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770 ANALYSIS OF GUT MICROBIOME IN PATIENTS RECEIVING ADOPTIVE T-CELL THERAPY (ACT) ACROSS DIFFERENT SOLID TUMOUR TYPES

Arantazu Barquin Garcia*, Sergio Ruiz, Paloma Navarro, Juan Francisco Rodriguez-Moreno, Elena Sevillano, Monica Yague, Sandra Amarilla, Jesus Garcia-Donas. *Centro Integral Oncológico Clara Campal, Madrid, Spain*

Background Tumour Infiltrating Lymphocytes (TILs) is a modality of ACT under development in solid tumours. Unfortunately, prior lymphodepletion is a key step that frequently requires the administration of antibiotics and antifungals for long periods of time. Although there is evidence that gut microbiome may influence tumour response in patients treated with checkpoint-inhibitors, it has not been extensively studied in ACT.¹

Methods Analysis of gut microbiome at three different times (T1: before lymphodepletion, T2: before TIL infusion and T3: day +15) has been performed in patients treated with ACT between 2018 and 2020. The composition and structure of the sampled microbial communities was assessed through the amplification and sequencing the V3-V4 variable regions of the 16S rRNA gene. The Illumina Miseq sequencing 300×2 approach was used. Taxonomic assignment of phylotypes was performed using a Bayesian Classifier trained with Silva database version 132 (99% OTUs full-length sequences). The following metrics were measured: observed OTUs (community richness), evenness (Pielou's index) and Shannon's diversity index. Differential abundance of taxa was tested using ANCOM test and Kruskal Wallis test.

Results A total of 21 patients have been treated with TILs between 2018 and 2020 at our institution. 67% were female. Median age was 43 (range 26–70 years). All patients had stage IV pre-treated solid tumours: 55% cervical cancer, 33% melanoma, 10% lung adenocarcinoma and 5% head and neck cancer. Median previous treatment lines was 3 (range 2–4). Analysis of gut microbiome has been performed in 3 of these patients: one achieved PR, one progressed and the third one suffered an unexpected death. 971 phylotypes were detected. Analysis revealed differences in terms of observed OTUs, evenness and Shannon's index when comparing T1 and T2 with T3. At T3 a tendency towards less diversity and evenness was observed when compared with T1 and T2 (H 3.0, p-value 0.083, not statistically significant). Comparing the distribution of considered taxa in ACT responders vs. non-responders, we observed significant differences for both class (Bacteroidia, Clostridia and Gammaproteobacteria) and order (Bacteroidales, Lactobacillales, Clostridiales and Enterobacteriales) levels.

Conclusions A deep change in gut microbiome composition along TILs therapy was observed. Though preliminary, differences between responders and non-responders were observed but should be confirmed in larger populations.

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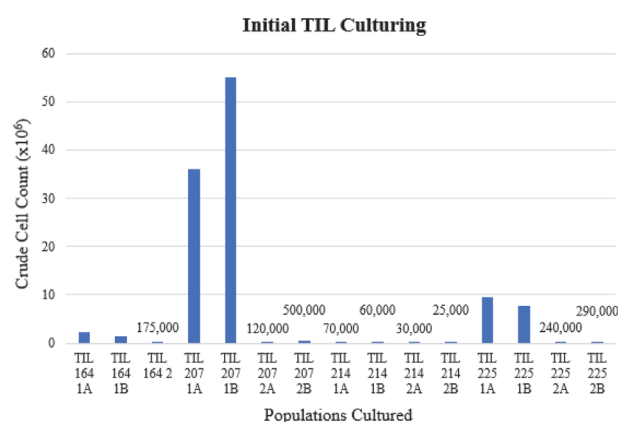
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771 CHARACTERIZATION OF TUMOR INFILTRATING IMMUNE CELLS FROM ADULT SOFT TISSUE SARCOMAS

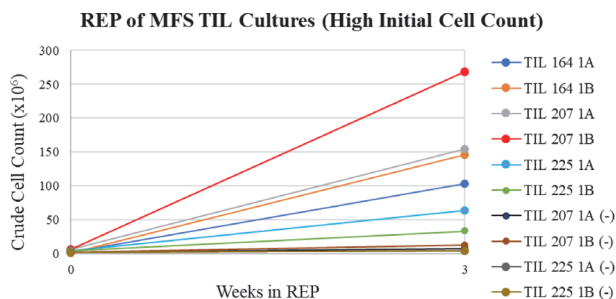
¹Jacky Chen*, ²Jay Wunder, ¹Nalan Gokgoz, ¹Irene Andrulis. ¹Lunenfeld Tanenbaum Research Institute, Toronto, Canada; ²Sinai Health System, Toronto, Canada

Background Sarcoma is a group of rare bone and soft tissue tumors with over 50 distinct subtypes. Survival rate ranges widely due to the lack of efficacious treatments. Immunotherapy, such as adoptive cell therapy (ACT), has drawn great interest due to its minimal toxicity. In ACT, tumor infiltrating lymphocytes (TILs) are isolated from patients, expanded, and autologously reinfused back. We recently observed TILs presence in Undifferentiated Pleomorphic Sarcoma (UPS) and Myxofibrosarcoma (MFS) tumors and found that tumor's PD-L1 overexpression is correlated with better clinical outcome in UPS but not MFS.¹ The Thelper1 inflammatory pathway was highly activated in the former subtype, which may explain the better outcome. These results illustrate the immunological differences where TILs may play a critical role. We hypothesize that there are phenotypic and functional differences between TILs of UPS and MFS that may be related to clinical outcomes. Sarcoma TILs are rare and challenging to culture



Abstract 771 Figure 1

Initial culturing of four primary MFS tumor cases with complete media (CM) over 4 weeks. Ten total cases were selected, five cases for each UPS and MFS sarcoma subtypes. To date, four MFS cases #164, 207, 214, and 225 have been processed. TIL populations were identified and categorized based on their growth rates and labelled as '1' or '2' representing 'fast' or 'slow' growing TILs, respectively. 'A' and 'B' represent technical replicates. Population TIL 164 '2' has no replicates. 15 populations were derived from the four MFS cases. TILs were cultured and expanded from tumor fragments in CM over 4 weeks in duration. CM consisted of Iscove's Modified Dulbecco's Medium, 6000 IU/mL IL-2, 10% human serum albumin, 25 mmol/L HEPES, 2mmol L-glutamine, 5.5x10⁻⁵ mol/L β-mercaptoethanol, 100 U/mL penicillin, and 100 μg/mL streptomycin. At week 4, cells were collected and counted with a hemocytometer. Only 6 populations achieved ≥ 1x10⁶ cells and are categorized as high initial cell count populations. 9 populations achieved <1x10⁶ cells and are categorized as low initial cell count populations. The 9 low initial cell count populations were further numbered with specific cell counts in figure 1 for clarity. These cell yields, with the exception of TIL 207 1A and 1B, are insufficient for characterization experiments.



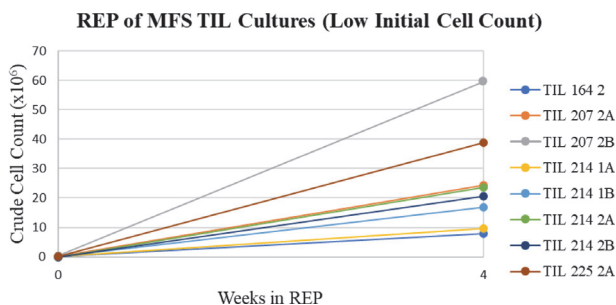
Abstract 771 Figure 2

REP treatment of populations with high initial cell count over 3 weeks. This graph corresponds to table 1. 6 out of 15 populations achieved $\geq 1 \times 10^6$ cells after 4 weeks of initial culturing and were subsequently REP-treated with anti-CD3/anti-CD28 coated magnetic Dynabeads (Life Technologies) on 24-well plates. REP-treated populations have trendlines with positive slopes across the 3 weeks of REP expansion, indicating positive growth rates. Negative controls (-) without REP treatments have near-flat trendlines indicating lack of growth. REP was successful in expanding all 6 TIL populations. At week 3 post-REP, cells were collected and counted via hemocytometer.

which impedes their studies. We first aim to robustly expand TILs to sufficient numbers.

Methods TILs are being expanded and cultured from UPS and MFS primary tumors with various PD-L1 levels. To initiate TIL culturing, bulk tumors were fragmented into 1mm, seeded at 1 fragment/well, and cultured in interleukin-2 supplemented complete media. Due to insufficient cell yields for characterization, rapid expansion protocol (REP) with anti-CD3/anti-CD28 co-stimulating beads was subsequently employed for further expansion.

Results Of 4 MFS cases processed to date, 15 TIL populations were derived and cultured (figure 1). Only 6 in 15 TIL cultures obtained $\geq 1 \times 10^6$ cells and are considered high initial cell count populations. 9 in 15 cultures obtained $< 1 \times 10^6$ cells and are considered low initial cell count populations. REP successfully expanded 14 out of 15 TIL populations, each



Abstract 771 Figure 3

REP treatment of populations with low initial cell count over 4 weeks. This graph corresponds to table 2. 9 out of 15 populations achieved $< 1 \times 10^6$ cells after 4 weeks of initial culturing and were subsequently REP-treated with anti-CD3/anti-CD28 coated beads on 96-well plates. REP-treated populations have trendlines with positive slopes across the 4 weeks of REP expansion, indicating positive growth rates. REP was successful in expanding 8 of 9 TIL populations. TIL 225 2B failed to expand (data not shown) due to cell fatigue and apoptosis during the initial tumor fragment culturing and not due to REP's capabilities or robustness. Negative controls were not established due to constraints with initial cell availability. At week 4 post-REP, cells were collected and counted via hemocytometer.

Abstract 771 Table 1

REP treatment of populations with high initial cell count over 3 weeks. Table 1 corresponds to graphed analysis in figure 2. 6 populations that achieved $\geq 1 \times 10^6$ cells after 4 weeks of initial culturing were treated with anti-CD3/anti-CD28 coated beads on 24-well plates over 3 weeks. Total cells seeded before REP, total cells collected after REP, and fold-expansion calculated are shown. At week 0, all populations were seeded at a density of 1×10^6 cells per well, except for TIL 164 1A and TIL 164 1B which were seeded at 1.1×10^6 cells and 1.4×10^6 cells per well, respectively. Populations were expanded via addition of beads at a bead to cell ratio of 1:1. At week 3 post-REP, cells were collected, counted, and fold-expansion was determined. Cell counts performed via hemocytometer. Negative controls (-) were only allocated for certain populations with sufficient initial cell availability.

Populations	Total Cells Seeded ($\times 10^6$)	Total Cells Collected ($\times 10^6$)	Fold-Expansion
TIL 164 1A	2.2	103.0	46
TIL 164 1B	1.4	146.0	103
TIL 207 1A	7.0	155.0	22
TIL 207 1B	7.0	268.0	38
TIL 225 1A	5.0	64.0	13
TIL 225 1B	4.0	33.5	8
TIL 207 1A (-)	2.0	8.0	4
TIL 207 1B (-)	2.0	12.7	6
TIL 225 1A (-)	2.0	4.5	2
TIL 225 1B (-)	2.0	4.3	2

Abstract 771 Table 2

REP treatment of populations with low initial cell count over 4 weeks. Table 2 corresponds to graphed analysis in figure 3. 9 populations that achieved $< 1 \times 10^6$ cells after 4 weeks of initial culturing were treated with anti-CD3/anti-CD28 coated beads on 96-well plates over 4 weeks. Total cells seeded before REP, total cells collected after REP, and fold-expansion calculated are shown. At week 0, populations were seeded at a density of 8×10^4 cells per well, except for populations with $< 8 \times 10^4$ cells in total, which were seeded at all available cells per well. For example, TIL214 1B was seeded at 6×10^4 cells per well. Populations were expanded via addition of beads at a bead to cell ratio of 1:1; once at week 0 and once more as re-stimulation at week 2. At week 4 post-REP, cells were collected, counted, and fold-expansion was determined. TIL225 2B did not yield any growth under 10X light microscope observation. Lack of cells and cell debris was observed; hence this population was not collected. Cell counts performed via hemocytometer. Negative controls were not established due to constraints with initial cell availability.

Populations	Total Cells Seeded ($\times 10^3$)	Total Cells Collected ($\times 10^6$)	Fold-Expansion
TIL 164 2	175	7.8	45
TIL 207 2A	120	24.3	203
TIL 207 2B	500	59.6	119
TIL 214 1A	70	9.6	137
TIL 214 1B	60	17.0	283
TIL 214 2A	30	23.5	783
TIL 214 2B	25	20.6	824
TIL 225 2A	240	38.8	162
TIL 225 2B	290	n/a	n/a

obtaining between 7.8 to 268.0 $\times 10^6$ cells (tables 1 and 2, figures 2 and 3).

Conclusions Sarcoma infiltrates are difficult to culture and their roles remain largely unstudied. Our results demonstrate anti-CD3/anti-CD28 co-stimulation's capability in expanding 93.3% of TILs and established a robust method of expansion. Future investigation of lineage markers, cytokine profiles, and cytotoxicity aims to identify immunological differences between UPS and MFS. TILs will be primed with memory-inducing cytokines (IL-7, IL-12, IL-15, IL-21) to modulate

differentiation state and enrich cellular stemness.² This would enhance TILs in vivo anti-tumor activity and prolong their survival. Elucidating TILs and their relations with tumor's PD-L1 expression would allow clinicians to appropriately recognize sarcoma's tumor immune environments and select the most desirable infiltrates for superior ACT.

Ethics Approval The study was approved by Mount Sinai Hospital's Ethics Board, approval number 01-0138-U.

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A POTENT AND OFF-THE-SHELF ONK CELL THERAPY PRODUCT TARGETS HER2+ CANCER CELLS AND RESISTS SUPPRESSIVE TUMOR MICROENVIRONMENT

¹Hao-Kang Li*, ²Ching-Wen Hsiao, ²Sen-Han Yang, ¹Hsiu-Ping Yang, ¹Tai-Sheng Wu, ¹Zih-Fei Cheng, ¹Chia-Yun Lee, ¹Yan-Liang Lin, ¹Yan-Da Lai, ²Sai-Wen Tang, ¹Janet Pan, ¹Wei-Lun Lo, ¹Shih-Chia Hsiao. ¹Acepodia Biotechnologies Ltd., New Taipei City, Taiwan, Province of China; ²Acepodia Biotech Inc., Vallejo, CA, USA

Background Autologous or allogeneic natural killer (NK) cells possess efficient cytotoxicity against tumor cells without severe side effects such as CRS or graft-versus-host disease (GvHD). In addition to chimeric antigen receptor (CAR) strategy, antibody-cell conjugates (ACC) platform provides more efficient way to arm NK cells with binding specificity and enhanced potency against target cells. In this work, we develop a NK cell therapy product ACE1702, a novel NK cell line oNK conjugated with trastuzumab, and assess its potency against HER2+ solid tumors.

Methods oNK cells were covalently conjugated with monoclonal antibody Trastuzumab after sublethal irradiation by our patented antibody-cell conjugates (ACC) platform to become our cryopreserved final product ACE1702 compliant with current good manufacturing practice (cGMP). Function of ACE1702 was validated by real-time xCELLigence analyzer and MTT assay in vitro. Efficacy of intraperitoneally (ip.) delivered ACE1702 was evaluated in tumor-bearing female immune compromised NSG mice. Characterization of ACE1702 was analyzed by flow cytometry.

Results We demonstrated that the trastuzumab-armed oNK cells, ACE1702, exerted human epidermal growth factor 2 (HER2) binding specificity and enhanced cytotoxicity against various types of cancer cells with different grade of HER2 expressions compared to control oNK cells in vitro. In vivo results in human ovarian cancer cell line SK-OV-3-bearing xenograft mouse model further supported the in vitro observations. Of note, ACE1702 also displayed a better cytotoxicity against HER2+ cancer cells than trastuzumab and its derived antibody-drug conjugate. ACE1702 also remained cytotoxicity against cancer cells in the suppressive tumor microenvironment. Characterization revealed a preferential expression of NK activation receptors, and conjugation of trastuzumab with cell membrane proteins responsible for NK activity capacitated ACE1702 with enhanced cytotoxicity. These results underscore the potency of ACE1702 in eradication of cancer cells.

Conclusions Here we introduced a novel trastuzumab-modified oNK cell product with enhanced specificity against myriad types of HER2+ cancers. Selective conjugation of trastuzumab with membrane proteins contributing to NK activation conferred ACE1702 with enhanced cytotoxicity even in the suppressive tumor microenvironment.

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Trial Registration None

Ethics Approval The animal study was conducted according to protocols approved by the Institutional Animal Care and Use Committee of Muragenics.

Consent None

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ADOPTIVE CELL THERAPY RESPONSE IN MELANOMA IS MEDIATED BY STEM-LIKE CD8 T CELLS

Sri Krishna*, Frank Lowery, Amy Copeland, Stephanie Goff, Grégoire Altan-Bonnet, Paul Robbins, Steven Rosenberg. *National Cancer Institute, Bethesda, MD, United States*

Background Adoptive T cell therapy (ACT) utilizing ex vivo-expanded autologous tumor infiltrating lymphocytes (TILs) can result in complete regression of human cancers.¹ Successful immunotherapy is influenced by several tumor-intrinsic factors.²⁻³ Recently, T cell-intrinsic factors have been associated with immunotherapy response in murine and human studies.⁴⁻⁵ Analyses of tumor-reactive TILs have concluded that anti-tumor neoantigen-specific TILs are enriched in subsets defined by the expression of PD-1 or CD39.⁶⁻⁷ Thus, there is a lack of consensus regarding the tumor-reactive TIL subset that is directly responsible for successful immunotherapies such as ICB and ACT. In this study, we attempted to define the fitness landscape of TIL-enriched infusion products to specifically understand its phenotypic impact on human immunotherapy responses.

Methods We compared the phenotypic differences that could distinguish bulk ACT infusion products (I.P.) administered to patients who had complete response to therapy (complete responders, CRs, N = 24) from those whose disease progressed following ACT (non-responders, NRs, N = 30) by high dimensional single cell protein and RNA analysis of the I.P. We further analyzed the phenotypic states of anti-tumor neoantigen specific TILs from patient I.P (N = 26) by flow cytometry and single cell transcriptomics.

Results We identified two CD8+ TIL populations associated with clinical outcomes: a memory-progenitor CD39-negative stem-like TIL (CD39-CD69-) in the I.P. associated with complete cancer regression (overall survival, P < 0.0001, HR = 0.217, 95% CI 0.101 to 0.463) and TIL persistence, and a terminally differentiated CD39-positive TIL (CD39+CD69+) population associated with poor TIL persistence post-treatment. Although the majority (>65%) of neoantigen-reactive TILs in both responders and non-responders to ACT were found in the differentiated CD39+ state, CR infusion products also contained a pool of CD39- stem-like neoantigen-specific TILs (median = 8.8%) that was lacking in NR infusion products (median = 23.6%, P = 1.86 x 10⁻⁵). Tumor-reactive stem-like T cells were capable of self-renewal, expansion, and persistence, and mediated superior anti-tumor response in vivo.

Conclusions Our results support the hypothesis that responders to ACT received infusion products containing a pool of stem-like neoantigen-specific TILs that are able to undergo prolific