

CD8+CD69+ EXPANDED TUMOR INFILTRATING LYMPHOCYTES FROM SOFT TISSUE SARCOMA HAVE INCREASED TUMOR-SPECIFIC FUNCTIONAL CAPACITY

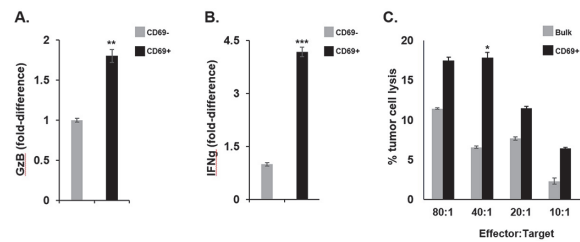
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Background Adoptive cell therapy (ACT) utilizing tumor infiltrating lymphocytes (TIL) has demonstrated durable responses in patients with metastatic melanoma and offers potential for other solid tumors. Preclinical experience with expanded TIL from soft tissue sarcoma (STS) demonstrates less frequent tumor-specific reactivity compared to melanoma samples, limiting the potential for efficacy.¹ We hypothesized that CD69+ TIL have increased tumor-specific reactivity, which can be manipulated in culture, thereby offering an opportunity to enhance the antitumor effect of this cellular immunotherapy product.

Methods Patients were enrolled on an IRB-approved protocol and TIL were expanded from fresh surgical specimens. After enzymatic digestion, tumor single cell suspensions were cultured in media containing 10% human serum and IL-2 (6000IU/mL). Expanded TIL were then enriched for CD8+ using magnetic bead isolation and CD69+ by flow cytometry cell sorting (FACS). After co-culture with autologous tumor digest, functional capacity was compared between bulk TIL and enriched TIL by evaluation of IFN-gamma (IFN γ) and Granzyme B (GzB) secretion. Capacity for direct tumor cytotoxicity was assessed by Cr51 assay after co-culture of autologous immortalized cell lines with expanded TIL subpopulations after enrichment.

Results Following co-culture with autologous tumor digest, CD69+ TIL demonstrated increased IFN γ secretion compared to CD69- TIL in 6 samples (1.4–4.2x, $p < 0.05$). CD8+ enriched TIL (75% compared to bulk) had higher relative IFN γ secretion in both CD69+ and CD69- subsets (4.2 and 5.8x, respectively, $p < 0.001$). Maximal IFN γ secretion was seen from TIL that were both CD69+ sorted and CD8+ enriched, demonstrating a synergistic effect (16.3x vs Bulk CD69-, 4.2x vs Bulk CD69+, 2.8x vs CD8 enriched CD69- ; $p < 0.001$). Functional capacity was also assessed by GzB secretion with similar results. CD69+ TIL had increased relative secretion (1.8–2.2x) compared to CD69- TIL ($p < 0.01$). CD8+ enriched TIL had increased relative GzB secretion in both CD69- and CD69+ sorted fractions (1.4x, 1.2x, respectively, $p < 0.05$). CD69+ sorted and CD8+ enriched TIL demonstrated an additive effect (2.6x vs Bulk CD69-, $p < 0.01$; 1.2x vs Bulk CD69+, $p < 0.05$; 1.8x vs CD8 enriched CD69-, $p < 0.01$). CD8+ enriched CD69+ sorted TIL had greater relative cytotoxicity (3x, $p < 0.05$) at 40:1 E:T ratio against autologous tumor cell lines compared to bulk expanded TIL (figure 1).

Conclusions TIL expanded from STS demonstrate greater tumor-specific functional capacity and cytotoxicity after CD8 enrichment and CD69+ FACS compared to bulk expanded TIL. These data validate the strategy to enhance CD8+CD69+ TIL during culture to yield a more efficacious cellular immunotherapy product.



Abstract 179 Figure 1 Functional capacity of CD69+ TIL is demonstrated by increased secretion of GzB (A) and IFN γ (B) after co-culture with autologous tumor digest. CD69+ TIL have greater cytotoxicity against autologous immortalized cell lines compared to bulk TIL at 40:1 E:T ratio (C).

Acknowledgements This work was funded by NIH K08CA252642

Trial Registration n/a

REFERENCE

1. Mullinax JE, Hall M, Beatty M, Weber AM, Sannasardo Z, Svrldin T, Hensel J, Bui M, Richards A, Gonzalez RJ, Cox CA, Kelley L, Mulé JJ, Sarnaik AA, Pilon-Thomas S. Expanded Tumor-infiltrating Lymphocytes From Soft Tissue Sarcoma Have Tumor-specific Function. *J Immunother* 2021 Feb-Mar 01;**44**(2):63–70.

Ethics Approval Abstract cites IRB-approved protocol in methods section.

Consent n/a

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.179>