Background: Dendritic cell (DC) vaccine therapies have so far demonstrated limited success as cancer therapeutics. Recently, cDC1 have been shown to support CD8 and CD4 activation, and both cell types were necessary for anti-tumor responses.\(^1\)\(^2\)

Methods: To test the ability of cDC1 to promote these responses, we designed and produced a stabilized Xcl1 protein molecule fused to Fc-chicken ovalbumin (OVA) as a model antigen that specifically engages and activates cDC1 by targeting the Xcr1-Xcl1 interaction.

Results: Xcl1-OVA was expected to activate the Xcr1 receptor and deliver antigen preferentially to cDC1 in vivo. After validating that the Xcl1-OVA protein bound to Xcr1 on murine cDC1 cells, we performed adoptive transfer of OT1 (CD8) and OT2 (CD4) cells into WT mice after inoculation with Xcl1-OVA. OT1 and OT2 expanded 20-fold and 14-fold respectively. To evaluate the effect of Xcl1-OVA treated cDC1 cells on T helper cell (Th) differentiation, naïve OT2 cells were transferred into Xcl1-OVA inoculated WT recipient mice and Th lineage differentiation was assessed by flow cytometry. Xcl1-stimulated cDC1s strongly induced Th1 and reduced Treg differentiation in spleen and lymph nodes. Xcl1-OVA inoculation resulted in 56% of transferred naïve OT2 cells differentiating to Th1 and only 5% into Treg. In comparison, equimolar amounts of soluble OVA resulted in 10% of transferred naïve OT2 cells differentiating to Th1 and 11% to Treg. Similar results were also observed in endogenous OVA-specific CD4 cells in the WT hosts. Activation of cDC1 in vivo was confirmed by increased CD40 and CD70 MFI on cDC1 in spleen and CD40, CD80 and CD86 MFI on cDC1 in lymph nodes as compared to control groups (polyIC, soluble OVA, Xcl1-Fc). Serum cytokines were measured after Xcl1-OVA stimulation; IL1α, IL9, IL12, IL17A and IL21 were all upregulated by Xcl1-OVA stimulation compared to controls. Finally, tumor regression was tested in OVA-transduced tumor models (MC38-OVA, EL4-OVA and B16-OVA). All three tumor models showed significantly more tumor regression in the Xcl1-OVA treated group compared to controls. Within the tumor infiltrating lymphocyte population, we saw reduced frequency of Tregs, and CD8 T cells showed evidence of Th1-helper activity as measured by expression of CX3CR1.

Conclusions: Our data suggests that targeting antigen to cDC1 via an activating receptor can improve CD8 and CD4 activation leading to enhanced anti-tumor immunity.

REFERENCES

1. Ferris, et al. cDC1 prime and are licensed by CD4+ T cells to induce anti-tumour immunity. *Nature* 2020 Aug;584(7822):624–629

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1143