Suppl Fig 1: Patient eligibility (pg 1 of 2)

5.1 Inclusion Criteria

5.1.1 Histopathological documentation of the diagnosis of synovial sarcoma and myxoid liposarcoma with metastatic or unresectable disease who have received an alkylating agent containing regimen (such as doxorubicin plus Ifosphamide).

This includes patients who received an alkylating agent as part of adjuvant therapy and then relapsed. Patients who were treated on the PICCASSO trial who have progressed will be allowed on the study.

"Unresectable disease" shall include patients with locally advanced disease where a surgery could be attempted but where this surgery would be mutilating, debilitating and would likely fail to result in long-term disease free survival. In this setting a patient might reasonably choose to undergo salvage/second line systemic therapy but could also pursue aggressive surgical options as standard of care.

5.1.2 Male or female subject, 18 or older and able to tolerate high-dose cyclophosphamide

5.1.3 NY-ESO-1 expression in >25% of tumor by IHC (at least 2+)

5.1.4 Expression of HLA-A0201. High resolution HLA typing performed at any experienced HLA lab will be accepted.

5.1.5 Zubrod performance status of '0-1' (Appendix B)

5.1.6 Patients with metastatic disease must have bi-dimensionally measurable disease by palpation on clinical exam, or radiographic imaging (CT scan). Please see section 9.3.1 regarding exceptions for patients with bi-dimensionally measurable but radiated locally advanced disease.

5.1.7 All patients must have an ECG. Patients with well-controlled diabetes who have an absolutely normal ECG, no cardiac history and with a performance status 0 require no further work up. Patients with history of cardiac disease, or with ongoing cardiac type chest pain must have a normal stress test within 182 days prior to treatment. Patients with diabetes who have any degree of shortness of breath or an abnormal ECG must undergo stress testing within 182 days prior to treatment.

5.1.8 Patients must have already been leukapheresed on either protocol 1246 or 2365 prior to entry into this study. Patients who are unable to have a leukapheresis product collected, for whatever reason, will be unable to participate in this study.

5.1.9 If there is a patient with an NY-ESO-1 expressing sarcoma who would be otherwise eligible for the trial, where there has been disagreement between pathologists regarding the histopathologic diagnosis, eligibility will be decided on by the PI.

5.1.10 Patients must have had NY-ESO-1 specific cells already in production. Patients must have NY-ESO-1 specific cells that have been generated and sorted. These cells may be either in the process of expansion or expanded and frozen at the time of enrollment.

5.1.11 Patients with definitively treated brain metastasis and patients with 4 or fewer untreated lesions less than 1 cm each will be included at the discretion of the PI.

5.1.12 Patients must be off metformin at least 2 weeks before receiving T cell therapy

5.2.13 Patients must have hemoglobin A1C < 8.5%

Suppl Fig 1: Patient eligibility (pg 2 of 2)

5.2 Exclusion Criteria

5.2.1 Patients for whom we are unable to generate NY-ESO-1 specific cells **5.2.2** Pregnant women, nursing mothers, men or women of reproductive ability who are unwilling to use effective contraception or abstinence. Women of childbearing potential must have a negative pregnancy test within two weeks prior to entry.
5.2.3 Serum creatinine > 1.5 mg/dL or Glomerular Filtration Rate >50.
5.2.4 Significant hepatic dysfunction (SGOT > 150 IU or > 3x upper limit of normal; bilirubin > 1.6 mg/dL; prothrombin time > 1.5 x control)
5.2.5 Most patients with metastatic sarcoma will have pulmonary metastasis and it is expected that the majority will have some mild to moderate baseline shortness of breath. These patients will be allowed on study so long as their ECOG performance status is 1. Patients with severe pulmonary dysfunction (≥ Grade 3 Respiratory disorders as defined by CTCAE v4) will not allowed on study until their condition improves. However, patients who have recently experienced a decrease in their pulmonary function will be required to undergo pulmonary function testing. Patients with a FEV1 < 1.5L or DLco (corr for Hgb) <50% will be excluded. Patients with a reversible cause of pulmonary dysfunction may undergo repeat testing and enroll if their PFT's meet criterion.
5.2.6 Significant cardiovascular abnormalities as defined by any one of the following:
active, symptomatic congestive heart failure
clinically significant hypotension
symptoms of coronary artery disease
presence of cardiac arrhythmias on EKG requiring drug therapy which has not been stable for at least 6 months
5.2.7 All patients must have an echo showing EF > 50% and normal troponin and CK MB.
5.2.8 Patients with symptomatic untreated brain metastasis or asymptomatic untreated brain metastasis > 1cm will not be allowed to participate. Additionally, patients with five or more untreated brain metastasis under 1 cm will not be allowed to participate. Treatment may include surgery or stereotactic radiation at the discretion of the patient's treatment team. Patients must be off steroids when starting therapy.
5.2.9 Patients with active infections or oral temperature > 38.2 C within 72 hours of study entry or systemic infection requiring chronic maintenance or suppressive therapy
5.2.10 Chemotherapeutic agents (standard or experimental or other immunosuppressive therapies) less than 3 weeks prior to T cell therapy (patients with bulky disease may undergo cytoreductive chemotherapy but treatment will be discontinued at least 3 weeks prior to T cell infusion). Patients may receive palliative radiation therapy two weeks prior to T cell infusion.
5.2.11 Clinically significant autoimmune disorders or conditions of immunosuppression. Patients with AIDS or HIV-1 associated complex or known to be HIV antibody seropositive or known to be recently PCR+ for hepatitis are not eligible for this study. Virology testing will be done within 6 months of T cell infusion. The severely depressed immune system found in these infected patients and the possibility of premature death would compromise study objectives.
5.2.12 Current treatment with steroids
5.2.13 Patients must not be receiving any other experimental drugs within 3 weeks of the initiation of treatment and must have recovered from all side effects of such therapy.
5.2.14 Patients who were not negative for HBV, HCV at the time of their leukapheresis on 1246 or 2365 must be retested to be sure they are PCR negative.

Supplementary Table 1

Antibody/Reagent	Fluorophore	Clone	Vendor	Concentration
CD8a	Pe Dazzle	RPA-T8	Biolegend	50ug/mL
CD27	BUV737	M-T271	BD Bioscience	0.2 mg/mL
CD39	APC Cy7	A1	Biolegend	100 ug/mL
CD45RA	BV605	HI 100	Biolegend	25 ug/mL
CD103	PECy7	BER ACT8	Biolegend	100 ug/mL
CCR7	AF700	G043H7	Biolegend	400 ug/mL
TIM-3	BV650	F38 2E2	Biolegend	100 ug/mL
PD1	BV786	EH12.1	BD Horizon	NA
SLAMF6	PE	NT-7	Biolegend	100 ug/mL
Ki67	BV711	Ki67	Biolegend	100 ug/mL
TCF-1	AF488	Rabbit mAB	Cell Signaling	100 ug/mL
TNFα	PerCPCY5.5	MAB11	Biolegend	60 ug/mL
IFNγ	BV510	B27	Biolegend	100 ug/mL
Granzyme B	Pac Blue	GB11	Biolegend	100 ug/mL
DCM	efluor405	NA	Invitrogen	NA
NY-ESO-1 Tetramer	APC	SLL MWI TOC	Fred Hutch	0.4 mg/ml

Supplementary Table 2

Cytokines	Vendor	Concentration
IL-2	Peprotech	Low dose:10 U/mL High Dose: 1000 U/mL
IL-15	Peprotech	Low dose:10 ng/mL High Dose: 1000 ng/mL
Anti-PD-1	Biolegend EH12.2H7	10 ug/mL

Detailed methodology regarding 3D Culture: GFP transduced 1765 MRCL cells were embedded in 2.5 mg/ml Collagen Type I (Corning) at a concentration of 2x 10^2 cells/µl in a solution with pH of ~ 7.5. 10µl of the above mix was injected into organoid chips (AIM Biotech) into each of the 4 injection ports.[14] Chips were then kept at 37° C for at-least 1h. Retronectin was injected into the lining ports and 1h later the lining ports were washed with PBS. 1.5x 10^4 mCherry transduced HUVEC cells were then injected into the each of the 4 lining ports. Chips were then kept at 37° C for at-least 1h. 1x 10^4 T cells each were injected into 2 lining ports. EBM media (Lonza) conditioned with cytokines was used to perfuse the chips. Chips were perfused by gravity flow as additional media was added into one-side(left) of the chips. Organoid chips were imaged using the Leica SP8 microscope. 49 Z stacks with 3µm step size were acquired with a 10X objective. GFP+ cells were identified and counted using Imaris software.

Suppl. Table 3 : Adverse Event (AE) tables (pg 1 of 3)

Body System	Initials	Body system	Adverse Event	Dur	Grade Onset	Grade Highest	Frequency	Expected	CTL	IFN- gamma	Cytoxan	IL-2
Blood and lymphatic system disorders	Cy1	Blood and lymphatic system disorders	Platelet count decreased	27	1	1	Intermittent	No	Unlikely	NA	Probable	NA
	Cy1	Blood and lymphatic system disorders	Anemia		1		Intermittent	No	Unlikely	NA	Probable	NA
	Cy2	Blood and lymphatic system disorders	Anemia	42	1	1	Intermittent	Yes	Unlikely	NA	Unlikely	NA
	Cy3	Blood and lymphatic system disorders	Anemia		1	1	Intermittent		Unlikely	NA	Probable	NA
	Cy4	Blood and lymphatic system disorders	Anemia		1		Intermittent	Yes	Unlikely	NA	Probable	NA
Cardiac disorders	Cy1	Cardiac disorders	Pre-existing prolonged QRS				Continuous	NA	NA	NA	NA	NA
GI disorders	Cy3	Gastrointestinal disorders	Nausea	11	1	1	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy4	Gastrointestinal disorders	Nausea	3	1	2	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy4	Gastrointestinal disorders	Vomiting	3	1	1	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy4	Gastrointestinal disorders	Nausea	21	1	1	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy4	Gastrointestinal disorders	Vomiting	18	1	1	Intermittent	Yes	Unrelated	NA	Probable	NA
	Cy4	Gastrointestinal disorders	Gastroesophageal reflux disease		1			Yes	Unrelated	NA	Possible	NA
General disorders and administration site conditions	Cy2	General disorders and administration site conditions	Fatigue		1	1	Intermittent	No	Unlikely		Unlikely	
	Cy3	General disorders and administration site conditions	Fatigue	13	1	1	Intermittent	Yes	Unlikely		Probable	
	Cy4	General disorders and administration site conditions	Fever	1	1	1	Continuous	Yes	Possible	NA	Probable	NA
	Cy4	General disorders and administration site conditions	Fatigue	59	1	1	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy4	General disorders and administration site conditions	Chills	1	1	1	Continuous	Yes	Possible	NA	Probable	NA
	Cy4	General disorders and administration site conditions	Fever	0	1	1	Continuous	Yes	Possible	NA	Probable	NA
	Cy4	General disorders and administration site conditions	Fatigue	10	1	1	Intermittent	Yes	Unlikely	NA	Probable	NA
Investigations	Cy1	Investigations	Lymphocyte count decreased		1	4	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy1	Investigations	Neutrophil count decreased	8	2	2	Intermittent	No	Unlikely	NA	Probable	NA
	Cy2	Investigations	Neutrophil count decreased	21	1	1	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy1	Investigations	Alkaline phosphatase increased	0	1	1	Continuous	No	Unlikely	NA	Possible	NA
	Cy2	Investigations	Lymphocyte count decreased		1	3	Intermittent	Yes	Unlikely	NA	Probable	NA

Suppl. Table 3 : AE tables (pg 2 of 3)

Body System	Initials	Body system	Adverse Event	Dur	Grade Onset	Grade Highest	Frequency	Expected	CTL	IFN- gamma	Cytoxan	IL-2
	Cy2	Investigations	Alkaline phosphatase increased	14	1	1	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy2	Investigations	Platelet count decreased	11	1	1	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy3	Investigations	Lymphocyte count decreased		1	3	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy3	Investigations	Aspartate aminotransferase increased	21	3	3	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy3	Investigations	Alkaline phosphatase increased	21	1	1	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy3	Investigations	Creatinine increased	1	1	1	Once	No	Unlikely	NA	Unlikely	NA
	Cy3	Investigations	Platelet count decreased	18	1	3+I19	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy4	Investigations	Creatinine increased	130	1	1	Intermittent	Yes	Unlikely	NA	Possible	NA
	Cy4	Investigations	White blood cell decreased	138	1	4	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy4	Investigations	Platelet count decreased	109	1	4	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy4	Investigations	Lymphocyte count decreased		2	4	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy4	Investigations	Blood bilirubin increased		1		Intermittent	No	Unlikely	NA	Possible	NA
	Cy4	Investigations	Neutrophil count decreased	90	2	2	Intermittent	Yes	Unlikely	NA	Probable	NA
Metabolism and nutrition disorders	Cy1	Metabolism and nutrition disorders	Hypoalbuminemia	7	2	2	Intermittent	No	Unrelated	NA	Unrelated	NA
	Cy1	Metabolism and nutrition disorders	Hypocalcemia	28	2	2	Intermittent	No	Unrelated	NA	Possible	NA
	Cy1	Metabolism and nutrition disorders	Hyponatremia	10	2	2	Intermittent	No	Unlikely	NA	Probable	NA
	Cy2	Metabolism and nutrition disorders	Hyponatremia	6	1	1	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy1	Metabolism and nutrition disorders	Hypokalemia	9	3	3	Intermittent	No	Unlikely	NA	Possible	NA
	Cy2	Metabolism and nutrition disorders	Hypocalcemia	18	1	1	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy3	Metabolism and nutrition disorders	Hypocalcemia	2	1	1	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy3	Metabolism and nutrition disorders	Hypermagnesemia	1	1	1	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy3	Metabolism and nutrition disorders	Hypoalbuminemia	2	1	1	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy3	Metabolism and nutrition disorders	Hyponatremia	1	1	1	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy4	Metabolism and nutrition disorders	Hypocalcemia	90	1	1	Intermittent	Yes	Unlikely	NA	Possible	NA
	Cy4	Metabolism and nutrition disorders	Hypoalbuminemia	89	2	2	Intermittent	No	Unrelated	NA	Unrelated	NA
	Cy4	Metabolism and nutrition disorders	Hypermagnesemia		1		Intermittent	No	Unlikely	NA	Possible	NA
	Cy4	Metabolism and nutrition disorders	Hyponatremia	90	1	1	Intermittent	Yes	Unlikely	Na	Possible	NA
	Cy4	Metabolism and nutrition disorders	Hypophosphatemia	75	1	1	Intermittent	No	Unrelated	NA	Unrelated	NA
	Cy4	Metabolism and nutrition disorders	Hypokalemia	88	1	1	Intermittent	No	Unlikely	NA	Probable	NA
Musculoskeletal and connective tissue disorders	Cy1	Musculoskeletal and connective tissue disorders	Back pain	3	1	1	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy2	Musculoskeletal and connective tissue disorders	Bone pain		2	2	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy4	Musculoskeletal and connective tissue disorders	Pain in extremity		1		Intermittent	No	Unrelated	NA	Unrelated	NA
Nervous system disorders	Cy1	Nervous system disorders	Peripheral motor neuropathy		1	1	Continuous	NA	NA	NA	NA	NA
	Cy2	Nervous system disorders	Dizziness	1	1	1	Once	No	Unlikely	NA	Unlikely	NA
	Cy3	Nervous system disorders	Headache	1	1	1	Intermittent	No	Unlikely	NA	Unlikely	NA

Suppl. Table 3: AE tables (pg 3 of 3)

Body System	Initials	Body system	Adverse Event	Dur	Grade Onset	Grade Highest	Frequency	Expected	CTL	IFN- gamma	Cytoxan	IL-2
	Cy4	Nervous system disorders	Headache	1	1	1	Continuous	Yes	Unlikely	Possible		NA
Psychiatric disorders	Cy1	Psychiatric disorders	Anxiety		2		Intermittent	No	Unrelated	NA	Unrelated	NA
	Cy3	Psychiatric disorders	Restlessness	1	1	1	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy4	Psychiatric disorders	Anxiety		1		Intermittent	No	NA	NA	NA	NA
Renal and urinary disorders	Cy4	Renal and urinary disorders	Hematuria	8	1	1	Intermittent	Yes	Unlikely	NA	Probable	NA
	Cy4	Renal and urinary disorders	Proteinuria		1		Once	Yes	Unlikely	NA	Probable	NA
Respiratory, thoracic and mediastinal disorders	Cy1	Respiratory, thoracic and mediastinal disorders	Sore throat	1	1		Continuous	No	Unlikely	NA	Possible	NA
	Cy2	Respiratory, thoracic and mediastinal disorders	Dyspnea		1	1	Intermittent	No	Unlikely	NA	Unlikely	NA
	Cy4	Respiratory, thoracic and mediastinal disorders	Nasal congestion	0	1	1	Once	No	Unlikely	NA	Possible	NA

Supplemental Fig 2: Additional Clinical Summary

Cy1

Cy1 was originally diagnosed with a myxoid/round cell liposarcoma of the thigh that was treated with resection and adjuvant radiation. He then had distant spine recurrence nearly 10 years later, treated again with resection and radiation. One year later he developed multifocal recurrence in the lung treated with AIM, and later single agent ifosfamide after developing cardiotoxicity with good response to chemotherapy. He was then treated on study with conditioning and cell infusion. Residual lung disease was resected 4 months after cell infusion. There was recurrence in the lung one year after cell infusion. Chemotherapy was reinitiated. His disease eventually continued to progress.

Cy2

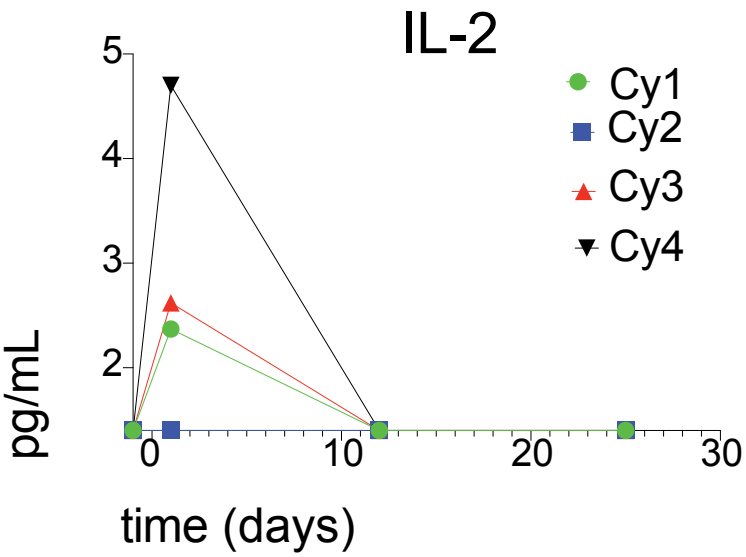
Cy2 presented with gross hematuria and was found to have renal synovial sarcoma with bilateral lung metastasis at diagnosis. He was treated with upfront resection of the renal mass followed by AIM for 6 cycles and then a treatment break. He had progression with disease in his abdomen about 6 months later and was treated on protocol with cell therapy. The rate of progression seemed to slow but there was PD by RECIST 2-3 months after receiving cell infusion. He subsequently received several different regimens and palliative radiation.

Cy3

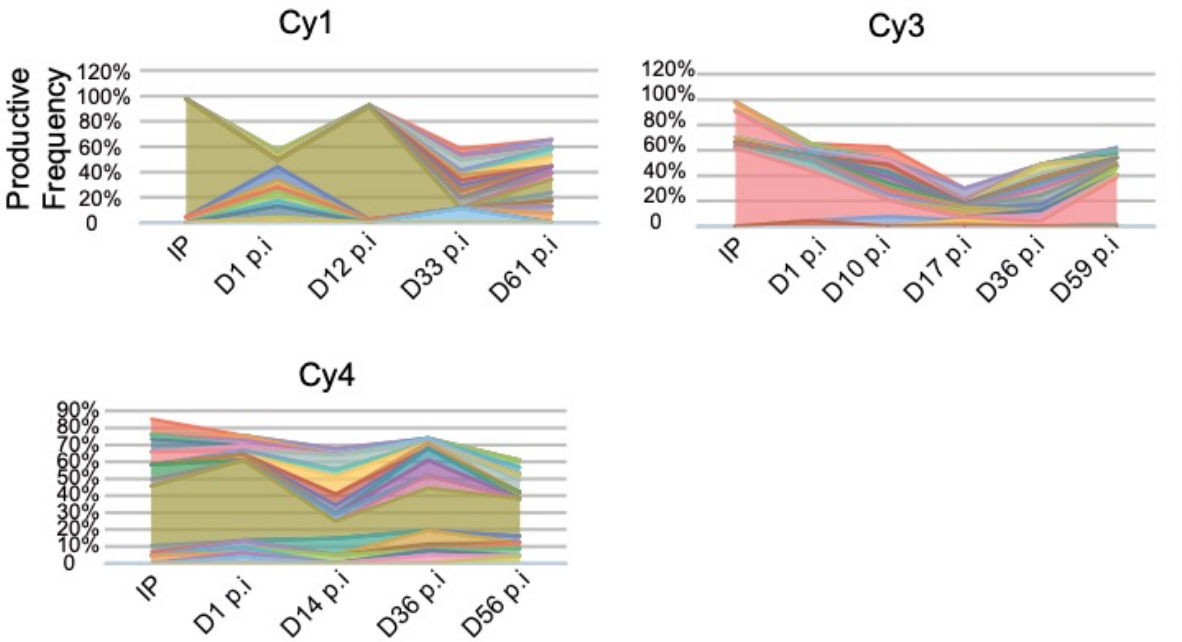
Cy3 was initially diagnosed with a synovial sarcoma of the distal lower extremity treated with a neoadjuvant ifosfamide, epirubicin, and sorafenib on a clinical trial with radiation. About 6 months after completion of chemotherapy, he had bilateral lung recurrence with rapid growth and he was treated with NY-ESO-1 specific T cells following HD Cy conditioning. He progressed 3 months later and started subsequent chemotherapy with trabectedin, pazopanib, and AIM for further progression before eventually developing brain metastasis.

Cy4

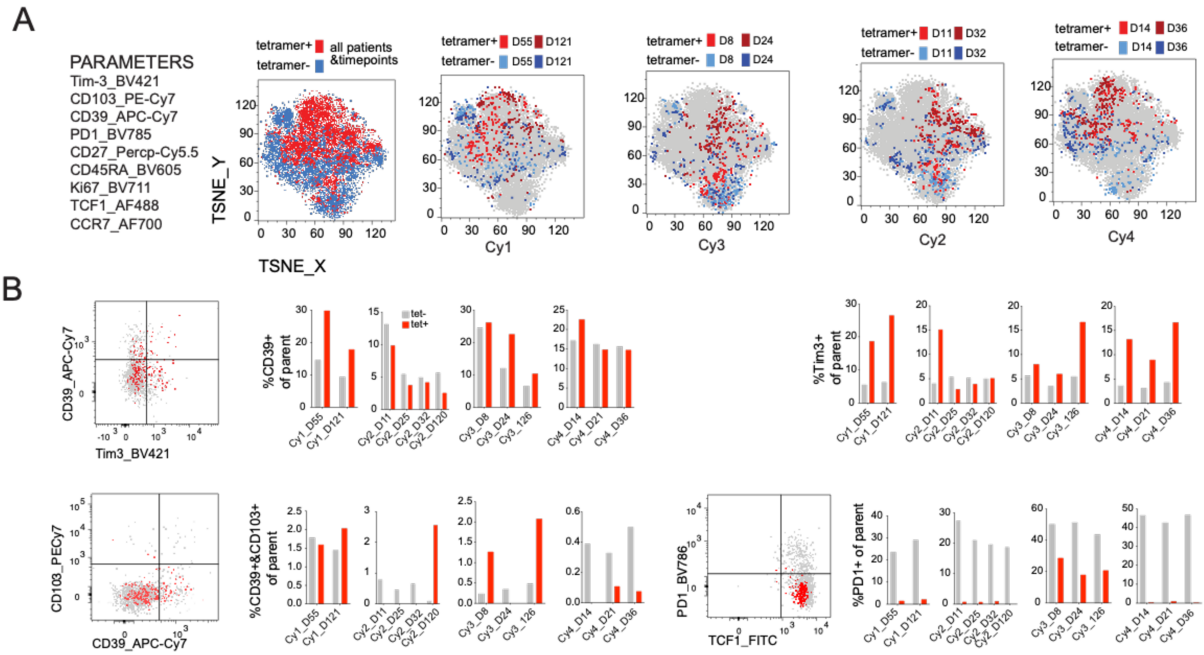
CY4 received neoadjuvant MAID, and then resection of 2 local recurrences. He was treated with gemcitabine+Docetaxel, sorafenib, capecitabine, erlotinib, and pazopanib over the years preceding enrolling on this study. He was treated with T-cells after developing widespread metastatic disease, received radiotherapy to a lesion in the pelvis, and then underwent conditioning and received a second infusion of cells 2 months after the first T-cell therapy. He received palliative radiation and eventually went on hospice care the following year.



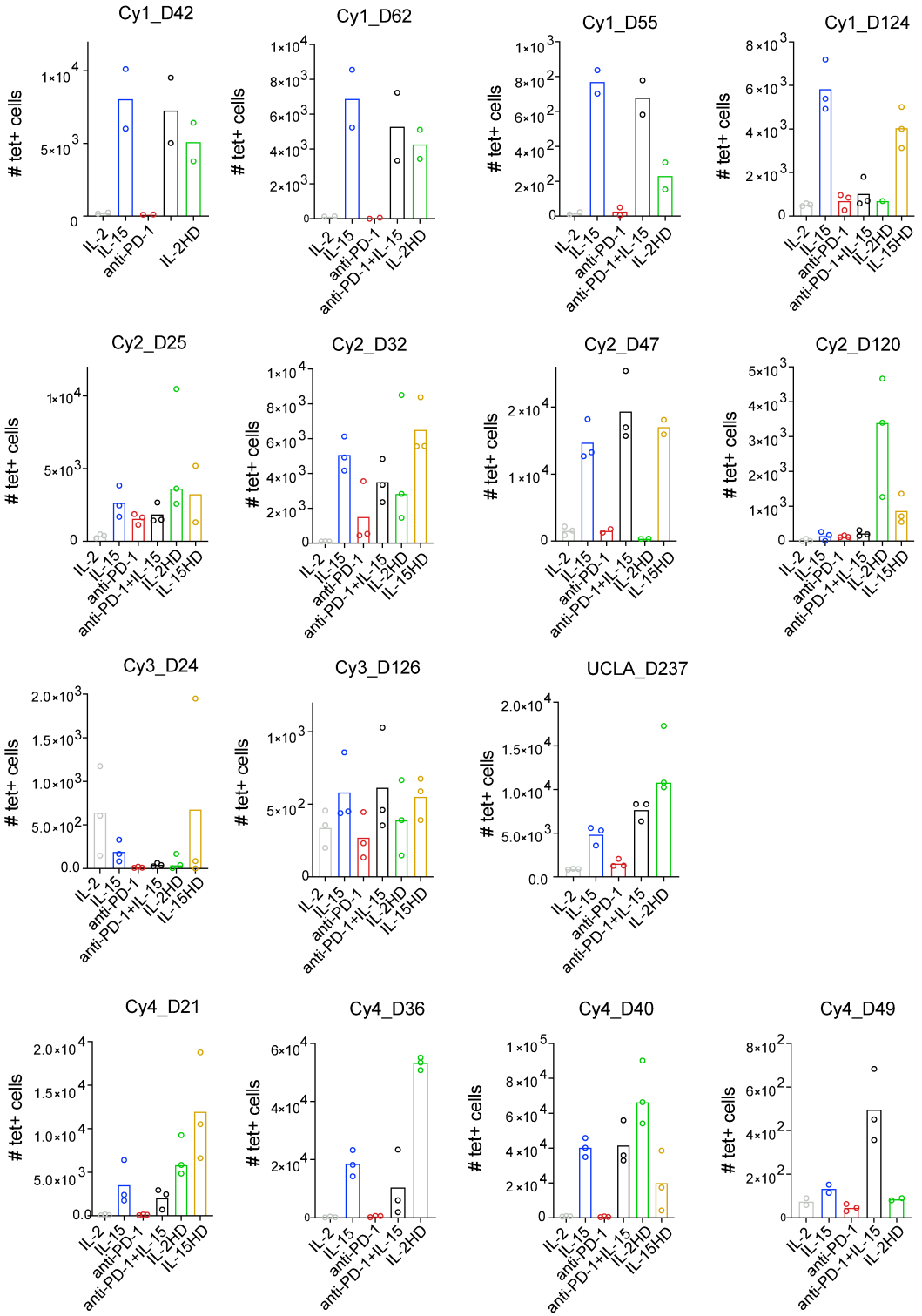
Suppl. Fig. 3. Serum IL-2 concentrations



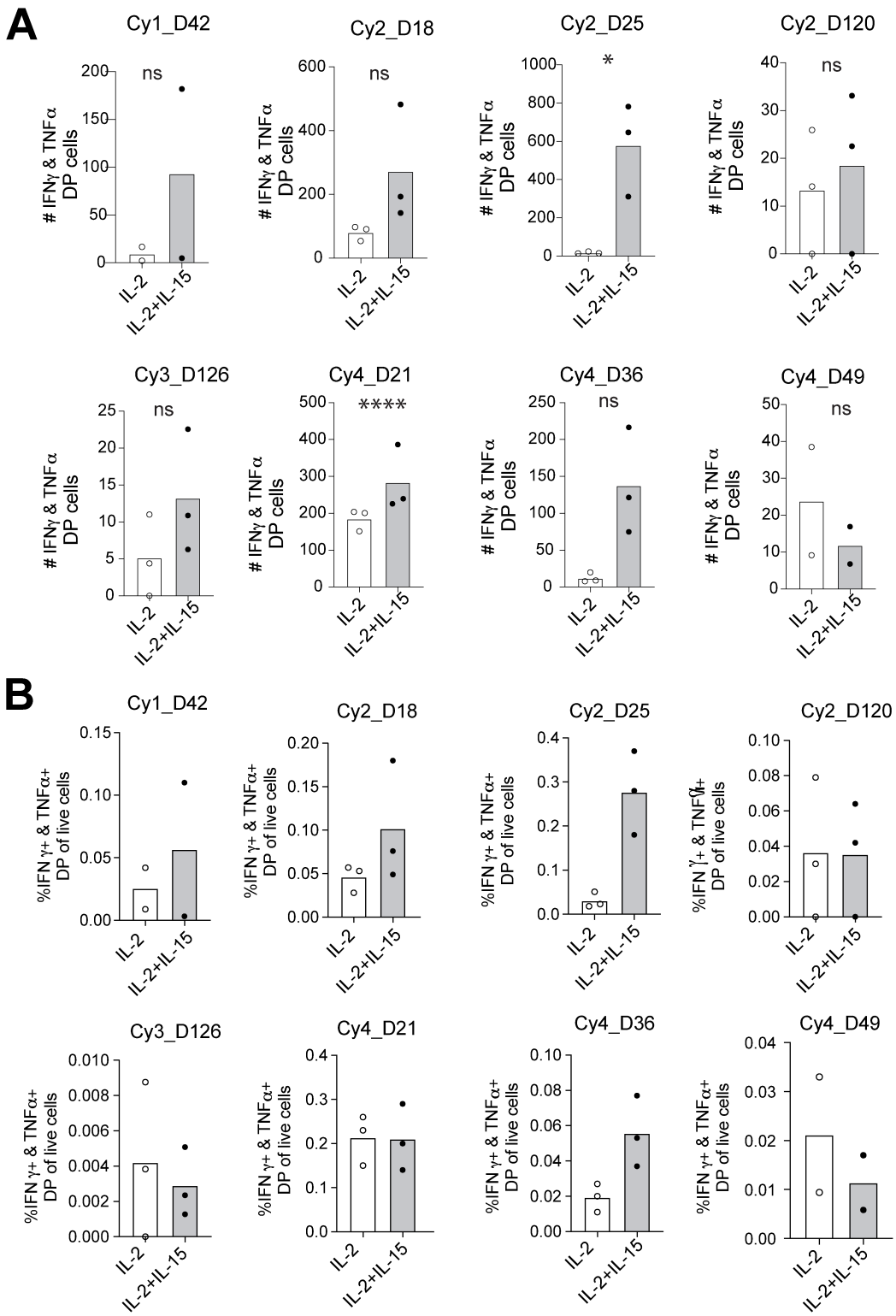
Suppl. Fig. 4. Persistence of dominant clones vary in patients. Sequencing of TCRβ chain depicting different T cell clones (each clone represented by a different color) prior to infusion and at various times post infusion for patient Cy1, Cy3 and Cy4.



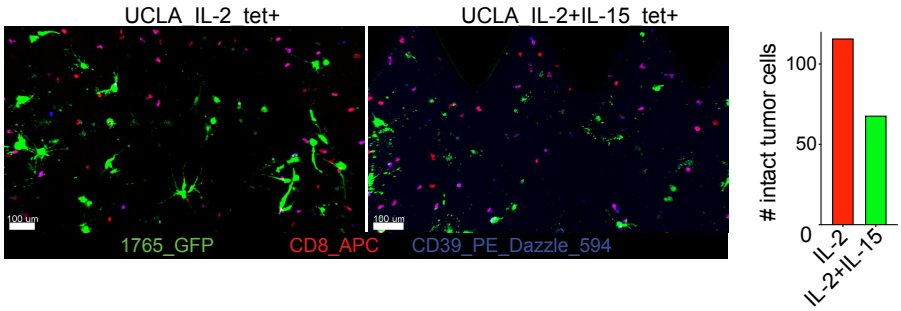
Suppl. Fig. 5. Phenotype of NY-ESO-1 specific T cells in patients' PBMCs. A) Representative t-SNE analysis of CD8+ cells from 4 patients. t-SNE cluster (grey) is generated from pooled CD8+ T cells from all patients and various time points post infusion. (Cy1:d55, d121; Cy2:d11, d25, d32, d120; Cy3:d8, d126; Cy4: d21, d28, d36, d49). t-SNE was run based on indicated parameters. Tet+ positive cells from each patient are overlaid on pooled cluster in shades of red and tetramer- cells are overlaid in shades of blue. B) Representative plot for expression of CD39,CD103, Tim3, PD1 in tet+ (red) and tet- (grey) cells. C) Frequency of CD39+, Tim3+, CD39&CD103+ and PD1+ cells of tet+ (red bars) and tet- (grey bars).



Suppl. Fig. 6. IL-15 and high does IL-2 expand NY-ESO1-1 specific T cells. 9-day culture of patients' PBMCs with NY-ESO-1 peptide in conditioned media. Bars show number of tetramer+ cells in cultures supplemented with IL-2 (grey), IL-2+IL-15 (blue), IL-2+anti-PD-1 (red), IL-2+IL-15+anti-PD-1 (black) and high dose IL-2 (green) and high dose IL-15 (orange). Each dot represents one technical replicate



Suppl. Fig. 7. IL-15 increases the number of IFNγ and TNFα producing cells. A) Number of IFNγ and TNFα double positive (DP) tet+ cells and B)Percentage of IFNγ and TNFα DP tet+ cells of of all live cells in patients' PBMC 9-day culture supplemented with IL-2 (clear bars with clear dots) or IL-2 plus IL-15 (grey bars with black dots). Each dot represents one technical replicate. **** $p < 0.0001$, * $p < 0.05$.



Suppl. Fig. 8: IL-15 increases cytotoxicity of NY-ESO-1 specific T cells. PBMCs of patient from UCLA trial cultured with NY-ESO-1 peptide and supplemented with IL-2 or IL-2 plus IL-15 for 7 days and tetr+ and tet- cells were sorted and injected into organoid chips. Number of GFP+ 1765 cells were counted 48h post T cell injection. Each bar represents one 3D chip. Experiment was performed once