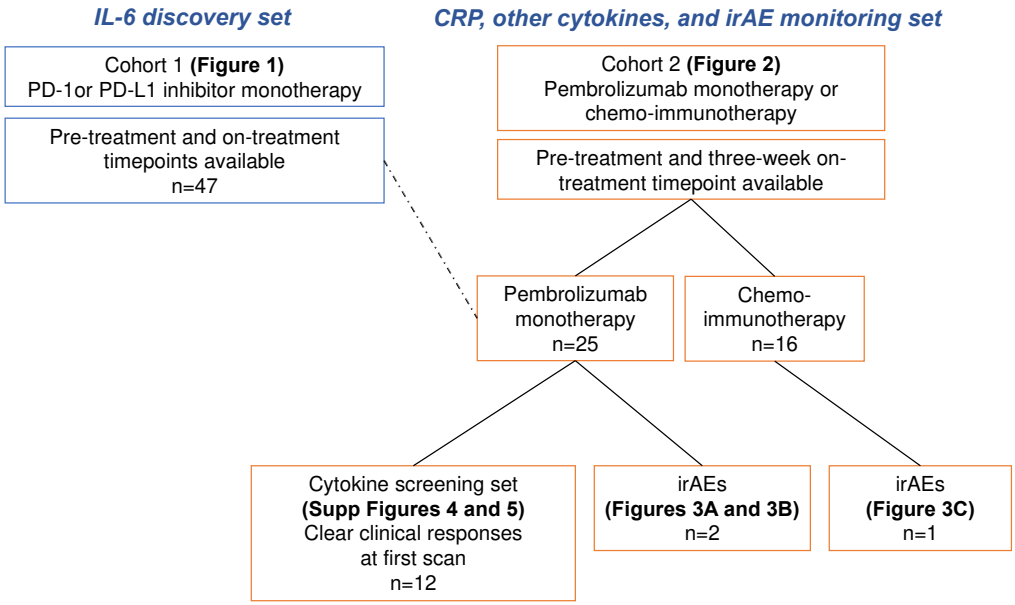
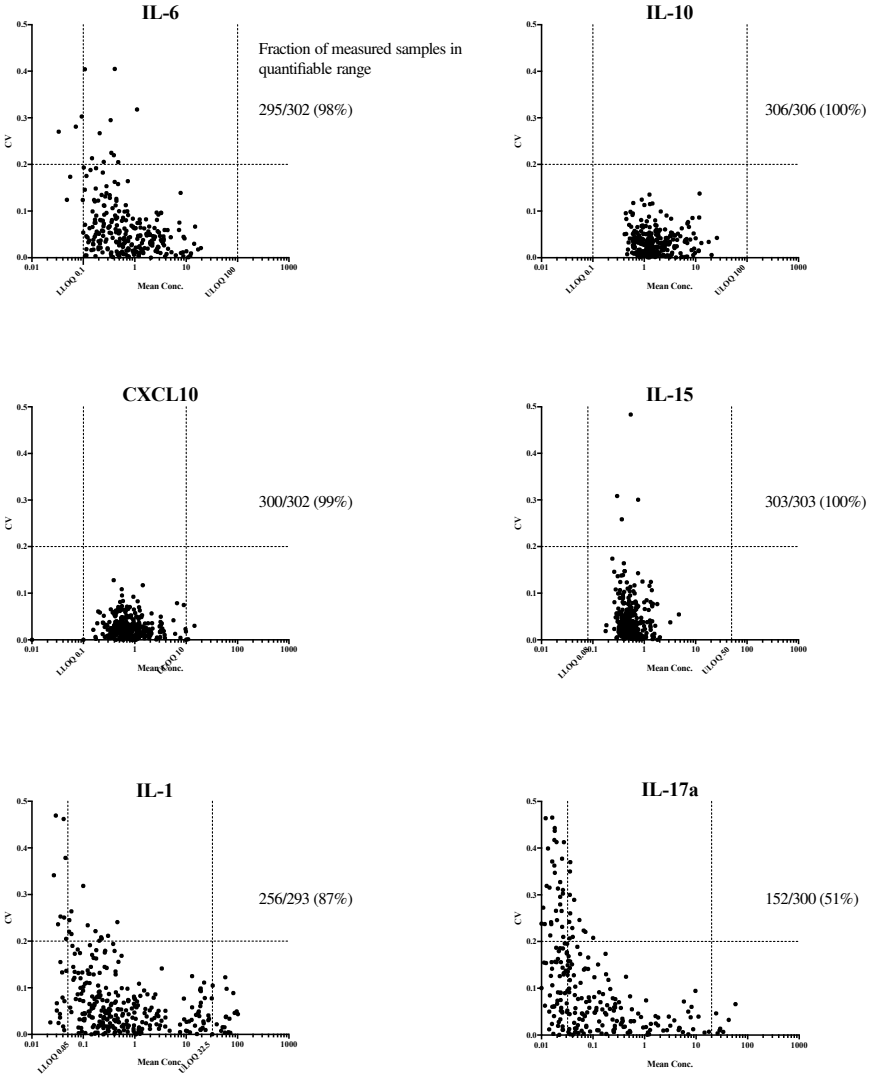
Supplemental material

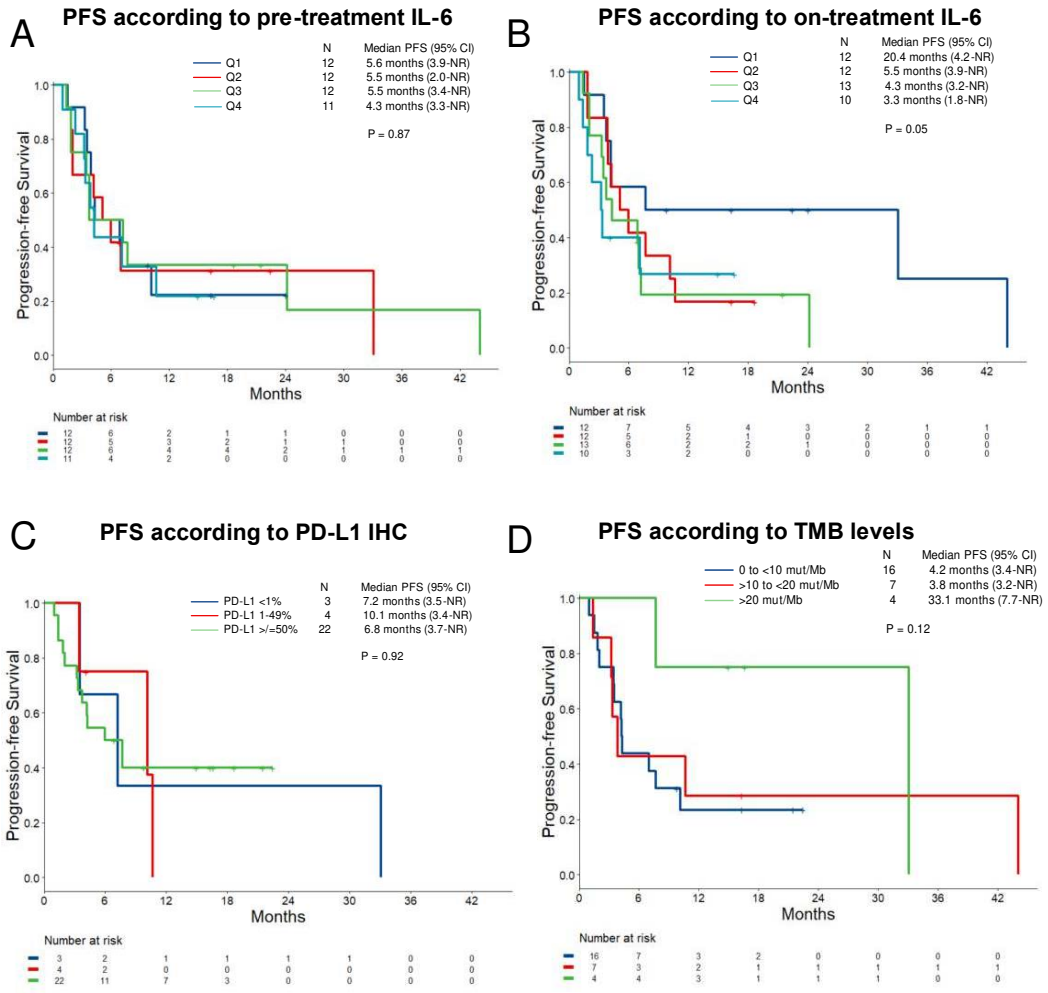
Supp Fig 1. Flow diagram outlining patient cohorts in this study. Dotted line represents that the 25 pembrolizumab monotherapy samples were included in both sets.



Supp Fig 2. Lower and upper limits of quantitation (vertical lines) were derived from calibrators and sample concentrations with less than 20% coefficient of variation (horizontal line). The fraction of plasma samples (including both pre- and on-treatment samples) is this reportable measuring range are reported in the top right quadrant. All cytokine levels below the lower limit of quantitation (LLOQ) or above the upper limit of quantiation (ULOQ) were set to the respective limit for the downstream analysis.



Supp Fig 3. Outcomes by IL-6, PD-L1, and TMB levels. A and B. PFS by IL-6 levels at either pre-(A) or on-treatment (B) according to quartile (Q1 lowest to Q4 highest). p=0.87 and p=0.05 comparing all quartiles by logrank test for trend. C and D. PFS by levels of PD-L1 IHC (C) and TMB (D). p=0.92 and p=0.12 comparing all levels by logrank test for trend.

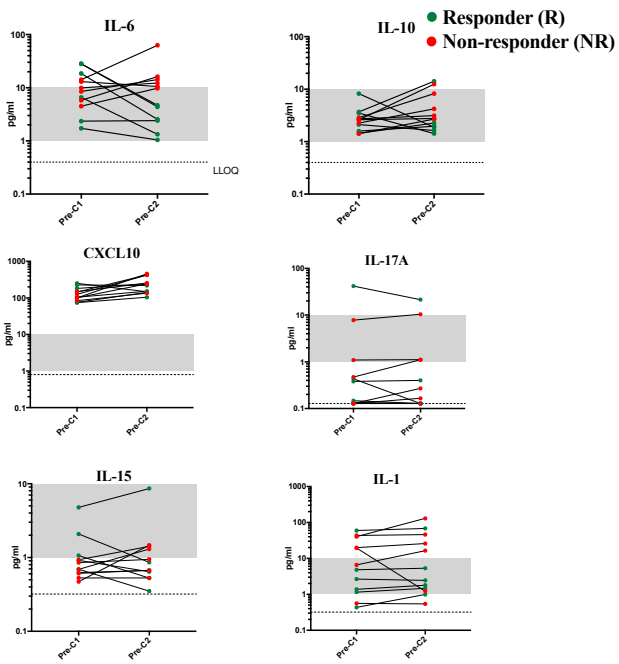


Supp Fig 4. Cytokine changes in 12 pembrolizumab monotherapy patients with early radiologic responses: clear decrease for responders or increase for nonresponders in tumor burden at first scan and on-treatment timepoints three weeks after first dose of therapy. A. Table indicates patient outcomes across the response categories. B. Absolute concentrations of these cytokines from pre-treatment to three weeks after one cycle of pembrolizumab. Each line represents one patient with responders in green and non-responders in red. The dotted horizontal line is the Simoa lower limit of quantitation (as shown in Supp Fig 1). The gray area represents the range of lower quantitation limits of other conventional immunoassays. C. Percentage change in the respective cytokine from pre-treatment to three weeks after first dose of therapy. Results from two independent experiments with different aliquots of the same plasma specimens are shown. Responder (R) Nonresponder (NR). **p<0.01, *p<0.05, ns not significant by Mann Whitney test.

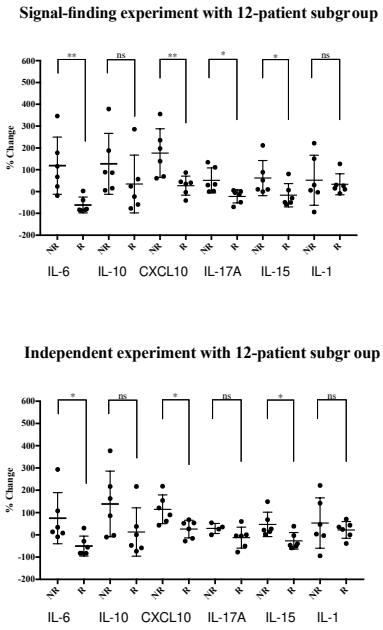
A

		Responders	Non-responders
Total number of patients		6	6
PFS months, range in months		1 to 4	8 to 22
Best Overall Response			
	PR	5	
	SD	1	4
	PD		2

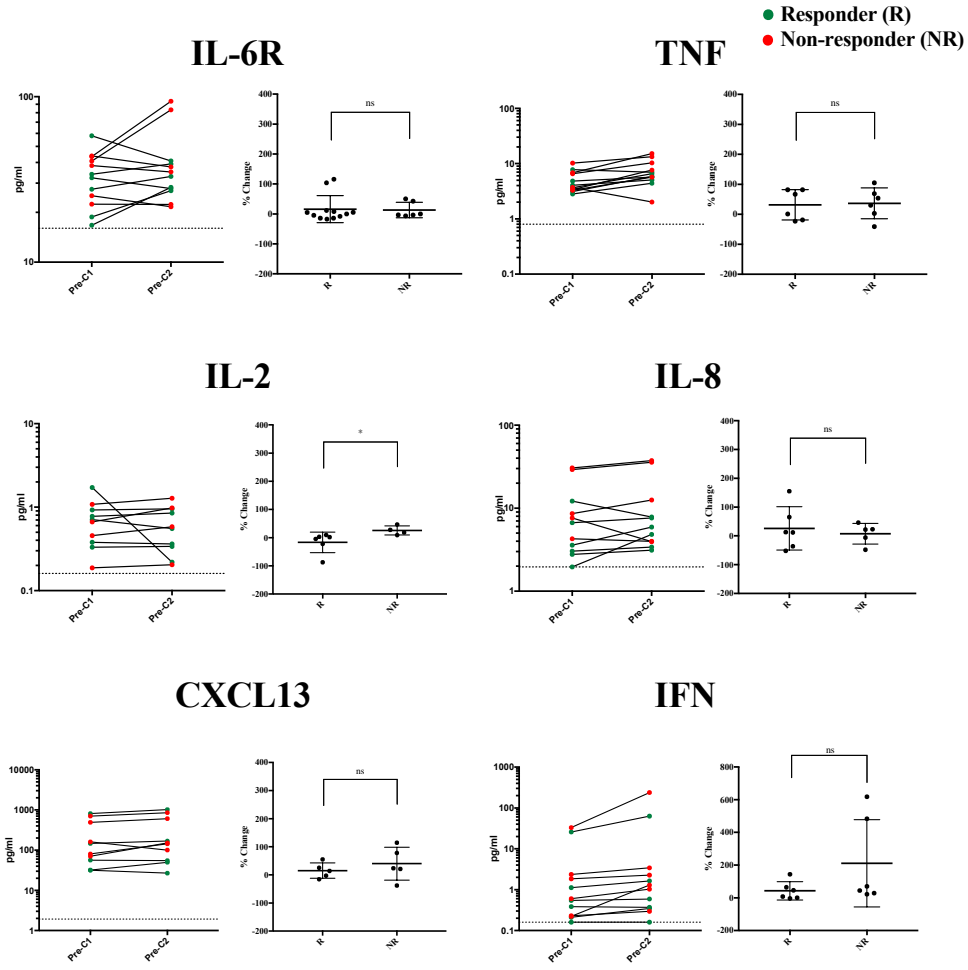
B



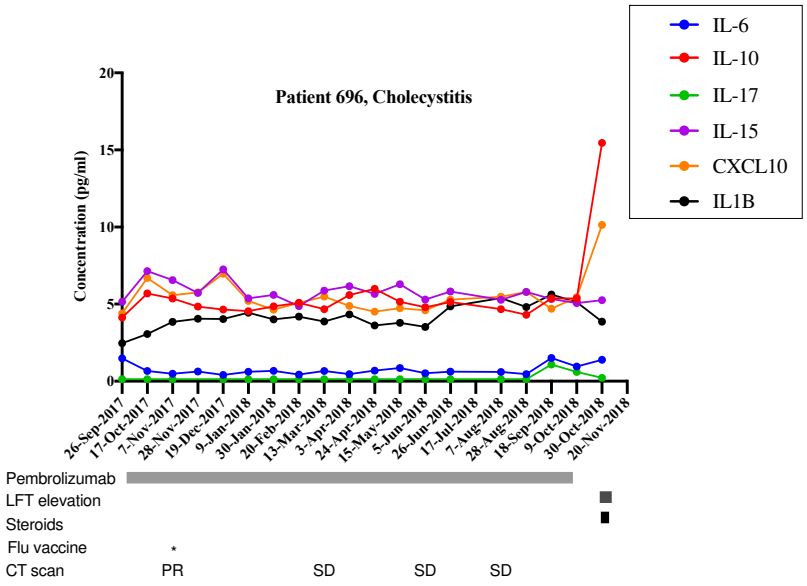
C



Supp Fig 5. Cytokine changes in twelve pembrolizumab monotherapy patients with early radiologic responses: clear increase for responders or decrease for nonresponders in tumor burden at first scan and on-treatment timepoints three weeks after first dose of therapy. As in Supp Fig 3, except these assays were not carried forward to testing across the entire patient cohort. *p<0.05, ns not significant by Mann Whitney test.



Supp Fig 6. Cytokine changes in relation to a severe infectious complication. The plots reflect concentrations of six cytokines (color key, top right) measured at three-week intervals, prior to each pembrolizumab infusion. Light gray bar reflects duration of pembrolizumab treatment, dark gray bar reflects liver function test elevation measured at onset of abdominal pain, and black bar reflects steroid treatment. Radiologic responses and imaging timepoints are indicated.



Supp Table 1. List of capture and detection antibodies and protein standards used for all Simoa assays.

Target	Capture Antibody	Detection Antibody	Protein Standard
IL-1β	Biolegend 508202	Biolegend 511703	R&D Systems 201-LB
IL-2	R&D Systems MAB602	R&D Systems MAB202	R&D Systems 202-IL
IL-6	R&D Systems MAB206	R&D Systems BAF206	R&D Systems 206-IL
IL-8	BD Biosciences 554716	BD Biosciences 554718	R&D Systems 208IL
IL-10	BioLegend 506802	BioLegend 501501	R&D Systems 217-IL
IL-15	R&D Systems MAB647	R&D Systems BAM247	R&D Systems 247-ILB
IL-17A	R&D Systems MAB317	R&D Systems BAF317	Biolegend 570509
IL-6Rα	R&D Systems DY227	R&D Systems DY227	R&D Systems DY227
CXCL10	BioLegend 524402	BioLegend 519403	R&D Systems 266-IP
CXCL13	R&D Systems DY801	R&D Systems DY801	R&D Systems DY801
IFNγ	BioLegend 507502	R&D Systems MAB285	R&D Systems 285-IF
TNFα	R&D Systems MAB610	AbCam ab9635	R&D Systems 210-TA

Supp Table 2. Simoa assay specifications for all Simoa assays including number of steps, incubation times, enzyme and detection antibody concentrations, sample dilution factors, and reagent volumes.

Target	Steps	Incubation Times (cadences)	Enzyme Concentration	Detection Antibody Concentration	Bead Volume	Sample Dilution Factor	Sample Volume	Detection Antibody Volume	Enzyme Volume
IL-1β	2	47-7	150 pM	0.3 µg/ml	25 µl	4	170 µl	20 µl	100 µl
IL-2	3	20-7-7	150 pM	0.6 µg/ml	25 µl	4	120 µl	100 µl	100 µl
IL-6	3	20-7-7	150 pM	0.3 µg/ml	25 µl	4	100 µl	100 µl	100 µl
IL-8	3	20-7-7	50pM	0.05 µg/ml	25 µl	6	100 µl	100 µl	100 µl
IL-10	3	20-7-7	150 pM	0.3 µg/ml	25 µl	4	100 µl	100 µl	100 µl
IL-15	3	20-7-7	150 pM	0.3 µg/ml	25 µl	4	100 µl	100 µl	100 µl
IL-17A	3	20-7-7	150 pM	0.3 µg/ml	25 µl	4	100 µl	100 µl	100 µl
IL-6Rα	3	20-7-7	150 pM	0.03 µg/ml	25 µl	100	100 µl	100 µl	100 µl
CXCL10	2	47-7	150pM	0.34 µg/ml	25 µl	8	100 µl	20 µl	100 µl
CXCL13	3	20-7-7	150pM	0.3 µg/ml	25 µl	6	100 µl	100 µl	100 µl
IFNγ	2	47-7	150 pM	0.3 µg/ml	25 µl	4	100 µl	20 µl	100 µl
TNFα	2	47-7	150 pM	0.22 µg/ml	25 µl	4	100 µl	20 µl	100 µl