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-gucan and inhibited the growth and branching of AF germlings 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 fungicidal 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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769 CAR T CELLS UNDERGOING EPIGENETIC REPROGRAMMING BY LOW-DOSE DECITABINE ENHANCES PERSISTENT ANTI-TUMOR EFFICACY IN VIVO

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Background Anti-CD19-directed chimeric antigen receptor (CAR) T-cell therapy has had a resounding effect on the treatment of B-ALL. However, CAR T cells have been less effective against B-cell non-Hodgkin lymphoma (B-NHL), in part because they become a exhausted state triggered by chronic antigen stimulation and characterized by upregulation of inhibitory receptors and loss of effector function.[1-4] It has recently been demonstrated that de novo DNA methylation promoted T-cell exhaustion, whereas methylation inhibition enhanced ICB-mediated T-cell rejuvenation in vivo.[5 6] FDA-approved DNA demethylating agents, such as decitabine (DAC), may provide a means to modify exhaustion-associated DNA methylation programs that restrict ICB-responsiveness.

Methods We treated CAR (CAR-CD19-expressing) T cells with low-dose DAC (dCAR T cells), to determine its effects on antitumor activities, exhaustion- and memory-associate cell phenotype change, cell cytokine production, and cell proliferation. Its impact on antitumor activities was evaluated in vitro functional assays and mouse in vivo studies. We also conducted western blot, flow cytometry, methylation analysis, RNA in situ hybridization and high throughput RNA sequencing to determine the underlying mechanisms of dCAR T cell function.

Results The low-dose, short-term DAC treatment in vitro enhanced the central memory (Tcm) population and the ration of CD4/CD8, and induced degradation of DNMT3a.CAR T cell treated by DAC developing into less-differention status by enhancing memory. dCAR T cells exhibit enhanced antitumour reactivity and the maintenance of a memory-like phenotype at low effector:target ratios. Especially shown by the 'stress test', the dCAR T cells at very low doses could efficiently control tumours with a very large burden, and have effective recall responses upon tumour re-challenge in vivo. Importantly, the dCAR T cells maintained a higher proportion of cells with a memory phenotype than did the CAR T cells under long-term tumour stimulation. Transcription of gene sets involved in memory maintenance, proliferation, cytokine production and anti-inhibitor processes was triggered by antigen-expressing target cells upon DAC exposure before antigen stimulation. dCAR T cells avoided the exhaustion programme induced during tumour cell stimulation; they did not upregulate the expression of genes encoding inhibitory receptors and retained relatively high expression of memory related transcription factors and genes.

Conclusions CAR T cells underwent DNA reprogramming after DAC treatment, which induced significant sustained cell expansion, cytotoxicity, and cytokine production and reduced exhaustion after antigen exposure.

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

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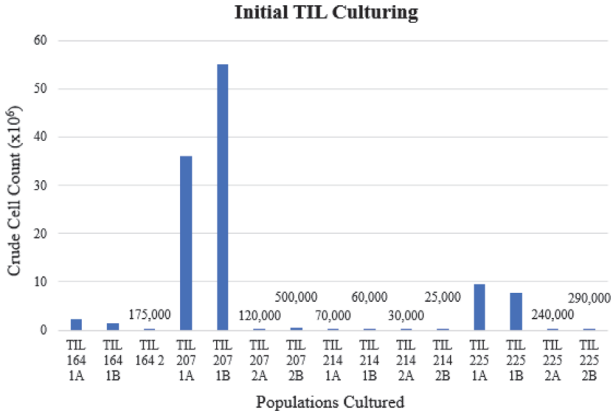
Background Sarcoma is a group of rare bone and soft tissue tumors with over 50 distinct subtypes. Survival rate ranges widely due to the lack of efficacious treatments. Immunotherapy, such as adoptive cell therapy (ACT), has drawn great interest due to its minimal toxicity. In ACT, tumor infiltrating lymphocytes (TILs) are isolated from patients, expanded, and autologously reinfused back. We recently observed 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.[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.