178 EXPANSION OF TUMOR-INFILTRATING LYMPHOCYTES AND MARROW-INFILTRATING LYMPHOCYTES FROM PEDIATRIC MALIGNANT SOLID TUMORS

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Background High-risk non-CNS pediatric malignant solid tumors (pMST) have unsatisfactory outcomes, and novel therapies are warranted. Adoptive cellular therapy (ACT) using tumor-infiltrating lymphocytes (TIL) has produced durable responses in melanoma, and improvements in TIL expansion have made ACT-TIL feasible for other solid tumors.[1–3] Preclinical mouse models suggest that T-cells from bone marrow (marrow-infiltrating lymphocytes, MIL) have antitumor reactivity offering another source for ACT.[4 5] To demonstrate feasibility of ACT in pMST we hypothesized that TIL/MIL can be expanded from these patients.

Methods Patients ≤21 years old undergoing standard-of-care pMST resection were enrolled on an IRB approved protocol. Fresh tumor (≥1 cm3) was collected and bone marrow (10 mL) was obtained when accessible from standard of care procedures. TIL/MIL were cultured in media containing IL-2 (6000 IU/mL). TIL were expanded from tumor fragment cultures (TFC, >1 mm3) or tumor digest. Select TIL samples were further expanded using a rapid expansion protocol (REP). Phenotype of expanded TIL (CD3, CD4, CD8 and CD56) was evaluated using flow cytometry. IFN- □ secretion, measured by ELISA assay, measured tumor-specific reactivity after co-culture with autologous tumor and TIL.

Results Twenty samples were obtained between March 2019-May 2021. Two samples were ineligible (final pathology not pMST), leaving 18 samples for analysis. Five marrow samples were collected. TIL were expanded from 14/18 samples (78%) through TFC with median 5.17 x 10^6 cells (range 1.86 x 10^6–3.21 x 10^8). Average phenotype (%) of TFC-TIL were CD3 (63.17), CD4 (21.46), CD8 (46.19) and CD56 (32.68). 9/10 (90%) of samples successfully underwent REP with median 9.35 x 10^7 cells(range 2.49 x 10^7–5.86 x 10^8) final viable TIL and average fold-change 718.6 (median 458.6). Average phenotype (%) of post-REP TIL were CD3 (96.04), CD4 (75.04), CD8 (19.17) and CD56 (0.43). TIL were expanded from TFC of therapy-naïve (8/10, 80%) and pretreated (chemotherapy and checkpoint immunotherapy) samples (5/8, 63%). Seven samples had sufficient tissue to test tumor-specific reactivity; all were non-reactive. MIL pre-REP was expanded from four samples with median 9.55 x 10^6 cells (range 8.00 x 10^5–1.00 x 10^7). Average phenotype of expanded MIL (%) were CD3 (45.17), CD4 (24.46), CD8 (36.15) and CD56 (28.21) (table 1).

Results of TIL and MIL expansion from 18 pMST samples. Abbreviations: Dx: diagnosis, pre-REP: pre-rapid expansion protocol, post-REP: post-rapid expansion protocol, PBMC: peripheral blood mononuclear cells, GNB: ganglioneuroblastoma, WT: Wilms tumor, OS: osteosarcoma, NB: neuroblastoma, IMT: inflammatory myofibroblastic tumor; ASPS: alveolar soft part sarcoma, SS: synovial sarcoma, ERMS: embryonal rhabdomyosarcoma, N: no systemic therapy, C: chemotherapy, I: immunotherapy, DNG: did not grow, N/A: not applicable, NR: non-reactive

Abstract 178 Table 1 Expansion of TIL from pMST

Sample	Dx	Age (years)	Staging	Disease Status	Sampling Timepoint	Sample Source	Prior Therapy	Tumor Fragment Culture (cells)	post-REP (cells)	Digest (cells)	MIL (cells)	Reactivity
1	GNB	3	Localized	Initial treatment	Upfront resection	Abdomen, primary site	N	DNG	N/A	none available	1.00E+07 pre-REP, 4.2E+06 post-REP	NR
2	WT	2	IV, favorable histology	Initial treatment	Upfront resection	Abdomen, primary site	N	4.69E+06	N/A	1.03E+09	N/A	NR
3	WT	1	IV, favorable histology	Initial treatment	Upfront resection	Abdomen, primary site	N	3.20E+06	4.01E+07	1.60E+08	N/A	NR
4	OS	15	Metastatic	Relapse	After salvage chemotherapy	Lung, metastatic site	C	6.00E+06	5.46E+07	7.25E+05	N/A	N/A
5	WT	7	IV, anaplastic	Initial treatment	Upfront resection	Abdomen, Lymph Node	N	2.50E+06	DNG	8.62E+06	N/A	N/A
6	NB	7	IV, high risk	Relapse	At relapse	CNS, metastatic site	C	5.65E+06	3.17E+08	4.21E+06	N/A	NR
7	IMT	14	Localized	Initial treatment	Upfront Resection	Lung, Primary site	N	3.21E+08	N/A	3.00E+07	N/A	N/A
8	ML	16	Stage III	Initial treatment	Upfront resection	Lower Extremity, Primary site	N	1.86E+06	N/A	1.00E+06	N/A	N/A
9	OS	14	Metastatic	Initial treatment	After neoadjuvant chemotherapy	Lower Extremity, Primary site	C	4.90E+07	N/A	none available	9.1E+06 pre-REP	N/A
10	ASPS	20	Metastatic	Relapse	After salvage therapy	CNS, metastatic site	I	DNG	N/A	2.08E+08	N/A	N/A
11	OS	13	Metastatic	Relapse	After salvage therapy	Lung, metastatic site	C	DNG	N/A	3.20E+06	N/A	N/A
12	ASPS	20	Metastatic	Relapse	After salvage therapy	CNS, metastatic site	I	DNG	N/A	5.52E+07	N/A	N/A
13	NB	7	IV, high risk	Initial treatment	Diagnostic biopsy	Abdomen, primary site	N	4.00E+06	9.35E+07	used in co-culture	7.26E+07 (post-REP only)	NR
14	SS	13	Stage II	Initial treatment	Upfront resection	Lower Extremity, Primary Site	N	3.10E+06	3.95E+07	used in co-culture	N/A	NR
15	ERMS	3	Stage III, group 2	Initial treatment	Upfront Resection	Abdomen, primary site	N	1.16E+08	3.22E+08	used in co-culture	N/A	NR
16	OS	14	Localized	Initial treatment	After neoadjuvant chemotherapy	Lower Extremity, Primary site	C	1.37E+08	4.47E+08	none available	8.00E+05 pre-REP	N/A
17	WT	4	Stage III, favorable histology	Initial treatment	Diagnostic biopsy	Abdomen, Primary site	N	5.40E+07	5.86E+08	8.92E+07	N/A	N/A
18	OS	12	Localized	Initial treatment	After neoadjuvant chemotherapy	Lower Extremity, Primary	C	3.5E+06	2.49E+07	4.00E+05	1.00E+07 pre-REP, 1.45E+08 post-REP	N/A

Conclusions This study demonstrates feasibility of pMST TIL expansion ex vivo. Due to tissue volume constraints inherent in pMST sampling, anti-tumor reactivity testing was not feasible for most patients. Determining optimal strategy for TIL-ACT in pMST will require further investigation regarding techniques for expanding tumor-specific TIL.

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Ethics Approval This study was approved by the Johns Hopkins All Children's Hospital IRB (#IRB00193453). Consent was obtained from the patient or parent, as appropriate for age, prior to participating in this study.

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