to neoantigen. The blood will be given to Dr. Schoenberger's group as fresh whole blood.
    - Arm A and B – samples should be collected during screening, within 28 days of treatment initiation, within 7 days of last vaccine dose, and within 7 days of Week 18
    - Arm C – samples should be collected during screening, within 28 days of treatment initiation, Week 8 within 7 days after vaccine administration, Week 32 (and within 12 weeks of last vaccine dose (+/- 3 days) and anti-PD1 antibody administration)
    - Arm D – samples should be collected during screening, within 28 days of treatment initiation, Week 8 within 7 days after vaccine administration, Week 32 (and within 12 weeks of last vaccine dose (+/- 3 days).
    - If additional vaccine doses are administered, additional samples may be collected per investigator, a sample should collected within 12 weeks of last vaccine dose (+/-3 days).
- An additional 50-75 mL whole blood (eight to ten 10mL heparin tubes) will be collected prior to the first dose of vaccine only in subjects enrolled to Arm B.
- 10 ml whole blood (cell-free DNA BCT) will be collected at Weeks 9-12.

Work Flow for Sample Collection:

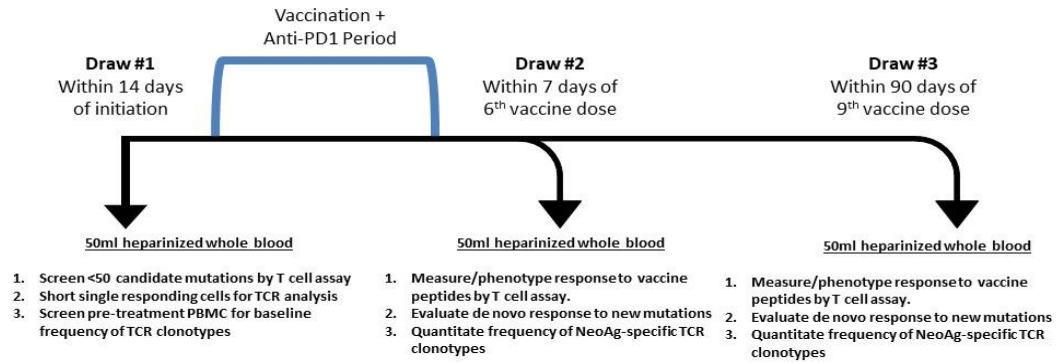
Arm A



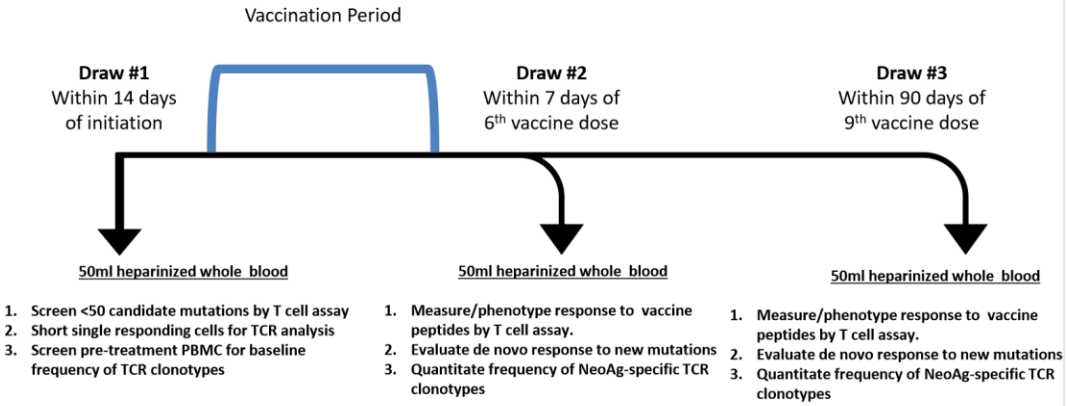
Arm B



Arm C



Arm D



Blood Collection

- At designated time points, the coordinator will obtain fresh whole blood in the appropriate number of heparin tubes.
- Upon start of vaccination period, blood draws should be obtained after the specified vaccine dose.

- Blood will be given to Dr. Schoenberger's group same day as fresh whole blood.

Tissue Procurement

- Biopsy
  - Patient will undergo biopsy in a clinic setting if needed
  - Coordinator will be present at time of biopsy to collect tissue
  - Tissue will be collected in pre-prepared RPMI provided by Dr. Schoenberger's group
  - Fresh tissue will be given to Dr. Schoenberger's group same day
  - See lab manual for specific instructions on tissue collection
- Surgery
  - Patient will undergo surgical resection
  - Coordinator will be present at time of surgery to collect tissue
  - Approximately 2 mg – 50 mg of fresh tissue will be collected in pre-prepared RPMI provided by Dr. Schoenberger's group
  - Fresh tissue will be given to Dr. Schoenberger's group same day
  - See lab manual for specific instructions on tissue collection

Distribution

- Call and/or email Dr. Schoenberger's group on day of sample collection (blood or tissue) @ Aaron Miller - amiller@lji.org/858-249-9097; Milad Bahmanof - milad@lji.org/858-886-6355;
- De-identified specimen will be released to designated personnel using chain of custody form. All personnel in the Schoenberger laboratory will remain blinded regarding subject identity or treatment arm allocation.
- If patient consented, additional blood aliquots or frozen solid specimen from Biorepository may be requested separately.

5.5.2 Assay Methodology

Overview:

Our objectives in monitoring the post-vaccination immune responses of trial patients will be threefold:
1) To determine whether T cell responses to the original vaccinating antigens have been preserved and/or potentiated.
2) To determine whether the treatment protocol results in diversification of immune responses to include tumor-expressed somatic mutations not detected as neoantigens in the pre-vaccine screen ("epitope spreading")
3) To determine whether the treatment protocol alters the cytokine polarity or inhibitory receptor expression of the responding neoantigen-specific T cells.

Methods:

Assay 1: Functional T cell assay from PBMC: We will perform a variation of the assay used in

Section 1.5 to identify the subset of somatic mutations confirmed as neoantigens by functional T cell assay (ELISPOT and CRA) following *in vitro* expansion to determine whether these responses persist in phenotype and magnitude in the treated patients. To do this, PBMC will be obtained from vaccinated patients at the time points listed above and an aliquot tested for recognition of the vaccinating peptides by ELISPOT (for IFN-γ and IL-5) either directly *ex-vivo* or after a 3 day co-culture with the individual vaccine peptides, with these time points chosen to reflect the presumed higher frequency with which neoantigen-specific T cells will be present in the peripheral blood of treated patients. Results will be reported as the frequency of cytokine-producing cells among total and positive controls will include responses against nominal recall antigens (CEF peptides, for CMV, EBV, and Flu).

Assay 2: Response diversification: In addition to measuring responses against the vaccine peptides, we will also include peptides comprising the set of tumor-expressed somatic mutations that did not elicit a T cell response in the original neoantigen screen in 14-day restimulation cultures using PBMC from treated patients to assess whether the antitumor immune response we expect to result from vaccination had led to de-novo priming of responses against additional neoantigen targets, presumably through enhanced tumor killing and release of tumor antigens in a classic 'abscopal effect'.

Assay 3: T cell clonotype assay from PBMC: The analysis in Section 1.5 will reveal the neoantigen targets for each patient's vaccine formulation on the basis of spontaneous T cell responses which are functionally detected on the basis of cytokine production in response to specific mutation-encoding peptides. As described, this analysis will also allow us to define the specific alpha and beta chains utilized in the TCRs (the 'clonotype") of the responding neoantigen-specific T cells by bioinformatic analysis of single sorted cells. These data can be used as a unique 'digital fingerprint' of the relevant clone within a larger population of cells, and in fact will be used to longitudinally measure the frequency of the responding neoantigen-specific T cells in peripheral blood at the time points listed above. To assess the frequency of neoantigen-specific clonotypes within the patient's peripheral lymphocyte pool, we will subject an aliquot of the PBMC obtained at each time point to ImmunSEQ analysis which uses highly-sensitive genomic sequencing and bioinformatics analysis software to give a quantitative assessment of the TCR clonotypes within a complex lymphocyte population. As we will know which specific paired TCR clonotypes belong to verified neoantigen-reactive T cells, we can utilize this approach to monitor the frequency of the specific T cell which we expect to be expanded by the vaccine.

5.5.3 Specimen Banking

Patient samples collected for this study will be retained at UCSD BTTSR for analysis and future cancer research. Specimens will be stored indefinitely or until they are used up. Samples will be labeled with the protocol number, subject's de-identified study number and collection date. The link between study number and medical record number will be viewed over a password secured encrypted server-client.

The study research coordinator will review their subject's medical record for demographic and clinical information pertaining to the subject's general medical history, diagnosis, and outcomes

of any treatments received. Samples and data extracted from the subject's medical record will be coded with a de-identified study number so that the subject's name and identifying information is removed. A log that links the subject's name and identifiers to the study number will be maintained in a secure database distinct from the secure database into which the subject's clinical information will be entered by study personnel.

The specimens, DNA, and their derivatives may have significant therapeutic or commercial value. The Informed Consent form contains this information and informs the subject that there is the potential for financial gain by UCSD, the investigator or a collaborating researcher or entity.

6.0 Measurement of Effect

6.1 Safety/tolerability

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the CTCAE version 5.0 (http://ctep.cancer.gov/reporting/ctc.html) for reporting of adverse events.

6.2 Antitumor Effect

Response and progression will be evaluated in this study using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [*JNCI* 92(3):205-216, 2000]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST v1.1 criteria (see Appendix B).

6.2.1 Best Overall Response

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation.

6.2.2 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of study treatment until objective tumor progression or death.

6.2.3 Time to Progression

Time to progression is defined as the duration of time from start of study treatment until objective tumor progression.

6.2.4 Overall Survival

Overall survival is defined as the duration of time from start of study treatment to death.

7.0 ADVERSE EVENTS

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal

laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Progression of the cancer under study or events which are unequivocally due to disease progression should not be reported as an AE during the study (unless it is considered to be drug related by the investigator).

Adverse events may occur during the course of the use of agents in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

All AEs, SAEs and other reportable safety events that occur after the consent form is signed but before treatment allocation must be reported by the investigator if the participant is receiving placebo run-in or other run-in treatment, if the event cause the participant to be excluded from the study, or if the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or a procedure.

● All AEs from the time of treatment allocation through 30 days following cessation of study treatment must be reported by the investigator.

● All pregnancies and exposure during breastfeeding, from the time of treatment allocation through 120 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy must be reported by the investigator.

Serious Adverse Events

● All AEs meeting serious criteria, from the time of treatment allocation through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy, whichever is earlier must be reported by the investigator.

● SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

● Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately by the investigator if the event is considered to be drug-related.

Investigators are not obligated to actively seek AE or SAE or other reportable safety events in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the Sponsor.

Definition of an Overdose for Pembrolizumab and Reporting of Overdose to the Sponsor and to Merck

For purposes of this study, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the participant should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported to the Sponsor within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229).

For the time period beginning when the consent form is signed until treatment allocation, any ECI, or follow up to an ECI, that occurs to any participant must be reported within 2 working days to Merck Global Safety if it causes the participant to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation through 90 days following cessation of treatment, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 2 working days to Merck Global Safety.

Events of clinical interest for this trial include:

1. An overdose of Merck product, as defined in Section Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

7.1 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care.

As far as possible, each adverse event should be evaluated to determine:

- duration (start and end dates)
- severity (grade)
- seriousness
- relationship to study agent
- action taken (i.e., none, study agent modification, medical intervention)
- outcome (i.e., resolved without sequelae, resolved with sequelae, ongoing)

Adverse events monitoring begins when the subject signs informed consent and ends 30 days following the last administration of study treatment or start of new anti-cancer therapy, whichever is earlier.

All patients experiencing an adverse event, regardless of its relationship to study drug will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any clinically significant abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

7.2 Severity

All adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The CTCAE v5.0 is available at http://ctep.cancer.gov/reporting/ctc.html

If no CTCAE grading is available, the severity of an AE is graded as follows:

Mild (grade 1): the event causes discomfort without disruption of normal daily activities.
Moderate (grade 2): the event causes discomfort that affects normal daily activities.
Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
Life-threatening (grade 4): the patient was at risk of death at the time of the event.

Fatal (grade 5): the event caused death.

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 5.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets. All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

7.3 Seriousness

A "serious" adverse event is defined in regulatory terminology as any untoward medical occurrence that:

1. **Results in death.**
   If death results from (progression of) the disease, the disease should be reported as a serious adverse event (SAE) itself.
2. **Is life-threatening.**
   The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
3. **Requires in-patient hospitalization or prolongation of existing hospitalization.**
   Note: Hospitalization (including hospitalization for an elective procedure) for a pre-existing condition which has not worsened does not constitute a serious adverse event.
4. **Results in persistent or significant disability or incapacity.**
5. **Is a congenital anomaly/birth defect**
6. **Is an important medical event**
   Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of "Serious Adverse Event".
   For example: allergic bronchospasm requiring intensive treatment in an emergency room

or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

7.4 Relationship

Attribution categories for adverse events in relationship to protocol therapy are as follows:

Definite – The AE *is clearly related* to the study treatment.
Probable – The AE *is likely related* to the study treatment.
Possible – The AE *may be related* to the study treatment.
Unlikely – The AE *is doubtfully related* to the study treatment.
Unrelated – The AE *is clearly NOT related* to the study treatment.

7.5 Evaluation of Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V5.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the participant, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of participant taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious	

	event of clinical interest and must be reported within 24 hours to the Sponsor and to Merck within 2 working days.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause Merck product or PSLP product to be discontinued?	
Relationship to Merck Product or PSLP product	Did Merck product or PSLP product cause the adverse event? The determination of the likelihood that Merck product or PSLP product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the test drug and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely an investigational product caused the adverse event (AE):	
	Exposure	Is there evidence that the participant was actually exposed to investigational product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of investigational product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck Product	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Sponsor's product; or (3) the trial is a single-dose drug trial); or (4) Sponsor's product(s) is/are only used one time.)
	Rechallenge	Was the participant re-exposed to Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Sponsor's product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY MERCK PRODUCT, OR IF REEXPOSURE TO MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE PARTICIPANT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of Merck product relationship).**	
Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to Merck product. The temporal sequence of the AE onset relative to the administration of Merck product is reasonable. The AE is more likely explained by Merck product than by another cause.	
No, there is not a reasonable possibility of Merck product relationship	Participant did not receive the Merck product OR temporal sequence of the AE onset relative to administration of Merck product is not reasonable OR the AE is more likely explained by another cause than the Merck product. (Also entered for a participant with overdose without an associated AE.)	

7.6 Reporting Requirements for Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

7.6.1 Expedited Reporting

A. The **Principal Investigator** must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug.

B. The Sponsor (or designee) will report all SAEs that are unexpected and considered related to the administration of the investigational agent to the appropriate health and regulatory authorities and Investigators in the form of an expedited safety report within 15 calendar days after receiving information on the SAE. The Investigators will notify their reviewing IRB and other committee(s) as required by institutional policies.

The Sponsor will also report to the appropriate health and regulatory authorities by facsimile, e-mail, or phone within 7 days of receiving the information, any unexpected life-threatening or fatal SAEs that are considered related to the investigational agent.

C. The **UCSD Human Research Protections Program (HRPP)** and **Moores Cancer Center Data and Safety Monitoring Board (DSMB)** must be notified within 10 business days of "any unanticipated problems involving risk to subjects or others" (UPR).

The following events meet the definition of UPR:

1. Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures.
2. Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
3. Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
4. Any new information (e.g., publication, safety monitoring report, and updated sponsor safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
5. Any breach in confidentiality that may involve risk to the subject or others.
6. Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.

D. The Sponsor (or designee) will report all SAEs that are unexpected and considered related to the administration of pembrolizumab to **Merck** within 2 working days. SAE reports and any other relevant safety information are to be forwarded to the **Merck Global Safety facsimile number: +1-215-661-6229**

7.6.2 Pregnancy and Lactation Reporting

Although pregnancy and infant exposure during breast feeding are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a participant (spontaneously reported to them) that occurs during the study.

Pregnancies and infant exposures during breastfeeding that occur after the consent form is signed but before treatment allocation must be reported by the investigator if they cause the participant to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and infant exposures during breastfeeding that occur from the time of treatment allocation through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, must be reported by the investigator.

If the subject or partner of a subject participating in the study becomes pregnant during the study or within 28 days of discontinuing study drug, the Investigator should report the pregnancy within 24 hours of being notified. A subject becoming pregnant while on study drug will immediately be withdrawn from the study and EOT study procedures will be performed.

The subject or partner should be followed by the Investigator until completion of the pregnancy. If the pregnancy ends for any reason before the anticipated date, the Investigator should notify designated safety personnel. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. SAE reporting procedures should be followed if the outcome of the pregnancy meets the criteria for immediate classification as an SAE (i.e. postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly).

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

7.6.3 Routine Reporting Requirements

A. The **IRB** must be notified of all adverse events, as required by institutional policies, at the time of the annual Continuing Review.

B. The **FDA** must be notified of all non-serious adverse events annually at the time of the annual report.

8.0 AGENT INFORMATION

8.1 Personalized vaccine

Storage and stability: PSLP lyophilized powder is stored in glass vials in the dark (away from light) at -20°C or lower. Once product has been fully prepared, it should be administered within a 2 hour timeframe. If there is a delay in administration, product will be kept at 4°C or colder. Product will be protected from light. The product must be administered within 24 hour timeframe. See Pharmacy Manual for additional details.

Preparation: The vaccine will not be prepared until sterility results are returned and the product is confirmed to be uncontaminated. Vaccine is prepared by thawing the appropriate quantity of vials of drug product at room temperature.

For Arm A and B: PSLP lyophilized powder will be dissolved in dimethylsulfoxide (DMSO) and admixed with sterile water for injection (WFI) or phosphate buffered saline (PBS) and adjuvant Montanide ISA-51 VG.

For Arm C and D: PSLP lyophilized powder will be dissolved in dimethylsulfoxide (DMSO) and admixed with sterile water for injection (WFI) or phosphate buffered saline (PBS). Poly-ICLC (Hiltonol®) adjuvant will be prepared in a separate syringe for administration.

The appropriate volume of product to achieve the target dose (100 µg per peptide) is drawn with a syringe for injection. See Pharmacy Manual for additional details.

Route of administration:

Arm A and Arm B: Subcutaneous injection.
Arm C and Arm D: Intramuscular injection. Note: The adjuvant should be administered in a separate syringe as an intramuscular injection concurrent with the peptide vaccine.

A dose of 100 µg per peptide PSLP vaccine will be administered by injection in any limb (left upper arm, right upper arm, left upper leg/thigh, or right upper leg/thigh). Watch patient for signs of allergic responses. Ensure that immediate treatment of severe allergic reactions are available prior to investigational drug administration, including staff well-trained in resuscitation, intravenous access for administration of fluids, antihistamines and corticosteroids, and epinephrine for intramuscular injection.

In case a subject experiences a DLT attributable to the investigational product, no additional doses of the investigational product will be given.

Side effects: Since the vaccine product administered in this study is unique for each subject, safety data are unavailable for the specific vaccine. However, a similar SLP vaccine directed against HPV, ISA101, has been administered in clinical trials and safety data are available. Side effects of the personalized vaccine are expected to be similar since the neoantigens, by definition, are not present in normal tissue.

Overall, the observations on the safety profile observed to date with ISA101/101b are summarized below and detailed in the IB:

- Most of the AEs in the CervISA study were expected toxicities reported to be related to chemotherapy or to complications associated with progression of cervical cancer.
- Dose-related injection site reactions (ISRs) to ISA101 were the most frequent AEs reported to be related to ISA101 with ISRs occurring in most patients who received ISA101. Most of the local ISRs were reported to be grade 1 to 2 in severity. One grade 3 ISR at 300µg/peptide has been reported. One patient refused additional ISA101 vaccination at 100µg/peptide due a grade 1 ISR.
- Dose-related systemic allergic reactions were reported particularly at the 300 µg/peptide dose. These may, in part, be related to the amount of Montanide administered, which is proportional to dose of the peptide vaccine.
- No new or unexpected safety concerns have been identified for ISA101 or ISA101b compared to the safety profile of the predecessor vaccine, HPV-16-SLP.
- There do not appear to be any unexpected or overlapping toxicities between ISA101/101b when used in combination with chemotherapy (e.g. paclitaxel and carboplatin) or anti-PD-1 therapy (e.g. Nivolumab).
- No autoimmune complications have been observed in relationship to administration of the vaccine.
- The safety profile and immunologic activity of ISA101b appears to be similar to ISA101.
- Based on the assessment of safety data in the initial dose escalation cohorts and the 2 expansion cohorts, as well as data on the HPV-16 specific T cell responses, the 100 µg/peptide dose level was selected as the recommended phase 2 dose for further studies in patients with advanced cancer.

8.1.1 Return and Retention of Study Drug

Upon completion or termination of the study, all unopened vials of IP must be returned to the Sponsor or Sponsor's designee, unless authorized by the Sponsor or Sponsor's designee to be destroyed at the study site. All vials of IP returned to the Sponsor or Sponsor's designee must be accompanied by the appropriate documentation and clearly identified by protocol number and study site number.

8.2 Pembrolizumab

When possible, pembrolizumab should be administered IV in a limb on contralateral to the site of vaccine administration.
Details on preparation and administration of IV pembrolizumab are provided in the Package Insert and Merck, Inc.'s Pembrolizumab (MK-3475) Pharmacy Manual for Investigational Studies.

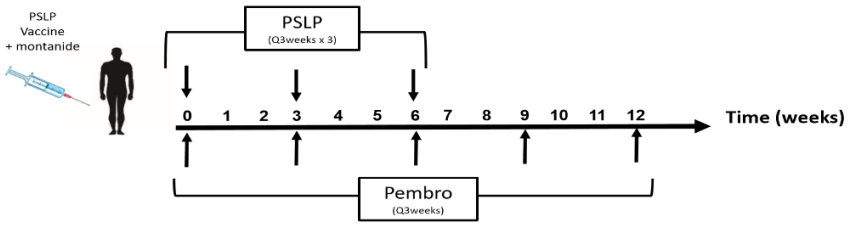
9.0 STATISTICAL CONSIDERATIONS

Statistical Analysis Plan

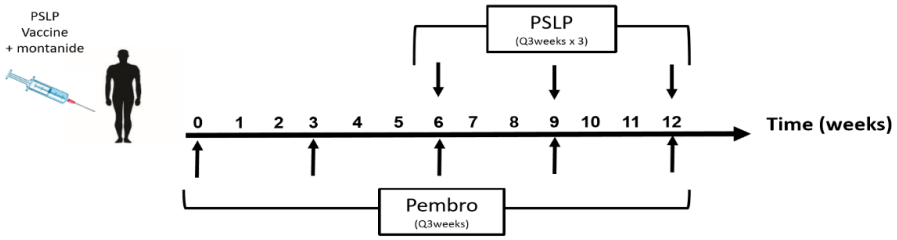
Study design

This is an open-label Phase 1b, sequential cohort, clinical study in adults with incurable solid tumors. Cohorts A and B will enroll 5 subjects to receive either: Arm A) personalized vaccine and anti- PD-1 administered concurrently at the start of study therapy or Arm B) anti-PD-1 antibody for 6 weeks followed by personalized vaccine therapy. Arm C will enroll 10 subjects to receive personalized vaccine and anti- PD-1 administered concurrently in a boosted schedule at the start of study therapy. An additional 5 subjects may be enrolled on to Arm C. Arm D will enroll 10 subjects to receive personalized vaccine alone, in a boosted schedule at the start of study therapy. Toxicities related to the personalized vaccine immunotherapy are expected to be consistent with prior clinical and non-clinical experience, based on the platform and unrelated to the expressed antigens. However, for additional safety monitoring subjects on each arm will be staggered to enroll a maximum of two subjects at a time and allow at least one week between subject pairs, i.e. enrollment of subject 1 and 2, followed by a one week interval, followed by enrollment of subjects 3 and 4, followed by a one week interval, etc.

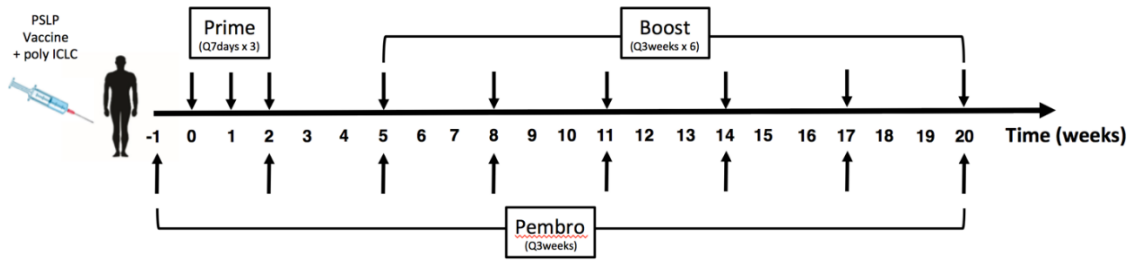
Arm A



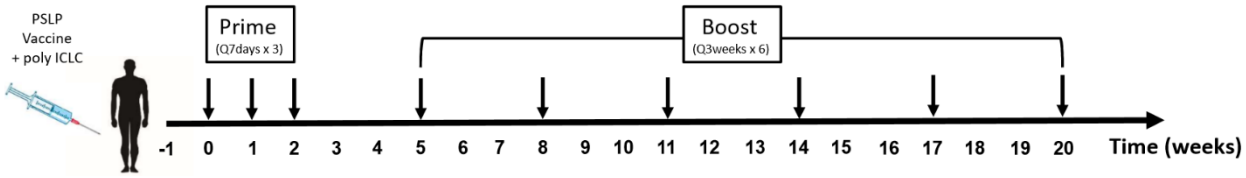
Arm B



Arm C



Arm D



Primary safety endpoints
Adverse events, reported overall and by grade, body system, attribution, and dose received.

Primary pharmacodynamic endpoint: neoantigen-specific T cell response, as measured by the T cell clonotype assay (Assay 3 below).

The primary pharmacodynamic outcome will be the change in neoantigen-specific clonotypes within the patient's peripheral lymphocyte pool. Validated personalized peptides will be injected for each patient. To assess the frequency of neoantigen-specific clonotypes within the patient's peripheral lymphocyte pool, we will subject an aliquot of the PBMC obtained at each time point to ImmunSEQ analysis. The within-patient change of TCR clonotypes corresponding to the pre-specified injected peptide sequences will be the primary outcome. Response to each peptide will be measured and the average value across injected peptides reported.

Primary efficacy hypothesis:

Concurrent administration of anti-PD1 antibody with vaccine therapy will result in a greater neoantigen-specific T cell response, than anti-PD1 therapy alone.

Hypothesis 1a: Draw #2 within-patient change from baseline for arms A and C (combination therapy) will be greater than Draw #2 within-patient change from baseline for arm B (anti-PD1 alone) and the corresponding Draw from arm D (anti-PD1 alone).

Key secondary efficacy hypotheses:

Administration of the vaccine, after priming with anti-PD1 therapy, will result in a quantifiable increase in neoantigen-specific T cell response.

Hypothesis 2: Arm B: post-sequential therapy (Draw #3) vs post- antiPD1 therapy alone (Draw #2) within-patient change will be greater than 0.

Boosted combination therapy will be better than evenly spaced combination therapy.

Hypothesis3: Arm C Post-sequential therapy (Draw #3) within-patient change from baseline will be greater than Arm A post-sequential therapy (Draw #3) within-patient change from baseline.

Additional efficacy hypotheses:

a. Concurrent combination therapy will be associated with increased neoantigen-specific T cell response. *Hypothesis*: Arms A and C: Post-combination therapy (Draw #2) within-patient change from baseline will be greater than 0.

b. Sequential combination therapy will be associated with increased neoantigen-specific T cell response. *Hypothesis*: Arm B: Post-sequential therapy (Draw #3) within-patient change from baseline will be greater than 0.

c. Sequential therapy will differ from combination therapy in neoantigen-specific T cell response
Hypothesis: Arms A and C post-combination-therapy (Draw #3) within-patient change from baseline will differ from Arm B Post-sequential therapy (Draw #3) within-patient change from baseline.

d. Anti-PD1 therapy alone will be associated with increase in neoantigen-specific T cell response
Hypothesis: Arm B Post anti-PD1 (Draw #2) within-patient change from baseline will be greater than 0.

Secondary efficacy endpoints

a. Objective response rate using RECIST 1.1.
b. Progression free survival – measured, in months, from date of enrollment until disease progression or death.
c. Overall Survival – measured, in months, from date of enrollment until disease progression or death.
d. Time to response – measured, in weeks, from date of first study treatment until best response assessed by RECIST 1.1.
e. Duration of response – measured, in weeks, from date of best response to date of progression assessed by RECIST 1.1.

Secondary feasibility endpoints

The proportion subjects with peptides sent for manufacture who receive at least one dose of PSLP vaccine

A consort-like diagram will be presented which displays the disposition of subjects screened for this trial according to feasibility outcomes. The manufacturing step is expected to be most important for cost and thus feasibility for future studies. Hence the primary feasibility endpoint is above. If fewer than half of subjects with peptides sent for manufacture are given a dose of vaccine, the feasibility criterion will not have been met. If fewer than 20% of eligible subjects whose tumor is sequenced are treated, the feasibility criterion will not have been met.

Exploratory Endpoints

- Response diversification assay
- Functional T cell assay
- Immune infiltrates in the tumor microenvironment including lymphoid and myeloid cell subsets, immune checkpoint expression, and other immunomodulatory molecules
- Genomic changes in the tumor

Analytic strategy

We will test the primary hypothesis at 0.05 alpha and test the key secondary hypotheses at the same familywise level only if the primary hypothesis is significant. This will control the type I error for both sets of hypotheses at 5%. To control the 2 key secondary hypotheses, we will use a Holm step-down test at 5% level.

The remaining secondary hypotheses will also be tested at familywise 5% level, using a Holm step-down test at familywise 5% level. This strategy will control the overall familywise error rate for the primary and key secondary outcomes at 5%, and will separately control the familywise error rate for the secondary hypotheses at 5%.

The secondary efficacy endpoints will be summarized by overall study arm, using an intent to treat analysis for sequential vs concurrent therapy. Because of the small sample sizes, no formal comparison between arms will be made for secondary outcomes. Exploratory outcomes will also be summarized by study arm. Subjects who are treated but have no efficacy evaluation following baseline will be considered treatment failures

Hypothesis tests will be carried out in R. For continuous endpoints, we will use t-tests on possibly log transformed data, with unequal variances if appropriate. Descriptive statistics will also be presented. Categorical variables will be summarized by treatment arm using frequency distributions: the number and percentage of non-missing observations will be given. Continuous variables will be summarized using standard quantitative statistics: the number of non-missing observations, mean, standard deviation, median and range (minimum and maximum observed values).

Sample Size and Power computations, primary and key secondary efficacy analysis

Primary Hypothesis: Hypothesis 1: Draw #2 within-patient change from baseline for Arms A and C (antiPD1-vaccine combination therapy) will be greater than with Draw #2 within-patient change from baseline for arm B (anti-PD1 alone).

Key Secondary Hypotheses: Hypothesis 2: Arm B: post sequential antiPD1-vaccine therapy (Draw #3) vs post- antiPD1-therapy alone (Draw #2) within-patient change in neoantigen specific T cell response will be greater than 0. Hypothesis 3: Arm B: post-sequential therapy (Draw #3) vs post- antiPD1 therapy alone (Draw #2) within-patient change will be greater than 0.

Stevanović et al [32] report on 2 subjects with HPV positive cervical cancer, treated with adoptive transfer of tumor infiltrating lymphocytes. Pre- and post- treatment PBMC samples from these 2 subjects were assayed for prevalence of 14 and 2 predefined neo-antigen specific T cell clones, respectively, using a similar TCR sequencing based assay. In both cases, consistent robust increases in relative abundance of the specific targeted T Cell clones were observed, of 3 to 4 orders of magnitude or more (Figure 3 C and D from the Stenanovic et al). A second study of treatment in cervical cancer with neoantigens (ASCO-SITC 2017, San Francisco, Abstract 140, Melief et al) show very large increases in antigen specific T Cell clones, with good reproducibility across the highest dose cohorts (5, 5b and 6), albeit measured with a different assay. Hence it is reasonable to power for very large effect sizes.

Arms A and B will accrue 5 subjects each; Arm C and D will accrue 10 subjects each.

We assume an approximately normal distribution for the final outcome measure, although it is likely skewed right. Using a two-sample, one-sided t-test with equal variances at 5% significance level to approximate power, with n=15 in combined Arms A and C vs 5 in Arm B, we have 80% power to detect a mean increase of about 1.33 standard deviations in the change in numer of neo-antigen specific clonotypes. As observed effect sizes appear to be much greater than this, we appear to be well-powered for Hypothesis 1.

For the two comparisons of the key secondary hypotheses, we again use a one-sided, one or two sample t-test with equal variances to approximate power. Here, at significance level 0.025 (to control for the 2 hypotheses) we have 80% power to detect an effect size of 1.7 standard deviations for Hypothesis 2 (within subject change for Arm B) and also of 1.7 standard deviations for Hypothesis 3 (Arm C vs Arm A). While we have no preliminary data on anti-PD1 therapy alone, it is likely that effect sizes much greater than this will be needed for clinical significance. Thus we are powered to detect meaningful pharmacodynamic effect sizes in this preliminary Phase 1b study.

Safety Analysis

Safety and tolerability data as well as demographic data will be summarized in tabular and/or graphical format for each treatment arm. The incidence of all laboratory test abnormalities and the median changes from baseline will be tabulated by treatment regimen and time point. The safety population will be used for these analyses.

Analysis populations

- The Safety population will include all randomized patients who took at least one dose of the study medication.
- The modified Intent-to-Treat (mITT) population will include all patients originally assigned to a study arm who took at least one dose of the study medication and who have at least one

efficacy evaluation following baseline.

- The Per Protocol (PP) population will include all subjects who:
  - took at least 90% of the assigned medication during the treatment period, and
  - did not have any major protocol deviations, and
  - completed efficacy measurements at baseline and at the end-of-study visit.

The primary population for all efficacy analyses is the mITT population, without imputation for missing values. Sensitivity analyses will also be performed using the PP populations for efficacy endpoints, using the mITT population with multiple imputation.

Adverse Events

Adverse events (AE) occurring after the start of study drug dosing at baseline (week 0) will be summarized descriptively for the safety population. All AEs will be coded according to system organ class (SOC) and preferred term (PT) using a Medical Dictionary for Regulatory Activities (MedDRA) dictionary. Summary tables showing the number of patients and percent within each category will be generated for each of the following types of adverse events and its relationship to study treatment (related to study treatment):

- All events
- Serious events
- Deaths
- Events leading to withdrawal
- Severe events

Laboratory Parameters

Laboratory parameters will be summarized by visit. Frequencies of high and low values with respect to the normal range will be displayed, as will shift tables comparing each treatment visit and baseline visit by time point and treatment group.

Pharmacodynamic Outcome measures

Assay 1: Functional T cell assay from PBMC: PBMC will be obtained from vaccinated patients at the time points listed above and an aliquot tested for recognition of the vaccinating peptides by ELISPOT (for IFN-γ and IL-5) either directly *ex-vivo* or after a 3 day co-culture with the individual vaccine peptides, with these time points chosen to reflect the presumed higher frequency with which neoantigen-specific T cells will be present in the peripheral blood of treated patients. Positive controls will include responses against nominal recall antigens (CEF peptides, for CMV, EBV, and Flu). The primary outcome will be the proportion of cytokine-producing cells in response to the individual vaccine peptides, among total PBMC.

Assay 2: Response diversification assay: In addition to measuring responses against the vaccine peptides, we will also include peptides comprising the set of tumor-expressed somatic mutations that did not elicit a T cell response in the original neoantigen screen in 14-day restimulation cultures using

PBMC from treated patients to assess whether the antitumor immune response we expect to result from vaccination had led to de-novo priming of responses against additional neoantigen targets. The outcome will be the proportion of cytokine-producing cells in response to any neoantigen, among total PBMC

Assay 3: T cell clonotype assay from PBMC: To assess the frequency of neoantigen-specific clonotypes within the patient's peripheral lymphocyte pool, we will subject an aliquot of the PBMC obtained at each time point to ImmunSEQ analysis which uses highly-sensitive genomic sequencing to give a quantitative assessment of the TCR clonotypes within a complex lymphocyte population. As we will know which specific paired TCR clonotypes belong to verified neoantigen-reactive T cells, we can utilize this approach to monitor *the frequency of the specific T cell clones which we expect to be expanded by the vaccine.*

10.0 STUDY MANAGEMENT

10.1 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed according to UCSD conflict of interest policy.

10.2 Institutional Review Board (IRB) Approval and Consent

The IRB should approve the consent form and protocol prior to any study-related activities. It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

10.3 Subject Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), subjects who have provided written informed consent must also sign a subject authorization to release medical information to the study and allow a Sponsor (or designee), and authorized regulatory authorities to have direct access to subject's medical information relevant to the study.

10.4 Data and Safety Monitoring/Auditing

In addition to adverse event monitoring and clinical oversight by the Study Chair, site principal investigator and co-investigators, quality assurance of the study will be performed by the UCSD Moores Cancer Center Clinical Trials Office internal monitor. Monitoring intervals will be dependent upon the number of patients enrolled and the complexity of the study.

This study will also use the UCSD Moores Cancer Center Data Safety and Monitoring Board (DSMB) to provide oversight in the event that this treatment approach leads to unforeseen toxicities. Data from this study will be reported annually and will include:

1) the protocol title, IRB protocol number, and the activation date of the study.
2) the number of patients enrolled to date
3) the dates of patient enrollment
4) a summary of all adverse events regardless of grade and attribution
5) a response evaluation for evaluable patients when available
6) a summary of any recent literature that may affect the ethics of the study.

10.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, investigators are required to conduct their research according to the plans reviewed and approved by the IRB.

10.5.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate apparent immediate hazards/risks to trial subjects without prior IRB approval. Any such emergency modification implemented must be noted and reported to the IRB along the lines of a protocol deviation or violation, depending on the nature of the modification.

10.5.2 Protocol Violations

Any unplanned variance from an IRB approved protocol is considered a violation and must be reported to the IRB in a timely fashion.

A. Major violations must be reported to the IRB within 10 working days of awareness of the violation.
Major violations include:
- Instances that have harmed or increased the risk of harm to one or more research participants.
- Instances that have damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

B. Minor violations may be reported to the IRB at the time of the continuing review.
Minor violations have no substantive effect on the risks to participants or on the scientific integrity of the research plan or the value of the data collected.

10.6 Protocol Amendments

Changes to the conduct of the study should be prepared as a protocol amendment.. Protocol amendments should also receive written IRB approval before implementation, except when necessary to eliminate immediate hazards to the patients or when the changes involve only logistical or administrative aspects of the trial (e.g., change of study monitor, telephone numbers). In this case, the Sponsor will amend and implement the protocol change and subsequently notify the regulatory authorities and/or the IRB, as appropriate. Note to File may be used for minor clarifications and included in future amendments so as long as it does not impact the safety.

10.7 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, correspondences, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

10.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms.

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Appendix A. Performance Status

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed < 50% of the time. 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	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead.

Appendix B. Response Evaluation Criteria in Solid Tumors (RECIST)

Tumor assessments will be made according to the schedule of assessments. Response and progression will be evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 guidelines [Eisenhauer et al. 2009].

1 Definitions

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

1.1 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray or as ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. *If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol*.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

1.2 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

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

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be

measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified

according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

1.3 Response Criteria

1.3.1 Evaluation of target lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

1.3.2 Evaluation of non-target lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status.

It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

1.3.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* ‘Non-CR/non-PD’ is preferred over ‘stable disease’ for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

1.3.4 Duration of overall response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

1.3.5 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

Appendix C: Contraceptive Guidance and Pregnancy Testing

Definition

Woman of Childbearing Potential (WOCBP): A woman of child-bearing potential is any female (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:

- Has not undergone a hysterectomy or bilateral oophorectomy; or
- Has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months)

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy

  Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
- Postmenopausal female
  - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    ▪
  - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Requirements

Male Participants:
Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following during the protocol:

- Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
- Use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described in Table 6 when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.

Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.**Female Participants:**

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in Table 6.

Table 6 Highly Effective Contraception Methods

Highly Effective Contraceptive Methods That Are User Dependent *Failure rate of <1% per year when used consistently and correctly.*
● Combined (estrogen- and progestogen- containing) hormonal contraception ○ Oral ○ Intravaginal ○ Transdermal ○ Injectable
● Progestogen-only hormonal contraception ○ Oral ○ Injectable
Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
● Progestogen- only contraceptive implant ● Intrauterine hormone-releasing system (IUS) ● Intrauterine device (IUD) ● Bilateral tubal occlusion
● **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
● **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)

Pregnancy Testing

WOCBP should only be included after a negative highly sensitive urine or serum pregnancy test. Following initiation of treatment, pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected; at the time points specified in the Schedule of Activities, and as required locally.

Pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected.