

LYMPHOMAS

## CK2 DRIVES IMMUNE CELL RECRUITMENT VIA CYTOKINE MODULATION IN HODGKIN LYMPHOMA

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**Introduction:** Classic Hodgkin Lymphoma (cHL) is characterized by a small population of malignant Hodgkin and Reed-Sternberg (HRS) cells that shape the tumor microenvironment (TME) via cytokines and chemokines secretion. These factors drive immune cells recruitment and establish an immunosuppressive milieu supporting neoplastic cells survival. The molecular basis of this aberrant secretory phenotype remains largely unknown. We recently identified the kinase CK2 as a critical regulator of cHL cell survival, whose inhibition induces apoptosis. Given CK2 involvement in signaling pathways controlling inflammation and cell migration, this study investigates its contribution to TME modulation in cHL.

**Methods:** cHL cell lines (HDLM-2, KM-H2, L-428, and L-540) were treated with 0, 5, or 10  $\mu$ M of the CK2 inhibitor CX-4945 (CX) for 24 or 48h. Migration assays were performed using T and B cells purified from healthy donors in fibronectin-coated transwells. Conditioned media (CM) from treated cHL cells was placed in the lower chambers. Migrated lymphocytes were quantified by flow cytometry. Cytokine levels in cHL supernatants after 24h of treatment were analyzed using a 25-plex array and validated by ELISA or by an Automated Immunoassay System for multiplex ELISA (ELLA).

**Results:** Our results revealed that CM from cHL cell lines showed a reduced chemoattractant effect on T and B cells

when CK2 was inhibited by CX. T-cell migration toward CM from HDLM-2, KM-H2, L-428, and L-540 cells after 24h of treatment with 5  $\mu$ M CX decreased by 12.1%, 18%, 30.2%, and 8.2% respectively ( $p < 0.05$ ), compared to the untreated condition. A further reduction was observed at 10  $\mu$ M (25.3%, 34%, 46.2%, and 30.2% respectively,  $p < 0.0001$ ). Similarly, the percentage of migrated B cells toward the CM from cHL cell lines treated with 5  $\mu$ M CX for 24h decreased of 9.8%, 14%, 6.75%, and 11.9% ( $p < 0.001$ ) compared to the untreated condition, with a more evident reduction at 10  $\mu$ M (9.6%, 18.5%, 13.4%, and 14.8%,  $p < 0.001$ ). Comparable effects were observed at 48h treatment. To investigate the CK2 role in shaping the cytokine milieu of the CM we performed an array analysis. Among the cytokines tested, IL-6, M-CSF, RANTES, TARC, TGF- $\beta$ 1, TNF- $\alpha$ , and VEGF levels were notably reduced in CM from CX-treated HDLM-2 and KM-H2 cells. The downregulation of IL-6, TNF- $\alpha$ , and VEGF was confirmed by single- or multiplex ELISA in all the four cHL cell lines.

**Conclusions:** This study demonstrates that CK2 inhibition impairs the ability of cHL cells to recruit T and B lymphocytes by altering their chemokine secretion profile. These findings suggest that CK2 may contribute to the tumor niche formation and immune evasion of HRS cells, supporting its potential as a therapeutic target in classic Hodgkin Lymphoma.