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Supplementary information

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# A therapeutic peptide vaccine for fibrolamellar hepatocellular carcinoma: a phase 1 trial

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## Methods

*Study design and treatment.* This was an open-label, single arm phase 1 clinical trial of a DNAJB1-PRKACA vaccine in combination with nivolumab and ipilimumab, in children or adults with advanced FLC (NCT03299946), conducted at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland. Patients were enrolled between April 2020 and September 2022. DNAJB1-PRKACA vaccine, consisting of a peptide encoding the DNAJB1-PRKACA fusion plus poly-ICLC adjuvant, is administered on weeks 0, 1, 2, 3, 6, 9 during the priming phase of the study. Nivolumab, 3 mg/kg, followed by ipilimumab, 1 mg/kg, were administered every 3 weeks for 4 doses during the priming phase. The first 3 evaluable patients age ≥18 years were treated with 3 doses of the neoantigen-targeted vaccine targeting the DNAJB1-PRKACA fusion kinase as monotherapy (Cycle 1) prior to combination therapy with nivolumab and ipilimumab. After completion of the priming phase, patients received nivolumab at a fixed dose of 480 mg every 4 weeks (one cycle course), for up to two years and booster vaccinations approximately every 3 cycles for up to one year from initial vaccination, until disease progression (defined according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1.), unacceptable toxicity or withdrawal. Dose interruptions and management of immunologic toxicities were in accordance with the protocol. Safety was continuously monitored for unacceptable toxicities.

*Correlative science*. Patients age ≥18 years underwent fresh tumor biopsy (subsequent passes flash frozen, formalin-fixed paraffin-embedded, and preserved in RNAlater) approximately 6 weeks after starting therapy, if considered safe and feasible by the Principal Investigator (PI). Tumor tissue from pediatric patients (age ≥ 12 and < 18 years old) was only obtained if the biopsy/surgery was performed on the child for clinical indications and there was additional material available for research purposes. All patients had research blood drawn at baseline, (prior to first dose of therapy) and prior to the administration of any study drug on D1 of each cycle during the priming phase. During the Maintenance phase, research blood was collected every other cycle (8 weeks +/- 7 days) starting from C6D1. If patients remained on study for > 6 months, research blood was collected every 8 or 12 weeks at the discretion of the PI. Research blood was also collected at the end of the treatment visit.

*Assessments.* Participants were seen every 3 weeks for clinical assessments, including a physical examination for vital signs, performance status (PS), hematology, and biochemistry tests on or within 72 hours before day 1 for the priming phase. During the maintenance phase, patients were seen monthly. Participants were evaluated for radiographic response every 8 weeks (+/- 7 days) for the first 6 months and then every 12 weeks subsequently. All AEs were monitored from registration until 28 days post-vaccine for vaccine-related toxicities and 90 days for immune checkpoint inhibitor-related toxicities. AEs and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0. SAEs that occurred within 100 days of the last treatment or before initiation of a new antineoplastic treatment were also followed and recorded. Following disease progression, patient follow-up took place within 28 days from the last dose (±7 days), with subsequent follow-up (by phone or email) every three months (+/- 2 weeks) until death, withdrawal consent, or study closure to monitor overall survival. Information on other cancer therapies after discontinuation from the study treatment was collected.: If patients discontinued without evidence of disease progression, they were followed until the start of new therapy, death, withdrawal consent, or study closure

*Study endpoints*. The primary trial endpoint were safety and neoepitope-specific T cells against the DNAJB1-PRKACA chimeric transcript in the peripheral blood at week 10 in patients. The safety endpoint was defined as the frequency of toxicities by grade by documenting the drug-related adverse events using the NCI CTCAE Version 5.0. The T cell response was evaluated by the fold change in interferon-producing DNAJB1-PRKACA chimeric transcript-specific CD8 and CD4 T cells after vaccination compared to the unstimulated at 10 weeks. Secondary endpoints include objective response rate (ORR), assessed by RECIST 1.1, duration of response, disease control rate (DCR), progression-free survival (PFS), and overall survival (OS). Exploratory objectives included biomarker and immunological analysis of serial tumor biopsies and peripheral blood samples.The evaluable population of the endpoint of T cell response consists of all patients who receive at least one dose of vaccine and have baseline and post-treatment T cell measures at 10 weeks. Previous studies showed 2-4-fold increase of IFN-γ-producing T cells after vaccination. We anticipated that our regimen would result in at least a 2.5-fold increase of IFN-γ-producing DNAJB1-PRKACA chimeric transcript-specific CD8 and CD4 T cells for 10 weeks with treatment in each cohort. We assumed, conservatively, that the coefficient of variation of IFN-γ-producing DNAJB1-PRKACA chimeric transcript-specific T cell density is 100%, and the correlation of the pre-and post-treatment measure is weak at 0.2. A sample size of 12 evaluable patients provided 81% power to detect a 2.5-fold increase based on a two-sided paired-sample t-test at significance level 0.05. If a subject was removed from study therapy prior to the 12-week immune response assessment, then additional patients will be enrolled to achieve 12 evaluable patients.

The overall response rate (ORR) was estimated as the proportion of subjects who have a complete response (CR) or partial response (PR) using RECIST 1.1 criteria at any time during the study. ORR was reported along with 95% confidence interval. The evaluable population for ORR includes all subjects who have received at least one dose of study drugs and have at least one post-baseline tumor assessment or discontinued due to toxicity or clinical progression prior to post-baseline tumor assessments. Duration of response (DOR) was calculated for subjects who achieved the best overall response of complete response (CR) or partial response (PR) with a vaccine targeting the FLC peptide vaccine in combination with nivolumab and ipilimumab. For such subjects, the duration of response was defined as the number of weeks from the start date of PR or CR (whichever response recorded first) and subsequently confirmed to the first date that recurrent or progressive disease or death was documented. DOR was summarized descriptively. PFS was defined as the time until the earlier date of either PD or death or was otherwise censored at the date of the last follow-up. OS was defined as time to death from any cause or otherwise censored at the date of the last follow-up for patients still alive at the time of analysis. OS curves, OS medians with 95% CIs, were estimated using the Kaplan-Meier methodology. PFS curves, PFS medians with 95% CIs, estimated using Kaplan-Meier methodology. A complete list of the end points and their definitions are provided in the protocol.

*Vaccine formulation and administration.* The study vaccine consists of 24-mer synthetic long peptides encoding the DNAJB1-PRKACA fusion junction in lyophilized form. Peptide was synthesized by JPT Technologies with sequence as follows: H-RKREIFDRYGEEVKEFLAKAKEDF-OH*TFA. The vaccine is a white powder supplied in single dose cryovials containing 0.5 mg FLC peptide.

The peptide was admixed (0.3mg/peptide) with 0.5mg TLR3 agonist poly-ICLC (Hiltonol; Oncovir) in saline prior to vaccine administration at the Investigational Drug Service Pharmacy at Johns Hopkins Hospital.

Poly-ICLC is a synthetic, nuclease-resistant complex of polyinosinic and polycytidylic acid, stabilized with poly-L-lysine and carboxymethylcellulose. Poly-ICLC for injection contains 1.8 mg/mL poly-L-lysine and 5 mg/mL sodium carboxymethylcellulose in 0.9% sodium chloride solution and is adjusted to pH 6-8 with sodium hydroxide. On the day of administration, poly-ICLC and 0.9% sodium chloride, USP are added to the FLC peptide resulting in a final vaccine concentration of 0.3 mg of peptide and 0.5 mg poly-ICLC in total volume of 3 mL. The vaccine was administered subcutaneously at three injection sites.

*Healthy donor PBMCs.* Healthy donor PBMCs were isolated from apheresis rings obtained from the St. Jude Blood Donor Center under Department of Pathology protocol BDC035. All apheresis rings were de-identified before release.

*IFNγ enzyme-linked immunosorbent spot (ELISpot) assay*. The frequency of IFNγ secreting cells was determined using Human IFN-γ (HRP) precoated stripwells (3420-4HST-2, Mabtech, Cincinnati OH) following the Johns Hopkins Immune Monitoring Core SOPs. The tissue culture medium was composed of RPMI-1640 (11875-093 Gibco, Paisley UK) supplemented with l-glutamine (25-005-Cl Mediatech Twerksbury MA), antibiotics (120-095-721 Quality Biological, Gaithersburg MD), 50 uM bME (Sigma, Burlington MA) and 10% Fetal Calf Serum (Hyclone, Logan UT). The peptide pool control (CTL-CEF-002) (CTL Shaker Heights, OH) and peptides (JPT, Berlin, Germany) including the full length 24mer fusion peptide (RKREIFDRYGEEVKEFLAKAKEDF) were used at 2 ug/ml with 200,000 fresh PBMC responders per well and an 18-hour incubation time. Developed assays were read using an iSpot Spectrum Analyzer (AID, Strassburg Germany) and reported as SFU (Spot Forming Units per million cells).

*CyTOF.* Patient PBMC samples from baseline (C1D1) and peak ELISpot response cycle were thawed and plated in a 48-well plate at $2x10^6$ cells per well. The PBMCs were rested at 37°C overnight and then cocultured with 2 ug/mL of the 24mer fusion peptide (RKREIFDRYGEEVKEFLAKAKEDF), 2 ug/mL of an irrelevant 21mer peptide (KRAS G12D; YKLVVVGADGVGKSALTIQLI, which was not detected on molecular profiling of the patient), CD3/CD28 dynabeads beads, or left unstimulated. After a 48-hour stimulation for all conditions, PBMCs were treated with BD GolgiStop™ (Cat. No 554724) for 5 hours and then washed with PBS before for CYTOF staining.

The PBMCs were dissociated in Maxpar® PBS (Standard BioTools) with 2mM EDTA. Cells were stained for viability using Cell-ID™ Cisplatin (Standard BioTools). Subsequently, the cells were quenched in RPMI with 10% FBS and 1 % of 1X Penicillin-Streptomycin and washed with Maxpar® Cell Staining Buffer (Standard BioTools). All samples were stained for 25 minutes at room temperature with a seven-choose-three metal barcoding scheme for up to 32 barcodes using CD45 at each channel. The samples were then washed and blocked with Fc block (Invitrogen) for 10 minutes at room temperature. The PBMCs were stained with a chemokine cocktail for 10 minutes followed by a surface stain antibody cocktail for an additional 30 minutes at room temperature and then fixed with the eBio™ Fixation/Permeabilization Concentrate/Diluent mixture (eBioscience). After two washes, the samples were stained with an intracellular stain antibody cocktail for 30 minutes at room temperature. After a wash, the samples were fixed using 16% Pierce™ Formaldehyde (Thermo Scientific) diluted in Maxpar® PBS to 1.6% PFA and stored until the day of acquisition. On acquisition day, all the PBMCs were stained with DNA Intercalator

Cell-ID™ Intercalator-Rh (Standard BioTools) for 30 minutes at room temperature. Samples were washed and resuspended in CAS + beads buffer (Standard BioTools) to allow the samples to go into the Helios™ mass cytometer. All antibodies (Supplementary Table 11), mass cytometry staining (Supplementary Table 12), and data processing was done as previously described[1].

CyTOF software (Standard BioTools) v6.7 was utilized for randomization, bead normalization, and bead removal of data from mass cytometry acquisition. FlowJo (BD) v10.9 was used to identify single cells and eliminate dead cells. The process of debarcoding the data involved selecting events that showed positivity for specific channel combinations exclusive to each barcode. After debarcoding, each separated sample was saved as an individual fcs file for further analysis. All antibodies, mass cytometry staining, and data processing was done as previously described.

*High-throughput TCR repertoire profiling with TIRTL-seq.* Peripheral blood TCR repertoires were characterized using TIRTL-seq as described previously[2]. Final libraries were sequenced on Illumina NovaSeq platform at read length 2x150bp.

*Raw TCR sequencing data processing.* Raw fastq files from each well were processed with mixcr - v 4.6.0[3] package using the analyze tool. Optional switches were set to analyze the 'generic-amplicon' preset with floating-left-alignment-boundary, floating-right-alignment-boundary for C segment and split-by-sample. All other parameters were set to default. Tag-pattern switch was used to match and split the samples based on plate barcodes incorporated during the PCR I step. Processing steps included demultiplexing raw reads by plate barcode, aligning V, D and J segments, assembling identical reads into clonotypes and frequency-based error-correction.

*TCR pairing with MAD-HYPE and T-SHELL.* TCR pairs were called from TIRTL-seq data as described previously[2] using T-SHELL and reimplemented MAD-HYPE algorithms.

*Identification of expanded/contracted clones.* To identify expanded/contracted clones between timepoints with TIRTL-seq we first average relative frequency (defined as sum of reads mapping to given CDR3β divided by sum of reads mapping to all CDR3βs) across all wells in a plate, including wells where the clone is not present. We filter out clones found in less than 5 wells in either time point to limit the number of comparisons. For each clonotype we calculated standard error of the mean on both timepoints, and called clones significantly expanding/contracting if $\log_2$ fold change between average frequencies on two timepoints is larger than 3 and 2.5 SEM intervals do not overlap. If clone has very low frequency on one of the timepoints and found in fewer than 3 wells we assumed that SEM is twice SEM of clones found in 3 wells at this time point, and pseudocount of $10^{-6}$ was added to all clonal frequencies to get reasonable fold change estimates.

*TCR repertoire analysis.* TCR similarity networks were constructed as described previously[4,5]. Briefly, to measure the distance between TCR α/β clonotypes, we used the TCRdist algorithm implementation from the conga python package[6]. Further analysis was performed using the R language for statistical computing, with merging and subsetting of data performed using the data.table and dplyr packages. stringdist and igraph R packages were used to build TCR similarity networks, and gephi software was used for TCR similarity network layout and visualization. When clustering all TCRs from TIRTL-seq, we found 127 TCRs that were similar (TCRdist≤120) to at least one of the known TCRs. We identified three clusters within these 134 total TCRs by performing dimension reduction via UMAP using the pre-computed TCRdist matrix and using HDBSCAN to cluster points in the two-dimensional UMAP space. We produced a heatmap of the

TCRdist values with hierarchical clustering of the clones, which agreed well with the HDBSCAN clustering. We also produced CDR3 sequence logos with ggseqlogo[7] and Sankey diagrams of V/J gene segment usage for each cluster.

*Hierarchical decision tree analysis.* Decision tree analysis was carried out with the rpart R package using the following variables as input: 1) ELISpot response (positive or negative, with positive response defined as at least 2.5-fold increase of IFN-γ-producing DNAJ-PKAc-specific CD8 and CD4 T cells relative to an unstimulated control); 2) CyTOF response (positive or negative, with positive consisting of a primarily Th response or a combined Th and Tc response); 3) change in number of expanded TCR clones (relative to baseline) from timepoint 2 to timepoint 3 (measured as log2FC); 4) change in TCRdist network connectivity from timepoint 2 to timepoint 3 (measured as change in number of edges in network). The model was built with classification method "Class"; parameters were controlled by setting a minimum of 3 observations to attempt a split and a complexity parameter of 0.1. Only ELISpot response, change in number of expanded clones, and change in TCRdist network connectivity were required to stratify responders, according to the analysis. After determining that the final branch of the decision tree (log2FC in number of expanded clones ≤ 6.4) was driven exclusively by a single patient, we elected to prune this branch and use a final decision tree metric with ELISpot response and change in TCRdist network connectivity as the only components. Area under the ROC curve for each component separately and the combined metric was calculated and plotted using the pROC R package. P-values were obtained by applying Fisher's exact test to the confusion matrix resulting from classifying with each component separately or with the combined metric.

*Antigen-specific T cell expansion.* Patient PBMCs were expanded in the presence of FLC peptide as described previously[48]. Briefly, cryopreserved PBMCs were thawed and plated in a 96-well plate at 1 x $10^5$ cells/well in 200 µL X-VIVO 15 serum-free hematopoietic cell medium (Lonza 04-418Q). On day 0 (same day as thaw), cells were supplemented with human GM-CSF (Miltenyi Biotec 130-095-372) at 1000 IU/mL, human IL-4 (R&D Systems 204-IL-010) at 500 IU/mL, and human Flt3-L (R&D Systems 308-FKN-025) at 50 ng/mL in X-VIVO 15. On day 1, cells were stimulated with either the 24mer vaccinated peptide or a pool of shorter DNAJ-PKAc peptides (GEEVKEFL, REIFDRYGEEV, REIFDRYGEEVKEF, REIFDRYGEEVKEFL, EEVKEFLAKAKEDFL; Genscript) at 1 µM each, R848 (InvivoGen tlrl-r848-5) at 10 µM, LPS (lipopolysaccharide, *Salmonella minnesota*; InvivoGen tlrl-smlps) at 100 ng/mL, and human IL-1β (R&D Systems 201-LB-010) at 10 ng/mL in X-VIVO 15. On days 2, 4, 7, and 10, cells were supplemented with human IL-2 (R&D Systems 202-IL-050) at 10 IU/mL, human IL-7 (R&D Systems 207-IL-025) at 10 ng/mL, and human IL-15 (PeproTech 200-15) at 10 ng/mL in R10 medium (RPMI 1640 supplemented with 10% human serum AB [Gemini Bio-Products 100-512], 10 mM HEPES, 0.1 mg/mL Gentamicin [ThermoFisher Scientific 15750-060], and 1X GlutaMAX [Gibco 35050-061]). On day 11, cells were pooled by stimulation condition and washed with PBS, then stained for activation and effector molecules as described below.

*Activation-induced marker (AIM) assay.* Pooled expanded PBMCs were pulsed with a stimulation cocktail containing either 1 µM 24mer vaccinated peptide or a pool of shorter DNAJ-PKAc peptides (GEEVKEFL, REIFDRYGEEV, REIFDRYGEEVKEF, REIFDRYGEEVKEFL, EEVKEFLAKAKEDFL; Genscript) at 1 µM each, and 1 µg/mL each of anti-human CD28 (BD Biosciences 555725), CD49d (BD Biosciences 555501), and CD40 (Miltenyi 130-094-133) made up in complete RPMI (RPMI 1640, 10% FBS, 2mM L-glutamine, 100 U/mL/100 µg/mL penicillin/streptomycin). An unstimulated negative control condition (complete RPMI, CD28,

CD49d, CD40) was included for each pool. Cells were incubated at 37 °C, 5% $CO_2$, for 6 hours. After incubation, IFNγ-producing cells were labeled using an IFNγ Cytokine Secretion Assay Kit (Miltenyi 130-054-202) according to the manufacturer's instructions. Cells were then washed with FACS buffer (PBS, 2% FBS, 2 mM EDTA), blocked using anti-human Fc block (Biolegend 422302) for 10 minutes at 4 °C, and stained with a cocktail of surface antibodies for 30 minutes at 4 °C. This staining cocktail included 2.5 µL Ghost Violet 510 viability dye (Tonbo Biosciences 13-080-T100), 2 µL TotalSeq-C anti-human hashtag antibody 5, 7, 8, or 10 (one per stimulation condition; Biolegend 394609, 394613, 394615, 394619), and 5 µL each of anti-human CD3 (Brilliant Violet 421-conjugated, Biolegend 344834, clone SK7), anti-human CD8 (Brilliant Violet 785-conjugated, Biolegend 344740, clone SK1), anti-human CD4 (PerCP/Cy5.5-conjugated, Biolegend 344608, clone SK3), anti-human CD154/CD40L (Brilliant Violet 605-conjugated, Biolegend 310826, clone 24-31), anti-human CD137/4-1BB (APC-conjugated, Biolegend 309810, clone 4B4-1), and anti-human CD19 (APC/Fire750-conjugated, Biolegend 302258, clone HIB19). After staining, cells were washed three times with FACS buffer, and live, CD3+ cells positive for IFNγ and/or CD40L were sorted on a BD Biosciences FACS Aria sorter. The gating strategy for this experiment is given in Supplementary Figure 3a.

*10x Genomics single-cell sequencing.* Sorted AIM-positive cells (i.e. cells positive for IFNγ and/or CD40L) for 24mer- and Pool-stimulated conditions were combined with 4000 AIM- unstimulated carrier cells. Combined cell populations were loaded into a single 10x Chromium NextGEM Single Cell 5' V(D)J v2 with Feature Barcoding reaction (10x Genomics 1000265, 1000190, 1000541, 1000252, 1000286, 1000215, 1000250). GEX, TCR, and surface feature (cell hashing) libraries were prepared according to the manufacturer's protocol. Libraries were sequenced on Illumina NovaSeq at read length 2x100bp for GEX libraries and 2x100-150bp for TCR and surface feature libraries.

*10x data analysis.* Raw data were processed using CellRanger version 7.0.1 and aligned to GRCh38-2020-A and vdj_GRCh38_alts_ensembl-7.0.0 Cell Ranger references. The resulting GEX matrices were analyzed using the Seurat R package version 5.0.1[8], and associated TCR clonotypes were added to each Seurat object via the AddMetaData function. Following standard quality control filtering, we discarded low quality cells (nFeatures<200 or >7500, MT% >5%) and non-T cells (CD3E<0), and normalized the data via the NormalizeData function using the default parameters. We then performed cell cycle regression to mitigate effects of cell cycle heterogeneity using the CellCycleScoring and ScaleData functions and found 2000 variable features via the FindVariableFeatures function. Cells were clustered with the resolution parameter set to 0.5. UMAP feature plots were visualized using Seurat.

*TCR cloning and expression.* The sequences of all TCRs cloned in this study are given in Supplementary Table 13; bolded TCRs in this table are validated fusion-specific clones identified in this study. Full-length TCRαβ sequences were reconstructed from V/J gene usage and CDR3 sequences using Stitchr[9]. TCRα and β chain sequences were modified to encode a T48C and S57C point mutation, respectively, to enhance pairing of the transgenic chains[10],. Modified TCRα and TCRβ sequences were joined by a 2A element from Porcine teschovirus-1 (P2A) or thosea asigna virus (T2A) and cloned into one of two lentiviral vectors: 1) pLV[Exp]-Puro-EF1A by Vector Builder or 2) SJL12-MND-GFP-P2A-mCherry (a kind gift from the St. Jude Vector Core Lab) and packaged into lentivirus. For the pLV[Exp]-Puro-EF1A vector, an additional silent mutation for the Cpf1 PAM site was made. When using the pLV[Exp]-Puro-EF1A system, transductions were carried out as follows: On day of transduction, 1 x $10^6$ E6.1 Jurkats containing the pASP90

reporter system (TCR-null, constitutive mCherry, NFAT-eGFP reporter[11]) were plated per well in a 24-well tissue-culture-treated plate in complete RPMI (RPMI 1640, 10% FBS, 100 U/mL/100 µg/mL penicillin/streptomycin) with 8 µg/mL polybrene. 100 µL lentivirus was thawed rapidly at 37 °C and added dropwise to each well to transduce Jurkats. The next day, 1 mL complete media was added to each condition. At 48 hours post transduction cell surface TCR expression was confirmed via flow cytometry using an antibody against human TCRαβ (BV780, Thermo Fisher Scientific 78-9986-42, clone IP26). Cells were then selected using 2ug/mL puromycin containing complete media. Once control cells were dead, transduced cells were grown in complete media and re-checked for TCR positivity via flow cytometry. The bulk population was single cell diluted and plated at 0.25, 0.5 and 1 cell per well of a 96 well flat bottom plate. Plate was checked for single cell populations every 2-3 days for the first week and they were then expanded up. Single cell clones were then verified to be TCR positive via flow cytometry. When using the SJL12-MND-GFP-P2A-mCherry system, transductions were carried out as follows: On day of transduction, 1 x $10^6$ 2D3 Jurkat J76.7 cells (TCR-null, CD8+, NFAT-eGFP reporter; a kind gift from Fumihiro Fujiki ,Department of Cancer Immunology, Osaka University Graduate School of Medicine, Suita, Japan)[12] per well were plated in a 12-well tissue-culture-treated plate in complete RPMI (RPMI 1640, 10% FBS, 2mM L-glutamine, 100 U/mL/100 µg/mL penicillin/streptomycin). Concentrated lentivirus was thawed rapidly at 37 °C and added dropwise to each well to transduce Jurkats. After >48 hours post-transduction, transduction was confirmed by expression of mCherry. All flow cytometry data were collected on the Beckman Coulter CytoFLEX or BD Symphony platforms and analyzed using FlowJo version 10.10.0 software (BD Biosciences).

*NFAT-GFP reporter assay.* TCR-expressing Jurkat cells were cocultured with autologous lymphoblastoid cell lines at a 1:1Jurkat:LCL ratio or with healthy donor PBMCs at a 1:5 Jurkat:PBMC ratio. For HLA blocking assays, LCLs were blocked with anti-pan-HLAII antibody (5 µg/1x$10^6$ cells; US Biological 367160, clone IVA12), anti-HLA-DP (5 µg/1x$10^6$ cells; Abcam ab20897, clone B7/21), anti-HLA-DQ (1 µg/1x$10^6$ cells; Novus Biologicals NBP2-45041, clone SPV-L3), or anti-HLA-DR (5 µg/1x$10^6$ cells; Biolegend 307666, clone L243) in cRPMI for 2 hours (37 °C, 5% $CO_2$) prior to coculture with Jurkats. Each well was pulsed with a stimulation cocktail containing either 10 µM FLC fusion peptide fused to HIV-tat to enhance uptake and presentation[13] (RKREIFDRYGEEVKEFLAKAKEDF-RKKRRQRRR, Genscript) or one of six tiling 15mer peptides crossing the fusion junction (REIFDRYGEEVKEFL, IFDRYFGEEVKEFLAK, DRYGEEVKEFLAKAK, RYGEEVKEFLAKAKE, YGEEVKEFLAKAKED, or GEEVKEFLAKAKEDF; Genscript) made up in complete RPMI (RPMI 1640, 10% FBS, 2mM L-glutamine, 100 U/mL/100 µg/mL penicillin/streptomycin), an unstimulated negative control cocktail (complete RPMI), or a PMA/Ionomycin positive control (1X Cell Stimulation Cocktail [eBioscience] in complete RPMI). Cells were incubated overnight (37 °C, 5% $CO_2$), washed with FACS buffer (PBS, 2% FBS, 2 mM EDTA), and NFAT-GFP expression of mCherry+ cells was assessed via flow cytometry on a custom-configured BD Symphony using FACSDiva software (Becton Dickinson). Data were analyzed using FlowJo version 10.10.0 software (BD Biosciences). A representative gating strategy for these experiments is given in Supplementary Figure 3b. NFAT-GFP+ frequencies were normalized to the unstimulated and positive controls for each sample according to the following equation: Normalized Frequency = (Sample Frequency - Unstimulated Frequency)/(Positive Control Frequency - Unstimulated Frequency).

*Whole transcriptome analysis.* Next generation sequencing (NGS) on primary, responding, and resistant specimens were performed on the Personalis NeXT Dx™ Tumor Profiling platform[14]. This is a whole exome and transcriptome assay with coverage across 20,000 genes (median

sequencing depth ~500X) with enhanced coverage of 247 cancer related genes (median sequencing depth >1500X). Analysis included augmented immunogenomics analysis based on the Personalis ImmunoID NeXT™, including enhanced targeting of HLA genes, MSI-related loci, TCR and BCR repertoire, and coverage of innate and adaptive immune pathways. Gene expression in transcripts per kilobase million (TPM) of key immunological genes involved in adaptive immune response, innate immune response, antigen presentation machinery, checkpoint modulation, chemokines, cytokines, repair and replication defects, and tumor associated antigens were assessed and visualized with corresponding heatmaps.

*HLA typing and neoepitope prediction.* HLA typing for all patients was conducted by at least one of the following methods: 1) typing at a clinical HLA typing facility, 2) typing based on tumor whole transcriptome data using arcasHLA[15], and/or 3) HLA imputation based on TCR repertoire sequencing as described previously[16] using the data generated by TIRTL-seq. Healthy donor PBMCs were HLA typed using AllType FASTplex NGS 11-Loci Amplification kit on DNA extracted from PBMCs. Briefly, high quality DNA was amplified in a multiplexed reaction for all major Class I and Class II HLA alleles. Data were sequenced according to manufacturer's instructions on a full MiSeq lane at 150x150bp. Data were then analyzed using TypeStream Visual NGS Analysis Software (OneLambda). HLA types are given in Supplementary Table 2. Neoepitope prediction was carried out using NetMHCpan 4.1 for class I HLAs and NetMHCIIpan 4.0 for class II HLAs[17]. A peptide was considered a putative strong binder for class I HLAs if its % rank was <0.5% and a putative weak binder if its % rank was <2%. A peptide was considered a putative strong binder for class II HLAs if its % rank was <1% and a putative weak binder if its % rank was <5%. All predicted fusion peptides for HLAs expressed by this patient cohort are listed in Supplementary Table 3.

*RNAscope.* Patient samples were formalin fixed and paraffin embedded (FFPE) prior to sectioning and mounting on Superfrost slides. Slides were stained with BaseScope™ Detection Reagent Kit v2-RED (catalog no. 323910) according to manufacturer's instructions (ACDbio). The BA-Hs-DNAJB1-PRKACA-Junc-C1 probe (catalog no. 1271391-C1) was used which targets base pairs 74-115 to validate its presence at the RNA level. For a negative control the BA-DapB-1zz negative control probe (catalog no. 701021) was used to target bacterial gene dapB. Slides were sectioned and scanned with the Nano Zoomer S210 Hamamatsu by the Johns Hopkins University Oncology Tissue Services (SKCCC). Images were quantified using QuPath-0.4.4 digital pathology software[18]. Cells were detected and subcellular fractionation was used to identify positive DNAJ-PKAc puncti. Total number of cells and puncti were calculated for five regions per lesion. Number of puncti per cell were calculated per region and graphed in GraphPad Prism 9. Significance between the lesions was calculated using a one-way ANOVA test.

*Immunohistochemistry (IHC).* FFPE slides were sectioned and stained with antibodies against HLA-I (Clone EMR8-5), HLA-II (Clone CR3/43), CD3 (Clone LN10), TIM3 (Clone BLR033F) and LAG3 (Clone 17B4), and imaged with the Nano Zoomer S210 Hamamatsu via the Johns Hopkins University Oncology Tissue Services (SKCCC). Images were quantified using the digital image analysis software HALO-v3.6.4134. Five representative regions of each lesion were annotated and the number of positive cells calculated per region calculated. Significance between lesions was calculated using a one-way ANOVA test.

*IMC Staining and Acquisition.* Immunohistochemical staining was performed through mass cytometry antibodies (Supplementary Table 12). Resected tumor slides were baked at 60°C for 2 hours, dewaxed in xylene, then rehydrated in a descending alcohol gradient (100%, 95%, 80%, and 70% EtOH). The slides were incubated in Antigen Retrieval Agent pH 9 (Agilent® S2367) at 96°C for 1 hour before blocking with 3% BSA in Maxpar® PBS at room temperature for 45

minutes. The mass cytometry antibody cocktail listed in Supplementary Table 11 was prepared and used to stain the slides at 4°C overnight. All custom antibodies were diluted to a concentration of 0.25 to 0.5mg/mL and were titrated empirically. Cell-ID™ Intercalator-Ir (Standard Biotools PN 201192A) was used for DNA labeling at a 1:400 dilution in Maxpar® PBS. Ruthenium tetroxide 0.5% Aqueous Solution (Electron Microscopy Sciences PN 20700-05) was diluted at 1:2000 in Maxpar® PBS and used as a counterstain. Images were acquired through the Hyperion Imaging System (Standard BioTools) at the Johns Hopkins Mass Cytometry Facility. After image acquisition, representative images were visualized and generated through MCD™ Viewer (Standard BioTools).

**References**


1. Sidiropoulos DN, Stein-O'Brien GL, Danilova L, et al. Integrated T cell cytometry metrics for immune-monitoring applications in immunotherapy clinical trials. *JCI Insight*. 2022;7(19). doi:10.1172/jci.insight.160398

2. Pogorelyy MV, Kirk AM, Adhikari S, et al. TIRTL-seq: Deep, quantitative, and affordable paired TCR repertoire sequencing. Published online October 31, 2024:2024.09.16.613345. doi:10.1101/2024.09.16.613345

3. Bolotin DA, Poslavsky S, Mitrophanov I, et al. MiXCR: software for comprehensive adaptive immunity profiling. *Nat Methods*. 2015;12(5):380-381. doi:10.1038/nmeth.3364

4. Mudd PA, Minervina AA, Pogorelyy MV, et al. SARS-CoV-2 mRNA vaccination elicits a robust and persistent T follicular helper cell response in humans. *Cell*. 2022;185(4):603-613.e15. doi:10.1016/j.cell.2021.12.026

5. Minervina AA, Pogorelyy MV, Kirk AM, et al. SARS-CoV-2 antigen exposure history shapes phenotypes and specificity of memory CD8+ T cells. *Nat Immunol*. 2022;23(5):781-790. doi:10.1038/s41590-022-01184-4

6. Schattgen SA, Guion K, Crawford JC, et al. Integrating T cell receptor sequences and transcriptional profiles by clonotype neighbor graph analysis (CoNGA). *Nat Biotechnol*. 2022;40(1):54-63. doi:10.1038/s41587-021-00989-2

7. Wagih O. ggseqlogo: a versatile R package for drawing sequence logos. *Bioinforma Oxf Engl*. 2017;33(22):3645-3647. doi:10.1093/bioinformatics/btx469

8. Hao Y, Stuart T, Kowalski MH, et al. Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nat Biotechnol*. 2024;42(2):293-304. doi:10.1038/s41587-023-01767-y

9. Heather JM, Spindler MJ, Alonso MH, et al. Stitchr: stitching coding TCR nucleotide sequences from V/J/CDR3 information. *Nucleic Acids Res*. 2022;50(12):e68. doi:10.1093/nar/gkac190

10. Kuball J, Dossett ML, Wolfl M, et al. Facilitating matched pairing and expression of TCR chains introduced into human T cells. *Blood*. 2007;109(6):2331-2338. doi:10.1182/blood-2006-05-023069

11. Bear AS, Blanchard T, Cesare J, et al. Biochemical and functional characterization of mutant KRAS epitopes validates this oncoprotein for immunological targeting. *Nat Commun*. 2021;12(1):4365. doi:10.1038/s41467-021-24562-2

12. Morimoto S, Fujiki F, Kondo K, et al. Establishment of a novel platform cell line for efficient and precise evaluation of T cell receptor functional avidity. *Oncotarget*. 2018;9(75):34132-34141. doi:10.18632/oncotarget.26139

13. Lu J, Wettstein PJ, Higashimoto Y, Appella E, Celis E. TAP-independent presentation of CTL epitopes by Trojan antigens. *J Immunol Baltim Md 1950*. 2001;166(12):7063-7071. doi:10.4049/jimmunol.166.12.7063

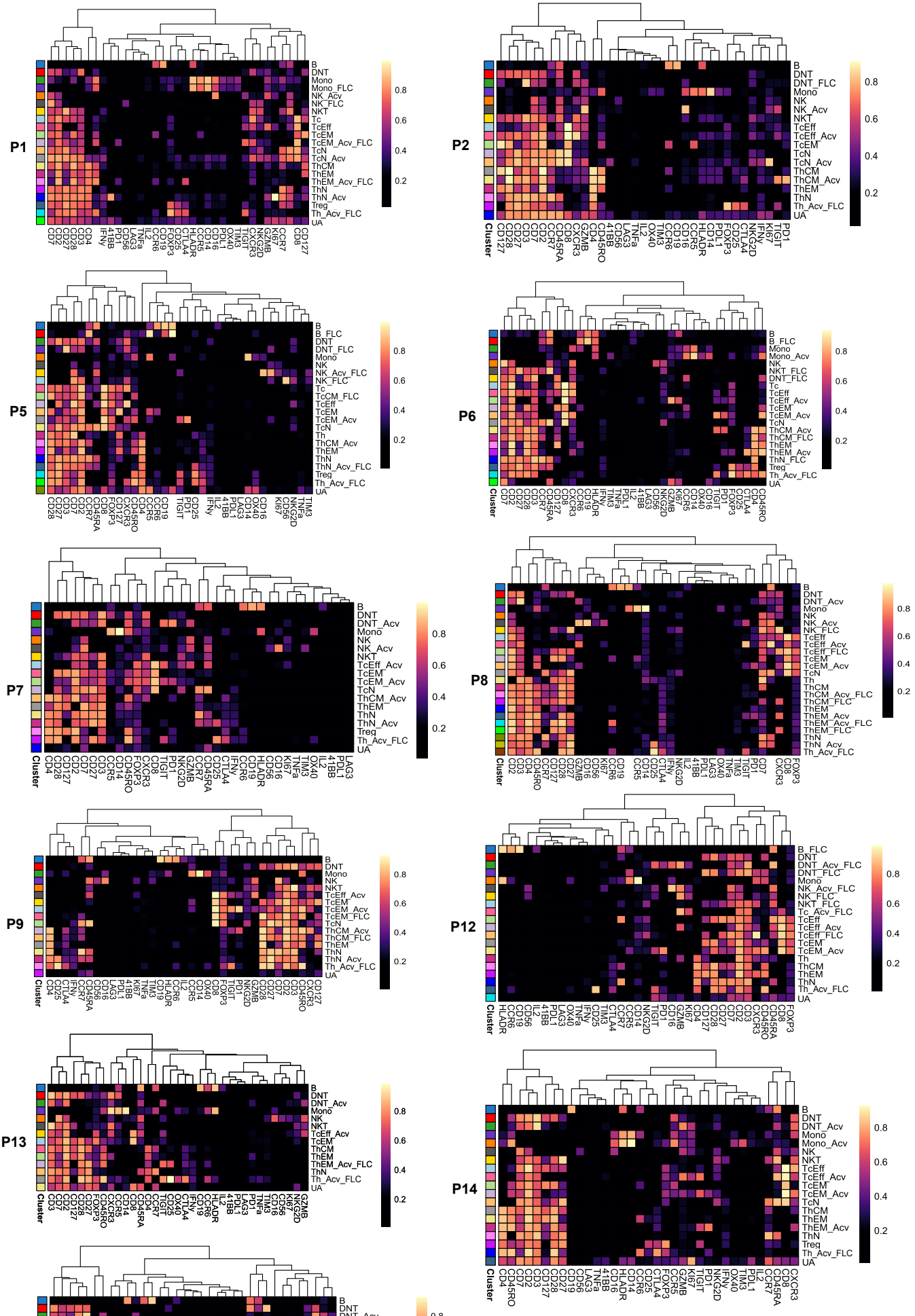
14. Patwardhan A, Harris J, Leng N, et al. Achieving high-sensitivity for clinical applications using augmented exome sequencing. *Genome Med*. 2015;7(1):71. doi:10.1186/s13073-015-0197-4

15. Orenbuch R, Filip I, Comito D, Shaman J, Pe'er I, Rabadan R. arcasHLA: high-resolution HLA typing from RNAseq. *Bioinforma Oxf Engl*. 2020;36(1):33-40. doi:10.1093/bioinformatics/btz474

16. Minervina AA, Komech EA, Titov A, et al. Longitudinal high-throughput TCR repertoire profiling reveals the dynamics of T-cell memory formation after mild COVID-19 infection. Krishna S, Davenport MP, Cowell L, eds. *eLife*. 2021;10:e63502. doi:10.7554/eLife.63502

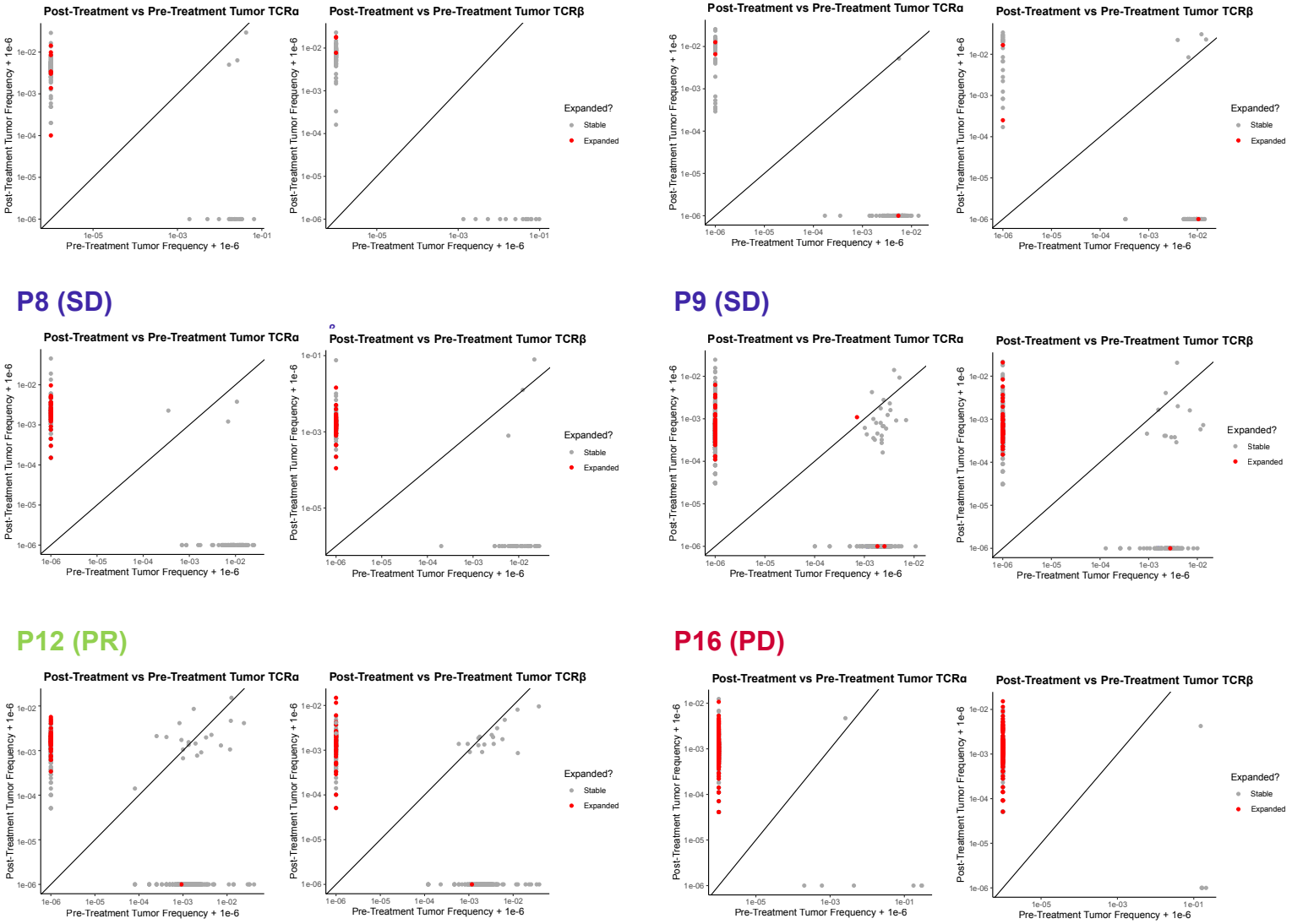
17. Reynisson B, Alvarez B, Paul S, Peters B, Nielsen M. NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. *Nucleic Acids Res*. 2020;48(W1):W449-W454. doi:10.1093/nar/gkaa379

18. Bankhead P, Loughrey MB, Fernández JA, et al. QuPath: Open source software for digital pathology image analysis. *Sci Rep*. 2017;7(1):16878. doi:10.1038/s41598-017-17204-5

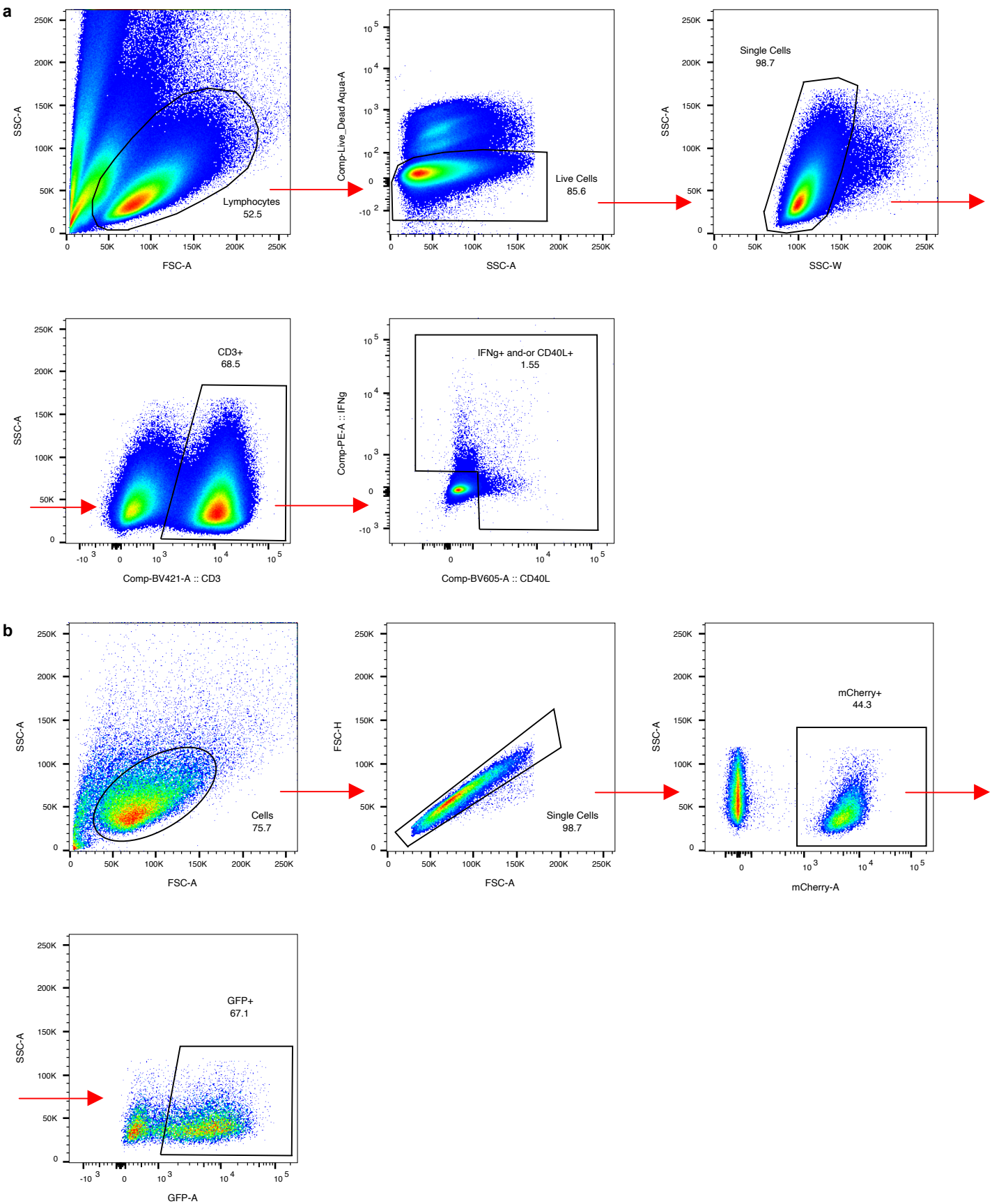



**Supplementary Figure 1.** Heatmap of the normalized expression for all markers used during FlowSOM from baseline and post-treatment samples using a lymphoid cell-oriented CyTOF panel. Cell subtypes listed on the right were assigned based on differential expression of the markers listed across the bottom

*ThEFF, effector T cells, ThEM, effector memory T cells. UnB, unstimulated Baseline, Un P, unstimulated post- treatment.*




**Supplementary Figure 2.** Scatterplots showing the relative intratumoral frequencies of TCR clones detected by TIRTL-seq for six patients who had paired pre- (x-axis) and post-treatment (y-axis) tumor biopsies. Clones that were statistically expanded relative to baseline in peripheral blood after two or more cycles of treatment (C3D1+) are red, and clones that were stable in peripheral blood are gray. Patient labels are color coded by clinical response (red=PD, blue=SD, green=PR).




**Supplementary Figure 3. a.** Gating strategy for AIM assay and 10X Genomics scRNAseq experiment. **b.** Gating strategy for all NFAT-GFP reporter TCR screening assays.

If appropriate for this study, a scanned copy of the signed consent form should be uploaded to the participant's Epic/EMR record.

Patient I.D. plate

# COMBINED PARENTAL PERMISSION/RESEARCH PARTICIPANT INFORMED CONSENT AND PRIVACY AUTHORIZATION FORM

**Protocol Title**: **A Pilot Study of a DNAJB1-PRKACA Fusion Kinase Peptide Vaccine Combined with Nivolumab and Ipilimumab for Patients with Fibrolamellar Hepatocellular Carcinoma**

**Main Consent**
**Cohort A: Patient #4 and Beyond**
**Cohort B: All**
**Cohort C: All**

**Application No.**: **IRB00222681**

**Principal Investigator**: **Mark Yarchoan, MD**
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You are being asked to join a research study. Participation in this study is voluntary. Even if you decide to join now, you can change your mind later.

If you are a parent or legal guardian of a child who may take part in this study, your permission is required for your child to participate. The assent (agreement) of your child may also be required. When we say "you" in this consent form, we mean you or your child.

## 1. Research Summary (Key Information):

The information in this section is intended to be an introduction to the study only. Complete details of the study are listed in the sections below. If you are considering participation in the study, the entire document should be discussed with you before you make your final decision. You can ask questions about the study now and at any time in the future.

The purpose of this research study is to determine the safety of the DNAJB1-PRKACA peptide vaccine (FLC peptide vaccine ) as well as to determine how the vaccine affects your immune system. This research study will enroll 3 different groups of people, called cohorts. Cohort A will be for people with Fibrolamellar Hepatocellular Carcinoma (FLC) who have never had treatment with a group of drugs called immune checkpoint inhibitors. Cohort B will be for people with FLC who have already received treatment with immune checkpoint inhibitors. Cohort C will be for people with cancers other than FLC whose cancer has the DNAJB1-PRKACA fusion. This study is the first to study this combination of vaccine and drugs in FLC and non-FLC cancer. Nivolumab (OPDIVO®) and ipilimumab (YERVOY®) are now approved for use in FLC and will no longer be supplied by BMS. The cost of those drugs will now be charged to participants/their insurance instead of being covered by the study.

If you are eligible to participate in this study based on the screening tests (described below), you will receive the FLC peptide vaccine weekly for the first cycle. The participants enrolled on this portion of the study will receive the combination of the FLC peptide vaccine, nivolumab and ipilimumab on day 1 of a 3 week cycle, for a total of 4 cycles (Cycle 1-4, priming phase).

After completing the priming phase, you will begin the maintenance phase of the study. During this phase, you will receive nivolumab on day 1 of a four week cycle, and FLC peptide vaccine every 12 weeks (see **Table 1, Section 3**).

You will have tests, exams and procedures that are part of your standard of care and for study purposes. You will be in this study as long as your cancer is not getting worse, you do not have bad side effects, and you wish to continue. There are risks to these study drugs that are described later in this document. Some risks could be serious and not all of the side effects/risks are known. There may or may not be a benefit to you from being in this study.

## 2. Why is this research being done?

This research is being done to evaluate the safety and the immune effects of combining the FLC peptide vaccine with immunotherapy agents, nivolumab and ipilimumab, in people with fibrolamellar hepatocellular carcinoma (FLC), which is metastatic (spread to other areas of the body) or unresectable (not able to be removed by surgery) and in non-FLC solid tumors. Another goal of the study is to learn more about how the immune system responds and how a tumor might respond to the drugs given in the study.

*Are there any investigational drugs?*

**FLC peptide vaccine**

Researchers have determined that many people with FLC have an underlying genetic defect that results in the abnormal (mutant) combination of two genes, the DNAJB1 and the PRKACA genes. Genes produce proteins that have several functions within the body. The combination of these two genes creates a new gene called a “chimeric” gene (DNAJB1-PRKACA fusion), which in turn produces an abnormal protein product. This genetic abnormality is not found in normal liver tissue or elsewhere in other organs.

FLC peptide vaccine is a vaccine that is made up small pieces (peptides) of the abnormal protein. This abnormal protein may cause cancer cells to grow and spread in the body. This study will test whether the FLC peptide vaccine is safe and whether it can teach the body’s immune system to recognize and stop cancer cells with mutant DNAJB1-PRKACA fusion kinase.

The use of the FLC peptide vaccine is investigational. The word "investigational" means that the FLC peptide vaccine is not approved for marketing by the Food and Drug Administration (FDA). The FDA is allowing the use of the FLC peptide vaccine in the study.

**Nivolumab (OPDIVO®)**
Nivolumab is an anti-PD-1 antibody. It works by attaching to and blocking a molecule called PD-1. PD-1 is a protein that is present on different types of cells in your immune system and controls parts of your immune system by shutting it down. Antibodies that block PD-1 can potentially prevent PD-1 from shutting down the immune system, thus allowing immune cells to recognize and destroy cancer cells. Nivolumab is approved by the FDA for the treatment of fibrolamellar hepatocellular carcinoma, melanoma, metastatic non-small cell lung cancer, advanced renal cell carcinoma and several other indications.

**Ipilimumab (YERVOY®)**
Ipilimumab is a human monoclonal antibody that acts against CTLA-4. An antibody against CTLA-4 can stop CTLA-4 from turning off the immune system, allowing the immune reaction to continue. The body's immune reaction may help the body to destroy cancer cells. Ipilimumab is approved by the FDA for the treatment of fibrolamellar hepatocellular carcinoma, melanoma, and advanced renal cell carcinoma among other conditions.

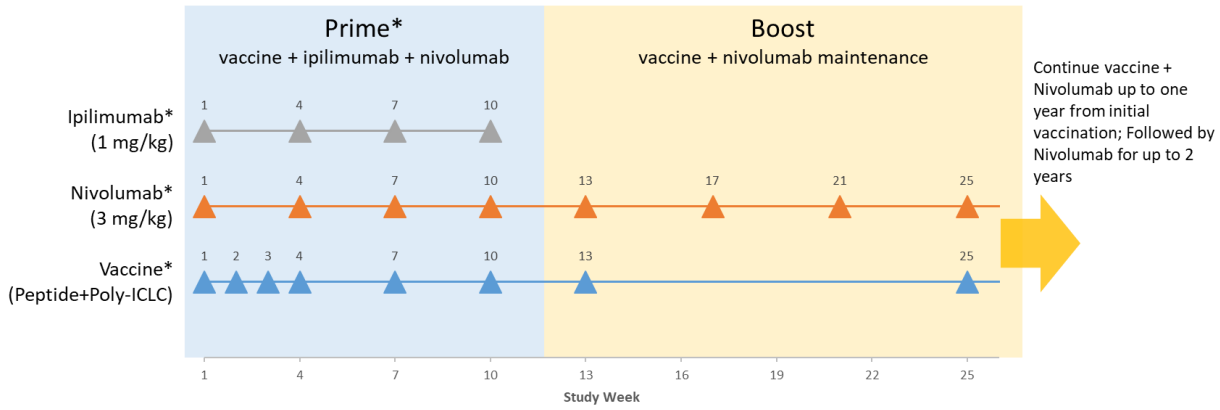
Nivolumab and ipilimumab have been administered to thousands of patients on clinical trials, alone or in combination with either drugs. However the effects of these drugs in combination with FLC peptide vaccine in fibrolamellar hepatocellular carcinoma is not known.

**Who can join this study?**
Cohort A and B: People 12 years or older with unresectable fibrolamellar hepatocellular carcinoma (FLC) may join.

Cohort C: People 18 years or older with unresectable solid tumors may join.

## 3. What will happen if you join this study?

The overall design of the study depicted below:




*Note: The first 3 evaluable participants 18 years or older will receive 3 doses of the FLC peptide vaccine as monotherapy (Cycle 1) prior to combination therapy with nivolumab and ipilimumab.

This is an open-label study, which means that you and your study doctor will know which study regimen you are assigned to receive. There is no placebo (a substance that looks like the study drug but does not contain any active drug) on this study. All patients receive the same regimen with FLC peptide vaccine plus nivolumab and ipilimumab.

If you agree to be in this study, we will ask you to do the following things:

The study will consist of the following parts:

- Screening evaluation
- Dosing period and response assessments
- Office visit after last dose
- Post-study follow-up

**Comprehensive Tumor Profiling**

During the pre-screen phase of the study, your tumor samples will be tested by an outside laboratory with The Caris Molecular Intelligence Comprehensive Tumor Profiling (Caris Life Sciences), or comparable molecular testing to determine whether your cancer has certain features, including the above mentioned DNAJB1-PRKACA fusion gene. We would like to perform research on tumor samples to see if there is a way to predict how people will respond to the study drugs, and carry out further research into the nature and cause of fibrolamellar hepatocellular carcinoma.

**Screening Visit**

You will be asked to sign this consent form after you have read it and all of your questions about the study have been answered. The tests listed below will be done to see if you qualify for the study. Not everyone who wants to be in the study will be able to join. These tests and procedures are likely to be part of your regular cancer care and may be done even if you do not take part in this study. If you have had some of these tests done recently, they may not have to be repeated.

The following tests will be performed:

- Review of your medical and cancer history including current and recent medications
- Physical exam and evaluation of your ability to perform everyday tasks
- Measurement of vital signs (blood pressure, heart rate, breathing rate, temperature, blood oxygen level), height and weight
- A computed tomography (CT) scan of your abdomen, chest, and pelvis. A CT scan uses x-rays and a computer to produce a 3-dimensional image of your body. In addition, the study doctor may decide that you need other tests such as an MRI (magnetic resonance imaging) to evaluate your cancer. If another CT scan was done 3 weeks or more prior to the study screening, information from that test may be collected and used.
- Blood tests and urine tests to assess your study eligibility and general health. The total amount of blood taken during this visit will be about 1 tablespoon and the total amount of urine will be about 3 ounces.
- Blood tests for pregnancy in women who are able to become pregnant. The pregnancy test must be negative for you to continue in the study.
- Resting electrocardiogram (ECG) that measures the electrical activity of your heart at rest.

In order to participate in this study, your tumor must be tested for the presence of the DNAJB1-PRKACA fusion gene if not tested previously. Archival surgical specimens may be used for testing. If you are less than 18 years old, we will use the archival biopsy sample (a biopsy sample that was already taken before

you decided to participate in the study). Tumor tissue from pediatric patients (age ≥ 12 and < 18 years old) may only be obtained if the biopsy or surgery is being performed on the child for other clinical reasons and there is additional tissue. Optional (research only) biopsies cannot be obtained on pediatric patients.

If you are 18 years or older, and an archival biopsy sample is not available, a fresh biopsy may be required. A biopsy is a procedure using a needle to remove a piece of tissue or a sample of cells from your body so that it can be analyzed in a laboratory and is used to diagnose or evaluate the cancer. The study doctor will explain this procedure and its risks in more detail with you. You will sign a separate consent form before the biopsy is taken. This will be a standard surgical consent form from the institution where the biopsy procedure takes place. Your tissue will be sent to a laboratory to see if the DNAJB1-PRKACA fusion gene is present in your tumor.

If the results are not available within 3 weeks from the screening visit, you may enroll and begin the study regimen. If you are later found not to have the DNAJB1-PRKACA fusion gene, the study doctor will determine if you should continue with ipilimumab and nivolumab (without vaccine), or if you should discontinue from all study drugs.

Individual results will be shared with you or your doctor.

In addition, we will collect tumor tissue samples from a previous surgery or biopsy to assess tumor characteristics and the immune system.

The study team will check the results of these screening tests to see if you meet the requirements to continue to take part in the study. If you would like to continue to participate in the study, you must also agree to the following:

- You will agree to attend all scheduled visits and procedures.
- Women who are capable of becoming pregnant must agree to use an acceptable method of highly effective contraception to prevent pregnancy for the entire time you are on the study and for 5 months after the last study drug. Men who have female partners who are capable of becoming pregnant, are pregnant, or are breastfeeding, must agree to use a barrier contraceptive method (a condom, for example) to prevent the transfer of fluids during the study and for 7 months after the last study drug.
- If you have certain medical history or conditions or are taking certain medications, you may not be able to participate or continue on the study.
- You will **not** take non-study chemotherapy or radiation within 14 days before getting any of the study drugs. **Note:** Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed after consultation with the study doctor while on the study.
- You will **not** take any non-study chemotherapy or immunotherapy (approved or investigational) during the study regimen period.
- You will **not** take steroids during the study or within 7 days before receiving a dose of study drug, unless required for the treatment of a drug-related side effect.
- You will **not** receive any live vaccines (vaccines that prevent disease), such as measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, typhoid [oral] vaccine, and Flu-Mist influenza vaccines, within 28 days prior to the first dose of trial drug and while participating on the trial.

The study investigator or staff will also explain other requirements for participation in the study. The screening visit will be performed within 21 days of the first study drug visit.

**Study Regimen Visits**

If you qualify for the study, you will then begin the dosing phase of this study. You will receive the following study drug regimen:

The order of administration is FLC peptide vaccine, nivolumab, and then ipilimumab.

- *FLC peptide vaccine*: The study doctor will review the results of your tests prior to each cycle and decide whether you should receive the study drug. If your results meet certain criteria, you will receive 3 injections of the FLC peptide vaccine, just under your skin of your upper thighs, arms and non-dominant arm. You might receive numbing cream over the areas where the injection will be done (lidocaine if you are not allergic) to reduce the pain from the injections. This will be done at least 1 hour before the injections. You will be monitored for 30 minutes after the injections to see if you have any negative reactions to the FLC peptide vaccine.

- *Nivolumab:* You will receive nivolumab after the FLC peptide vaccine unless you have side effects and the study doctor feels that you should not receive the nivolumab. The nivolumab is infused into your vein. This infusion will last about 30 minutes and you will be monitored for 30 minutes after the infusion.

- *Ipilimumab:* You will receive ipilimumab after nivolumab unless you have side effects and the study doctor feels that you should not receive the ipilimumab. The ipilimumab is also infused into your vein. This infusion will last 30 minutes you will be monitored for 30 minutes after the infusion.

  If you have a side effect that requires a procedure as part of your medical care, we will collect the tissue or cell samples to evaluate your cancer and your body's response to the study drugs.

**Priming Phase** ***(first 5 cycles; cycle= 3 weeks)***

**Study Visit Cycle 1**

Your first on study visit will be on Day 1 of Cycle 1. You will receive the FLC peptide vaccine, nivolumab and ipilimumab. You will have the following tests done:

- A short medical history documenting any changes in medications or health problems since the screening visit, performance status, and vital signs (blood pressure, heart rate,), temperature, pulse oximetry (blood oxygen level), and weight
- A brief physical examination
- A urine pregnancy test for women who are able to become pregnant
- Blood for safety and research purposes. The total amount of blood taken during this visit will be about ½ of a cup.

You will return the next 2 weeks (**Day 8 and Day 15**) to receive the FLC peptide vaccine alone. A review of any health issues you experienced since the last visit and any medications that you took since the last visit and collect your vital signs (blood pressure, heart rate, temperature).

**Study Visit Cycle 2, 3 and 4**

You will come back every 3 weeks to receive the FLC peptide vaccine, nivolumab, and ipilimumab.

On day 1 of each cycle, you will have the following tests done. Some of these tests may be done up to 3 days before the visit:

- Review of any changes in your health or medications since your last visit
- Physical exam and evaluation of your ability to perform everyday tasks

- Measurement of vital signs (blood pressure, heart rate, temperature, blood oxygen level) and weight
- Blood for safety and research purposes. The total amount of blood taken during this visit will be about 1/2 of a cup. Additional research blood draws may be obtained at the discretion of the PI for patients with clinical responses of interest.
- Urine pregnancy test for women who are able to become pregnant
- CT scans of the chest, abdomen and pelvis (or MRI if you are allergic to IV contrast) will be done to check the size of your tumor(s) during the study. This will be done at screening and at Cycle 4.
- If you are 18 years old or older, you may have a tumor biopsy to remove a small piece of your tumor using a needle will be performed about 6 weeks after initiation of therapy, prior to Cycle 3 Day 1. An optional tumor biopsy may be performed at the time of progression. All biopsies are optional and at the discretion of the PI.

**Maintenance phase *(Cycle 5 and beyond, cycle = 4 weeks)***
On day 1 of each cycle, you will receive nivolumab on day 1 of a four week cycle and FLC peptide vaccine every 12 weeks you will have the following tests done. Some of these tests may be done up to 3 days before the visit:

- Review of any changes in your health or medications since your last visit
- Physical exam and evaluation of your ability to perform everyday tasks
- Measurement of vital signs (blood pressure, heart rate, temperature, blood oxygen level) and weight
- Blood for safety and research purposes. The total amount of blood taken during this visit will be about 1/2 cup. Blood will be collected at Cycle 5 and every 8-12 weeks during the maintenance phase. Additional research blood draws may be obtained at the discretion of the PI for patients with clinical responses of interest.
- Urine pregnancy test for women who are able to become pregnant
- CT scans of the chest, abdomen and pelvis (or MRI if you are allergic to IV contrast) will be done to check the size of your tumor(s) during the study. This will be done at Cycle 5 and every 8 weeks thereafter. If there is no evidence that your tumor has worsened, the study doctor might decide to get these tests done every 12 weeks.

Maintenance therapy with nivolumab may continue for up to two additional years.

If the radiographic assessment of your cancer by CT, MRI or lung X-ray shows that your cancer may have worsened, you may be asked to have another radiographic assessment later to confirm. The study doctor will discuss your options during the period between scans, which may include the following:

- Discontinue from the study and instead receive an approved, licensed drug which may shrink your tumor, delay progression of your cancer, provide symptom relief or prolong survival, or;
- You may be eligible to receive a different investigational drug on another study.
- If you are 18 years old or older, an additional research biopsy may be done if there is disease progression.

**Follow-Up Visit**
The following evaluations will be performed during a clinic visit after your last dose of study drug (about 30 days after last dose of study drug):

- Review of any changes in your health or medications since your last visit
- Physical exam and evaluation of your ability to perform everyday tasks

- Measurement of vital signs (blood pressure, heart rate, breathing rate, temperature, blood oxygen level) and weight
- Blood for safety and research purposes. The total amount of blood taken during this visit will be about 1/2 cup.
- Resting electrocardiogram (ECG) that measures the electrical activity of your heart at rest.
- CT scans of the chest, abdomen and pelvis (or MRI if you are allergic to IV contrast) will be done to check the size of your tumor(s) during the study. This will not be repeated if you have already had one within 6 weeks.

**Post-Study Follow Up**

After the follow-up visit, you will return for a visit at 100 days after your last dose of study drug (or before your start a new treatment for your cancer, whichever comes first) to ask about your health and medications. Your doctor is responsible to ensure that you receive the appropriate standard of care or other treatment required to treat your condition.

If you do not have recurrence of your cancer prior to stopping the study drugs, we will continue to review any changes in your health or medications since your last visit and collect copies of your CT scans until 1) you may start a new anti-cancer therapy, 2) your cancer gets worse, 3) withdrawal of your consent or 4) the study closes, whichever occurs first.

If you have growth of your cancer, the study staff will contact you by phone or email as per standard of care to ask about your health and other cancer therapies.

**Incidental Findings**

As part of this research study, you will undergo an imaging procedure. A qualified professional will review your research imaging. This research imaging will not include the full diagnostic information that you would get if your primary doctor referred you for imaging.

There is a possibility that while reviewing your imaging we may see an unexpected abnormality. This is called an "incidental finding."

We will let you know if we see such an incidental finding. Depending on the type of incidental finding, we may contact you by mail, email, or phone. In the case of a potential serious emergency, someone may go to your home.

A qualified person (usually a member of the research team) will talk to you if there is an incidental finding. You do not have an option to decline information about an incidental finding from an imaging procedure. If you want, we will give information about this incidental finding to your primary doctor or we will refer you to an appropriate doctor for further evaluation.

What could happen if there is an incidental finding?

- An incidental finding may cause you to feel anxious.
- Since a report of the incidental finding will be part of your medical record, it will be available to those accessing your medical record for your clinical care and may affect your current or future life or health insurance coverage. This risk will vary depending on the type of insurance plan involved.

The costs for any care that may come from the incidental finding, such as the need to see a doctor to diagnose or treat an incidental finding, will not be paid for by this research study. These costs would be your or your insurance company's responsibility.

**Will research test results be shared with you?**
We will share with you the results regarding the presence DNAJB1-PRKACA fusion gene mutations in your tumor samples collected prior to the screening process for this study or during the study tumor biopsy.

This study involves also other research tests that we do not expect will be useful for your clinical care. We will not share these results with you.

**How long will you be in the study?**
As long as your cancer is not getting worse, you do not have bad side effects, and you wish to continue, you will continue nivolumab and the vaccination for up to one year from initial vaccination. Maintenance therapy with nivolumab may continue for up to two additional years.

## 4. What happens to data and biospecimens that are collected in the study?

If you join this study, your data and biospecimens will be used to answer the research question and your data will be used to publish the findings of this study. Examples of biospecimens include blood, tissue, saliva, urine, bone marrow, cells, etc. The specific types of biospecimens that will be collected in this study may include the following: blood, tumor tissue, and cells. Most biospecimens contain DNA, which is the genetic code for each person.

You will not own the data and/or biospecimens collected from you as part of this research study. If researchers use them to create a new product or idea, including those that may have commercial value, you will not benefit financially from these efforts.

This study involves genetic testing on samples that you provide. The Genetic Information Nondiscrimination Act (GINA) is a federal law that helps reduce the risk of discrimination by health insurers or employers based on your genetic information. GINA does not protect you from discrimination if you apply for other types of insurance (such as life, disability or long-term care). GINA also does not protect you against discrimination based on an already-diagnosed genetic condition or disease.

Genetic information is unique to you and your family. Even without your name or other identifiers, it may be possible to identify you or other members of your family with your genetic information.

Johns Hopkins follows procedures so that people who work with your DNA information for research cannot discover it belongs to you, unless you have given consent. However, new techniques may be developed that in the future make it easier to link your genetic data to you, so we cannot promise that your genetic information will never be linked to you.
Johns Hopkins researchers and their collaborators may use the data/biospecimens collected in this study for future research purposes and may share some of the data/biospecimens with others. Future research may include:

- Genetic research: Study of human DNA to find out what genes and environmental factors contribute to diseases. Each cell contains your complete DNA.
- Gene sequencing: Gene sequencing of your DNA provides researchers with the code to your genetic material.
- Cell line creation: Cell lines can be grown in the laboratory. This allows researchers to have an unlimited supply of your cells in the future without asking for more samples from you.

Because science constantly advances, we do not yet know what future testing may include. This future research may be unrelated to the current study and may include outside collaborators. If biospecimens

are tested/used in ways not described above, further IRB review and approval may be needed and the IRB will determine whether additional consent is required.

Sharing data and/or biospecimens is part of research and may increase what we can learn from this study. Often, data/biospecimen sharing is required as a condition of funding or for publishing study results. It also is needed to allow other researchers to validate study findings and to come up with new ideas. Your data and/or biospecimens may be shared directly with researchers at Johns Hopkins and other institutions, for-profit companies sponsors, government agencies, other research partners. Some of our research partners may be international. Your data and/or biospecimens may also be put in government or other databases/repositories. .

We (Johns Hopkins) will do our best to protect and maintain your data/biospecimens in a safe way. One of the ways we protect data/biospecimens is by limiting the uses of the information and the type of information that is shared, especially your personal information. This may occur through data/specimen sharing agreements and review by oversight groups within Johns Hopkins.

If data/biospecimens are used or shared with types of information that may be likely to identify, you such as your name, address or medical record number, further institutional review and approval would be required. In these cases, Johns Hopkins will review whether additional consent from you is required.

Generally, if your data/biospecimens are used/shared without any personal identifiers or with information that is less likely to identify you (such as the date of a procedure), further review and approval is not needed.

Data/biospecimen sharing could change over time, and may continue after the study ends.

The use and sharing of your data is required for participation in this research study. If you are not comfortable with the use and sharing of your data/biospecimens in future research without further consent, you should not participate in this study.

This research includes sequencing of some, or all of your genes. Sequencing all of your genes is called whole genome sequencing. Genes are found in every cell in your body and they provide instructions to your body for how to grow and live. Genes have information that is unique to you and your family. It is now possible to figure out the makeup of every gene in your body by a process called sequencing. Since your genes are unique, it may be possible to identify you or other relatives by gene sequencing, even without your name or personal information. It is also possible that gene sequencing can identify genetic changes that cause, or put you at risk, for medical conditions.
Your tissue sample may be used to make a living tissue sample (called a "cell line") that can be grown in the laboratory. This allows researchers to have an unlimited supply of your cells in the future without asking for more samples from you. Each cell contains your complete DNA.

*What you should know about the cell lines that will be derived in the course of this study?*

- There is the possibility that your cells or the derived cell lines might be used in research that will involve genetic manipulation of the cells or the mixing of human and non-human cells in animal models.
- The cell lines may be shared with researchers both inside and outside of Johns Hopkins, including commercial partners.
- The cell lines may be used to develop treatments for a variety of diseases and conditions.

We may use the cells taken from your tumor to make what is sometimes called an "organoid". An organoid is an organized cluster of cells, grown in the lab, which mimic parts of an organ's structure and function. Organoids can be used to help understand diseases and treatments for them.

**Genomic Data Sharing**
Genomic studies, including genome-wide association studies (GWAS), examine genetic differences in the entire human genome (the complete set of human genes) and the association between these genetic differences and health conditions.

As part of this study, we will collect information about your health and your individual genes. This information will be sent to a National Institutes of Health (NIH) designated data repository that includes genomic and other data from studies funded by the NIH.

The aim of collecting this information is to look for genetic connections that may:

- Increase the likelihood of getting a certain disease (such as asthma, cancer, diabetes, heart disease or mental illness) or a condition (such as high blood pressure or obesity);
- Affect the progress of a certain disease or condition;
- Affect treatments (medicines, etc.) that work for certain diseases in some people, but not in others.

We or our collaborators will remove direct identifiers (such as your name or date of birth) and instead code your information before sending it to the repository**.** The NIH will never receive this code or the identifiers we have removed.

The repository is a controlled-access repository. This means that your individual de-identified data is only available to researchers who apply to the NIH. The NIH will review data requests for scientific merit and for methods to protect data and methods to ensure data will be used for the approved purpose. We will not always know what types of health-related research will be done with the data that are sent to the repository. Information from Johns Hopkins participants that is sent to the repository will only be shared with researchers at other not-for-profit organizations (for example, other academic institutions).

Genomic summary results (GSR) data for non-sensitive studies may be made available by NIH without controlled-access. GSR data does not include information about you as an individual, but consists of statistical information calculated using your data combined with data from other people.

**What are the risks to your privacy?**
There may be risks to your privacy and the privacy of your relatives from storing your information in the repository. Although the NIH takes measures to protect privacy, we do not know how likely it is that your identity could become re-connected with your genetic and health information.

If your genetic information were re-identified, personal information about you, your health and your risk of disease could become known to others. This could present unknown risks. Current federal law will help protect you from genetic discrimination in health insurance and employment.

**Are there benefits to sharing your genetic information?**
There is no direct benefit to you from placing your genetic information in the repository. Allowing researchers to study your genetic information may lead to a better understanding of how genes affect health. This may help other people in the future.

## 5. What are the risks or discomforts of the study?

The study drugs may cause one or more side effects. Listed below are the possible side effects that can be caused by study drugs

**FLC peptide vaccine**

The investigational FLC peptide vaccine with poly-ICLC adjuvant used in the study may cause side effects that are mild and a minor inconvenience, or it could case life-threatening reactions or death. You will be watched closely for side effects. Some of the suspected risks of the investigational FLC peptide vaccine and adjuvant are listed below.

**Likely**

- Redness or swelling in the area where the injection was given lasting a few days at most
- Soreness at the site of the injection
- Mild flu-like symptoms such as fever, chills, rash, aches and pains, nausea, headache, dizziness, and fatigue

**Less likely**

- In rare circumstances, it is possible that an allergic reaction to the study regimen may occur. An allergic reaction could result in hives, rash, difficulty breathing, low blood pressure, or severe blood circulation and breathing problems. These reactions are usually reversible but in rare cases could lead to permanent disability or death. If you think you are having allergic reaction including shortness of breath, hives, closing of your throat, or tingling and/or swelling of your lips, face, or tongue, seek emergency medical attention immediately

**Nivolumab (OPDIVO®)**

Nivolumab may cause one or more of the side effects listed below. This information is based on data from cancer subjects in other clinical trials with nivolumab. In addition, there may be side effects that are not yet known that may occur. You should tell your doctor or nurse right away about any possible side effects you experience.

**Very common side effects of nivolumab are: [≥ 1/10 or ≥10%]**

- Diarrhea
- Fatigue or lacking energy
- Itching
- Rash
- Thyroid gland function decreased; including increased thyroid stimulating hormone (a lab test associated with abnormal thyroid function)

**Common side effects of nivolumab include: [≥ 1/100 to < 1/10 or ≥ 1% to <10%]**

- Abdominal pain
- Alkaline phosphatase increased: lab test result associated with liver or bone abnormalities
- Allergic reaction/hypersensitivity, a reaction related to the infusion of the medicine. The symptoms may include but are not limited to fever, rash, pain, swelling
- Amylase increased: lab test result associated with pancreas inflammation
- Chills
- Constipation
- Cough

- Creatinine increased: lab test result associated with decreased kidney function
- Decreased appetite
- Dizziness or vertigo (feeling off balance which can lead to dizziness)
- Dry mouth
- Dry skin
- Fever
- Headache
- Inflammation of the colon or small intestine
- Inflammation of the mouth
- Infusion related reaction
- Lab tests results associated with abnormal liver function: ALT increased, AST increased, Bilirubin increased, and Gamma-glutamyl transferase (GGT) increased
- Lipase increased: lab test result associated with pancreas inflammation
- Loss of color (pigment) from areas of skin
- Low levels of sodium in the blood
- Low red blood cell counts (anemia): this may make you feel weak and tired
- Lung inflammation (pneumonitis), inflammation of the tissues of the lung. While many patients with X-ray or CT (Computed Tomography) abnormalities have not developed any symptoms, some patients have developed mild to severe symptoms of their lung inflammation. Signs and symptoms of lung inflammation may include difficulty breathing, pain or discomfort while breathing, chest pain, cough, shortness of breath, increased rate of breathing, fever, low blood oxygen levels, or fatigue
- Nausea
- Pain in the muscles, bones, ligaments, tendons, and nerves
- Shortness of breath
- Swelling, including face, arms, and legs
- Thyroid gland function increased; including decreased thyroid stimulating hormone (a lab test associated with abnormal thyroid function.
- Tingling, burning, numbness or weakness, possibly in arms, legs, hands and feet
- Upper respiratory tract infection: common viral/bacterial infection that affects the nose, throat, and airways
- Vomiting

**Uncommon side effects of nivolumab include: [≥ 1/1,000 to < 1/100 or ≥0.1% to <1%]**

- A condition in which not enough oxygen passes from your lungs into your blood, or when your lungs cannot properly remove carbon dioxide (respiratory failure).
- A disease involving abnormal collections of inflammatory cells (granulomas) in organs such as lungs, skin, and lymph nodes (sarcoidosis)
- A skin disorder that's considered to be an allergic reaction to medicine or an infection. Symptoms may include symmetrical, red, raised skin areas that can appear all over the body, more noticeable on the fingers and toes. These patches often look like "targets" (dark circles with purple-grey centers) (erythema multiforme)
- Abnormally excessive sweating involving the arms, legs, hands and feet, underarms, and face, usually unrelated to body temperature or exercise
- Blistering of the skin or mouth caused by the immune system attacking healthy tissue (pemphigoid)
- Decreased secretion of hormones produced by adrenal glands
- Dehydration

- Diabetes complications: resulting in excess acid in the blood
- Dry eye
- Flu-like symptoms (which may include fever, chills, cough, sore throat, runny or stuffy nose, muscle or body aches, headaches, feeling tired)
- Hair loss
- Heart rate increased
- Heart rhythm abnormal
- High blood pressure
- Hives
- Increased blood sugar or a disease that results in too much sugar in the blood (diabetes)
- Inflammation of the eye
- Inflammation of the kidney
- Inflammation of the lining of bronchial tubes, which carry air to and from the lungs (bronchitis)
- Inflammation of the liver
- Inflammation of the muscle
- Inflammation of the pancreas
- Inflammation of the pituitary gland
- Inflammation of the stomach
- Inflammation of the thyroid gland
- Joint pain or stiffness
- Kidney function failure, kidney disease
- Low blood pressure
- Low platelet counts (thrombocytopenia): this may increase your risk for skin bruising, nose bleeds, and bleeding from the gums.
- Low white blood cell counts (neutropenia): these put you at higher risk for infection
- Under active function of the pituitary gland
- Skin disease with thickened patches of red skin, often silvery scales (Psoriasis)
- Trouble falling asleep (Insomnia)
- Vision blurred

**Rare side effects of nivolumab include: [≥ 1/10,000 to < 1/1,000 or ≥0.01% to <0.1%]**

- Autoimmune hemolytic anemia: (AHA) a malfunction of the immune system that produces autoantibodies, which attack red blood cells as if they were foreign substances to the body
- Cranial nerve disorder
- Disease caused by the body's immune system attacking healthy organs (auto-immune disorder)
- Disorder of the lymph nodes which causes the lymph nodes to become enlarged, inflamed and painful, commonly affecting lymph nodes of the neck and possibly associated with fever or muscle and joint pains (histiocytic necrotizing lymphadenitis or Kikuchi lymphadenitis)
- Double vision
- Drug induced liver injury
- Guillain-Barre syndrome: An autoimmune disorder associated with progressive muscle weakness or paralysis
- Hemophagocytic lymphohistiocytosis (HLH) syndrome: A disease that may affect your body's defense system, called immune system. Certain white blood cells may attack other blood cells. These abnormal blood cells collect in your spleen and liver, causing these organs to enlarge. The symptoms may include fever, rash, and low blood cell counts (hemophagocytic

lymphohistiocytosis (HLH) syndrome)
- Inflammation of the blood vessels
- Inflammation of the brain
- Inflammation of the heart
- Inflammation of the lining of the brain and spinal cord
- Lung infiltrates, associated with infection and inflammation
- Myasthenic syndrome, myasthenia gravis: A neuromuscular disease that may cause weakness of eye, face, breathing, and swallowing muscles
- Pericarditis: A swelling and irritation of the thin saclike membrane surrounding the heart (pericardium)
- Polymyalgia rheumatic: An inflammatory disorder that causes muscle pain and stiffness, especially in the shoulders
- Rhabdomyolysis: Muscle fiber released into the blood stream which could damage your kidneys
- Rosacea: Acne-like skin condition resulting in redness of face
- Rupture of the intestine/hole in the intestine or stomach
- Severe allergic reaction may include but not limited to high grade fever, rash, swelling, difficulty breathing or pain
- Stevens Johnson syndrome: Inflammatory disorder of skin and mucous membranes, resulting in blistering and shedding of skin
- Toxic epidermal necrolysis: A severe skin disease characterized by blistering and peeling of the top layer of skin resembling a severe burn

The following events have been identified during post-approval use of nivolumab. Because reports are voluntary from a population of unknown size, an estimate of frequency cannot be made.
- A condition that occurs when donor bone marrow or stem cells attack the recipient (graft-versus-host-disease)
- A disease that may affect several parts of the body, including the eyes, ears, nervous system, and skin. The symptoms may include eye swelling, pain and/or blurred vision; hearing loss, ringing in the ears; and /or loss of skin color (Vogt-Koyanagi-Harada [VKH] disease)
- Solid organ transplant rejection

**Lung Inflammation (pneumonitis)**: It is possible that nivolumab may cause inflammation of the tissues of the lung. This adverse effect has been reported in participants treated with nivolumab. While many participants with x-ray or CT (Computed Tomography) abnormalities have not developed any symptoms, some participants have developed mild to severe symptoms and in rare cases, death has occurred as a result of their lung inflammation. Signs and symptoms of lung inflammation may include difficulty breathing, pain or discomfort while breathing, chest pain, cough, shortness of breath, increased rate of breathing, fever, low blood oxygen levels, or fatigue.

Your study doctor and nurse will watch you closely for changes in your ability to breathe and for other signs or symptoms that might show you are developing this type of lung inflammation and will perform regular tests including physical exams, measurement of oxygen levels through non-invasive testing (with a pulse oximeter, which is a clip that rests on your finger), blood tests, chest x-rays and/or CT scans.

Complications, including fatal events, have occurred in participants who received allogeneic hematopoietic stem cell transplantation (HSCT) before or after nivolumab.

Complications, including rejection, have also been reported in participants who have received an organ or tissue transplant. Treatment with nivolumab may increase the risk or rejection of the organ or tissue transplant.

**Please inform your study doctor or nurse <u>AT ONCE</u> if you experience any of the following:**

- Any new or increased shortness of breath;
- Any new or increased chest pain;
- Any new or increased pain/difficulty while breathing
- Any new or increased cough or any significant change in your type of cough; for example, any new or increased mucous or blood in your cough;
- Any change in the amount of oxygen you require;
- Any fever, fatigue, or other symptoms that occur at the same time as any changes to your breathing or other lung symptoms.

If you start to develop symptoms, your study doctor will ask you to return to the clinic for additional tests, which could include a physical exam, measurement of oxygen levels, blood tests, chest x-rays, and/or CT scans. You will be monitored very closely for changes in your overall lung symptoms, monitoring may require hospitalization. You may require specific treatment in order to control pneumonitis. You may also be seen by a special doctor called a pulmonologist, who has special training to be an expert in how your lungs work.

Prolonged treatment with medicines that suppress inflammation, sometimes needed to manage the side effects of nivolumab treatment, may lower your body's ability to fight off certain infections (called opportunistic infections). These infections may require treatment with antibiotics or antifungal medications and may be fatal.

**<u>Nivolumab (OPDIVO®) Combined with Ipilimumab (YERVOY )</u>**

Nivolumab combined with ipilimumab for advanced melanoma has been studied in another clinical trial. Currently, there have been no new side effects seen with this combination, which have not been identified in other trials separately evaluating nivolumab or ipilimumab. However, while no new side effects have been reported, the combination of the study drugs has shown a large number (28-60 %) of subjects with serious side effects. There may be side effects that are not yet known that may occur.

You should tell your doctor or nurse right away about any possible side effects you experience.

**Very common side effects of nivolumab combined with ipilimumab are: [≥ 1/10 or ≥10%]**

- ALT and/or AST increased: lab test results associated with abnormal liver function
- Decreased appetite
- Diarrhea
- Fatigue-feeling tired or lack of energy
- Fever
- Hypothyroidism/Hyperthyroidism
- Lipase increased
- Nausea
- Skin Itching
- Skin Rash

**Common side effects of nivolumab combined with ipilimumab include: [≥ 1/100 to < 1/10 or ≥ 1% to <10%]**

- Abdominal pain
- Adrenal insufficiency
- Alopecia
- Amylase increased
- Anemia
- Arthritis
- Blood alkaline phosphatase increased
- Blood creatine increased
- Blood bilirubin increased
- Blood thyroid stimulating hormone increased/decreased
- Chills
- Colitis
- Constipation
- Cough
- Dehydration
- Diabetes
- Dizziness
- Dry eye
- Dry mouth
- Dry skin
- Dyspnea
- Edema
- Headache
- Hepatitis
- Hypopituitarism
- Hyperglycemia
- Hypersensitivity
- Hyponatremia
- Hypophysitis
- Hypotension
- Influenza-like illness
- Infusion-related reaction
- Insomnia
- Musculoskeletal pain
- Pancreatitis
- Pneumonitis
- Peripheral neuropathy
- Renal failure
- Stomatitis: inflamed, sore mouth
- Thrombocytopenia
- Thyroiditis
- Upper respiratory tract infection
- Vision blurred
- Vitiligo: skin loses pigment cells

- Vomiting

**Uncommon side effects of nivolumab combined with ipilimumab include: [≥ 1/1,000 to < 1/100 or ≥ 0.1% to <1%]**

- Abdominal pain
- Alkaline phosphatase increased: lab test result associated with liver or bone abnormalities
- Arrhythmia
- Bronchitis
- Cranial nerve disorder
- Diabetic ketoacidosis
- Diplopia: double vision
- Drug-induced liver injury
- Encephalitis
- Erythema multiforme
- Eye inflammation
- Gastritis
- Hives
- Hypertension
- Hyperhidrosis: inflammation of the pituitary gland
- Lung infiltration
- Myocarditis
- Myasthenic syndrome
- Myositis
- Nephritis
- Neutropenia
- Pemphigoid
- Polymyalgia rheumatica
- Respiratory failure
- Sarcoidosis
- Tachycardia: heart rate that is too fast

**Rare but potentially serious side effects of nivolumab combined with ipilimumab include (≥ 1/10,000 to < 1/1,000 or ≥0.01% to <0.1%]**

- Anaphylactic reaction
- Autoimmune disorder
- Autoimmune hemolytic anemia
- Demyelination: damage to the protective covering (myelin sheath) that surrounds nerves
- Diabetic Coma
- Guillain-Barre syndrome
- Hemophagocytic lymphohistiocytosis (HLH) syndrome
- Intestinal perforation
- Meningitis
- Pericarditis
- Rhabdomyolysis
- Rosacea

- Stevens-Johnson syndrome
- Toxic epidermal necrolysis
- Vasculitis
- Vogt-Koyanagi-Harada disease

**You should call the study doctor or nurse immediately if you have any of the above symptoms**

**Other side effects**

**Infusion related side effects:** Nivolumab and ipilimumab are types of drugs called antibodies. Very rarely, some symptoms may occur 10 minutes to 4 hours after the infusion of antibodies. Mild to moderate swelling and itching at the infusion site can occur. Mild headache, flushing, dizziness, and nausea can also occur. Moderate symptoms include chest pain, shortness of breath, fever, and change in blood pressure. These symptoms can become more severe with partial obstruction of the airways, severe flushing, and joint stiffness. Delayed symptoms usually occur between 24 hours to 14 days after administration. The delayed symptoms include rash, flu-like symptoms, stiffness of the joints, pain in the joints, headache, and muscle pain

**Immune related side effects:** Nivolumab and Ipilimumab are both immunotherapies. An immunotherapy is a treatment that acts by enhancing the function of your immune system. However, in rare cases, immunotherapy may result in an overreaction against your healthy tissues and organs; this is called an autoimmune reaction. This means that the study drug may cause your immune system to not only attack the cancer cells, but also normal healthy cells in your body, and potentially may cause an autoimmune disease. These immune-related side effects, or side effects resulting from these immune reactions may have been included in the previous lists above but are explained in more detail here with possible symptoms you may experience.

- Uveitis: inflammation of the eyes that may cause eye redness, eye pain, sensitivity to light, blurred vision, decreased vision, and floating spots in your vision
- Hypophysitis: inflammation of the pituitary gland that may cause low blood sugar, dehydration, weakness, dizziness, and other symptoms
- Hypothyroidism: underactive thyroid gland activity that may lead to fatigue, weight gain, intolerance to cold, and infrequent or hard stools, in addition to other symptoms
- Hyperthyroidism: overactive thyroid gland activity that may lead to fatigue, weakness, muscle aches, anxiety, weight loss, intolerance to heat, in addition to other symptoms
- Pneumonitis: inflammation of the lungs that may lead to fatigue, shortness of breath, cough, and/or low blood oxygen levels.
- Peritonitis: inflammation of the membrane lining the abdominal wall and covering the abdominal organs caused by leakage or hole in the intestines
- Encephalitis: inflammation of the brain that may result in weakness, fatigue, problems with coordination, disorientation, headache, fever, nausea, in addition to other symptoms
- Hepatitis: inflammation of the liver that may result in nausea, fatigue, abdominal pain, impart a yellowish hue to your skin or eyes, or darkness of your urine
- Pancreatitis: inflammation of the pancreas that may lead to high levels of lipase and that may be accompanied by nausea, vomiting, and abdominal pain
- Colitis or Proctitis: inflammation of the lining of the colon or rectum, usually resulting in diarrhea and possibly abdominal pain.
- Arthritis: painful swelling, inflammation and stiffness of the fingers, wrists, arms and legs

- Myocarditis: life-threatening inflammation of the heart muscle which could result in chest pain, swelling of the legs, weakness, difficulty breathing, and or altered hear rate or rhythm.
- Aseptic Meningitis: inflammation of the lining of the brain and/or the spinal cord, which could result in symptoms such as headache, fever, increased sensitivity to noise and light, visual changes or altered level of consciousness.
- Nephritis: inflammation of the kidney which could result in symptoms such as pain in the kidney area or abdomen, changes in frequency or appearance of the urine, swelling of the body (most commonly the legs and feet), and pain with urination.

**You should call the doctor or nurse immediately if you have any of the above symptoms.**

In addition, blood tests will be performed prior to each study drug administration to check if there is evidence of inflammation of the liver, called hepatitis, which may not be causing any symptoms. In several cases, treatment has had to be held until the inflammation has resolved, usually with steroid treatment. In one case severe life-threatening liver dysfunction occurred which was not responsive to treatment.

**Risks of Other Study Procedures**

You may feel discomfort during some of these tests or may experience some inconveniences. Some may also have risks, which may include:

- Blood samples: Taking blood may cause discomfort, bleeding, or bruising where the needle enters the body. In rare cases, it may result in fainting. There is a small risk of infection.
- IV line: may cause discomfort, irritation, mild bruising, bleeding, leakage of drug solution, and rarely, infection, nausea, and lightheadedness.
- Tumor Biopsy: Your doctor will discuss the risks associated with obtaining tissue samples. Having biopsies performed may cause pain, bruising, bleeding, redness, low blood pressure, vasovagal reaction (lightheadedness, low blood pressure, sweating), swelling and/or infection. An allergic reaction to the anesthetic may occur. A scar may form at the biopsy site. Inadvertent injury to other tissues, organs, blood vessels and/or nerves may also occur.

You may get tired or bored when we are asking you questions or you are completing questionnaires. You do not have to answer any question you do not want to answer.

While no significant risks have been found from the use of MRI scans, you may be bothered by the noise made by the MRI scanner and by feelings of being closed in (claustrophobia).

**Imaging Procedures**

This research study includes exposure to radiation from x-rays or gamma rays. This radiation exposure is for research purposes only and is not part of your medical care. X-rays and gamma rays from natural or medical sources can damage the genetic material (DNA) in your cells. At low doses, the body is usually able to repair the damage.

The radiation exposure that you will get in this research study is 2.4 rem (a rem is a unit of absorbed radiation). This is more than the 0.3 rem that the average person in the United States gets each year from natural sources like the sun, outer space, air, food and soil. It is less than the 5 rems of radiation that is allowed each year for people who are exposed to radiation in their jobs.

The radiation exposure described here is what you will get from this research study only. It does not include any exposure you may have received or will receive from other tests outside of this study that are

a part of your medical care. Radiation risk builds up with each exposure. You should think about your own history of radiation exposure from tests (like x-rays or CT scans) in deciding about the radiation in this study. If you have questions about the total amount of radiation you will be receiving, you should ask your doctor.

**Identifiable private information**
There is the risk that information about you may become known to people outside this study.

**Interaction with COVID-19 Vaccine**
There is no known interaction of the study drug with the COVID-19 vaccine.

**Unknown risk**
There may be other side effects or discomforts that are not known at this time. You will be told about any new risks that become known during this research study.

## 5. Are there risks related to pregnancy?

This research may hurt an embryo or fetus in ways we do not currently know. Laboratory and animal studies have not been conducted to determine possible reproductive risks related to the FLC peptide vaccine, nivolumab and ipilimumab. The use of the FLC peptide vaccine, nivolumab and ipilimumab in pregnant women has not been formally studied in clinical studies.

**For women:**
Pregnancy could possibly result in birth defects or death to the embryo or fetus that is conceived while you are receiving the study drugs or after you receive the last study drug. It is, therefore, very important that you do not become pregnant.

If you are a woman who is still able to get pregnant (in other words, pre-menopausal and not surgically sterilized), you must either use two forms of birth control or remain abstinent in order to avoid pregnancy from the time you start study drug dosing until 5 months receiving your last dose of study drug. If you do become pregnant during this time, you must inform your study doctor immediately.

If you are a woman who is able to become pregnant, you will have a pregnancy test at the time of enrollment in this study, and within 3 days prior to each dose of study drugs.

Females should not breastfeed while receiving the study drugs and up to 5 months from the last dose of study drug.
If you do become pregnant while in this research study, you must agree to inform your study doctor immediately, so that you can be withdrawn from the research study. Your doctor will follow up with you to determine the pregnancy outcome.

**For men:**
For men, you must agree not to cause pregnancy during the study and for a period of 7 months after the last dose of study drug. Pregnancy could possibly result in birth defects or death to the embryo or fetus that is conceived while you are receiving the study drugs or after you receive the last study drug. It is, therefore, very important that you do not cause a pregnancy.

If you are a man who is still able to get a woman pregnant (in other words, not surgically sterilized), you must agree to the following for the duration of the study drug dosing and 7 months after the last dose:

1. Communicate your participation in this clinical trial and the contraceptive requirement to your sexual partner(s) who is a woman capable of becoming pregnant. Together with your partner, you must either use two forms of birth control (one highly effective and the other either highly effective or less highly effective method) or remain abstinent in order to prevent a woman from becoming pregnant or prevent fetal exposure to study drug if your partner is already pregnant, from the time you start study drug dosing. One of these methods should be a condom. Men who are not capable of fathering a child (for example, surgical removal of both testicles) only need to use a condom.
2. Tell the study doctor if your partner becomes pregnant during the clinical trial. While your sexual partner is pregnant or breastfeeding you must agree to remain abstinent from penile vaginal intercourse or use a male condom during each episode of penile penetration.
3. Refrain from donating sperm.

It is unknown whether this research may hurt an embryo or fetus.

## 6. Are there benefits to being in the study?

You may or may not benefit from being in this study. If the drug works, you may have some benefit. If the drug does not work, you may not benefit. If you take part in this study, you may help others in the future.

## 7. What are your options if you do not want to be in the study?

If you decide not to join this study, other options are available. You do not have to join this study to get treatment. You can receive the usual treatment for your cancer without being in the study. You may be eligible for other research studies. You should talk to your study doctor about other options.

You do not have to join this study. If you do not join, your care at Johns Hopkins will not be affected.

## 8. Will it cost you anything to be in this study?

You will receive a separate Insurance and Research Participant Financial Responsibility Information Sheet. This Sheet will give you the following information:

- The procedures, tests, drugs or devices that are part of this research and that will be paid for by the study (no cost to you).
- The procedures, tests, drugs or devices that will be billed to you and/or your health insurer. If you have health insurance, you will be responsible for any co-pays or deductibles not covered by your insurance.

**The cost of this molecular profiling by Caris will be billed to your health insurance. The study will pick up any costs of testing that are not covered by your insurance, so there is no cost to you.**

## 9. Will you be paid if you join this study?

No.

## 10. Can you leave the study early?

- You can agree to be in the study now and change your mind later.
- If you wish to stop, please tell us right away.
- Leaving this study early will not stop you from getting regular medical care.

If you leave the study early, Johns Hopkins may use or share your health information that it has already collected if the information is needed for this study or any follow-up activities.

## 11. Why might we take you out of the study early?

You may be taken out of the study if:

- Staying in the study would be harmful.
- You need treatment not allowed in the study.
- You fail to follow instructions.
- You become pregnant.
- The study is cancelled.
- There may be other reasons to take you out of the study that we do not know at this time.

If you are taken out of the study early, Johns Hopkins may use or give out your health information that it has already collected if the information is needed for this study or any follow-up activities.

## 12. How will your privacy be maintained and how will the confidentiality of your data be protected?

**HIPAA Authorization for Disclosure of Protected Health Information**

**What information is being collected, used, or shared?**

To do this research, we will need to collect, use, and share your private health information. By signing this document, you agree that your health care providers (including both Johns Hopkins Medicine and others) may release your private health information to us, and that we may use any and all of your information that the study team believes it needs to conduct the study. Your private information may include things learned from the procedures described in this consent form, as well as information from your medical record (which may include information such as drug, alcohol or STD treatment, genetic test results, or mental health treatment).

**Who will see, use or share the information?**

The people who may request, receive, or use your private health information include the researchers and their staff who may be a part of Johns Hopkins Health System, Johns Hopkins University or the Johns Hopkins Applied Physics Laboratory. Additionally, we may share your information with other people at Johns Hopkins, for example if needed for your clinical care or study oversight and BMS, its affiliates and employees. To improve coordination of your research and clinical care, some information about the study you join will be included in your electronic medical record.

By signing this form, you give permission to the research team to share your information with others outside of Johns Hopkins. This may include the sponsor of the study and its agents or contractors, outside providers, study safety monitors, government agencies, other sites in the study, data managers and other agents and contractors used by the study team.

We try to make sure that everyone who sees your information keeps it confidential, but we cannot guarantee that your information will not be shared with others. If your information is disclosed by your health care providers or the research team to others, federal and state confidentiality laws may no longer protect it.

**Do you have to sign this Authorization?**

You do not have to sign this Authorization, but if you do not, you may not join the study.

**How long will your information be used or shared?**
Your Authorization for the collection, use, and sharing of your information does not expire. Additionally, you agree that your information may be used for similar or related future research studies.

**What if you change your mind?**
You may change your mind and cancel this Authorization at any time. If you cancel, you must contact the Principal Investigator in writing to let them know by using the contact information provided in this consent form. Your cancellation will not affect information already collected in the study, or information that has already been shared with others before you cancelled your authorization.

**How will your information be protected?**
The following procedures will be used to protect the confidentiality of data and samples collected and stored for research purposes:

- Only authorized persons will be granted access
- Only authorized persons may enter and view study data
- Passwords and system IDs will not be shared
- Physical security of the workstation/files will be maintained
- Adequate back-up plan is in effect
- Staff trained on data entry system and importance of security procedures
- Workstations with databases will not be left unattended

## 13. What is an Electronic Medical Record (EMR) and what research information may be included in the EMR?

An Electronic Medical Record (EMR) is an electronic version of your medical chart. If you do not already have an EMR at Johns Hopkins, one may be created for this study. Some information from this study will be put in your EMR. (Examples include your consent form, test results, and scheduled procedures as well as any communications with the study team or assessments completed through MyChart, a portal used by patients to access their EMR). This information will be visible to any of your providers who view your EMR.

The information in your EMR may also be used and shared consistent with other medical information about you as described in the Johns Hopkins Notice of Privacy Practices.

Information within your EMR can be accessible to others (e.g., health insurance company, life insurance company, disability provider, third-parties specified in this consent). It is possible this information could be used to make decisions about coverage.

If you have any questions about what information may be added to your EMR from participating in this research, please ask the study team. If you do not want information from this research study included in your medical record you should not participate in this study.

## 14. Will the study require any of your other health care providers to share your health information with the researchers of this study?

As a part of this study, the researchers may ask to see your health care records from your other health care providers.

## 16. What does a conflict of interest mean to you as a participant in this study?

A researcher has a financial or other interest in this study.

In some situations, the results of this study may lead to a financial gain for the researcher and/or Johns Hopkins University. This financial interest has been reviewed in keeping with Johns Hopkins' policies. It has been approved with certain conditions, which are intended to guard against bias in how the study is conducted, how the results are analyzed, and how participants are protected.

If you have any questions about this financial interest, please talk to Christy Salamone at 410-614-0267. This person is a member of the study team, but does not have a financial interest related to the study. You may also call the Office of Policy Coordination 410-361-8667 for more information. The Office of Policy Coordination reviews financial interests of researchers and/or Johns Hopkins.

## 17. What treatment costs will be paid if you are injured in this study?

Johns Hopkins does not have a program to pay you if you are hurt or have other bad results from being in the study. However, medical care at Johns Hopkins is open to you as it is to all sick or injured people.

The costs for any treatment or hospital care you receive as the result of a study-related injury that are not covered by a health insurer will be billed to you.

By signing this form, you will not give up any rights you have to seek compensation for injury.

## 18. What other things should you know about this research study?

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time. If you would like to review the information for this study, or a summary of the results, ask the study team doctor for the ClinicalTrials.gov study registration number.

During the study, we will tell you if we learn any new information that might affect whether you wish to continue to participate.

**What is the Institutional Review Board (IRB) and how does it protect you?**
This study has been reviewed by an Institutional Review Board (IRB), a group of people that reviews human research studies. The IRB can help you if you have questions about your rights as a research participant or if you have other questions, concerns or complaints about this research study. You may contact the IRB at 410-502-2092 or jhmeirb@jhmi.edu.

**What should you do if you have questions about the study, or are injured or ill as a result of being in this study?**
Call the principal investigator, Dr. Mark Yarchoan at 410-614-1058. If you wish, you may contact the principal investigator by letter. The address is on page one of this consent form. If you cannot reach the principal investigator or wish to talk to someone else, call the IRB office at 410-502-2092.

**If you have an urgent medical problem** or think you are injured or ill because of this study, call 911 or go to your local emergency department. You should also call Dr. Mark Yarchoan at 410-614-1058 during regular office hours and 410-955-4331 after hours and on weekends.

## 19. Assent Statement

This research study has been explained to my child in my presence in language my child can understand. He/she has been encouraged to ask questions about the study now and at any time in the future.

## 20. Optional Study Components:

This part of the consent form is about optional component(s) of the study that you can choose to take part in or not. You can still take part in the main study even if you say "no" to this/these optional component(s).

**Future Contact**

We would like your permission for our research team to contact you in the future. Please note that your decision below does not prevent other researchers at Johns Hopkins from contacting you about other research.

**Please sign and date your choice below:**

**YES** ☐ _____ _____
Signature of Participant Date

**NO** ☐ _____ _____
Signature of Participant Date

## 21. What does your signature on this consent form mean?

Your signature on this form means that you have reviewed the information in this form, you have had a chance to ask questions, and you agree to join the study. You will not give up any legal rights by signing this consent form.

## WE WILL GIVE YOU A COPY OF THIS SIGNED AND DATED CONSENT FORM

Signature of Participant (Print Name) Date/Time

Signature of Person Obtaining Consent (Print Name) Date/Time

Signature of Parent/Legal Guardian/Court-Appointed Representative (Print Name) Date/Time
**FOR CHILD PARTICIPANT**

Description of relationship to child research participant (for example: parent, legal guardian, court-appointed representative)

Signature of Interpreter/Witness to Consent Procedures (Print Name) Date/Time
(Required for studies enrolling non-English speakers using the short form process or otherwise as determined required by the IRB)

**I have received the separate Insurance and Research Participant Financial Responsibility Information Sheet.**

Signature of Participant, LAR or Parent/Guardian (Print Name) Date/Time

**NOTE: A COPY OF THE SIGNED, DATED CONSENT FORM MUST BE KEPT BY THE PRINCIPAL INVESTIGATOR AND A COPY MUST BE GIVEN TO THE PARTICIPANT. IF APPROPRIATE FOR THIS STUDY, A SCANNED COPY OF THE SIGNED CONSENT FORM SHOULD BE UPLOADED TO THE PARTICIPANT'S EPIC/EMR RECORD (UNLESS NO MEDICAL RECORD EXISTS OR WILL BE CREATED).**

## DOCUMENTATION OF PHYSICIAN/ADVANCED PRACTICE PROVIDER CONSENT PROCESS

**My signature below indicates that I have discussed the risks, benefits, and alternatives, answered any questions, and believe the participant is able to make an informed choice to join the study.**

Signature of Physician/Advanced Practice Provider (Print Name) Date/Time

Signature of Participant (Print Name) Date/Time

Signature of Parent/Legal Guardian/Court-Appointed Representative (Print Name) Date/Time
**FOR CHILD PARTICIPANT**

Description of relationship to child research participant (for example: parent, legal guardian, court-appointed representative)

Signature of Interpreter/Witness to Consent Procedures (Print Name) Date/Time
(Required for studies enrolling non-English speakers using the short form process or otherwise as determined required by the IRB)

**NOTE: A COPY OF THE SIGNED, DATED CONSENT FORM MUST BE KEPT BY THE PRINCIPAL INVESTIGATOR AND A COPY MUST BE GIVEN TO THE PARTICIPANT. IF APPROPRIATE FOR THIS STUDY, A SCANNED COPY OF THE SIGNED CONSENT FORM SHOULD BE UPLOADED TO THE PARTICIPANT'S EPIC/EMR RECORD (UNLESS NO MEDICAL RECORD EXISTS OR WILL BE CREATED).**

If appropriate for this study, a scanned copy of the signed consent form should be uploaded to the participant's Epic/EMR record.

Patient I.D. plate

# RESEARCH PARTICIPANT INFORMED CONSENT AND PRIVACY AUTHORIZATION FORM

**Protocol Title**: **A Pilot Study of a DNAJB1-PRKACA Fusion Kinase Peptide Vaccine Combined with Nivolumab and Ipilimumab for Patients with Fibrolamellar Hepatocellular Carcinoma**

**Pregnant Partner Consent**

**Application No.:** **IRB00222681**

**Principal Investigator:** **Mark Yarchoan, MD**
**Sidney Kimmel Comprehensive Cancer Center,**
**Johns Hopkins University**
**1650 Orleans Street, Room 4M07**
**Baltimore MD 21287**
**410-614-1058/Fax 410-614-9006**
**mark.yarchoan@jhmi.edu**

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You are being asked to join a research study. Participation in this study is voluntary. Even if you decide to join now, you can change your mind later.

If you are a parent or legal guardian of a child who may take part in this study, your permission is required for your child to participate. The assent (agreement) of your child may also be required. When we say "you" in this consent, we mean you and your child.

## 1. Research Summary (Key Information):

The information in this section is intended to be an introduction to the study only. Complete details of the study are listed in the sections below. If you are considering participation in the study, the entire document should be discussed with you before you make your final decision. You can ask questions about the study now and at any time in the future.

You are being asked to take part in this follow-up only research study because you became pregnant while the biological father of your child was participating in a study called "A pilot study of a DNAJB1-PRKACA fusion kinase peptide vaccine combined with nivolumab and ipilimumab for patients with fibrolamellar hepatocellular carcinoma." The purpose of this consent form is to ask your permission to

obtain medical information on your pregnancy, its outcome and, if appropriate, the birth and health of the child (until the child is 8 weeks of age), in order to learn more about any possible effects. Details of what will be collected are outlined below. There are no medical risks associated with the collection of information about your pregnancy. There is the risk that information about you may become known to people outside this study. There are no benefits or costs to you related to providing this information.

## 2. Why is this research being done?

This research is being done to gather medical information about your pregnancy and, if applicable, the birth and health of your child. We want to determine if the study drugs your male partner received (DNAJB1-PRKACA peptide vaccine, nivolumab, and ipilimumab) have any effect on your pregnancy and/or the health of your child.

Laboratory and animal studies have not been conducted to determine possible reproductive risks related to the DNAJB1-PRKACA peptide vaccine, nivolumab, and ipilimumab. One case has been identified of a male patient receiving nivolumab with a female partner who became pregnant. The pregnancy was uneventful and at birth, the infant was slightly underweight.

**Who can join this study?**

People who become pregnant while the biological father of their child was participating in a research study called: "A pilot study of a DNAJB1-PRKACA fusion kinase peptide vaccine combined with nivolumab and ipilimumab for patients with fibrolamellar hepatocellular carcinoma."

## 3. What will happen if you join this study?

If you agree to be in this study, we will ask you to do the following things:

We will ask you for your permission to review your and your child's medical records relating to your pregnancy, the delivery of your child, if applicable, and the health of your child up to 8 weeks of age.

We will ask you for the names and telephone numbers of the doctor(s) who take care of you during your pregnancy. We will ask you for names and telephone numbers of the doctor(s) who take care of your child. Information from your baby's medical records that will be collected will include any medical concerns and the baby's height and weight.

If your doctor's or the child's doctor's contact information changes, please tell the study doctor.

**How long will you be in the study?**

Your involvement will last until about 8 weeks after the birth of your child or termination of your pregnancy.

## 4. What happens to data that are collected in the study?

If you join this study, your data will be used to answer the research question and your data will be used to publish the findings of this study. You will not own your research data. If researchers use your data to create a new product or idea, including those that may have commercial value, you will not benefit financially from those efforts.

*How will your data be shared now and in the future?*

Johns Hopkins researchers and their collaborators may use the data collected in this study for future research purposes and may share some of the data with others.

Sharing data is part of research and may increase what we can learn from each study. Often, data sharing is required as a condition of funding or for publishing study results. It also is needed to allow other researchers to validate study findings and to come up with new ideas. Your data may be shared directly with researchers at Johns Hopkins and other institutions, for-profit companies sponsors, government agencies, other research partners. Some of our research partners may be international. Your data may also be put in government or other databases/repositories.

Because science constantly advances, we do not yet know what future use of research data may include. This future research may be unrelated to the current study and may include outside collaborators.

We (Johns Hopkins) will do our best to protect and maintain your data in a safe way. One of the ways we protect data is by limiting the uses of the information and the type of information that is shared, especially your personal information. This may occur through data sharing agreements and review by oversight groups within Johns Hopkins.

If data are used or shared with types of information that may be likely to identify, you such as your name, address or medical record number, further institutional review and approval would be required. In these cases, Johns Hopkins will review whether additional consent from you is required. Generally, if your data are used/shared without any personal identifiers or with information that is less likely to identify you (such as the date of a procedure), further review and approval is not needed.

Data sharing could change over time, and may continue after the study ends.

The use and sharing of your data is required for participation in this research study. If you are not comfortable with the use and sharing of your data in future research without further consent, you should not participate in this study.

## 5. What are the risks or discomforts of the study?

There are no medical risks associated with the collection of information about your pregnancy.

There is the risk that information about you and/or your child may become known to people outside this study.

## 6. Are there benefits to being in the study?

There is no direct benefit to you from being in this study. The information from this research study may help researchers understand if there is any effect to embryos/fetuses fathered by men who receive these study drugs.

## 7. What are your options if you do not want to be in the study?

You do not have to join this study. If you do not join, your/your child's care at Johns Hopkins will not be affected.

## 8. Will it cost you anything to be in this study?

The regular medical care costs related to your pregnancy and the birth and care of your baby will be billed to you and/or your health insurance in the usual way.

## 9. Will you be paid if you join this study?

No.

## 10. Can you leave the study early?

- You can agree to be in the study now and change your mind later.
- If you wish to stop, please tell us right away.
- Leaving this study early will not stop you from getting regular medical care.

If you leave the study early, Johns Hopkins may use or share your health information that it has already collected if the information is needed for this study or any follow-up activities.

## 11. How will your privacy be maintained and how will the confidentiality of your data be protected?

**HIPAA Authorization for Disclosure of Protected Health Information**

**What information is being collected, used, or shared?**

To do this research, we will need to collect, use, and share your private health information. By signing this document, you agree that your health care providers (including both Johns Hopkins Medicine and others) may release your private health information to us, and that we may use any and all of your information that the study team believes it needs to conduct the study. Your private information may include things learned from the procedures described in this consent form, as well as information from your medical record (which may include information such as drug, alcohol or STD treatment, genetic test results, or mental health treatment).

**Who will see, use or share the information?**

The people who may request, receive, or use your private health information include the researchers and their staff who may be a part of Johns Hopkins Health System, Johns Hopkins University or the Johns Hopkins Applied Physics Laboratory. Additionally, we may share your information with other people at Johns Hopkins, for example if needed for your clinical care or study oversight and BMS, its affiliates and employees . To improve coordination of your research and clinical care, some information about the study you join will be included in your electronic medical record.

By signing this form, you give permission to the research team to share your information with others outside of Johns Hopkins. This may include the sponsor of the study and its agents or contractors, outside providers, study safety monitors, government agencies, other sites in the study, data managers and other agents and contractors used by the study team.

We try to make sure that everyone who sees your information keeps it confidential, but we cannot guarantee that your information will not be shared with others. If your information is disclosed by your health care providers or the research team to others, federal and state confidentiality laws may no longer protect it.

**Do you have to sign this Authorization?**

You do not have to sign this Authorization, but if you do not, you may not join the study.

**How long will your information be used or shared?**

Your Authorization for the collection, use, and sharing of your information does not expire. Additionally, you agree that your information may be used for similar or related future research studies.

**What if you change your mind?**

You may change your mind and cancel this Authorization at any time. If you cancel, you must contact the Principal Investigator in writing to let them know by using the contact information provided in this

consent form. Your cancellation will not affect information already collected in the study, or information that has already been shared with others before you cancelled your authorization.

**How will your information be protected?**

The following procedures will be used to protect the confidentiality of data collected and stored for research purposes:

- Only authorized persons will be granted access
- Only authorized persons may enter and view study data
- Passwords and system IDs will not be shared
- Physical security of the workstation/files will be maintained
- Adequate back-up plan is in effect
- Staff trained on data entry system and importance of security procedures
- Workstations with databases will not be left unattended

## 12. Will the study require any of your other health care providers to share your health information with the researchers of this study?

As a part of this study, the researchers may ask to see your health care records from your other health care providers. You will be asked to give us a list of other health care providers that you use.

## 13. What does a conflict of interest mean to you as a participant in this study?

A researcher has a financial or other interest in this study.

In some situations, the results of this study may lead to a financial gain for the researcher and/or Johns Hopkins University. This financial interest has been reviewed in keeping with Johns Hopkins' policies. It has been approved with certain conditions, which are intended to guard against bias in how the study is conducted, how the results are analyzed, and how participants are protected.

If you have any questions about this financial interest, please talk to Christy Salamone at 410-614-0267. This person is a member of the study team, but does not have a financial interest related to the study. You may also call the Office of Policy Coordination 410-361-8667 for more information. The Office of Policy Coordination reviews financial interests of researchers and/or Johns Hopkins.

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A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time. If you would like to review the information for this study, or a summary of the results, ask the study team doctor for the ClinicalTrials.gov study registration number.

During the study, we will tell you if we learn any new information that might affect whether you wish to continue to participate.

**What is the Institutional Review Board (IRB) and how does it protect you?**

This study has been reviewed by an Institutional Review Board (IRB), a group of people that reviews human research studies. The IRB can help you if you have questions about your rights as a research participant or if you have other questions, concerns or complaints about this research study. You may contact the IRB at 410-502-2092 or jhmeirb@jhmi.edu.

**What should you do if you have questions about the study?**
Call the principal investigator, Dr. Mark Yarchoan at 410-614-1058. If you wish, you may contact the principal investigator by letter. The address is on page one of this consent form. If you cannot reach the principal investigator or wish to talk to someone else, call the IRB office at 410-502-2092.

## 15. Assent Statement

This research study has been explained to my child in my presence in language my child can understand. He/she has been encouraged to ask questions about the study now and at any time in the future.

## 16. What does your signature on this consent form mean?

Your signature on this form means that you have reviewed the information in this form, you have had a chance to ask questions, and you agree to join the study. You will not give up any legal rights by signing this consent form.

## WE WILL GIVE YOU A COPY OF THIS SIGNED AND DATED CONSENT FORM

| Signature of Participant | (Print Name) | Date/Time |
|---|---|---|
| Signature of Person Obtaining Consent | (Print Name) | Date/Time |
| Signature of Parent/Legal Guardian/Court-Appointed Representative **FOR CHILD PARTICIPANT** | (Print Name) | Date/Time |

Description of relationship to child research participant (for example: parent, legal guardian, court-appointed representative)

| Signature of Interpreter/Witness to Consent Procedures | (Print Name) | Date/Time |
|---|---|---|

(Required for studies enrolling non-English speakers using the short form process or otherwise as determined required by the IRB)

**NOTE: A COPY OF THE SIGNED, DATED CONSENT FORM MUST BE KEPT BY THE PRINCIPAL INVESTIGATOR AND A COPY MUST BE GIVEN TO THE PARTICIPANT. IF APPROPRIATE FOR THIS STUDY, A SCANNED COPY OF THE SIGNED CONSENT FORM SHOULD BE UPLOADED TO THE PARTICIPANT'S EPIC/EMR RECORD (UNLESS NO MEDICAL RECORD EXISTS OR WILL BE CREATED).**

# TEST METHOD VALIDATION REPORT

Retrospective ELISpot Test Method Validation Report FLC 04-07-25

# TEST METHOD VALIDATION PLAN

IMC-ETMVP-FLC-VT-final_03-24-25

# PURPOSE

The purpose of this document is to detail the ELISpot Test Method Validation Plan using the results from ELISpot tests performed for the FLC Vaccine Trial (J19140).

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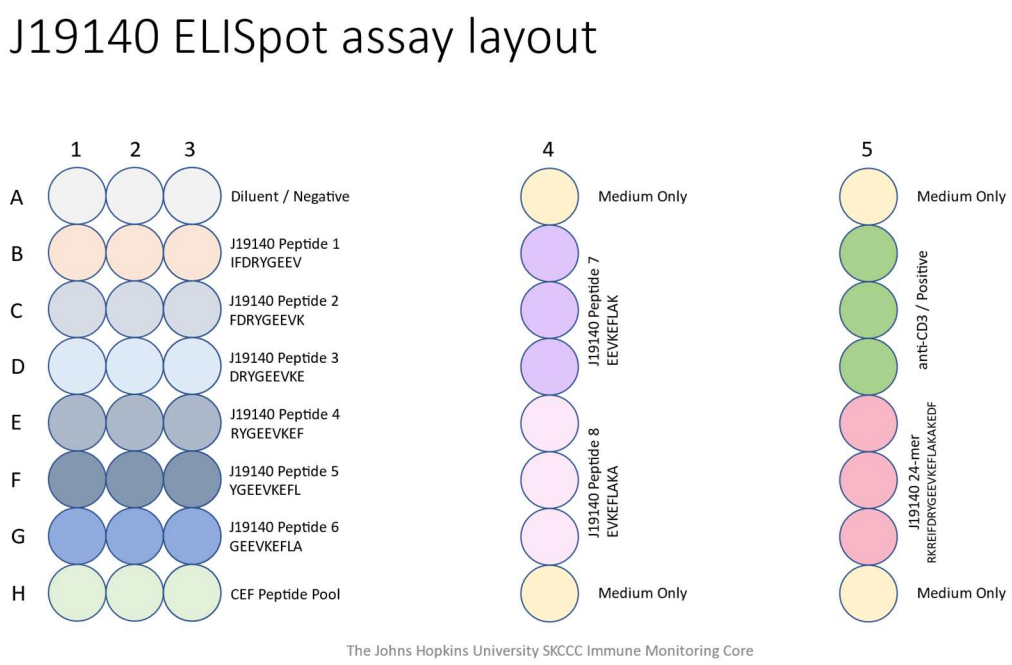
## SYSTEM DESCRIPTION

IMC performs ELISpot testing for Fresh and Cryopreserved cells for a clinical trial that include an FLC vaccine component. All tests/assays are performed using predefined layouts and critical materials/reagents by qualified staff. All tests/assays undergo both technical and QA reviews prior to report/result release.

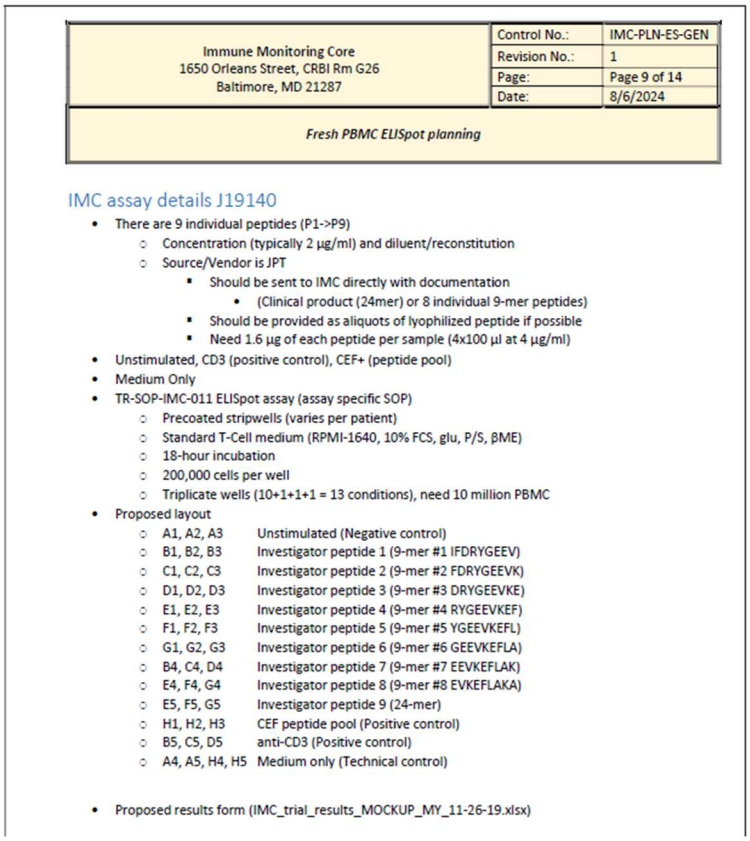
The FLC trial (J19140) involves the use of a peptide vaccine (a single 24-mer peptide with the driver mutation).

Figure 1




The ELISpot assay design was in place prior to the initiation of the trials and measures the frequency of IFNγ secreting PBMC to the vaccine peptide component and smaller peptides covering the driver mutation individually (2 µg/ml) and includes the appropriate controls (aCD3, CEF peptide pool, unstimulated, medium only). The responders were plated at 200,000 cells per well in triplicate.

Figure 2



| Immune Monitoring Core<br>1650 Orleans Street, CRBI Rm G26<br>Baltimore, MD 21287 | Control No.: | IMC-PLN-ES-GEN |
|---|---|---|
| | Revision No.: | 1 |
| | Page: | Page 9 of 14 |
| | Date: | 8/6/2024 |

*Fresh PBMC ELISpot planning*

IMC assay details J19140

- There are 9 individual peptides (P1->P9)
  - Concentration (typically 2 µg/ml) and diluent/reconstitution
  - Source/Vendor is JPT
    - Should be sent to IMC directly with documentation
      - (Clinical product (24mer) or 8 individual 9-mer peptides)
    - Should be provided as aliquots of lyophilized peptide if possible
    - Need 1.6 µg of each peptide per sample (4x100 µl at 4 µg/ml)
- Unstimulated, CD3 (positive control), CEF+ (peptide pool)
- Medium Only
- TR-SOP-IMC-011 ELISpot assay (assay specific SOP)
  - Precoated stripwells (varies per patient)
  - Standard T-Cell medium (RPMI-1640, 10% FCS, glu, P/S, βME)
  - 18-hour incubation
  - 200,000 cells per well
  - Triplicate wells (10+1+1+1 = 13 conditions), need 10 million PBMC
- Proposed layout
  - A1, A2, A3 Unstimulated (Negative control)
  - B1, B2, B3 Investigator peptide 1 (9-mer #1 IFDRYGEEV)
  - C1, C2, C3 Investigator peptide 2 (9-mer #2 FDRYGEEVK)
  - D1, D2, D3 Investigator peptide 3 (9-mer #3 DRYGEEVKE)
  - E1, E2, E3 Investigator peptide 4 (9-mer #4 RYGEEVKEF)
  - F1, F2, F3 Investigator peptide 5 (9-mer #5 YGEEVKEFL)
  - G1, G2, G3 Investigator peptide 6 (9-mer #6 GEEVKEFLA)
  - B4, C4, D4 Investigator peptide 7 (9-mer #7 EEVKEFLAK)
  - E4, F4, G4 Investigator peptide 8 (9-mer #8 EVKEFLAKA)
  - E5, F5, G5 Investigator peptide 9 (24-mer)
  - H1, H2, H3 CEF peptide pool (Positive control)
  - B5, C5, D5 anti-CD3 (Positive control)
  - A4, A5, H4, H5 Medium only (Technical control)
- Proposed results form (IMC_trial_results_MOCKUP_MY_11-26-19.xlsx)

All source documentation including labnotes and reports are available on the network drive (Data->[J19140]->->[studyID]->[test date]). Information from historical assays were collected in a spreadsheet (collected_KRAS_VT_data.xlsx) and all values are easily traceable to source documentation.

Separate sheets were added for Precision-Sensitivity-Specificity analysis of the entire dataset and analysis of subsets for robustness.

For ELISpot assays, there are several important considerations when evaluating assay performance and determining when the signal (reported values - spots) is reliably detected over noise.

The limit of Blank (LOB) is determined by counting spots in wells that contained only medium (no cells) and were otherwise processed identically to wells containing cells. LOB should always be zero for ELISpot assays and values above zero indicate deficiencies with reagents or execution.

The limit of detection (LOD) is the threshold where antigen specific responses can be reliably detected. LOD is typically determined by assigning a value of 2 x SFU (spot forming units) of unstimulated PBMC responders. This is an arbitrary but commonly accepted threshold.

The lower limit of quantification (LLOQ) is typically determined by assigning the threshold where the %CV >= 30% for the replicate wells. In practice this is commonly in the range of 20 to 30 SFU for normal control PBMC.

The upper limit of quantification (ULOQ) is dependent on many factors and therefore difficult to assign a threshold to in ELISpot assays. It is common to include a very strong positive stimulator (aCD3, PHA, TPA, etc) that will activate enough responders that the wells are saturated for ELISpot assays using PBMC responders. When images are acquired, it is important to assign TNTC (too numerous to count – effectively exceeding the ULOQ) when spots merge and cannot be reliably counted.

The analyzer (both hardware and software components) used to acquire well images from the assay plates and selection of spot counting parameters have significant impacts on the ability to discriminate and count spots. The IMC has well defined SOPs for analyzer use, has extensive training for parameter selection, maintains documentation with examples of expected and problematic wells. More importantly, the IMC workflow contains a technical review of all reports/results by a different staff member qualified to perform the test/assays. This workflow is critical in establishing and maintaining high quality and longitudinally consistent agreement concerning the discrimination of spots.

## SPECIMENS

Tests/assays are scheduled in advance through coordination with the clinical team and Fresh PBMC are accepted only from IMC qualified upstream sources (TRCS/CPC-IP is the only qualified provider for the specimens in these trials). Cryopreserved cells are used as an alternate path when the tests/assays could not be scheduled for a specimen.

Specimens are drawn prior to treatment and at defined intervals over the course of treatment with the option of additional time points at the discretion of the investigator. The majority of ELISpot assays are run using fresh/real-time PBMC but there is an alternate path for using cryopreserved PBMC.

For this retrospective validation, the specimen identifiers used for the assays were captured in the lab documentation.

## CRITICAL REAGENTS

Reagents for the ELISpot assays are provided by the IMC.

Table 1

| Description | Primary Vendor/Product# | Alternate Vendor/Product# |
|---|---|---|
| Precoated IFNγ ELISpot Stripwell Kit | Mabtech / 3420-4HST-2 | N/A |
| RPMI-1640 | Gibco 11875-093 | Cellgro 10-040-CV |
| FCS | Hyclone SH30071.03 | N/A |
| l-glutamine (200 mM) | Gibco 25030-081 | Cellgro 25-005-CI |
| Antibiotics (Pen. / Stept.) (100X) | QB / 120-095-721 | Cellgro 30-001-CL |
| BME (2-mercaptoethanol) 14M | Sigma / M-3148 | Sigma / M-7522 |
| CEF (peptide pool) | CTL/PA-CEF-002 | Mabtech / 3615-1 |

Peptides used for the stimulations are provided by the investigators. There are batches of the six separate lyophilized peptides in 0.5 mg aliquots sourced from the same manufacturer (JPT) of the clinical vaccines used in the clinical trials. Reconstitution is performed and documented by the IMC, aliquots are assigned a 6-month expiration window and stored at -80C.

For this retrospective validation, the vendor/product/lot numbers for all critical reagents used for the assays were captured in the lab documentation.

## EQUIPMENT

All IMC equipment is restricted to use by core staff only. Equipment operation, maintenance, calibration, OQ/PQ is performed and documented under the IMC SOP infrastructure. Assay documentation includes the specific equipment used.

Table 2

| Description | Primary Vendor/Product# | Alternate Vendor/Product# |
|---|---|---|
| Analyzer | AID / iSpot Spectrum | CTL / S6UNVM2 |

For this retrospective validation, the specific equipment used for the assays were captured in the lab documentation.

For this retrospective validation, acquisition and analysis was performed using the AID analyzer for both J1994 and J2177 trials and the CTL analyzer was used for the J2456 trial. An initial examination of historical plates when the new CTL analyzer was acquired partway through the J1994 and J2177 trials revealed sensitivity issues when acquiring aged plates. Therefore, the decision was made to also acquire all plates going forwards on the CTL analyzer in real time to facilitate possible future comparison for matching spot parameter selection.

## SOPs

There are SOPs for all IMC assays, and assays are performed following SOPs. The relevant SOPs for these ELISpot assays are: thawing of cells/PBMC, counting of cells/PBMC, and the ELISpot assay.

Table 3

| Control Number | SOP Title |
|---|---|
| TR-SOP-IMC-009 | Cell Counting |
| TR-SOP-IMC-010 | Cryopreservation and Thawing of Cells |
| TR-SOP-IMC-011 | ELISpot Assay |

For this retrospective validation, the SOPs used for the assays were captured in the lab documentation.

## EXECUTION/IMPLEMENTATION

N/A (This is a retrospective validation so details pertaining to staff/reagents/equipment/SOPs/etc selection cannot be determined in advance)

## VALIDATION COMPONENTS FOR ANALYSIS – SPECIFICITY

Specificity can be assessed in this plan (determination of ability to differentiate true responses from background). The assays include negative controls in addition to the stimulation conditions. Raw well image data, spot counting parameters, reported results are available for the assays IMC performed.

Table 4

| SFU | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
|---|---|---|---|---|---|---|---|---|---|---|
| mean | 675.0 | 11.1 | 36.1 | 15.0 | 27.3 | 18.8 | 52.5 | 16.7 | 20.0 | 71.8 |
| min | 5.0 | 5.0 | 5.0 | 15.0 | 5.0 | 5.0 | 13.3 | 16.7 | 20.0 | 11.7 |
| max | 2228.3 | 23.3 | 83.3 | 15.0 | 93.3 | 36.7 | 91.7 | 16.7 | 20.0 | 351.7 |
| n | 77 | 3 | 3 | 1 | 5 | 4 | 2 | 1 | 1 | 22 |

These tests were not designed to measure specificity, but partial information can be determined by comparing the responses. Each test of stimulation or peptide was capable of not generating a response and was capable of generating low to very strong responses across the tested specimens. In all cases the number of detected responses was different for each stimulation or peptide. There were very few responses to the smaller fragments of the vaccine peptide. Importantly, the response pattern was different for each of the patients/responders to the different peptides and the CEF pool (table 5).

Table 5

| Observation | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
|---|---|---|---|---|---|---|---|---|---|---|
| n | SFU | SFU | SFU | SFU | SFU | SFU | SFU | SFU | SFU | SFU |
| 1 | 640.0 | | | | | | | | | |
| 2 | 473.3 | | | | | | | | | |
| 3 | 66.7 | | | | | | | | | |
| 4 | 33.3 | | | | | 5.0 | | | | |
| 5 | 700.0 | | | | | | | | | 61.7 |
| 6 | 1388.3 | | | 15.0 | | | | | | |
| 7 | 1721.7 | | | | | | | | | |
| 8 | 2045.0 | | | | | | | | | |
| 9 | 180.0 | | | | | | | | | |
| 10 | 118.3 | | | | | | | | | |
| 11 | 161.7 | | | | | | | | | |
| 12 | 1671.7 | | | | | | | | | |
| 13 | 120.0 | 5.0 | | | | | | | | |
| 14 | 425.0 | | | | | | | | | |
| 15 | 363.3 | | | | | | | | | |
| 16 | 1641.7 | | | | | | | | | |
| 17 | 676.7 | | | | | | | | | |
| 18 | 195.0 | | | | | | | | | 28.3 |
| 19 | 446.7 | | | | | | 13.3 | 16.7 | | 23.3 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 353.3 | | | | | | | | | |
| 21 | 2228.3 | | | | | | | | | |
| 22 | 218.3 | | | | | | | | | 21.7 |
| 23 | 96.7 | | 5.0 | | | | | | | |
| 24 | 878.3 | | | | 13.3 | | | | | |
| 25 | 181.7 | | 20.0 | | | 36.7 | | | | 28.3 |
| 26 | 426.7 | | | | | | | | | |
| 27 | 1965.0 | | | | | | | | | |
| 28 | 323.3 | | | | | | | | | |
| 29 | 573.3 | | 83.3 | | 93.3 | | 91.7 | | | 120.0 |
| 30 | 150.0 | | | | | | | | | |
| 31 | 55.0 | | | | | | | | | 16.7 |
| 32 | 115.0 | | | | | | | | | |
| 33 | 175.0 | | | | | | | | | |
| 34 | 1668.3 | | | | | | | | | |
| 35 | 1843.3 | | | | 11.7 | | | | | |
| 36 | | | | | | | | | | |
| 37 | 125.0 | | | | | | | | | |
| 38 | 45.0 | | | | | | | | | |
| 39 | 1631.7 | | | | | | | | | |
| 40 | | | | | | | | | | |
| 41 | 1510.0 | | | | | | | | | 90.0 |
| 42 | | | | | | | | | | |
| 43 | 1001.7 | | | | 13.3 | | | | | 176.7 |
| 44 | | | | | | | | | | |
| 45 | 1291.7 | | | | | | | | | 148.3 |
| 46 | | | | | | | | | | |
| 47 | | | | | | | | | | |
| 48 | 585.0 | | | | | | | | | 78.3 |
| 49 | | | | | | | | | | |
| 50 | 26.7 | | | | | | | | | 31.7 |
| 51 | | | | | | | | | | |
| 52 | | | | | | | | | | |
| 53 | | 23.3 | | | | 28.3 | | | 20.0 | |
| 54 | | | | | | | | | | 118.3 |
| 55 | 195.0 | | | | | | | | | |
| 56 | | | | | | | | | | |
| 57 | 330.0 | | | | | | | | | |
| 58 | 106.7 | | | | | | | | | |
| 59 | | | | | | | | | | |
| 60 | 1525.0 | | | | | | | | | |
| 61 | 1176.7 | | | | | | | | | 11.7 |
| 62 | 1420.0 | | | | | | | | | |
| 63 | | | | | | | | | | |
| 64 | 5.0 | | | | | | | | | |
| 65 | 1546.7 | | | | | | | | | |
| 66 | 241.7 | | | | | | | | | |
| 67 | | | | | | | | | | |
| 68 | | 5.0 | | | 5.0 | | | | | |
| 69 | 371.7 | | | | | | | | | 26.7 |
| 70 | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 71 | | | | | | | | | | |
| 72 | 193.3 | | | | | | | | | |
| 73 | 863.3 | | | | | | | | | 20.0 |
| 74 | 78.3 | | | | | | | | | |
| 75 | | | | | | | | | | 50.0 |
| 76 | 505.0 | | | | | | | | | 351.7 |
| 77 | | | | | | | | | | |
| 78 | 400.0 | | | | | | | | | 30.0 |
| 79 | 50.0 | | | | | | | | | |
| 80 | 765.0 | | | | | 5.0 | | | | |
| 81 | 386.7 | | | | | | | | | 63.3 |
| 82 | 583.3 | | | | | | | | | |
| 83 | | | | | | | | | | |
| 84 | | | | | | | | | | |
| 85 | | | | | | | | | | |
| 86 | 580.0 | | | | | | | | | |
| 87 | 695.0 | | | | | | | | | |
| 88 | 785.0 | | | | | | | | | |
| 89 | 1421.7 | | | | | | | | | |
| 90 | 785.0 | | | | | | | | | |
| 91 | 808.3 | | | | | | | | | |
| 92 | 26.7 | | | | | | | | | |
| 93 | | | | | | | | | | 38.3 |
| 94 | 825.0 | | | | | | | | | |
| 95 | 781.7 | | | | | | | | | |
| 96 | 21.7 | | | | | | | | | 45.0 |
| 97 | | | | | | | | | | |
| 98 | 493.3 | | | | | | | | | |
| 99 | | | | | | | | | | |
| 100 | 1458.3 | | | | | | | | | |
| 101 | 528.3 | | | | | | | | | |
| 102 | 671.7 | | | | | | | | | |
| 103 | 811.7 | | | | | | | | | |

## VALIDATION COMPONENTS FOR ANALYSIS – PRECISION

Precision can be assessed in this plan (determination of ability to provide consistent results within an assay but not across identical assay runs). The assays are run in triplicate wells. Raw well image data, spot counting parameters, reported results are available for the assays IMC performed.

Table 6

| %cv | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
|---|---|---|---|---|---|---|---|---|---|---|
| mean | 7.1 | 8.2 | 12.5 | 0.0 | 18.1 | 11.9 | 11.9 | 17.3 | 0.0 | 17.1 |
| min | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 17.3 | 0.0 | 4.3 |
| max | 28.6 | 24.7 | 25.0 | 0.0 | 24.7 | 27.0 | 27.0 | 17.3 | 0.0 | 29.4 |
| n | 77 | 3 | 3 | 1 | 5 | 4 | 4 | 1 | 1 | 22 |

Above the LLOQ the variability is well within the expected range for all peptides and stimulations.

These tests were not designed to measure precision, but partial information can be determined by comparing the responses above and below the LLOQ. Individual tables below separate detectable responses above and below the LLOQ for each peptide or stimulation.

Table 7

| | CEF | |
|---|---|---|
| | Detectable Responses | |
| %cv | below LLOQ | above LLOQ |
| mean | 71.2 | 7.1 |
| min | 33.1 | 0.0 |
| max | 173.2 | 28.6 |
| n | 12 | 77 |

Table 8

| | Pep1 | |
|---|---|---|
| | Detectable Responses | |
| %cv | below LLOQ | above LLOQ |
| mean | 93.7 | 8.2 |
| min | 34.6 | 0.0 |
| max | 173.2 | 24.7 |
| n | 13 | 3 |

Table 9

| | Pep2 | |
|---|---|---|
| | Detectable Responses | |
| %cv | below LLOQ | above LLOQ |
| mean | 97.4 | 12.5 |
| min | 68.9 | 0.0 |
| max | 173.2 | 25.0 |
| n | 8 | 3 |

Table 10

| | Pep3 | |
|---|---|---|
| | Detectable Responses | |
| %cv | below LLOQ | above LLOQ |
| mean | 95.9 | 0.0 |
| min | 43.3 | 0.0 |
| max | 173.2 | 0.0 |
| n | 11 | 1 |

Table 11

| | Pep4 | |
|---|---|---|
| | Detectable Responses | |
| %cv | below LLOQ | above LLOQ |
| mean | 84.2 | 18.1 |
| min | 34.6 | 0.0 |
| max | 173.2 | 24.7 |
| n | 11 | 5 |

Table 12

| | Pep5 | |
|---|---|---|
| | Detectable Responses | |
| %cv | below LLOQ | above LLOQ |
| mean | 118.2 | 11.9 |
| min | 50.0 | 0.0 |
| max | 173.2 | 27.0 |
| n | 5 | 4 |

Table 13

| | Pep6 | |
|---|---|---|
| | Detectable Responses | |
| %cv | below LLOQ | above LLOQ |
| mean | 112.0 | 17.1 |
| min | 34.6 | 12.6 |
| max | 173.2 | 21.7 |
| n | 7 | 2 |

Table 14

| | Pep7 | |
|---|---|---|
| | Detectable Responses | |
| %cv | below LLOQ | above LLOQ |
| mean | 110.7 | 17.3 |
| min | 31.5 | 17.3 |
| max | 173.2 | 17.3 |
| n | 13 | 1 |

Table 15

| | Pep8 | |
|---|---|---|
| | Detectable Responses | |
| %cv | below LLOQ | above LLOQ |
| mean | 92.5 | 0.0 |
| min | 33.3 | 0.0 |
| max | 173.2 | 0.0 |
| n | 11 | 1 |

Table 16

| | Pep9 | |
|---|---|---|
| | Detectable Responses | |
| %cv | below LLOQ | above LLOQ |
| mean | 76.5 | 17.1 |
| min | 40.5 | 4.3 |
| max | 173.2 | 29.4 |
| n | 22 | 22 |

As expected, while low frequency counts can be detected they have greater variability when close to the noise floor.

## VALIDATION COMPONENTS FOR ANALYSIS – SENSITIVITY

Sensitivity can be assessed in this plan (determination of ability to detect a signal over a defined range). The assays are run in triplicate wells. Raw well image data, spot counting parameters, reported results are available for the assays IMC performed.

Table 17

| SFU | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
|---|---|---|---|---|---|---|---|---|---|---|
| mean | 675.0 | 11.1 | 36.1 | 15.0 | 27.3 | 18.8 | 52.5 | 16.7 | 20.0 | 71.8 |
| min | 5.0 | 5.0 | 5.0 | 15.0 | 5.0 | 5.0 | 13.3 | 16.7 | 20.0 | 11.7 |
| max | 2228.3 | 23.3 | 83.3 | 15.0 | 93.3 | 36.7 | 91.7 | 16.7 | 20.0 | 351.7 |
| n | 77 | 3 | 3 | 1 | 5 | 4 | 2 | 1 | 1 | 22 |

Each stimulation or peptide was capable of not generating a response and was capable of generating low to very strong responses across the tested specimens. The figures below indicate the distribution and range of responses for each of the peptides or stimulators.

Figure 1

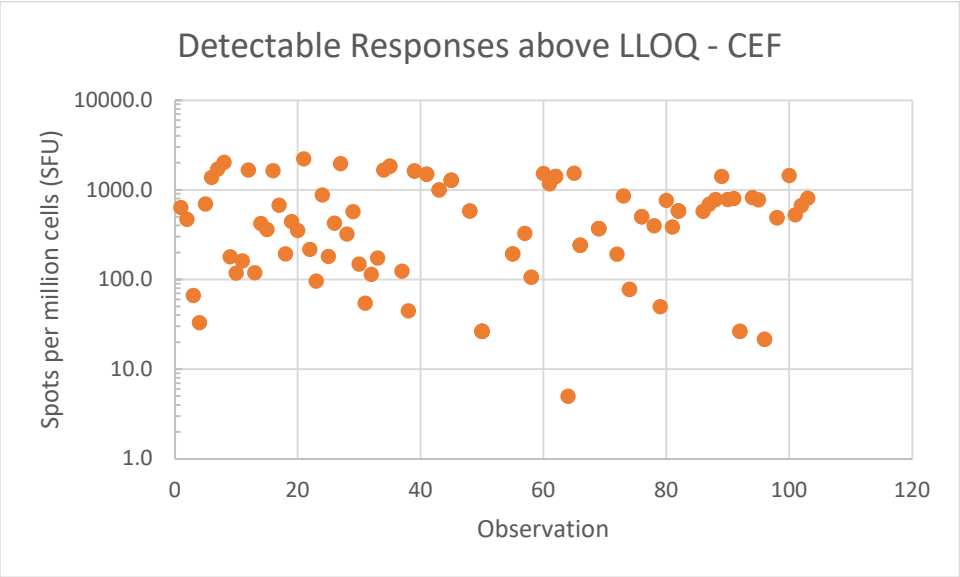



Figure 2

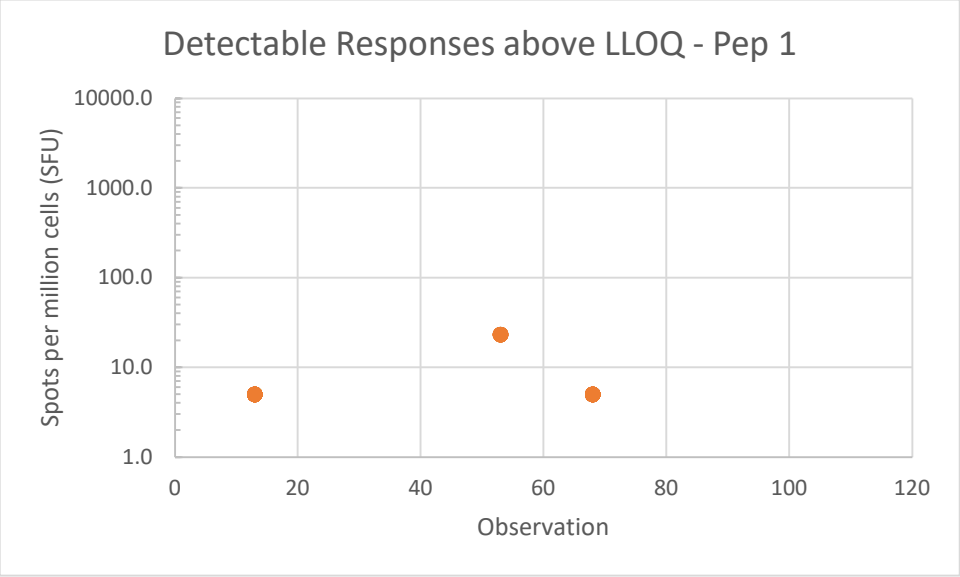


Figure 3

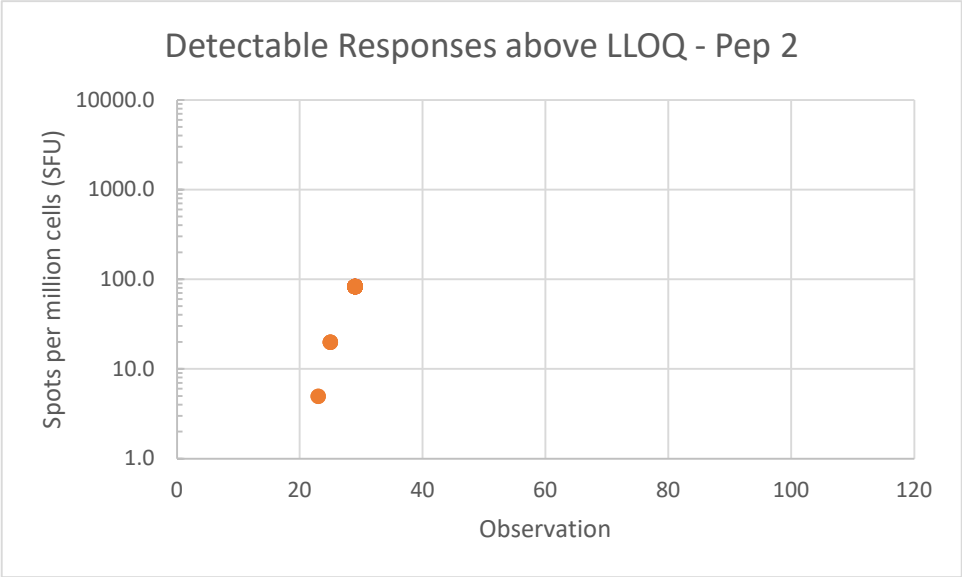



Figure 4

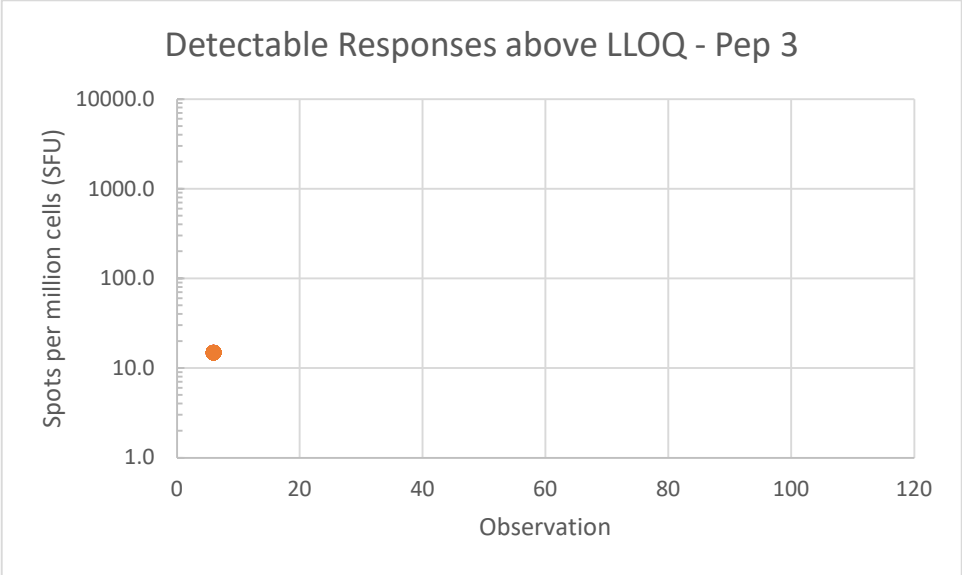


Figure 5

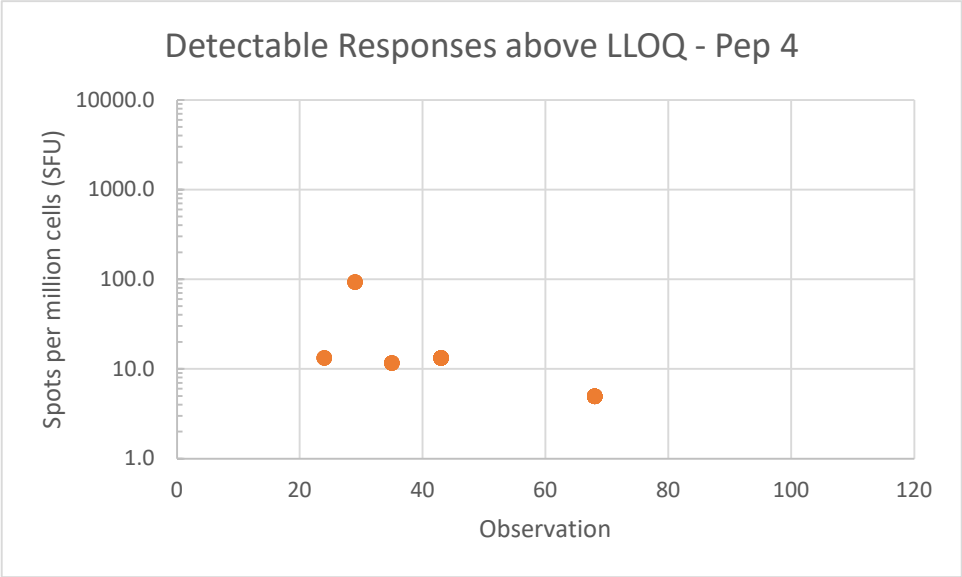



Figure 6

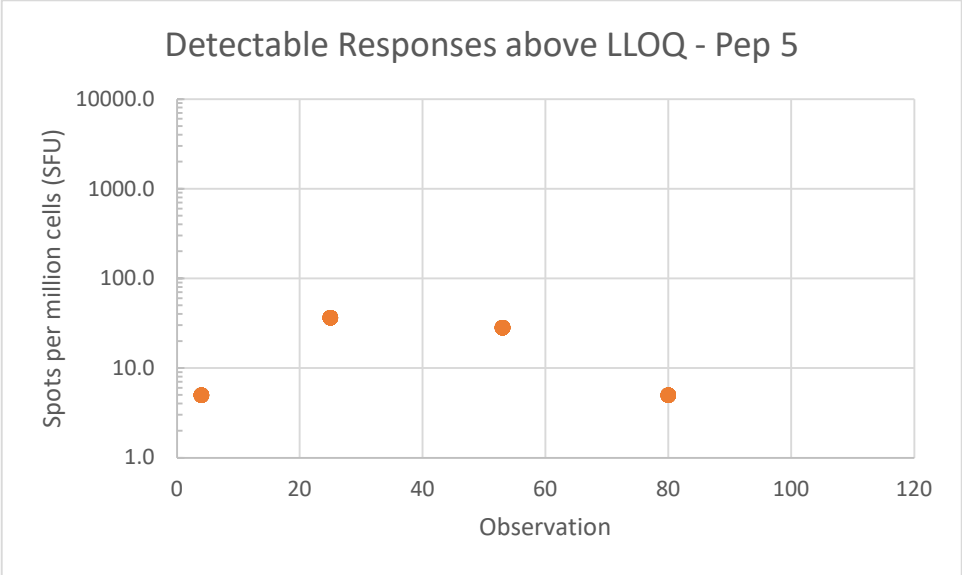


Figure 7

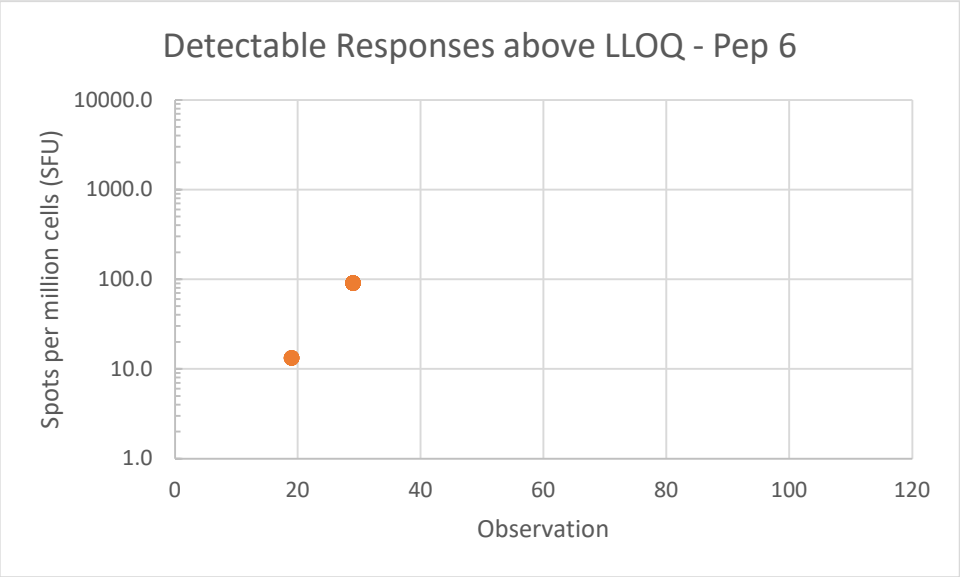



Figure 8

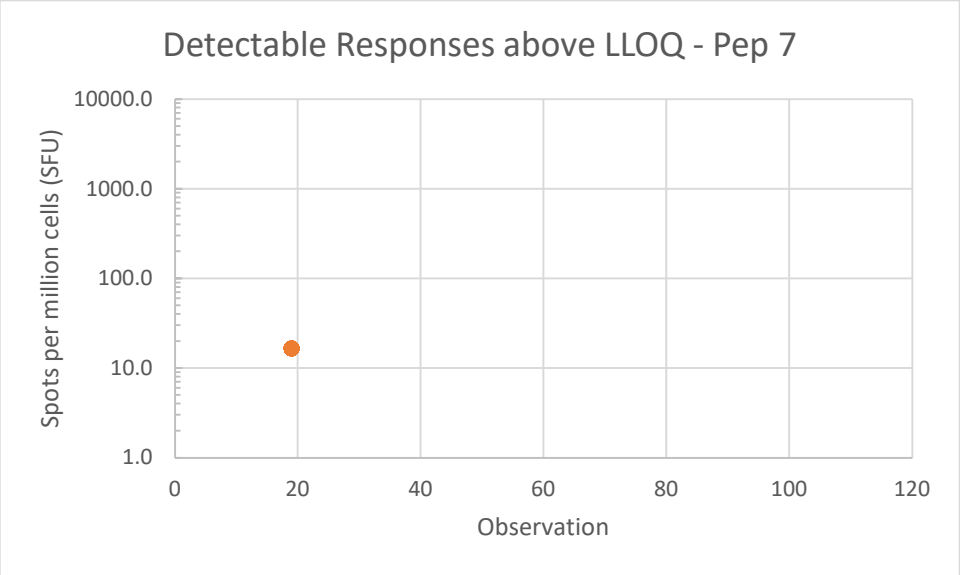


Figure 9

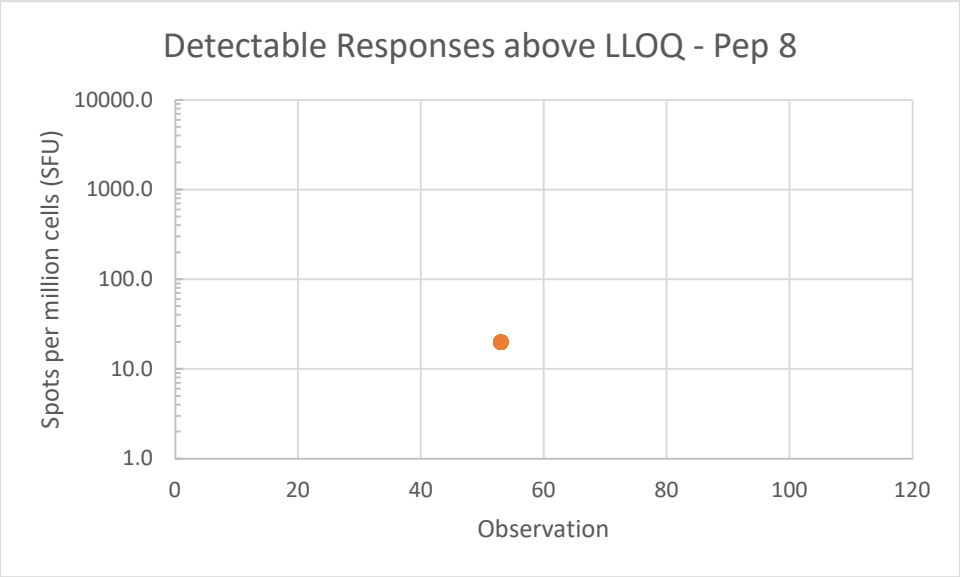
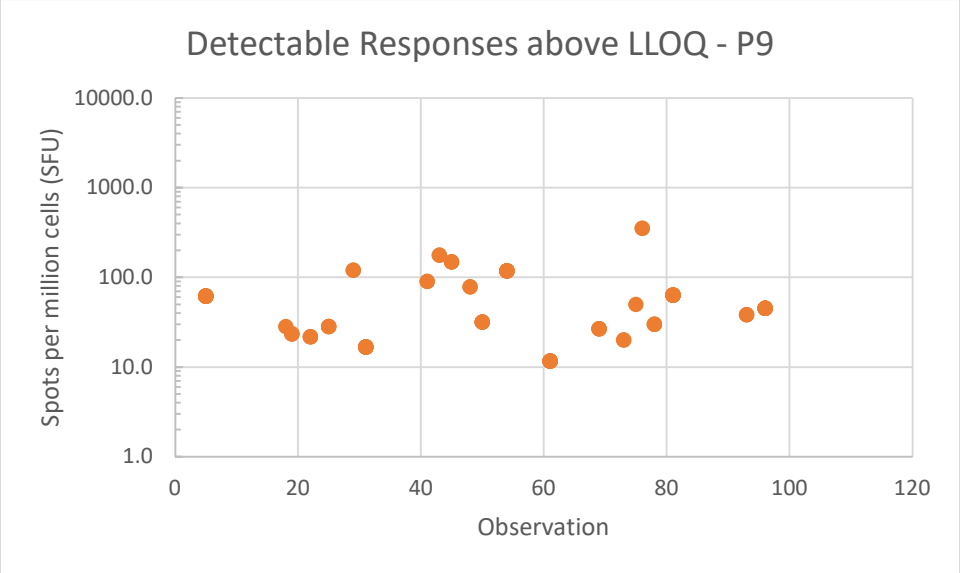


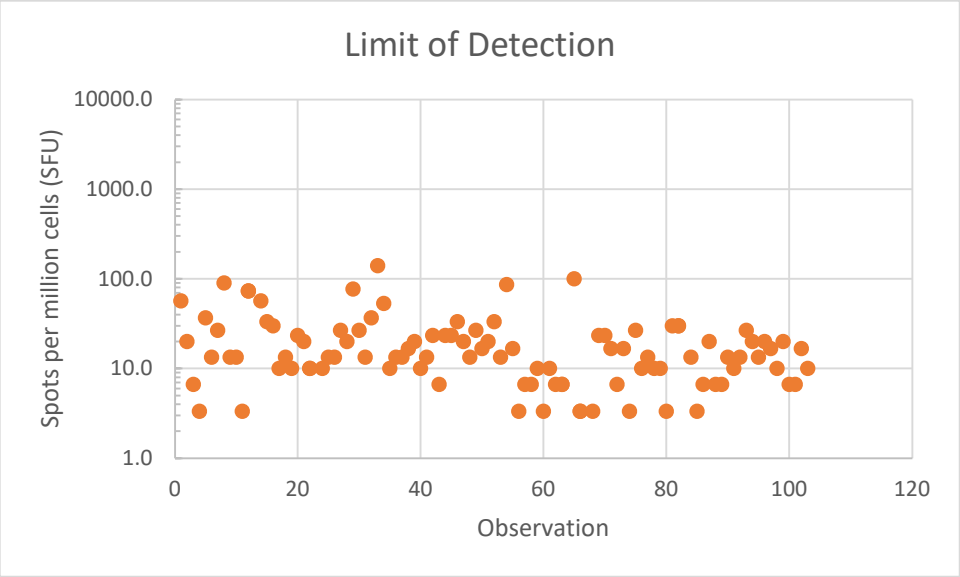

Figure 10




The range of detectable responses (above LLOQ) was broad and not limited to only high spot count responders. The irrelevant peptide failed to generate detectable responses in most specimens.

Because the specimens were from patients treated with immune checkpoint inhibitors it is important to consider the limit of detection.

Figure 11




Some specimens will be from periods where significant background elevation occurs and the appropriate threshold for considering a detectable response meaningful is higher than specimens from baseline or normal individuals. We use LLOQ as 2 x SFU for unstimulated responders (this may cause some true responses to be marked undetected due to the elevated noise floor at some time points when using immune checkpoint inhibitors).

## VALIDATION COMPONENTS FOR ANALYSIS – ROBUSTNESS

Robustness can be assessed in this plan (determination of ability to remain consistent despite minor variations in protocols). Robustness of our SOP to changes in staff executing the assay and changes in reagents (lot#) can be assessed in this retrospective validation.

Staff performing the tests

While not designed to test robustness, there were the staff members that performed these ELISpot assays/tests. While all specimens were different, the subpopulations of the specimens tested by individual staff members had similar characteristics for the detectable responses to the individual peptides, stimulations with the peptide pool and the LOD and were not distinguishable from the whole population.

Table 18

| Detectable Responses > LLOQ - ALL TESTS | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SFU | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 675.0 | 11.1 | 36.1 | 15.0 | 27.3 | 18.8 | 52.5 | 16.7 | 20.0 | 71.8 |
| min | 5.0 | 5.0 | 5.0 | 15.0 | 5.0 | 5.0 | 13.3 | 16.7 | 20.0 | 11.7 |
| max | 2228.3 | 23.3 | 83.3 | 15.0 | 93.3 | 36.7 | 91.7 | 16.7 | 20.0 | 351.7 |
| n | 77 | 3 | 3 | 1 | 5 | 4 | 2 | 1 | 1 | 22 |

| Detectable Responses > LLOQ - Gizzi | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SFU | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 646.2 | 23.3 | #DIV/0! | #DIV/0! | #DIV/0! | 16.7 | #DIV/0! | #DIV/0! | 20.0 | 42.5 |
| min | 55.0 | 23.3 | 0.0 | 0.0 | 0.0 | 5.0 | 0.0 | 0.0 | 20.0 | 11.7 |
| max | 1668.3 | 23.3 | 0.0 | 0.0 | 0.0 | 28.3 | 0.0 | 0.0 | 20.0 | 78.3 |
| n | 18 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 4 |

| Detectable Responses > LLOQ - Thoburn | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SFU | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 690.7 | 5.0 | 36.1 | 15.0 | 27.3 | 20.8 | 52.5 | 16.7 | #DIV/0! | 78.3 |
| min | 5.0 | 5.0 | 5.0 | 15.0 | 5.0 | 5.0 | 13.3 | 16.7 | 0.0 | 20.0 |
| max | 2228.3 | 5.0 | 83.3 | 15.0 | 93.3 | 36.7 | 91.7 | 16.7 | 0.0 | 351.7 |
| n | 51 | 2 | 3 | 1 | 5 | 2 | 2 | 1 | 0 | 18 |

| Detectable Responses > LLOQ - Koch | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SFU | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 639.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| min | 26.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| max | 825.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| n | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

The %cv for the specimens tested by the individual staff members had similar characteristics for the detectable responses for the individual peptides, stimulations and the peptide pool and the LOD and were not distinguishable from the whole population.

Table 19

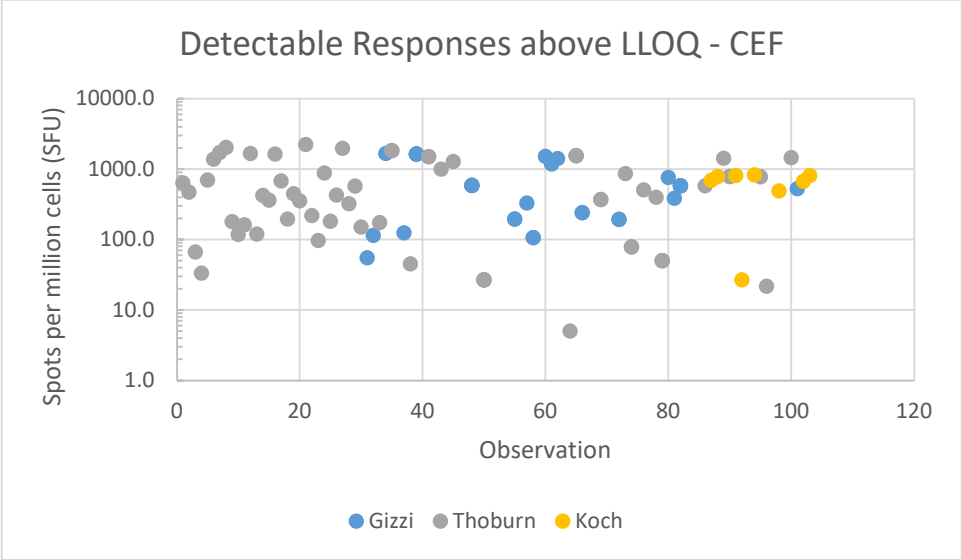
| Detectable Responses > LLOQ - ALL TESTS | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| %cv | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 7.1 | 8.2 | 12.5 | 0.0 | 18.1 | 11.9 | 11.9 | 17.3 | 0.0 | 17.1 |
| min | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 17.3 | 0.0 | 4.3 |
| max | 28.6 | 24.7 | 25.0 | 0.0 | 24.7 | 27.0 | 27.0 | 17.3 | 0.0 | 29.4 |
| n | 77 | 3 | 3 | 1 | 5 | 4 | 4 | 1 | 1 | 22 |

| Detectable Responses > LLOQ - Gizzi | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| %cv | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 7.5 | 24.7 | 0.0 | 0.0 | 0.0 | 13.5 | 13.5 | 0.0 | 0.0 | 15.2 |
| min | 2.7 | 24.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 9.1 |
| max | 18.2 | 24.7 | 0.0 | 0.0 | 0.0 | 27.0 | 27.0 | 0.0 | 0.0 | 24.7 |
| n | 18 | 1 | 0 | 0 | 0 | 2 | 2 | 0 | 1 | 4 |

| Detectable Responses > LLOQ - Thoburn | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| %cv | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 6.5 | 0.0 | 12.5 | 0.0 | 18.1 | 10.4 | 10.4 | 17.3 | 0.0 | 17.6 |
| min | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 17.3 | 0.0 | 4.3 |
| max | 17.3 | 0.0 | 25.0 | 0.0 | 24.7 | 20.8 | 20.8 | 17.3 | 0.0 | 29.4 |
| n | 51 | 2 | 3 | 1 | 5 | 2 | 2 | 1 | 0 | 18 |

| Detectable Responses > LLOQ - Koch | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| %cv | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 10.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| min | 1.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| max | 28.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| n | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Figure 12




The distributions of the detected responses for the specimens tested by individual staff members covered similar ranges and were not distinguishable from the whole population. Because most of the vacciene responses were infrequent, CEF was used for this comparison.

PBMC quality used for the tests

While not designed to test robustness, there was a range of quality for the specimens used in these ELISpot assays/tests. These specimens come from patients on cancer vaccine trials and there are multiple reasons that the PBMC may had RBC contamination after isolation.
Table 20

| Quality | Meaning of grade | Visual Characteristics |
|---|---|---|
| Excellent | cells look perfect, 1 or less RBC per 10 WBC, less than 1 PLT per WBC, no debris | clear/translucent |
| Ok | cells look typical, around 1 RBC per 2 or 3 WBC, less than 2 PLT per WBC, no debris | clear/translucent or light pink |
| Suboptimal | cells look typical, around 1 or 2 RBC per WBC, less than 2 PLT per WBC, no debris | pink |
| Poor | cells look typical, around 3 to 4 RBC per WBC, more than 2 PLT per WBC, no debris | red or pink/red |
| Very poor | cells look typical, more than 4 RBC per WBC, more than 2 PLT per WBC, minor debris/clumping | red |

| Quality | Impact on IMC workflow | Impact on Techncial Time |
|---|---|---|
| Excellent | none | none |
| Ok | none | none |
| Suboptimal | may require changes to counting, may increase difficulty analyzing results/images | may increase technical time |
| Poor | requires change to counting, increases error in counting, increases difficulty analyzing results/images | Increased techncial time |
| Very poor | requires change to counting, increases error in counting, increases difficulty analyzing results/images, possible assay failure | Increased techncial time |

We can create two groups using the quality information (Acceptable Quality PBMC = AQP and Low Quality PBMC = LQP).

While all specimens were different, the subpopulations of the specimens tested (AQP or LQP) had similar characteristics for the detectable responses to the individual peptides, stimulations with the peptide pool and the LOD and were not distinguishable from the whole population.

Table 21

| Detectable Responses > LLOQ - ALL TESTS | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SFU | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 675.0 | 11.1 | 36.1 | 15.0 | 27.3 | 18.8 | 52.5 | 16.7 | 20.0 | 71.8 |
| min | 5.0 | 5.0 | 5.0 | 15.0 | 5.0 | 5.0 | 13.3 | 16.7 | 20.0 | 11.7 |
| max | 2228.3 | 23.3 | 83.3 | 15.0 | 93.3 | 36.7 | 91.7 | 16.7 | 20.0 | 351.7 |
| n | 77 | 3 | 3 | 1 | 5 | 4 | 2 | 1 | 1 | 22 |

| Detectable Responses > LLOQ - PBMC - Acceptable Quality | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SFU | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 599.1 | 14.2 | 36.1 | 15.0 | 40.0 | 18.8 | 52.5 | 16.7 | 20.0 | 75.9 |
| min | 5.0 | 5.0 | 5.0 | 15.0 | 13.3 | 5.0 | 13.3 | 16.7 | 20.0 | 16.7 |
| max | 2045.0 | 23.3 | 83.3 | 15.0 | 93.3 | 36.7 | 91.7 | 16.7 | 20.0 | 351.7 |
| n | 70 | 2 | 3 | 1 | 3 | 4 | 2 | 1 | 1 | 20 |

| Detectable Responses > LLOQ - PBMC - Low Quality (heavy RBC contamination) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SFU | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 1433.8 | 5.0 | 0.0 | 0.0 | 8.3 | 0.0 | 0.0 | 0.0 | 0.0 | 30.8 |
| min | 785.0 | 5.0 | 0.0 | 0.0 | 5.0 | 0.0 | 0.0 | 0.0 | 0.0 | 11.7 |
| max | 2228.3 | 5.0 | 0.0 | 0.0 | 11.7 | 0.0 | 0.0 | 0.0 | 0.0 | 50.0 |
| n | 7 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |

The %cv for the specimens tested in the AQP and LQP groups had similar characteristics for the detectable responses for the individual peptides, stimulations and the peptide pool and the LOD and were not distinguishable from the whole population.

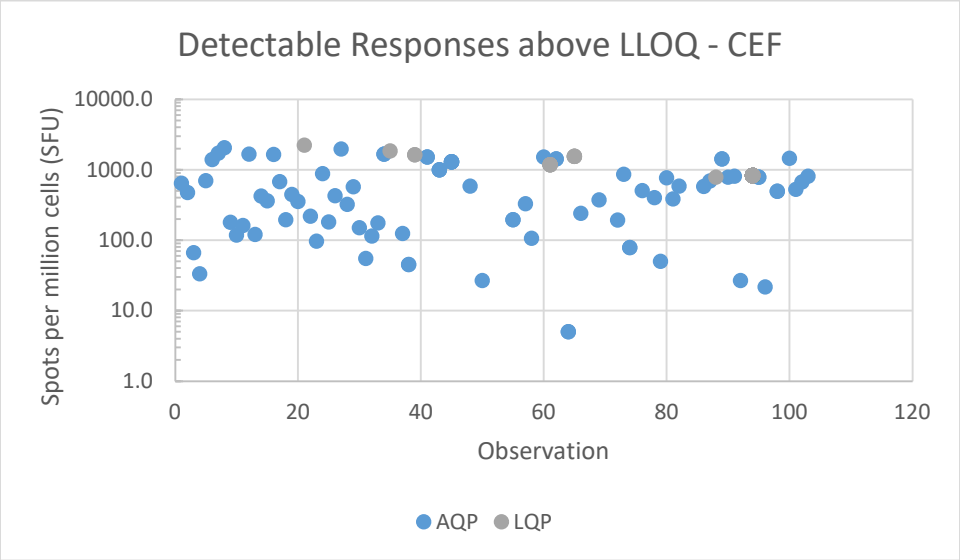
Table 22

| Detectable Responses > LLOQ - ALL TESTS | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| %cv | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 7.1 | 8.2 | 12.5 | 0.0 | 18.1 | 11.9 | 11.9 | 17.3 | 0.0 | 17.1 |
| min | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 17.3 | 0.0 | 4.3 |
| max | 28.6 | 24.7 | 25.0 | 0.0 | 24.7 | 27.0 | 27.0 | 17.3 | 0.0 | 29.4 |
| n | 77 | 3 | 3 | 1 | 5 | 4 | 4 | 1 | 1 | 22 |

| Detectable Responses > LLOQ - PBMC - Acceptable Quality | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| %cv | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 7.4 | 12.4 | 12.5 | 0.0 | 21.9 | 11.9 | 11.9 | 17.3 | 0.0 | 16.3 |
| min | 0.0 | 0.0 | 0.0 | 0.0 | 21.7 | 0.0 | 0.0 | 17.3 | 0.0 | 4.3 |
| max | 28.6 | 24.7 | 25.0 | 0.0 | 22.3 | 27.0 | 27.0 | 17.3 | 0.0 | 29.4 |
| n | 70 | 2 | 3 | 1 | 3 | 4 | 4 | 1 | 1 | 20 |

| Detectable Responses > LLOQ - PBMC - Low Quality (heavy RBC contamination) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| %cv | CEF | Pep1 | Pep2 | Pep3 | Pep4 | Pep5 | Pep6 | Pep7 | Pep8 | Pep9 |
| mean | 4.4 | 0.0 | 0.0 | 0.0 | 12.4 | 0.0 | 0.0 | 0.0 | 0.0 | 25.6 |
| min | 1.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 24.7 |
| max | 11.9 | 0.0 | 0.0 | 0.0 | 24.7 | 0.0 | 0.0 | 0.0 | 0.0 | 26.5 |
| n | 7 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |

Figure 13




The distributions of the detected responses for both the AQP and LQP specimens covered similar ranges and were not distinguishable from the whole population. It is possible that the ability to detect weaker

responses was compromised by LQP specimens but there are not enough LQP specimens to be confident.

## VALIDATION CONCLUSION

The IMC ELISpot test method used for the FLC vaccine trial has consistently achieved high quality results evaluated in this retrospective validation. The assay is sensitive, specific, precise, and robust to operator, qualified reagents, and PBMC quality (RBC contamination) with stronger responses. There were not enough LQP specimens to be confident in the ability to detect weaker responses.

While not designed to assess precision, the IMC ELISpot test method used for the FLC vaccine trial was able to provide consistent results within an assay for the peptide vaccine component and the peptide pool. There were not enough responses to the shorter peptides that covered the mutation in the vaccine peptide to be confident that they were all functional for cells that could respond in the specimens (this was a screen for CD8 responses limited to class 1 MHC – the vaccline 24-mer peptide was the actual test).

While not designed to assess sensitivity, the IMC ELISpot test method used for the FLC vaccine trial was able to detect a signal over a range (LLOQ defined by tests where detectable spots had a %cv <= 30) for each of the six peptide vaccine components and the peptide pool. The range of detectable responses was broad and not limited to only high spot count responders.

While not designed to measure specificity, the IMC ELISpot test method used for the used for the FLC vaccine trial was able detect different responses for the peptide used in the vaccine and the peptide pool. While not covered in this retrospective validation, the baseline responses (pre vaccination) were essentially negative and some of the patients developed significant responses to the peptide in the vaccine over the trial. Importantly, the response pattern was different for each of the patients/responders to the different peptides and the CEF pool

While not designed to assess robustness, the IMC ELISpot test method used for the FLC vaccine trial was able to provide consistent results within an assay for the vaccine component and the peptide pool when performed by three different staff members. The subpopulations of the specimens run by the individual staff members were indistinguishable from the whole population of specimens tested.

While not designed to assess robustness, the IMC ELISpot test method used for the FLC vaccine trial was able to provide consistent results within an assay for each of the six peptide vaccine components and the peptide pool when performed both acceptable quality PBMC (AQP) and low quality PBMC (LQP). The characteristics of the subpopulations of the AQP and LQP specimens were indistinguishable from the whole population of specimens tested for significant responses. The potential compromise of the ability to detect weaker responses in poor quality specimens was not able to be determined because the number of LQP specimens was low.