Background T-cell-inflamed tumor microenvironments (TME) are pre-requisite for immunotherapy efficacy; however, the drivers for promoting T cell priming and infiltration within the TME remain incompletely understood. Conventional type I dendritic cells (cDC1) are indispensable for anti-tumor immunity. Several key studies have documented the paradoxical localization of cDC1 within tumor stroma. We hypothesized that conserved provisional matrix signals emanating from peritumoral stroma remodeling play an unrecognized role in this paradox and license cDC1 to promote T-cell repriming and tumor entry.

Methods We performed multiplex IHC in human lung cancer biopsies using antibodies against anti-VCAN proteolysis neoepitope DPEAAE, cDC1 (XCR1+), CD8+ and CD4+ T cells. Multiparametric flow cytometry was used to characterize stroma-licensed cDC1. Therapeutic anti-PD1 antibodies were used in a preclinical model of lung cancer (LLC).

Results We found that in human T-cell inflamed cancers, even though T cells penetrated the tumor core, cDC1 remained confined within tumor-adjacent stroma recurrently demonstrating versican (VCAN) proteolysis, an extracellular matrix modification typically associated with provisional matrix remodeling in embryonic development and wound healing. VCAN proteolysis releases a bioactive N-terminal fragment, versikine. Using multi-parametric flow cytometry, we found that versikine-exposed cDC1, but not cDC2 or monocyte-derived (Mo)-DC, overexpressed CD40 whereas all three DC subtypes overexpressed PD-L1. Moreover, cDC1, but not other tumor DC subtypes, expanded in absolute numbers in versikine-replete tumors. Versikine sensitized refractory LLC tumors to anti-PD1 immunotherapy and produced a survival benefit in vivo, suggesting that versikine overcomes immune checkpoint inhibitor resistance. Human lung cancers demonstrated a predominant localization of CD4+ and CD8+ T cells within peritumoral stroma. Consistent with CD40-mediated cDC1 licensing models, we observed enhanced antigen-specific T cell responses using the model antigen OVA in versikine-replete TME.

Conclusions Peritumoral stroma has traditionally been viewed as an immune barrier. Our data provide an alternative view and demonstrate that peritumoral stroma mimicking embryonic or wound provisional matrix remodeling instead promotes cDC1 abundance and activation. "Stroma-licensed" cDC1 may represent a conserved homeostatic response against nascent tumors during immune surveillance ("elimination" and "equilibrium"). Progressive loss of stromal proteolytic remodeling (e.g., versikine loss) and transition into stromal fibrosis are associated with immunoediting "escape". Stroma-licensed cDC1 overexpress CD40 and localize near stromal CD4+ and CD8+ T cells, raising the possibility that CD4 T-cells may provide CD40L-CD40-mediated "help" in stromal locations. These data provide novel insights into the role of stroma in regulating the nature of the T-cell-inflamed TME and may permit optimized designs for T-cell penetration into solid tumors.

Acknowledgements Supported through grants R01CA252937 and R01CA230275 and the Robert J. Shillman Foundation.

Ethics Approval The study was approved by UCSD IACUC #S19109.