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

1Jonathan Metts, 2Jonathan Hensel, 3Alejandro Alfaro, 4Brook Olmo, 5Shari Pilon-Thomas, 1John 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 Preclinical mouse models suggest that T-cells from bone marrow (marrow-infiltrating lymphocytes, MIL) have antitumor reactivity offering another source for ACT.4 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 CD3 (63.17), CD4 (21.46), CD8 (46.19) and CD56 (28.21) (Table 1). TIL 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 x 10⁶ cells (range 1.86 x 10⁶–3.21 x 10⁸). 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 10⁷ cells (range 2.49 x 10⁷–7.86 x 10⁸) 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 10⁶ cells (range 8.00 x 10⁵–1.00 x 10⁷). Average phenotype of expanded MIL (%) were CD3 (45.17), CD4 (24.46), CD8 (36.15) and CD56 (28.21) (Table 1).


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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REFERENCES

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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