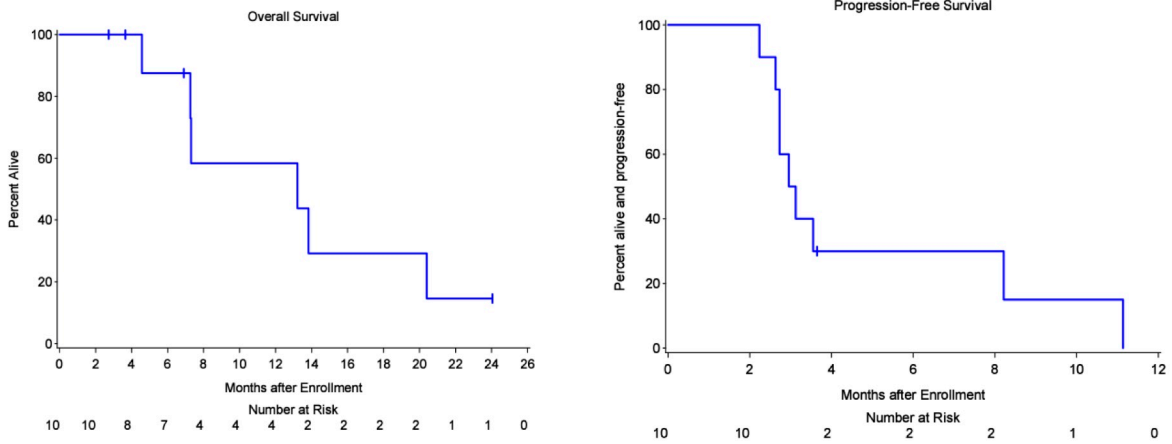
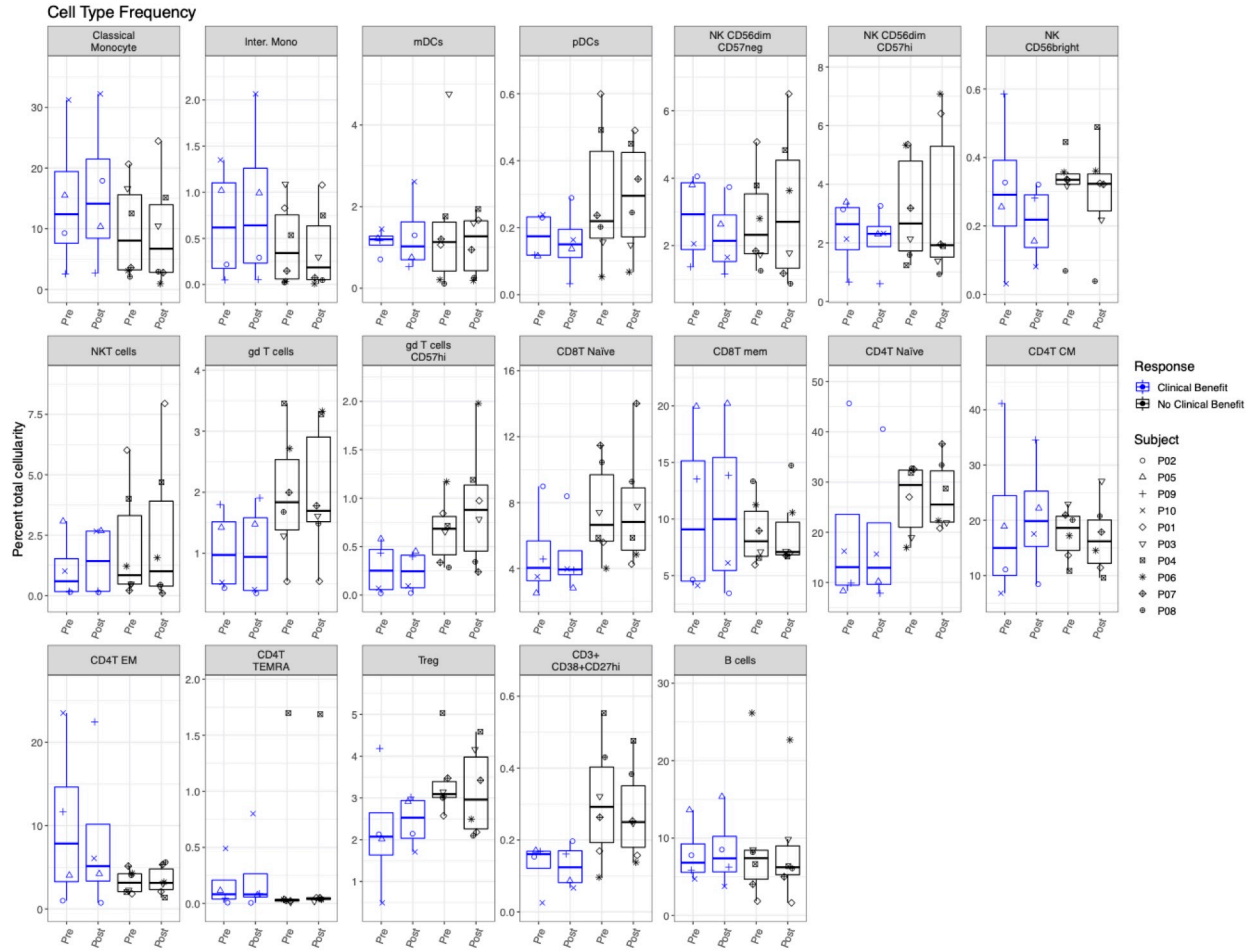


Supplemental Figure 1



Kaplan-Meier estimates of overall and progression-free survival. Median overall survival (OS) was 13.2 months (90% CI: 7.3 – 20.4) and median progression-free survival (PFS) was 3.0 months (90% CI: 2.6-8.2)

Supplemental Figure 2 Cell type frequencies, cellularity



Changes in cell type frequencies of CB and NCB over the course of treatment

Supplemental Table 1 Prior systemic therapy

Prior Systemic Therapy	Number of Patients	No Clinical Benefit	Clinical Benefit
Ipi-Nivo	8	4	4
Nivo monotherapy	1	1	0
Pembro monotherapy	1	1	0
Ipi monotherapy	1	1	0
CMP-001	1	1	0
Temozolomide	1	0	1
Dabrafenib/Trametinib	1	0	1
Chemotherapy*	3	1	2

Abbreviations: Ipi, Ipilimumab; Nivo, Nivolumab; Pembro, Pembrolizumab

*Carboplatin/Paclitaxel, Cyclophosphamide/IRX-2, Cisplatin.

Supplemental Table 2 Treatment-related adverse events

System-Organ Class	Description	CTCAE Grade			
		1	2	3	4
		n=105	n=52	n=18	n=2
Blood and lymphatic system disorders	Anemia	2	-	-	-
	Eosinophilia	1	-	-	-
	Leukocytosis	-	-	1	-
Endocrine disorders	Hypothyroidism	-	1	-	-
Eye disorders	Decreased Close Visual Acuity	1	-	-	-
	Dry Eye	1	-	-	-
	Eye Pain	1	-	-	-
Gastrointestinal disorders	Abdominal Pain	-	1	1	-
	Constipation	2	-	-	-
	Diarrhea	4	-	-	-
	Dry Mouth	2	-	-	-
	Dyspepsia	-	1	-	-
	Dysphagia	1	-	-	-
	Gastrointestinal Pain	1	-	-	-
	Hemorrhoids	1	-	-	-
	Mucositis Oral	-	1	-	-
	Nausea	2	1	-	-
	Oral Dysesthesia	1	-	-	-
	Rectal Hemorrhage	1	-	-	-
	Vomiting	2	1	1	-
General disorders and administration site conditions	Chills	1	-	-	-
	Edema Limbs	2	-	-	-
	Fatigue	3	3	1	-
	Fever	-	-	1	-
	Flu Like Symptoms	1	-	-	-
	Infusion Related Reaction	1	-	-	-
Infections and infestations	Pain	1	-	-	-
	Encephalitis Infection	-	-	-	1
	Lung Infection	-	-	1	-
	Primary Tumor- Oropharynx	-	-	1	-
	Sinusitis	-	1	-	-
Injury, poisoning, and procedural complications	Urinary Tract Infection	-	1	-	-
	Fall	1	-	-	-
Investigations	Hernia	-	1	-	-
	Alanine Aminotransferase Increased	4	-	-	-
	Alkaline Phosphatase Increased	1	-	-	-
	Aspartate Aminotransferase Increased	3	1	-	-
	Blood Bilirubin Increased	1	-	-	-

	Blood Lactate Dehydrogenase Increased	1	-	-	-
	Creatinine Increased	3	-	-	-
	Increased Bun	1	-	-	-
	INR Increased	-	1	-	-
	Lipase Increased	2	1	-	-
	Lymphocyte Count Decreased	1	3	-	-
	Neutrophil Count Decreased	2	-	-	-
	Platelet Count Decreased	2	-	-	-
	Platelet Count Increased	1	-	-	-
	Serum Amylase Increased	1	1	-	-
	Weight Loss	3	-	-	-
Metabolism and nutrition disorders	Anorexia	-	3	-	-
	Dehydration	-	1	-	-
	Glucose Intolerance	1	-	-	-
	Hyperkalemia	2	-	-	-
	Hypoalbuminemia	-	3	-	-
	Hypocalcemia	-	1	-	-
	Hypokalemia	1	-	-	-
	Hyponatremia	5	-	-	-
	Hypophosphatemia	1	2	-	-
Musculoskeletal and connective tissue disorders	Hypoproteinemia	1	-	-	-
	Arthralgia	2	2	-	-
	Arthritis	1	-	-	-
	Avascular Necrosis	-	1	-	-
	Back Pain	1	-	-	-
	Flank Pain	-	-	1	-
	Generalized Muscle Weakness	1	-	-	-
	Left Chest Wall Pain	-	1	-	-
Neoplasms benign, malignant, and unspecified (incl cysts and polyps)	Neck Pain	1	-	2	-
	Pain in Extremity	2	-	-	-
Nervous system disorders	Tumor Pain	1	-	-	-
	Dizziness	2	-	-	-
	Dysesthesia	-	1	-	-
	Headache	2	4	2	-
Psychiatric disorders	Paresthesia	-	1	-	-
	Confusion	1	-	-	-
Renal and urinary disorders	Insomnia	1	1	-	-
	Hematuria	3	-	-	-
	Proteinuria	2	4	-	-
Respiratory, thoracic and mediastinal disorders	Allergic Rhinitis	-	1	-	-
	Cough	2	-	-	-
	Dyspnea	1	1	-	-
	Epistaxis	1	-	-	-
	Hoarseness	1	-	-	-
	Nasal Congestion	1	-	-	-

	Pulmonary Edema	-	1	-	-
	Rhinorrhea	1	-	-	-
	Sore Throat	1	-	-	-
Skin and subcutaneous tissue disorders	Alopecia	1	-	-	-
	Eczema- Right Palm	1	-	-	-
	Photosensitivity	1	-	-	-
	Pruritus	3	-	-	-
	Psoriasis	-	1	-	-
	Rash	1	-	-	-
	Rash Maculo-Papular	-	1	-	-
Vascular disorders	Hypertension	-	2	6	1
	Thromboembolic Event	-	1	-	-

Abbreviations: CTCAE, common terminology criteria for adverse events

Supplemental Table 3 Cytokine measurements values

Analyte pg/mL	Clinical benefit	Non-clinical benefit	p-value
IL-1b	91.4 (1.0 – 679.9)	89.6 (1.0 – 566.7)	0.97
IL-3	889.8 (5.1 – 3909.6)	142.2 (1.0 – 1210.2)	0.61
IL-4	215.6 (10.6 – 668.0)	90.6 (19.6 – 467.4)	0.91
IL-5	9.4 (1.0 – 31.8)	6.3 (1.0 – 28.3)	0.81
IL-6	8.6 (2.2 – 24.7)	7.1 (1.1 – 16.7)	0.61
IL-7	24.7 (1.6 – 115.1)	10.0 (3.2 – 64.8)	0.99
IL-10	7.1 (1.0 – 22.5)	3.7 (1.0 – 13.5)	0.99
IL-12p70	1023.5 (30.5 – 3563.8)	308.6 (9.1 – 2694.0)	0.91
IL-13	38038.0 (27648.0 – 57338.0)	36280.0 (1.0 – 53411.0)	0.91
CXCL10	109.4 (71.9 – 258.8)	128.4 (97.6 – 470.4)	0.35
FGFb	35.4 (1.1 – 120.0)	5.2 (0.3 – 58.4)	0.76
Flt-3 ligand	303.3 (83.0 – 647.7)	170.4 (60.6 – 223.5)	0.48
HGF	273.2 (55.7 – 364.4)	187.1 (91.6 – 953.5)	0.91
PD-L1	55.1 (28.3 – 107.1)	63.5 (30.1 – 84.3)	0.76
PDGF-AB	1637.5 (936.1 – 4138.6)	1097.7 (489.3 – 1905.5)	0.27
TGFa	199.2 (1.6 – 432.1)	57 (6.7 – 164.2)	0.91
TNFa	64.4 (1.0 – 347.1)	32.5 (2.0 – 241.8)	0.99
TNFb	120.2 (1.0 – 555.8)	29.0 (1.0 – 335.3)	0.97
VEGFa	113.5 (29.1 – 418.6)	86.0 (27.8 – 318.0)	0.99

All data are presented in median (range) pg/mL.

Supplemental Table 4 Mass cytometry antibody panel

Metal	Marker	Clone
142Nd	CD19	HIB19
143Nd	CD45RA	HI100
144Nd	CD69	FN50
145Nd	CD4	RPA-T4
146Nd	CD8a	RPA-T8
147Sm	CD11c	Bu15
148Nd	ICOS	C398.4A
149Sm	CD56	NCAM16.2
150Nd	LAG3	11C3C65
151Eu	CD123	6H6
152Sm	TCR $\gamma\delta$	11F2
153Eu	CXCR5	RF8B2
154Sm	CD3	UCHT1
155Gd	CD27	L128
156Gd	CD14	HCD14
158Gd	CD33	WM53
159Tb	TIGIT	MBSA43
160Gd	MIP1b	D21-1351
161Dy	Tbet	4B10
162Dy	FoxP3	259D/C7
163Dy	CD57	HCD57
164Dy	IL-17a	N49-653
165Ho	IFNg	B27
166Er	NKG2D	ON72
167Er	CCR7	G043H7
168Er	Ki-67	B56
169Tm	CD25	2A3
170Er	CTLA-4	14D3
171Yb	CCR5	NP6G4
172Yb	CD38	HIT2
173Yb	HLA-DR	L243
174Yb	PD-1	EH12.2H7
175Lu	CCR4	L291H4
176Yb	CD127	A019D5
209Bi	CD16	3G8

Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-Human CD19 (HIB19)-142Nd—100 Tests (1:100)	Fluidigm	Cat#3142001B
Anti-Human CD45RA (HI100)-143Nd—100 Tests (1:100)	Fluidigm	Cat#3143006B
Anti-Human CD69 (FN50)-144Nd—100 Tests (1:100)	Fluidigm	Cat#3144018B
Anti-Human CD4 (RPA-T4)-145Nd—100 Tests (1:100)	Fluidigm	Cat#3145001B
Anti-Human CD8 (RPA-T8)-146Nd—100 Tests (1:100)	Fluidigm	Cat#3146001B
Anti-Human CD11c (Bu15)-147Sm—100 Tests (1:100)	Fluidigm	Cat#3147008B
Anti-CD278/ICOS (C398.4A)-148Nd—100 Tests (1:100)	Fluidigm	Cat#3148019B
Anti-Human CD56/NCAM (NCAM16.2)-149Sm—100 Tests (1:100)	Fluidigm	Cat#3149021B
Anti-Human CD223/LAG-3 (11C3C65)-150Nd—100 Tests (1:100)	Fluidigm	Cat#3150030B
Anti-Human CD123/IL-3R (6H6)-151Eu—100 Tests (1:100)	Fluidigm	Cat#3151001B
Anti-Human TCRgd (11F2)-152Sm—100 Tests (1:100)	Fluidigm	Cat#3152008B
Anti-Human CD185/CXCR5 (RF8B2)-153Eu—100 Tests (1:100)	Fluidigm	Cat#3153020B
Anti-Human CD3 (UCHT1)-154Sm—100 Tests (1:200)	Fluidigm	Cat#3154003B
Anti-Human CD27 (L128)-155Gd—100 Tests (1:100)	Fluidigm	Cat#3155001B
Anti-Human CD14 (HCD14)-156Gd—100 Tests (1:100)	Fluidigm	Cat#3156019B
Anti-Human CD33 (WM53)-158Gd—100 Tests (1:100)	Fluidigm	Cat#3158001B
Anti-Human TIGIT (MBSA43)-159Tb—100 Tests (1:100)	Fluidigm	Cat#3159038B
Anti-Human/Mouse MIP1beta (D21-1351)-160Gd—100 Tests (1:100)	Fluidigm	Cat#3160013B
Anti-Human/Mouse Tbet (4B10)-161Dy—100 Tests (1:100)	Fluidigm	Cat#3161014B
Anti-Human FoxP3 (259D/C7)-162Dy—50 Tests (1:100)	Fluidigm	Cat#3162024A
Anti-Human CD57 (HCD57)-163Dy—100 Tests (1:200)	Fluidigm	Cat#3163022B
Anti-Human IL-17A (N49-653)-164Dy—100 Tests (1:100)	Fluidigm	Cat#3164002B

Anti-Human IFN γ (B27)-165Ho—100 Tests (1:100)	Fluidigm	Cat#3165002B
Anti-Human CD314/NKG2D (ON72)-166Er—100 Tests (1:100)	Fluidigm	Cat#3166016B
Anti-Human CD197/CCR7 (G043H7)-167Er—50 Tests (1:100)	Fluidigm	Cat#3167009A
Anti-Ki-67 (B56)-168Er—100 Tests (1:100)	Fluidigm	Cat#3168007B
Anti-Human CD25 (2A3)-169Tm—100 Tests (1:100)	Fluidigm	Cat#3169003B
Anti-Human CD152/CTLA-4 (14D3)-170Er—100 Tests (1:100)	Fluidigm	Cat#3170005B
Anti-Human CD195/CCR5 (NP-6G4)-171Yb—50 Tests (1:50)	Fluidigm	Cat#3171017A
Anti-Human CD38 (HIT2)-172Yb—100 Tests (1:100)	Fluidigm	Cat#3172007B
Anti-Human HLA-DR (L243)-173Yb—100 Tests (1:200)	Fluidigm	Cat#3173005B
Anti-Human CD279/PD-1 (EH12.2H7)-174Yb—100 Tests (1:100)	Fluidigm	Cat#3174020B
Anti-Human CD194/CCR4 (L291H4)-175Lu—50 Tests (1:50)	Fluidigm	Cat#3175035A
Anti-Human CD127/IL-7Ra (A019D5)-176Yb—100 Tests (1:100)	Fluidigm	Cat#3176004B
Anti-Human CD16 (3G8)-209Bi—100 Tests (1:100)	Fluidigm	Cat#3209002B
Chemicals, peptides, and recombinant proteins		
Ficoll-Paque™ Plus	Sigma-Aldrich	Cat#17144003
Dimethyl Sulfoxide (DMSO)	Fisher BioReagents	Cat#BP231-100
Gibco™ Phosphate-Buffered Saline (PBS)	Thermo Fisher Scientific	Cat#10010023
Antibiotic-Antimycotic (100 \times)	Gibco	Cat#15240096
Bovine Serum Albumin (BSA), 30% \pm 2% in 0.85% NaCl	Sigma-Aldrich	Cat#A7284
Sodium Azide, 10% (w/v) solution in Ultra-Pure H ₂ O	Teknova	Cat#S0209
RPMI Medium 1640 [+] L-glutamine	Thermo Fisher Scientific	Cat#11875093
Gibco™ Premium Plus Fetal Bovine Serum, heat inactivated, qualified (FBS)	Thermo Fisher Scientific	Cat#10438026
UltraPure™ DNase/RNase-Free Distilled Water	Invitrogen	Cat#10977023
ViaStain™ AOPI Staining Solution	Nexcelom Biosciences	Cat#CS2-0106-5mL
Cell-ID™ Intercalator-103Rh—500 μ M	Fluidigm	Cat#201103A
Cell-ID™ Intercalator-191/193Ir—125 μ M	Fluidigm	Cat#201192B
Maxpar® Cell Acquisition Solution	Fluidigm	Cat#201237

Maxpar® Cell Staining Buffer	Fluidigm	Cat#201068
Maxpar® 10× Barcode Perm Buffer	Fluidigm	Cat#201057
EQ™ Four Element Calibration Beads	Fluidigm	Cat#201078
eBioscience™ Permeabilization Buffer (10×)	Invitrogen	Cat#00833356
eBioscience™ Fixation/Permeabilization Concentrate	Invitrogen	Cat#00512343
eBioscience™ Fixation/Permeabilization Diluent	Invitrogen	Cat#00522356
Human TruStain FcX™ (FcR Blocking Solution)	BioLegend	Cat#422302
Pierce™ 16% Formaldehyde Solution (w/v), Methanol-free	Thermo Fisher Scientific	Cat#28906
CyTOF® Tuning Solution, 250 mL	Fluidigm	Cat#201072
Critical commercial assays		
Cell-ID™ 20-Plex Pd Barcoding Kit	Fluidigm	Cat#201060
Biological samples		
Human PBMCs isolated from whole blood clinical samples (sex, age as required par study)	Any Supplier	N/A
Software and algorithms		
FlowJo v.10	Becton Dickinson & Company	FlowJo
GraphPad Prism v9.2	GraphPad Software, LLC	GraphPad Prism
CyTOF Software v7.0.8493	Fluidigm	CyTOF Software
Other		
CoolCell™ LX Cell Freezing Vial Containers	Corning Inc.	Cat#07210001
15 mL Polypropylene Centrifuge Tubes	Corning Inc.	Cat#430052
50 mL Polypropylene Centrifuge Tubes	Corning Inc.	Cat#430829
Cellometer® SD100 Cell Counting Chamber Slides	Nexcelom Biosciences	CHT4-SD100-014
70 µm Cell Strainer, Polypropylene Frame	Biologix	Cat#15-1070
Eppendorf® 1.5 mL Safe-Lock Microcentrifuge Tubes, natural	Eppendorf	Cat#022363204
96-well Polystyrene V-Bottom Plate, untreated	Corning Inc.	Cat#3896
Polystyrene 96-well Microplate Corner Notch Lid	Corning Inc.	Cat#3930
Falcon® 5 mL Round Bottom Polypropylene Tubes (FACS tubes)	Corning Inc.	Cat#352063
Falcon® 5 mL Round Bottom Polystyrene Tubes with 35 µm Cell Strainer Snap Caps	Corning Inc.	Cat#352235
ThermoFisher Scientific™ Sorvall™ Legend™ XTR Refrigerated Centrifuge, 120VAC	Thermo Fisher Scientific	Cat#75004521

Fisherbrand™ Mini-Centrifuge 100–240 V, 50/6-Hz Universal Plug, Grey	Thermo Fisher Scientific	Cat#12-006-901
Fisher Scientific Digital Vortex Mixer	Thermo Fisher Scientific	Cat#0215370
Thermo Scientific™ Precision™ Circulating Water Bath	Thermo Fisher Scientific	Cat#TSCIR19
Cellometer® Auto 2000 Cell Viability Counter	Nexcelom Biosciences	Auto 2000
Fluidigm Helios™ Mass Cytometer	Fluidigm	N/A
Nebulizer for Helios™ Mass Cytometer	Fluidigm	Cat#107144
WB Injector for Helios™ Mass Cytometer	Fluidigm	Cat#107950

Complete RPMI

Reagent	Final concentration	Amount
RPMI Medium 1640 (1×)	n/a	500 mL
FBS	10%	50 mL
Antibiotic-Antimycotic (100×)	1%	5 mL
Total	n/a	555 mL

Store at 4°C for up to 2 weeks.

CyFACS

Reagent	Final concentration	Amount
PBS	n/a	500 mL
30% BSA	0.5%	8.3 mL
5% Sodium Azide	0.02%	2 mL
Total	n/a	510.3 mL

Store at 4°C for up to 6 weeks.

FoxP3 Fixation/Permeabilization Buffer

Reagent	Final concentration	Amount
Fixation/Permeabilization Concentrate	25%	8 mL
Fixation/Permeabilization Diluent	75%	32 mL
Total	n/a	40 mL