related to mesenchymal transition in GBM such as NF- κ B and CEBPB were accessible from normal to tumor-associated microglia. On the other hand, tissue-associated macrophages exhibited enhanced calcium-regulated NFAT TF accessibility. Tumor-associated IWP and IWR myeloid cells also showed a gain of DGE of apoptosis and a reduction of proliferation-related genes.

Conclusions Our studies demonstrate that in addition to the previous dogma of myeloid mediated immune suppression that contributes to tumor immune escape, epigenomic reprogramming in the brain TIME leads to unexpected activation of transcriptional pathways that can trigger transdifferentiation and cell death of myeloid cells further promoting tumor progression. In summary, we provide an unparalleled epigenomic landscape of glioma-associated myeloid cells that may have translational implications.

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Trial Registration NA

Ethics Approval The brain tumor/tissue samples were collected as per MD Anderson internal review board (IRB)-approved protocol numbers LAB03-0687 and, LAB04-0001. One non-tumor brain tissue sample was collected from a patient undergoing neurosurgery for epilepsy as per Baylor College of Medicine IRB-approved protocol number H-13798. All experiments were compliant with the review board of MD Anderson Cancer Center, USA.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal

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CRISPR-MEDIATED IN SITU EDITING OF LIVER RESIDENT MACROPHAGES FOR TREATING LIVER CANCERS

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Background Liver cancer is one of the leading cause of cancer death worldwide with limited treatment options. The liver accommodates the largest population of tissue resident macrophages in the body, namely Kupffer cells. Immune deviation of hepatic immune responses from anti-tumor towards pro-tumor is crucial for cancer progression. This process is closely correlated with the functional polarization of these macrophages. In situ genome editing of liver resident macrophage with intention to shift macrophage function to stimulate anti-tumor immune responses is promising in treating liver cancers.

Methods We have previously shown that Kupffer cells quickly capture and phagocytose circulating bacteria, making bacteria as a potential liver macrophage-specific deliver vector. Taking advantages of the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 technology, we have established a bacteria mediated genome editing methods for liver resident macrophages in vivo.

Results We used a non-pathogenic *Escherichia coli* (*E. coli*) strain as a deliver vector for the CRISPR-Cas9 plasmids, essentially all liver resident macrophages but neither liver sinusoids endothelial cells nor hepatocytes were shown to taken up the bacteria, indicating the robustness and specificity of *E. coli*-mediated plasmid delivery. To test the genome editing efficiency, we chose VSIG4, Tim-4 and F4/80 that were highly expressed by Kupffer cells and validated the gene knockout/knockdown effects using intravital imaging. Expression of these receptors by Kupffer cells diminished by more than 90%. Simultaneously editing of multiple genes was also achieved with a slightly decreased efficiency when compared to single gene editing. The acute inflammatory responses and the hepatotoxicity caused by bacteria were ameliorated by pre-immunization with the same *E. coli* strain, and can be further minimized by using a mutant *E. coli* strain that processed a modified LPS structure, which dramatically decreased the TLR-4 mediated inflammatory signaling and improved the safety of this method. Moreover, we have shown that not only embryonically-derived Kupffer cell but also monocyte-derived liver macrophages could be edited. The applications of this approach in treating primary liver cancers and liver metastasis are under investigation.

Conclusions Taken together, we have established a rapid, efficient and convenient method to achieve in situ genome editing of liver resident macrophages in vivo. By targeting essential genes that instruct macrophage polarization, this method could be used as immunotherapy for liver diseases, including cancers.

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Immune cell types

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STRUCTURAL DIFFERENCE CAUSED BY MUTATED RESIDUES IS CORRELATED WITH IMMUNOGENICITY OF NEOANTIGENS AND SPECIFICITY OF REACTIVE T CELLS

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Background Host T-cell response is limited to only a small fraction of nonsynonymous mutations; however, the molecular properties of those immunogenic neoantigens remain elusive.

Methods Here, we interrogated the HLA class I ligandome of a microsatellite instability (MSI)-type cancer cell line using a proteogenomic approach, and found an immunogenic 9-mer neoantigen, AKF9. The AKF9 was a non-anchor type neoantigen that harbored a single amino-acid substitution (Asn > Lys) at position 8, which did not affect the HLA-binding affinity.

Results In order to assess a determinant of the immunogenicity, we prepared a panel of AKF9 variants with substitutions at position 8, and found that CD8+ T-cell responses were biased toward residues with structural difference from the wild-type. Interestingly, a substitution with moderate structural change (Asp) also induced reactive T cells; however, in contrast to the others, induced T cells frequently cross-reacted to the wild type HLA ligand. To validate these findings, we used in silico prediction of accessible surface areas and scored the difference between neoantigens and wild types (Δ ASA). Evaluation of reported clinical datasets demonstrated that patient T-

cell induction was positively correlated with Δ ASA values, while cross-reactivity of induced T cells was inversely correlated.

Conclusions Our results indicate that dissimilarity is key for both T-cell induction and discrimination from self. Δ ASA may help predict immunogenic non-anchor type neoantigens inducing specific T-cell response from a variety of cancer mutation pools.

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836 RELEASING THE RESTRAINTS OF V γ 9V δ 2 T-CELLS IN CANCER IMMUNOTHERAPY

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Background V γ 9V δ 2 T-cells are a subset of cells with a crucial role in immunosurveillance which can be activated and expanded by multiple means to stimulate effector responses, often exploited in cancer immunotherapy. Little is known about the expression of checkpoint molecules on this cell population and whether the ligation of these molecules can regulate their activity. The aim of this study was to assess the expression of activatory and inhibitory markers on V γ 9V δ 2 T-cells to assess potential avenues of regulation to target with immunotherapy.

Methods PBMCs were isolated from healthy donors and the expression of activatory and inhibitory receptors was assessed on V γ 9V δ 2 T-cells by flow cytometry at baseline, following 24 hours activation and 14 days expansion using zoledronic acid (ZA) and Bacillus Calmette-Guerin (BCG), both with IL-2. Activation and expansion of V δ 2 cells was assessed by expression of CD69 and by frequency of V δ 2 cells, respectively. Production of effector molecules was also assessed following coculture with various tumour cell targets. The effect of immune checkpoint blockade on V γ 9V δ 2 T-cells was also assessed.

Results V γ 9V δ 2 T-cells constitutively expressed high levels of NK-associated activatory markers NKG2D and DNAM1 which remained high following stimulation with ZA and BCG. V γ 9V δ 2 T-cells expressed variable levels of checkpoint inhibitor molecules at baseline with high levels of BTLA, KLRG1 and NKG2A and intermediate levels of PD1, TIGIT and VISTA. Expression of checkpoint receptors were modulated following activation and expansion with ZA and BCG with decreased expression of BTLA and upregulation of numerous markers including PD1, TIGIT, TIM3, LAG3 and VISTA. Expression of these markers is further modulated upon coculture with tumour cell lines with changes reflecting activation of these cells with V γ 9V δ 2 T-cells expressing inhibitory receptors PD1 and NKG2A producing the highest level of TNF.

Conclusions Our data reveals unique characteristics of V δ 2 in terms of their expression of immune checkpoints, which provide a mechanism which may be utilised by tumour cells to subvert V γ 9V δ 2 T-cell cytotoxicity. Our work suggests different profiles of immune checkpoints dependent on the method of stimulation. This highlights importance of expansion method in the function of V γ 9V δ 2 T-cells. Furthermore, this work suggests important candidates for blockade by immune checkpoint therapy in order to increase the successful use of V γ 9V δ 2 T-cells in cancer immunotherapy.

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837 INTERLEUKIN-10 DRIVES THE DEVELOPMENT OF T REGULATORY TYPE 1 (TR1) CELLS AND IS A TARGET FOR IMMUNOTHERAPY

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Background In recent years, immunotherapy has become a common tool of cancer treatment. In order to define therapeutic targets, it is necessary to understand mechanisms of tumor-induced immunosuppression. In malignant B-cell lymphoma, the effects of the anti-inflammatory cytokine interleukin-10 (IL-10) remain poorly understood.

Methods To investigate the role of IL-10 in a tumor microenvironment, we used λ -MYC-transgenic mice that spontaneously develop B-cell lymphoma. The experiments were performed either in vivo or in vitro and the cell samples were then analyzed by flow cytometry.

Results In MYC tumors, CD4+Foxp3- effector T cells maintained the expression of interferon- γ (IFN- γ), yet became exhausted. Within this population we found a cell fraction of unknown origin coexpressing IFN- γ and IL-10 that increased during disease progression. These cells turned out to be T regulatory type 1 (Tr1) cells, which are known to be immunosuppressive. When exposing homogeneous IFN- γ -producing T helper type 1 (Th1) cells to a MYC tumor milieu in vitro, part of these cells started to express both, IFN- γ and IL-10, and showed an increased level of programmed cell death protein 1 (PD-1). Notably, these changes diminished when an IL-10 neutralizing monoclonal antibody (mAb) was added to the coculture, indicating that IL-10 is necessary for the Tr1 development and is involved in the upregulation of PD-1. In line with these results, we treated λ -MYC mice with anti-IL-10 mAb. This therapy not only led to significantly prolonged survival but also decreased expression of PD-1 on effector T cells and increased proliferation of cytotoxic T cells.

Conclusions In summary, these results showed the importance of IL-10 for the tumor immune escape in lymphoma. IL-10 induced a conversion from Th1 to Tr1 cells and elevated levels of PD-1. Both effects were diminished after IL-10 ablation. Thus, targeting IL-10 might be a promising new approach of immunotherapy.

Ethics Approval All animal studies were approved by Regierung von Oberbayern, approval number 55.2-1-54.

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838 PHENOTYPIC AND FUNCTIONAL SIGNATURES OF PERIPHERAL AND TUMOR-RESIDENT $\gamma\delta$ T CELLS ARE INFORMATIVE FOR OUTCOME OF CHECKPOINT BLOCKADE IN MELANOMA

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Background Immune checkpoint blockade (ICB) set a milestone in cancer immunotherapy, but still only a fraction of patients responds. Thus, there is an urgent need for biomarkers predicting outcome, and also for understanding the responsible mechanisms. $\gamma\delta$ T cells constitute a numerically minor subset of 1-10% of the peripheral T cell compartment in healthy people and have a major role in defense against multiple microbial and non-microbial challenges. Unlike the majority of T cells, $\gamma\delta$ T cells bind their ligands in an MHC-