sought to develop fungus-specific chimeric antigen receptor (CAR) T cells as a novel immune augmentation strategy to treat IFIs including invasive aspergillosis. To target fungal pathogens, we fused the pattern-recognition receptor Dectin-1 to activate T cells via chimeric CD28 and CD3-ζ domains upon binding to β-1,3-glucan carbohydrates in the fungal cell wall(3). The generated Dectin-1 CAR+ T cells showed high specificity for β-1,3-glucan and inhibited the growth and branching of AF germinals in an in-vitro co-culture assay. However, we found poor efficacy of Dectin-1 CAR+ T cells against mature AF hyphae, likely due to changes in the fungal cell wall that hamper T-cellular binding to β-1,3-glucan carbohydrates. To overcome this limitation, we have recently developed an AF-specific CAR (AF-CAR) based on a monoclonal antibody which recognizes a surface epitope of mature AF hyphae.

Methods Lentiviral vectors were used to generate AF-CAR expressing T cells from human peripheral blood mononuclear cells. Heat killed AF-293 hyphae was used for co-culture studies with No DNA T cells, and AF-CAR expressing T cells. Cell clusters, binding with AF hyphae were noticed in AF-CAR incubated wells whereas no such cell cluster were observed in NoDNA T cells incubated wells.

Results When co-incubated with AF hyphae, AF-CAR+ T cells efficiently targeted mature hyphae and formed lytic synapses with hyphal filaments. The released cytolytic granules damage hyphae and controls branch node formation. Furthermore, exposure to AF hyphae stimulated significant upregulation of activation markers CD69 and CD154 on AF-CAR+ T cells. The activated CAR T cell secretes proinflammatory cytokines which can boost innate immune system to fight against IFI.

Conclusions In summary, these results indicate that we have successfully generated a novel anti-Aspergillus CAR construct with good in-vitro targeting efficacy against mature AF hyphae. After thorough evaluation of fungidal activity, cytokine response patterns, and release of cytotoxic mediators, we plan to embark on preclinical tolerability and efficacy studies in a murine model of invasive pulmonary aspergillosis. Thus, we report the production of Aspergillus specific CAR T cells to provide long term protection to immunocompromised patients, such as AML patients and HSCT recipients, from invasive Aspergillus infections.

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Ethics Approval This study was approved by IBC committee, University of Texas MD Anderson Cancer Center, Houston, Texas, 77030.

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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.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 approach was used. Taxonomic assignment of phyotypes 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.

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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CHARACTERIZATION OF TUMOR INFILTRATING IMMUNE CELLS FROM ADULT SOFT TISSUE SARCOMAS

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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’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.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 and expansion.