Methods In vitro stimulated Cbl-b/-/+ or Cbl-b/-/- Thy1.1+ P14 TCR-transgenic CD8+ T cells were adoptively transferred into B16-gp33 melanoma-bearing Thy1.2+ FoxP3-GFP/DTR transgenic mice treated with or without diphtheria toxin (n = 15). Tumor size and overall survival were measured. Congenically labelled T cells from tumor, draining lymph node, and spleen were comprehensively profiled using flow cytometry. To further examine the biological mechanism of Treg resistance, we performed in vitro Treg suppression assays and RNA-sequencing.

Results Adoptively transferred tumor-specific Cbl-b/-/- effector CD8+ T cells mediated superior control over tumor growth and increased overall survival in comparison to the wild-type counterpart. Depletion of FoxP3+ cells increased the quantity and percentage of CD25+ 4-1BB+ expressing P14 Thy1.1+ CD8+ T cells in the tumor, whereas the effect of FoxP3+ cell depletion was negligible with Cbl-b deficient CD8+ T cells. Cbl-b deficiency also attenuated sensitivity to Treg cell-mediated suppression in vitro. Transcriptomic analyses suggested that Cbl-b regulates pathways associated with cytokine production and cellular proliferation. Specifically, hyper-secretion of IFN-γ by Cbl-b deficient CD8+ T cells attenuated suppression by Treg cells. In murine models of adoptive T cell therapy, Cbl-b deficient CD8+ T cells were less susceptible to suppression by Treg cells in the tumor through the effects of IFN-γ.

Conclusions We demonstrate that adoptively transferred effector CD8+ T cells are susceptible to regulation by Treg cells in the tumor, and that ablation of Cbl-b abrogates Treg cell-mediated suppression. We highlight the therapeutic implications of targeting Cbl-b in the context of ACT.

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Results Single cell-based analysis of RNAseq data revealed two sets of genes discriminating between different subpopulations of melanoma cells and covering most melanoma cells. Set 1 was shown to be AXL high/MITF low and expressed PRAME, whereas set 2 was shown to be AXL low/MITF high and expressed melanoma lineage markers. The 6-color multiplex immunofluorescence panel could discriminate different melanoma subpopulations, thereby giving information on melanoma heterogeneity. Image analyses of melanoma phenotypes and immune infiltrates is still ongoing. These analyses also include the topographical location of different melanoma cell subpopulations with respect to immune cells, and their changes after immunotherapy.

Conclusions Melanoma heterogeneity pre- and post-immunotherapy can be analyzed by 6-color multiplex immunofluorescence.
related to mesenchymal transition in GBM such as NF-kB and CEBPB were accessible from normal to tumor-associated microglia. On the other hand, tissue-associated macrophages exhibited enhanced calcium-regulated NEAT 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 data 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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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 take up the bacteria, indicating the robustness and specificity of E. coli-mediated plasmid delivery. To test the genome editing efficiency, we chose VSG4, 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

835 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 (Asp > 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 (AASA). Evaluation of reported clinical datasets demonstrated that patient T-