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

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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.

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.

REFERENCE

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

1Jacky Chen*, 2Jay Wunder, 1Nalan Gokgoz, 1Irene Andrusis, 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 TIL's 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. The T cell 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 labeled as fast and slow. Analysis of gut microbiome at three different times (T1: before lymphodeple- tion, 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.

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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 fast and slow. Population TIL 164 'A' and 1B had 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 low initial cell count populations. 9 populations achieved >1x10⁶ cells and are categorized as high initial cell count populations.
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 ≥1x10⁶ cells and are considered high initial cell count populations. 9 in 15 cultures obtained <1x10⁶ cells and are considered low initial cell count populations. REP successfully expanded 14 out of 15 TIL populations, each obtaining between 7.8 to 268.0 x10⁶ 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 their capabilities or ‘s capability in expanding TILs to sufficient numbers.
A POTENT AND OFF-THE-SHELF ONK CELL THERAPY
ADOPTIVE CELL THERAPY RESPONSE IN MELANOMA IS
2020; the potency of ACE1702 in eradication of cancer cells. ACE1702 with enhanced cytotoxicity. These results underscore
NK activity capacitated NK activation receptors, and conjugation of trastuzumab with antibody-drug conjugate. ACE1702 also remained cytotoxicity
(graft mouse model further supported the in vitro observations. Of note, ACE1702 also displayed a better cytotoxicity
expressions compared to control oNK cells in vitro. In vivo.

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772 A POTENT AND OFF-THE-SHELF ONK CELL THERAPY PRODUCT TARGETS HER2+ CANCER CELLS AND RESISTS SUPPRESSIVE TUMOR MICROENVIRONMENT
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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-body 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 novel 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 vivo 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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773 ADOPTIVE CELL THERAPY RESPONSE IN MELANOMA IS MEDIATED BY STEM-LIKE CD8 T CELLS
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Background Adoptive T cell therapy (ACT) utilizing ex vivo-expanded autologous tumor infiltrating lymphocytes (TILs) can result in complete regression of human cancers. Successful immunotherapy is influenced by several tumor-intrinsic factors. Recently, T cell-intrinsic factors have been associated with immunotherapy response in murine and human studies.

Methods We compared the phenotypic differences that could distinguish bulk ACT infusion products (IP) 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 IP. We further analyzed the phenotypic states of anti-tumor neoantigen-specific TILs from patient IP (N = 26) by flow cytometry and single cell transcriptomics.

Results We identified two CD8+ TIL populations associated with complete outcomes: a memory-progenitor CD39-negative stem-like TIL (CD39-CD69-) in the IP 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