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

¹Jonathan Metts*, ²Jonathan Hensel, ²Alejandro Alfaro, ²Brook Olmo, ²Shari Pilon-Thomas, ²John Mullinax, ¹Ivanna Leon. ¹Johns Hopkins All Children's Hospital, St Petersburg, FL, USA; ²Moffitt Cancer Center, Tampa, FL, USA

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- γ 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 5.17×10^6 cells (range 1.86×10^6 – 3.21×10^8). 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 9.35×10^7 cells (range 2.49×10^7 – 5.86×10^8) 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 9.55×10^6 cells (range 8.00×10^5 – 1.00×10^7). 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	Diagnosis	Sampling	Sample Source	Prior Therapy	Tumor Fragment Culture (cells)	post-REP (cells)	Digest (cells)	MIL (cells)	Reactivity
1	GNB	3	Localized	Initial treatment	Uphill resection	Abdomen, primary site	N	DNG	N/A	none available	1.00×10^7 pre-REP, 5.35×10^6 post-REP	NR
2	WT	2	IV, favorable histology	Initial treatment	Uphill resection	Abdomen, primary site	N	4.60×10^6	N/A	1.00×10^8	N/A	NR
3	WT	1	IV, favorable histology	Initial treatment	Uphill resection	Abdomen, primary site	N	3.20×10^6	4.01×10^7	1.60×10^8	N/A	NR
4	OS	15	Metastatic	Relapse	After salvage chemotherapy	Lung, metastatic site	C	6.00×10^6	5.40×10^7	7.20×10^8	N/A	N/A
5	WT	7	IV, anaplastic histology	Initial treatment	Uphill resection	Abdomen, lymph node	N	2.50×10^6	DNG	8.63×10^8	N/A	N/A
6	NB	7	IV, high-risk	Relapse	At relapse	CNS, metastatic site	C	5.60×10^6	3.17×10^8	4.21×10^8	N/A	NR
7	IMT	14	Localized	Initial treatment	Uphill resection	Lung, Primary site	N	3.21×10^6	N/A	3.00×10^7	N/A	N/A
8	NB	16	Stage III	Initial treatment	Uphill resection	Lower Extremity, Primary site	N	1.80×10^6	N/A	1.00×10^8	N/A	N/A
9	OS	14	Metastatic	Initial treatment	After neoadjuvant chemotherapy	Lower Extremity, Primary site	C	4.90×10^7	N/A	none available	9.11×10^6 pre-REP	N/A
10	ASPS	20	Metastatic	Relapse	After salvage therapy	CNS, metastatic site	I	DNG	N/A	2.00×10^8	N/A	N/A
11	OS	13	Metastatic	Relapse	After salvage therapy	Lung, metastatic site	C	DNG	N/A	3.20×10^8	N/A	N/A
12	ASPS	20	Metastatic	Relapse	After salvage therapy	CNS, metastatic site	I	DNG	N/A	5.51×10^7	N/A	N/A
13	NB	7	IV, high-risk	Initial treatment	Diagnostic biopsy	Abdomen, primary site	N	4.00×10^6	9.30×10^7	used in co-culture	7.20×10^7 (post-REP only)	NR
14	SS	13	Stage II	Initial treatment	Uphill resection	Lower Extremity, Primary site	N	3.10×10^6	3.90×10^7	used in co-culture	N/A	NR
15	ERMS	3	Stage II, group 2	Initial treatment	Uphill resection	Abdomen, primary site	N	1.10×10^6	2.22×10^8	used in co-culture	N/A	NR
16	OS	14	Localized	Initial treatment	After neoadjuvant chemotherapy	Lower Extremity, Primary site	C	1.37×10^6	4.47×10^8	none available	8.00×10^6 pre-REP	N/A
17	WT	4	Stage III, favorable histology	Initial treatment	Diagnostic biopsy	Abdomen, Primary site	N	5.40×10^7	5.80×10^8	8.90×10^7	N/A	N/A
18	OS	12	Localized	Initial treatment	After neoadjuvant chemotherapy	Lower Extremity, Primary	C	3.51×10^6	2.49×10^7	4.00×10^8	1.00×10^7 pre-REP, 1.40×10^6 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.

Acknowledgements The authors would like to thank Swim Across America (www.swimacrossamerica.org) and the Ocala Royal Dames (www.ocalaroyaldames.org) for their generous support of this work.

REFERENCES

- Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* 2015;**348**(6230):62–68.
- Hall M, Mullinax JE, Royster E, et al. Expansion and characterization of tumor-infiltrating lymphocytes from human sarcoma. *Journal of Immunotherapy of Cancer* 2015;**3**(Suppl. 2):19.
- Mullinax JE, Hall M, Beatty M, et al. Expanded tumor-infiltrating lymphocytes from soft tissue sarcoma have tumor-specific function. *J Immunother* 2021;**44**(2):63–70.
- Feuerer M, Beckhove P, Bai L, et al. Therapy of human tumors in NOD/SCID mice with patient-derived reactivated memory T cells from bone marrow. *Nat Med* 2001;**7**(4):452–458.
- Feuerer M, Rocha M, Bai L, et al. Enrichment of memory T cells and other profound immunological changes in the bone marrow from untreated breast cancer patients. *Int J Cancer* 2001;**92**(1):96–105.

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.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.178>