Supplementary files

Media used for experiments

Culture medium (CM)

Medium containing RPMI 1640 (Gibco 72400-021), penicillin/streptomycin (Gibco 15140-122), Fungizone (E.R. Squibb & Sons Ltd 49182), 10% heat-inactivated human AB serum (Sigma-Aldrich H4522-100ML), and 6,000 IU/mL IL-2 (Proleukin ®, Novartis 004184).

Tumor medium (R10)

Medium containing RPMI 1640, penicillin/streptomycin, 10% fetal bovine serum (Gibco 10270-106), and Solu-cortef (Pfizer H02AB09).

Enzyme digest medium

Medium containing RPMI 1640, penicillin/streptomycin, 1mg/mL collagenase (Sigma-Aldrich), and 0.0125 mg/mL dornase alpha (Pulmozyme, Roche 148213).

Antibodies used for flow cytometry

Antibody and fluorochrome	Provider	Catalog no.
7AAD PerCP	BD Biosciences	559925
BTLA PE	BD Biosciences	558485
CCR7 FITC	R&D systems	FAB197F
CCR7 PE-Cy7	BD Biosciences	557648
CD127 Pe-Cy7	BD Biosciences	560822
CD137 APC	Biolegend	309810
CD137 PE	BD Biosciences	555956
CD16 FITC	DAKO	F7011
CD25 BV421	BD Biosciences	564033
CD27 PE	BD Biosciences	340425
CD28 APC	BD Biosciences	559770
CD3 AmCyan	BD Biosciences	339186
CD3 BV510	BD Biosciences	740202
CD3 FITC	BD Biosciences	345763
CD4 APC	BD Biosciences	345771
CD4 BV510	BD Biosciences	562970
CD4 PerCP	BD Biosciences	345770
CD45RA APC	BD Biosciences	550855
CD45RA FITC	BD Biosciences	335039
CD45RO PE	BD Biosciences	347967
CD56 PE	BD Biosciences	345810
CD56 PE Cy7	BD Biosciences	557747
CD57 FITC	BD Biosciences	555619
CD62L APC	BD Biosciences	559772
CD62L APC Cy7	BioLegend	304814
CD69 Pe-Cy7	BD Biosciences	557745
CD8 BV421	BD Biosciences	562428
CD8 PB	DAKO	PB984
CD8 PerCP	BD Biosciences	345774
FoxP3 PE	eBioscience	12-4776-42
Gamma-Delta TCR PE	Biolegend	331212
LAG3 FITC	LS Biosciences	LS-B2237
PD1 Pe-Cy7	BD Biosciences	561272
TIM3 APC	eBioscience	17-3109-42

Supplementary figure legend

Figure S1

Bar plot showing median expansion time of Young TILs for each sarcoma subtype. Each open circle represents one patient sample.

Figure S2

Scatter plot showing percentage of αβ T cells, γδ T cells, and NK cells in the Young TIL and REP TIL populations, and scatter plot showing percentage of naïve T cells (CD3+CD45RO-CCR7+), central memory T cells (CD3+CD45RO+CCR7+), effector memory T cells (CD3+CD45RO+CCR7-), and effector T cells (CD3+CD45RO-CCR7-) in the Young TIL and REP TIL populations.

Figure S3

Microscope images of established tumor cell lines. The described malignant characteristics were also present in the tumor at the time of surgery. **A)** Photograph showing many vacuolated "lipoblast-like/lipoblast" pleomorphic cells in a sample from a pleomorphic liposarcoma (SAR-30). **B)** Photograph showing pleomorphic cell population with many mitotic figures in a sample from an inflammatory myofibroblastic sarcoma (SAR-26). **C)** Photograph showing mononuclear and multinuclear giant cells in a sample from an undifferentiated pleomorphic sarcoma (SAR-14).

Figure S4

Additional phenotypic data of TIL stimulated with CD3, 41BB or a combination of these. **A)** Scatter plot showing percentage of γδ T cells in the Young TIL populations for the four analyte groups. **B)** Scatter plot showing percentage of αβ T cells in the REP TIL populations for the four analyte groups. **C)** Bar plots showing median percentage of $CD4^+$ and CD8+ T cells expressing CD27, CD28, 4-1BB, PD-1, LAG3, BTLA, and TIM-3 in REP TILs stimulated with only IL-2 (white), IL-2+anti-CD3 (blue), IL-2+4-1BB (black), and IL-2+anti-CD3+4-1BB (red). Each dot represents one patient sample. **D-G)** Bar plots showing median NPX values as measures of CCL4, CD70, CSF-1, and CD83 concentrations in the supernatants on day 5 of expansion. Each open circle represents one patient sample.

Figure S5

Graph showing an example of cytolysis from a TIL sample with high reactivity (SAR-26) as a function of time using xCelligence. In this sample we tested if PD-1 blocking, LAG3 blocking, or 4-1BB stimulation had any effect on cytolysis. The arrow indicates the time point of addition of the REP TILs.

Figure S6

FACS plots showing gating strategies for TIL phenotype analyses.