Abstract 194 Figure 1 Major clusters of cells derived from 8 patient skin punch biopsies visualized by tSNE (n=7280). Differential gene expression analysis guided by established lineage markers revealed three keratinocyte clusters (KC1-KC3), two fibroblast clusters (FB1, FB2), smooth muscle cells (SMC), two endothelial cell clusters (VEC, LEC), melanocytes (MEL), sweat gland cells (SG), macrophages/dendritic cells (MAC-DC) and T cells (TC). Ranked by abundance, patient skin exhibited KC>FB>EC>MAC-DC>SMC>TC>SG>MEL

Conclusions Single-cell RNAseq is both feasible and informative in cell-specific transcriptome analysis of fresh non-lesional non-sun exposed LN skin biopsies. 10X genomics significantly increases cell numbers and facilitates identification of major cell clusters compared to Fluidigm C1. The expression of fibroblasts and genes reflective of fibrotic and interferon-related pathways support the application of this approach to study readily accessible tissue for biomarkers related to disease progression.

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SEQUENTIAL BLOCKADE OF OX40L FOLLOWED BY PD-1 ALTERED THE SUPPRESSIVE FUNCTION AND PROLIFERATION OF CD4+ REGULATORY T CELLS IN LUPUS MICE

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Background In systemic lupus erythematosus (SLE), dysregulated production of autoantibodies is a consequence of disrupted T cell homeostasis. We have published that blockade of

Programmed death-1 (PD-1), a negative regulator in T cells, limits helper T cell (Th) activation, restores regulatory T cell (Treg) suppression, and reinstates immune cell function. We have also shown that attenuated PD-1 expression in Treg down-regulates OX40L, which helps restore the suppressive capacity of Treg. However, concurrent administration of different immunotherapies may negate positive outcomes. We hypothesize that the sequence of blocking PD-1 and OX40L influences the induction and sustainability of Treg suppressivity.

Methods We treated 8-week-old BWF1 mice with a neutralizing Ab against PD-1 or OX40L intraperitoneally. At age 25 weeks when anti-dsDNA began to rise, mice were treated with either anti-PD1 or anti-OX40L that they had not received at age 8 weeks. OX40L, Foxp3 and PD-1 expression on CD4 +CD25+Treg from spleens, apoptosis of Treg and CD4 +CD25 Th were measured by flow cytometry. Serum production of IFN (Th1), IL4 (Th2), IL17a (Th17) and TGF- (Treg), and anti-dsDNA (B cells) were measured by ELISA. The survival of these mice were compared to those treated with anti-PD1 alone at 8 weeks, which we previously demonstrated prolonged survival with delayed onset of proteinuria.

Results Anti-OX40L suppressed Th function and proliferation independent of Foxp3 expression in Treg with decreased anti-dsDNA production. Subsequent blockade of PD-1 in anti-OX40L-treated mice generated more PD1^{hi}Treg with increased TGF- production; it sustained Treg suppressivity and delayed onset of proteinuria when compared to mice treated with anti-PD1 alone. Conversely, sequential blockade of anti-OX40L in anti-PD1-treated mice did not promote Treg survival and their disease inconsistently progressed: these mice had predominantly PD1^{hi} or PD1^{hi}-Treg, and antagonistic OX40L could not restore their suppressivity.

Conclusions Effective induction of Treg is associated with low expression of PD-1 and OX40L, which permits Treg to survive and perform cell suppressive function. Combination of Abs targeting OX40L and PD-1 can improve Treg function and survival outcomes, but it is determined by the timing and sequence of Ab administration: blocking OX40L followed by PD-1 has an additive effect which is not observed when the order of Abs given was reversed. OX40L and PD-1 signaling communicate sequentially with Treg to regulate its suppressive capacity and survival to achieve peripheral tolerance in SLE, suggesting that treatment with one immunotherapy could change the biology of T-cell signaling such that another immunotherapy may lose its efficacy or has unexpected negative outcome.

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IMMUNE PATHOLOGY CAUSED BY INTRINSIC LOSS OF CONTROL IN CD4 T CELLS

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Background Naïve T cells constantly see self, need to avoid spontaneous immunity, yet be primed to react to foreign antigens when an infection occurs. Both CD4 +and CD8+ primary T cells exhibit tonic signaling (Myers et al., 2017b) and continuous interactions of the TCR with self-p/MHC generate tonic signals (Stefanova et al., 2002). T cells lacking the central adapter molecule LAT cause a spontaneous lymphoproliferative T helper 2 (TH2) cell syndrome in mice. Thus, LAT