**Supplementary material**

**Supplementary Table S1. Patient eligibility**

|  |  |
| --- | --- |
| Inclusion Criteria | Exclusion Criteria |
| A diagnosis of synovial sarcoma and myxoid/ round cell liposarcoma | Active infection requiring oral or intravenous antibiotics |
| Male or female subject, 18 or older | 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. |
| A superficial tumor easily and safely accessible for a research biopsy or are being considered for resection or biopsy of their tumor as part of standard of care and have recent pathology. | Serum creatinine > 1.5 mg/dL or Glomerular Filtration Rate <50. |
| Zubrod performance status of ‘0-2’ or Karnofsky score >60%. (Appendix A) | Significant hepatic dysfunction (SGOT > 150 IU or > 3x upper limit of normal; bilirubin > 1.6 mg/dL; prothrombin time > 1.5 x control). |
| No treatment with systemic anti-cancer treatment (chemotherapy or biologics) within 2 weeks of starting interferon gamma. | Known CNS metastasis. Once CNS metastasis have been treated these patients may participate if they are otherwise good trial candidates. |
| Patients with a history of coronary artery disease must have had a normal stress test within 180 days of starting IFNγ. | Current treatment with steroids (must be discontinued 1 week before starting IFNγ). |
| Must have been off metformin for at least 2 weeks prior to starting IFNγ. | Hemoglobin A1C >8.5%. |
| No use of full dose, therapeutic anti-coagulation. However, low dose warfarin for catheter prophylaxis or acetylsalicylic acid are acceptable. | Uncontrolled hypertension, BP >150/100mmHg. Patients with elevated BP may enroll once BP is corrected. |
| No thrombotic event within 6 months (deep vein thrombosis, pulmonary embolism) of starting IFNγ. | NY-ESO-1 specific T cell therapy within 1 year of starting on the trial. |
|  | New (<6 months) cardiac arrhythmia (EKG should be performed within 2 weeks of starting IFNγ. |
|  | History of clinically significant congestive heart failure. |

**Supplementary Table S2. Flow cytometry multicolor panel:**

|  |  |  |  |
| --- | --- | --- | --- |
| Marker | Color | Clone | Company |
| CD3 | PerCP/Cy5.5 | SK7 | BioLegend |
| CD4 | FITC | OKT4 | StemCell |
| CD8 | PE-Texas Red | 3B5 | Life Technologies |
| CD11b | PE | ICRF44 | BioLegend |
| CD14 | APC | 61D3 | Invitrogen |
| CD45 | V450 | HI30 | BD Horizon |
| CD56 | Pe-Cy7 | CMSSB | Invitrogen |
| CD163 | BV711 | GHI/61 | BD Horizon |
| CD279 | BV786 | EH12.1 | BD Horizon |
| HLA-ABC | AF700 | W6/32 | BioLegend |
| HLA-DR | APC/Cy7 | L243 | BioLegend |
| PD-L1 | BV605 | 29E.2A3 | BioLegend |
| Fixable Viability Dye | eFlour 506 | None | Invitrogen |

**Supplementary Table S3. Summary of adverse events occurred during treatment**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Category (CTCAE)** | **Adverse Event/Toxicity** | **Number (N) and Grade of Events** | | | | | | |
| **N** | **N** | **N** | **N** | **N** | **Total Number of Incidences** |
| **Category** | **AE Name** | **1** | **2** | **3** | **4** | **5** | **#** |
| General disorders and administration site conditions | Flu-like symptoms | 5 | 0 | 0 | 0 | 0 | 5 |
| General disorders and administration site conditions | Fatigue | 4 | 1 | 0 | 0 | 0 | 5 |
| General disorders and administration site conditions | Chills | 4 | 0 | 0 | 0 | 0 | 4 |
| Nervous system disorders | Headache | 4 | 0 | 0 | 0 | 0 | 4 |
| General disorders and administration site conditions | Fever | 2 | 0 | 0 | 0 | 0 | 2 |
| Musculoskeletal and connective tissue disorders | Body Aches | 2 | 0 | 0 | 0 | 0 | 2 |
| General disorders and administration site conditions | Injection site pain | 2 | 0 | 0 | 0 | 0 | 2 |
| Nervous system disorders | Dizziness | 1 | 0 | 0 | 0 | 0 | 1 |
| Psychiatric disorders | Insomnia | 1 | 0 | 0 | 0 | 0 | 1 |
| General disorders and administration site conditions | Night Sweats | 1 | 0 | 0 | 0 | 0 | 1 |
| Musculoskeletal and connective tissue disorders | Arthralgia | 1 | 0 | 0 | 0 | 0 | 1 |
| General disorders and administration site conditions | Malaise | 1 | 0 | 0 | 0 | 0 | 1 |
| Nervous system disorders | Paresthesia | 1 | 0 | 0 | 0 | 0 | 1 |
| Musculoskeletal and connective tissue disorders | Bone pain | 1 | 0 | 0 | 0 | 0 | 1 |
| Musculoskeletal and connective tissue disorders | Neck pain | 1 | 0 | 0 | 0 | 0 | 1 |
| General disorders and administration site conditions | Injection site erythema | 1 | 0 | 0 | 0 | 0 | 1 |
| Gastrointestinal disorders | Dry mouth | 1 | 0 | 0 | 0 | 0 | 1 |
| Infections and infestations | Upper Respiratory Infection | 0 | 1 | 0 | 0 | 0 | 1 |
| Gastrointestinal disorders | Diarrhea | 1 | 0 | 0 | 0 | 0 | 1 |
| Infections and infestations | Urinary Tract Infection | 0 | 0 | 1 | 0 | 0 | 1 |
| General disorders and administration site conditions | Biopsy site pain | 0 | 1 | 0 | 0 | 0 | 1 |

**Supplementary Fig. S1**



**Supplementary Fig. S1. Profiles of patients’ serum tumor antigen specificity.** Antibodies against 29 tumor antigens have been accessed in patients’ serum by Serametrix. Patient #1, #5 and #8 had significantly more serum antibody specificity post treatment.

**Supplementary Fig. S2**



**Supplementary Fig. S2. IFN𝛄 treatment non-responder and moderate responder didn’t have increased antigen processing and presentation capability.** Unlike major responder patient #6, tumor cells from non-responder patient #7 and moderate responder patient #8 had decreased and no change in antigen processing and presentation capacity, respectively. This observation is consistent with tumor surface HLA-ABC expression trend.

**Supplementary Fig. S3**

**Supplementary Fig. S3. Tumor cells from our cohort are positive for CT antigens.** Tumor mRNA expression levels measured by micro array show that tumors are positive for the following tumor antigens, NY-ESO-1, PRAME and MAGE-A4. Negative lines were drawn according to the average expression level of the respective genes in CD8+ TILs. No significant change of the tumor antigen expression level was observed. Based on Wilcoxon matched-pairs signed rank test, p values equal to 0.25, 0.75 and >0.9999 for NY-ESO-1, PRAME and MAGE-A4 respectively.

**Supplementary Fig. S4**



**Supplementary Fig. S4. Significant increase of tumor apoptosis from a major responder.** GSEA analysis of patients #6, #7 and #8’s tumor cells with the BIOCARTA CASPASE PATHWAY reveals a significant increase of tumor apoptosis from the major responder patient #6, no significance was observed from non-responder or moderate responder.

**Supplementary Fig. S5**



**Supplementary Fig. S5. Patients mainly have biased CD8 over CD4 T cells infiltration in the tumor.** Except for patient #1 who had more CD4+ infiltrates in the tumor, the other 6 patients have up to 4.5 times more CD8 T cell infiltrates than CD4 T cells. IFN𝛄 treatment doesn’t change the tumor infiltrating CD8:CD4 T cells ratio.

**Supplementary Fig. S6**



**Supplementary Fig. S6. Profiles of patients’ serum cytokine.** Patients’s serum weremeasured by ProcartaPlex Immunoassays for 79 cytokines, chemokines and other molecules. Red indicates high values, and blue indicates low values.