Methods Combining genomics, phenotypic and functional analyses in various physiologically and clinically relevant settings, we investigated the key factors leading to T-cell dysfunction. Specifically, we evaluated the impact of repeated stimulations using CD3/CD28-coated beads or antigen-loaded dendritic cells in human T-cell long term cultures, and BCMA-expressing cells for anti-BCMA CAR T cells. We also examined mouse antigen-specific T cells during chronic lymphocytic choriomeningitis virus (LCMV) infection as well as datasets obtained from circulating T cells from acute myeloid leukemia (AML) patients.

Results We identified telomere-independent cellular senescence as a central aspect of exhausted PD-1-expressing T cells following repeated stimulations. Mechanistically, it is associated the induction of p16INK4a. Additionally, we found that cellular senescence features are partly regulated by the activation of caspase-8, through a non-apoptotic function of this molecule not previously described in T cells.

Conclusions We thus conclude that caspase-8 may regulate the balance between apoptosis and proliferation by protecting T cells from cellular senescence. Senescence-associated mechanisms may be seen as key players in T-cell dysfunction occurring following repeated stimulations and as such should be considered as novel immune checkpoints impeding the success of T-cell adoptive immunotherapy in humans.

Ethics Approval This study was approved by the local Maison-neuve-Rosemont Hospital research ethics authorities and participants' informed consent was obtained (CÉR2020-2141 and CÉR13030).

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517 REGULATORY T CELL FUNCTIONAL IDENTITY IS SUSTAINED BY A GLUCOSE:LACTATE AXIS THAT IS EXPLOITED IN THE TUMOR MICROENVIRONMENT

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Background Regulatory T (Treg) cells are vital for preventing autoimmunity but are a major barrier to robust cancer immunity as the tumor microenvironment (TME) recruits and promotes their function. The deregulated cellular metabolism of tumor cells leads to a metabolite-depleted, hypoxic, and acidic TME. While the TME impairs the effector function of highly glycolytic tumor infiltrating CD8 T cells, Treg cell suppressive function is maintained. Further, studies of in vitro induced and ex vivo Treg cells reveal a distinct metabolic profile compared to effector T cells. Thus, it may be that the altered metabolic landscape of the TME and the increased activity of intratumoral Treg cells are linked.

Methods Flow cytometry, isotopic flux analysis, Foxp3 driven Cre-lox, glucose tracers, Seahorse extracellular flux analysis, RNA sequencing.

Results Here we show Treg cells display heterogeneity in terms of their glucose metabolism and can engage an alternative metabolic pathway to maintain their high suppressive function and proliferation within the TME and other tissues. Tissue derived Treg cells (both at the steady state and under inflammatory conditions) show broad heterogeneity in their ability to take up glucose. However, glucose uptake correlates

with poorer suppressive function and long-term functional stability, and culture of Treg cells in high glucose conditions decreased suppressive function. Treg cells under low glucose conditions upregulate genes associated with the uptake and metabolism of the glycolytic end-product lactic acid. Treg cells withstand high lactate conditions, and lactate treatment prevents the destabilizing effects of high glucose culture. Treg cells utilize lactate within the TCA cycle and generate phosphoenolpyruvate (PEP), a critical intermediate that can fuel intratumoral Treg cell proliferation in vivo. Using mice with a Treg cell-restricted deletion of lactate transporter Slc16a1 (MCT1) we show MCT1 is dispensable for peripheral Treg cell function but required intratumorally, resulting in slowed tumor growth and prolonged survival.

Conclusions These data support a model in which Treg cells are metabolically flexible such that they can utilize 'alternative' metabolites present in the TME to maintain their suppressive identity. Further, our studies support the notion that tumors avoid immune destruction not only by depriving effector T cells of essential nutrients, but also by metabolically supporting regulatory T cells.

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518 EPIGENETIC DYSFUNCTION OF TERMINALLY EXHAUSTED TUMOR INFILTRATING T CELLS

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Background Tumor-infiltrating CD8+ T cells have been characterized by their exhausted phenotype with decreased cytokine expression and increased expression of co-inhibitory receptors, such as PD-1 and Tim-3. These receptors mark the progression towards exhaustion from a progenitor stage (PD-1Low) to a terminally exhausted stage (PD-1+Tim-3+). While the epigenetics of tumor-infiltrating T cells are unique compared to naïve, effector, and memory populations, how the chromatin landscape changes during this progression has not been described.

Methods Using a low-input ChIP-based assay called Cleavage Under Targets and Release Using Nuclease (CUT&RUN), we profiled the histone modifications at the chromatin of tumor-infiltrating CD8+ T cell subsets to better understand the relationship between the epigenome and the transcriptome as TIL progress towards terminal exhaustion.

Results We have identified two epigenetic characteristics unique to terminally exhausted cells. First, we found a substantial increase in the number of genes that exhibit bivalent chromatin marks, defined by the presence of both activating (H3K4me3) and repressive (H3K27me3) epigenetic modifications that inhibit gene expression. In contrast to stem cells which exhibit bivalency, bivalent genes in terminally exhausted T cells are not associated with plasticity and represent aberrant hypermethylation in response to tumor hypoxia. Secondly, we have also identified a unique set of enhancers, characterized by H3K27ac that do not drive gene expression. These enhancers are enriched for AP-1 transcription factors, whereas enhancers that correlate with gene transcription are enriched for nuclear receptor (NR) transcription factors. Intriguingly, while most AP-1 and NR transcription factors are not expressed in terminally exhausted cells, we found that Batf, an inhibitory AP-1 family member, and Nr4a2, a NR known to promote both exhaustion and modify chromatin were

specifically expressed in terminally exhausted cells. These data suggest the balance of Batf and Nr4a2 may modulate the enhancer landscape to promote terminal exhaustion, while hypoxia simultaneously promotes hypermethylation and gene repression.

Conclusions Our study defines for the first time the features of epigenetic dysfunction in tumor-mediated T cell exhaustion and deepens our understanding of the epigenetic regulation of gene expression. These observations are the bases for future work that will elucidate that factors that drive progression towards terminal T cell exhaustion at the epigenetic level and identify novel therapeutic targets to restore effector function of tumor T cells and mediate tumor clearance.

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519 DIACYLGLYCEROL KINASE ζ LIMITS IL-2-DEPENDENT CONTROL OF PD-1 EXPRESSION IN TUMOR-INFILTRATING T LYMPHOCYTES

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Background Tumors evade T cell responses targeting them through the upregulation of tolerance-inducing mechanisms. One of the best characterized is that of PD-1/PD-1L engagement, that in healthy CD8+ T cells limits cytotoxic responses against self-antigens and that tumors employ to neutralize T cell attack. Antibody-based therapies aimed to block the PD-1/PD-1L axis have rendered notable results, but most patients eventually develop resistance. This failure is attributed to CD8+ T cells achieving an exhausted phenotype where recovery is hardly feasible. The dysfunctional phenotype of tumor-infiltrating T cells is largely triggered by the unbalance of diacylglycerol (DAG)- and Ca²⁺-regulated signals that results in alteration of the transcriptional T cell program. DAG kinase (DGK) ζ -dependent DAG consumption contributes to hypofunctional T cell states while DGK ζ deficiency facilitates tumor rejection in mice without apparent adverse autoimmune effects. In spite of its therapeutic potential, little is known about DGK ζ function in human T cells and there are not isoform-specific inhibitors targeting this DGK isoform.

Methods Here we used a human triple parameter reporter (TPR) cell line to examine the consequences of DGK ζ depletion in the transcriptional restriction imposed by PD-1 ligation. We also investigated the effect of DGK ζ deficiency in the expression dynamics of PD-1, as well as the impact of the absence of this DGK isoform in the in vivo growth of a MC38 adenocarcinoma cell line.

Results We demonstrate that DGK ζ depletion enhances DAG-regulated transcriptional programs, favoring IL-2 production and limiting PD-1 expression. Diminished PD-1 expression and enhanced expansion of cytotoxic CD8+ T cell populations is also observed even in the context of immunosuppressive milieus and correlates with the failure of MC38 adenocarcinoma cells to form tumors in DGK ζ -deficient mice.

Conclusions Our results suggest the relevance of DGK ζ as a therapeutic target on its own as well as a biomarker of CD8+ T cell dysfunctional states.

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520 THE IMMUNE LANDSCAPE OF PEDIATRIC TUMORS

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Background It is now well established that the immune system has a substantial role in controlling cancer growth and progression. Immunotherapy is quickly coming to the forefront of cancer treatment, however the implementation of immunotherapy in pediatric solid cancers, which classically display a low mutational load, is hindered by insufficient understanding of the determinants of cancer immune responsiveness in children. In order to better understand tumor-host interplay, we sought to characterize solid pediatric cancers based on immunological parameters¹ using analytes extracted from gene expression data.

Methods We performed single sample GeneSet Enrichment Analysis for 105 immune signatures previously described on 5 pediatric tumors (410 patients) from TARGET dataset¹ to identify coherent signature modules. Then we clustered samples according to representative signatures¹ and compared survival across clusters. We completed the analysis by analyzing the enrichment of immune subpopulations and the expression of the immune checkpoints. The degree of dysregulation of

Abstract 520 Figure 1 Immune subtypes of pediatric solid tumors
A. Spearman Correlation matrix of 105 cancer immune signatures showing 5 main modules.
B. Spearman's correlation of the 105 cancer immune signatures, identifies separation of the 5 immune signatures in different clusters
C. Distribution of cancer types within immune subtypes. The percentage of samples belonging to each tumor is shown in colors.
D. Distribution of immune subtypes within TARGET pediatric tumors. The percentage of samples belonging to each immune subtype is shown in colors.
E. Distributions of signature scores within the six immune subtypes (rows), with dashed line indicating the median.
F. Kaplan-Meier OS curve for the 6 immune subtypes (S1-S6) showing different outcomes.