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CHS-114: a Cytolytic Anti-CCR8 Antibody that Depletes Tumor-Infiltrating Regulatory T Cells as a Treatment for Head and Neck Squamous Cell Carcinoma

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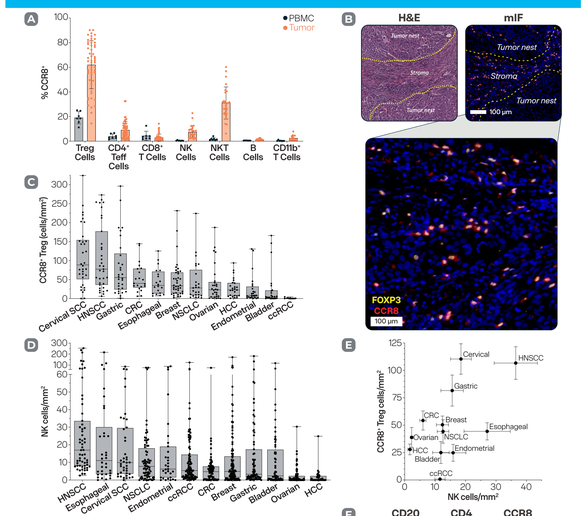
Background

- FOXP3⁺ regulatory T (Treg) cells play a crucial role in orchestrating immune responses across several tissues, including the tumor microenvironment (TME).
- Intratumoral Treg cells support an immunosuppressive TME and their increased frequency correlates with poor clinical prognosis.
- Chemokine receptor 8 (CCR8) is highly upregulated by intratumoral Treg cells compared to their peripheral counterparts and other immune cell types.
- Monoclonal antibodies targeting CCR8 have been developed to deplete tumor Treg cells. This represents an attractive strategy to reshape the TME and enhance anti-tumor immune responses.
- CHS-114 is an afucosylated, human IgG1 antibody that selectively targets human CCR8 and preferentially induces depletion of CCR8⁺ Treg cells and not T effector (Teff) cells.
- These preclinical studies aimed to characterize the expression of CCR8 in human tissues and study the effects of CHS-114 mediated depletion of CCR8⁺ Treg cells.
- CHS-114 is currently in a Phase 1 clinical study (NCT05635643).

Intratumoral Treg Cells Highly Express CCR8 and Are Enriched in HNSCC Tumors

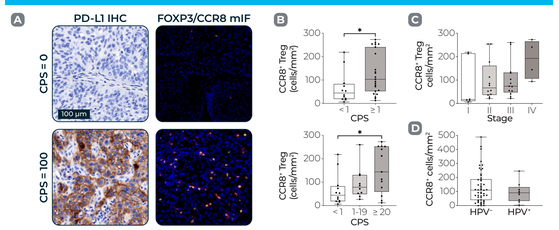


(A) CCR8⁺ cell frequency in immune cell subsets as determined by flow cytometry in PBMC and tumor samples. (B) Multiplex immunofluorescence (mIF) highlights abundant CCR8⁺ Treg cells in the TME of HNSCC. Representative images at 10x (H&E and low-power mIF) and 20x magnification (high-power mIF). (C) The number of CCR8⁺ Treg cells per mm² in 12 types of solid tumors. (D) The number of NK cells per mm² in 12 types of solid tumors. (E) Comparison of CCR8⁺ Treg density and NK cells density in 12 types of solid tumors. (F) Representative IHC on serial sections of a gastric cancer sample shows numerous CCR8⁺ lymphocytes within the T-cell region of tertiary lymphoid structures (TLS) in the TME (13x magnification).

Abbreviations: ADCP = antibody-dependent cellular phagocytosis; CCR8 = chemokine receptor 8; CFSE = Carboxyfluorescein succinimidyl ester; CPS = combined proportional score; CR = complete response; ctrl = control; DTC = dissociated tumor cells; FFPE = formalin-fixed paraffin-embedded; H&E = hematoxylin and eosin; HNSCC = head and neck squamous cell carcinoma; IFN-γ = interferon gamma; IHC = immunohistochemistry; IV = intravenous; k_a = association rate constant; k_d = dissociation rate constant; K_D = dissociation equilibrium constant; MDM = monocyte-derived macrophage; MFI = mean fluorescence intensity; mIF = multiplex Immunofluorescence; MoDC = monocyte-derived dendritic cells; NK = natural killer; NSCLC = non-small cell lung cancer; NSG = NOD scid gamma; PD-1 = programmed cell death protein 1; PD-L1 = programmed cell death ligand 1; PBMC = peripheral blood mononuclear cells; TAM = tumor-associated macrophages; TAN = tumor-associated neutrophils; Tconv = conventional T cell; Teff = effector T cell; TIL = tumor-infiltrating lymphocytes; TLS = tertiary lymphoid structures; TMA = tumor microarray; TME = tumor microenvironment; Treg = regulatory T cell.

Statistics: * = p < 0.05; ** = p < 0.01; *** = p < 0.001; **** = p < 0.0001; when not shown, comparisons are not statistically significant.

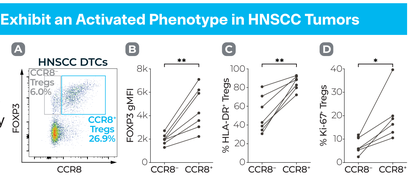
CCR8⁺ Treg Levels Correlate with PD-L1 Status but not Tumor Stage or HPV Status in HNSCC



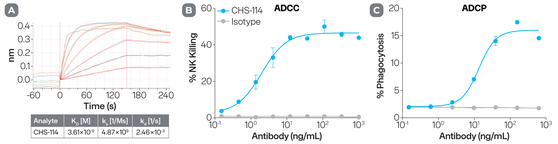
Levels of CCR8⁺ Treg cells are more abundant in HNSCC samples that express PD-L1, but do not appear to correlate with clinical stage of disease or HPV status. A) Representative images of IHC for PD-L1 and mIF for FOXP3 and CCR8 at 20x magnification. Quantitative analysis of density of CCR8⁺ Treg cells (by mIF) based on (B) PD-L1 CPS score or (C) disease stage and of (D) CCR8⁺ cells (by IHC) based on HPV status (n = 35-59 samples).

CCR8⁺ Tregs Exhibit an Activated Phenotype in HNSCC Tumors

A) Representative FOXP3 and CCR8 expression in CD4⁺ T cells from a dissociated primary HNSCC tumor sample. B) FOXP3 geometric MFI level C) HLA-DR⁺ frequency and D) Ki-67 expression measured in CCR8⁺ and CCR8⁻ Treg cells in dissociated HNSCC tumor samples (n = 7).



CHS-114 Binds to hCCR8 and Induces ADCC and ADCP Activity

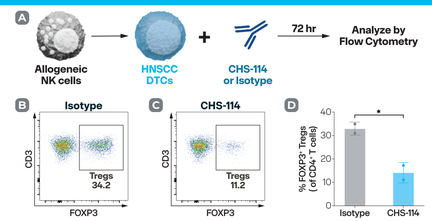


(A) ForteBio sensorgram depicting exposure of the CHS-114-loaded sensor to a recombinant human CCR8 extracellular domain Fc-fusion protein. The resulting equilibrium dissociation constant (K_D), association rate constant (k_a), and dissociation rate constant (k_d) are shown. (B) CFSE-labeled Raji-hCCR8 cells were co-cultured with NK cells, treated with serial dilutions of CHS-114 or an isotype control antibody for 4 h at 37° C, and analyzed by flow cytometry. Percent NK killing represents the frequency of Viability Dye⁺CFSE⁺Raji-hCCR8 cells. (C) CFSE-labeled Raji-hCCR8 cells were co cultured with monocyte-derived macrophages, treated with serial dilutions of CHS-114 or an isotype control antibody for 3 h at 37° C, and analyzed by flow cytometry. Percent phagocytosis represents the frequency of CD14⁺CFSE⁺ macrophages.

CHS-114 Ex Vivo Treatment Activates HNSCC NK Cells and Induces IFN-γ Secretion

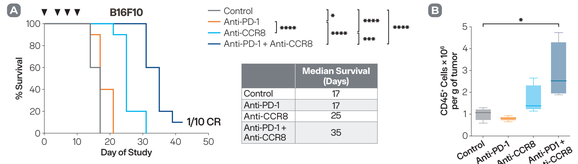
A) Intratumoral NK cell activation was measured by the upregulation of 4-1BB expression after a 24-hr incubation with 10 µg/mL CHS-114 or an isotype control antibody in dissociated HNSCC tumors. B) Secretion of IFN-γ was measured at 24 and 72 hr in the supernatant of dissociated HNSCC tumor cells following incubation with 10 µg/mL CHS-114 or isotype control antibody (n = 1-3 per group).

CHS-114 Depletes Intratumoral Tregs in Dissociated HNSCC Samples



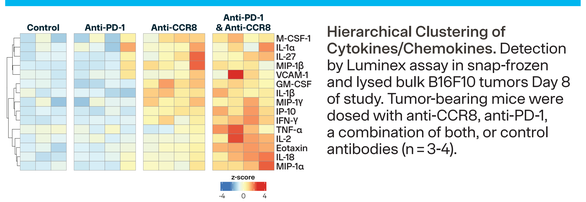
A) Allogeneic NK cells isolated from PBMC were added to dissociated primary HNSCC tumor tissues with either 10 µg/mL CHS-114 or an isotype control antibody and incubated at 37° C for 3 days. B-C) Representative Treg gating of FOXP3⁺ cells on total CD4⁺ T cells. D) Quantitation of remaining Treg cells following treatment with 10 µg/mL CHS-114 or isotype control (n = 2).

Anti-CCR8 and Anti-PD-1 Combination Treatment of Tumor-Bearing Mice Enhances Overall Survival and Promotes Expansion of Effector T Cells in B16F10 Tumors



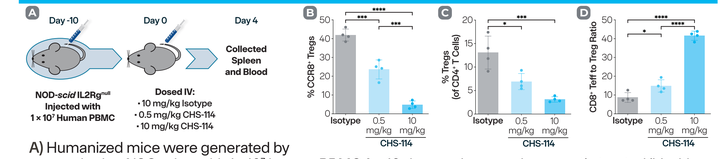
A) Survival of mice bearing subcutaneous B16F10 tumors treated with anti-murine CCR8.mIgG2a, anti-murine PD-1, or control antibodies. B-C) Cytometric analyses and average frequency, respectively, of tumor-infiltrating lymphocytes (TIL) on Day 8 of study. D) Representative IHC images of infiltrating CD8⁺ T cells in B16F10 tumors on Day 8 of study. E) Density of infiltrating CD8⁺ T cells. (Treatment initiated 6 days post-implantation, mean tumor volume of 71 mm³; A: n = 10 per group; B-E: n = 4 per group.)

Anti-CCR8 + Anti-PD-1 Combination Treatment Enhances Production of Pro-Inflammatory Cytokines and Chemokines in B16F10 Tumors



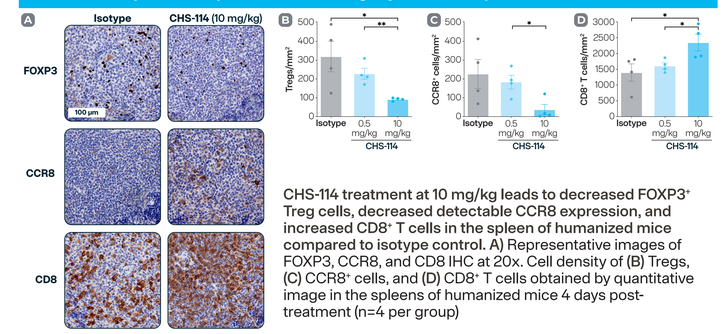
Hierarchical Clustering of Cytokines/Chemokines. Detection by Luminex assay in snap-frozen and lysed bulk B16F10 tumors Day 8 of study. Tumor-bearing mice were dosed with anti-CCR8, anti-PD-1, a combination of both, or control antibodies (n = 3-4).

CHS-114 Depletes CCR8⁺ Tregs In Vivo



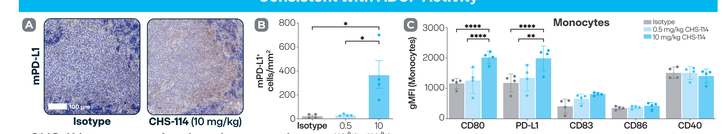
A) Humanized mice were generated by reconstituting NSG mice with 1 × 10⁷ human PBMC for 10 days and were subsequently treated IV with 10 mg/kg isotype control, 0.5 mg/kg CHS-114 or 10 mg/kg CHS-114. Graphs show B) quantitation of detectable CCR8⁺ Tregs, C) total Treg frequencies, and D) ratio of CD8⁺ Teff cells to Treg cells in the peripheral blood 4 days post-treatment by flow cytometry (n = 4 per group).

Dose-Dependent Depletion of CCR8⁺ Tregs by CHS-114 in Spleen of Humanized Mice



CHS-114 treatment at 10 mg/kg leads to decreased FOXP3⁺ Treg cells, decreased detectable CCR8 expression, and increased CD8⁺ T cells in the spleen of humanized mice compared to isotype control. A) Representative images of FOXP3, CCR8, and CD8 IHC at 20x. Cell density of (B) Tregs, (C) CCR8⁺ cells, and (D) CD8⁺ T cells obtained by quantitative image in the spleens of humanized mice 4 days post-treatment (n=4 per group)

Dose-Dependent Activation of Myeloid Cells in Response to CHS-114 Treatment Consistent with ADCP Activity



CHS-114 treatment leads to increased expression of murine PD-L1 in the spleen. A) Representative images of mPD-L1 IHC at 10x. B) Cell density obtained by quantitative image analysis in the spleens of humanized mice 4 days post-treatment (n = 4 per group). C) Flow cytometric analysis of CD11b⁺ Ly6c⁺ splenic monocytes (n = 4 per group). CD80 and PD-L1 were induced on monocytes in response to 10 mg/kg CHS-114. Other activation markers, CD83, CD86 and CD40, were not induced with either dose of CHS-114.

Conclusions

- CCR8 expression is highly enriched within the TME and predominantly expressed on intratumoral Treg cells.
- CCR8⁺ Tregs are abundant in HNSCC tumors, exhibit an activated phenotype, and their frequency correlates with PD-L1 status.
- In dissociated HNSCC tumors, CHS-114 activates NK cells and specifically induces cytotoxicity against tumor-infiltrating Tregs.
- The mechanism of action is demonstrated in humanized mice: CHS-114 depletes human CCR8⁺ Tregs in vivo, resulting in the expansion of CD8⁺ T cells and activation of murine myeloid cells.
- Anti-CCR8 and anti-PD-1 combination treatment improves overall survival in a checkpoint inhibitor-resistant melanoma mouse model by promoting expansion of CD8⁺ T cells in the TME.
- Molecular epidemiology and in vitro studies highlight HNSCC as a potentially relevant indication to evaluate the therapeutic activity of an CHS-114 as a monotherapy or in combination with an anti-PD-1 antibody.
- CHS-114, a CCR8-specific cytotoxicity-inducing antibody that preferentially depletes CCR8⁺ Treg cells and not Teff cells, is currently being evaluated in a Phase 1 clinical trial (NCT05635643).