Uncovering the dual Impact of CuEt: T-Cell signaling and tumour immunogenicity

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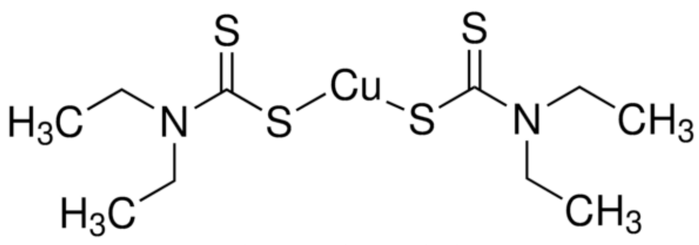
Introduction

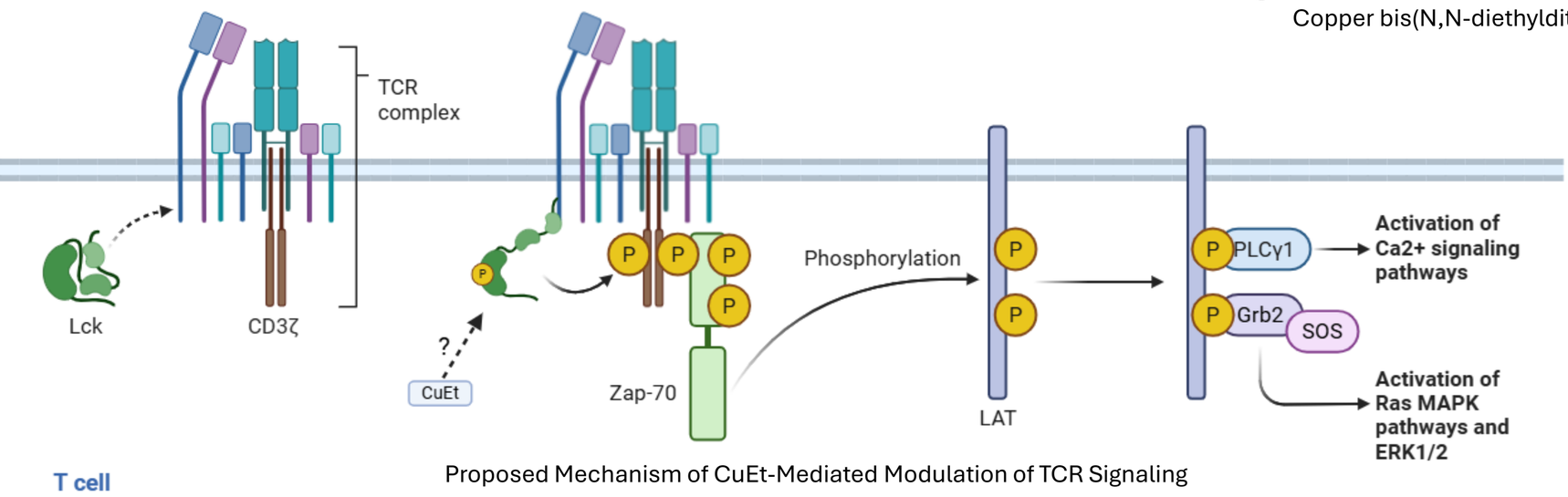
Effective anti-tumor immunity depends on strong cytotoxic lymphocytes and immunogenic cell death. Tumors often evade this by altering antigen presentation and suppressing T-cell activation, leading to exhaustion. Thus, strategies that weaken tumor resistance while boosting immune function are essential. Copper diethyldithiocarbamate (CuEt), the active metabolite of Disulfiram, shows copper-dependent anti-cancer activity through proteotoxic stress. At low concentrations, CuEt enhances $CD8^+$ T-cell cytotoxicity by upregulating perforin and NKG2D and activating ERK1/2 signaling, thereby sensitizing tumor cells to immune killing. Since T-cell receptor (TCR) signaling depends on kinase phosphorylation such as LCK, which activates the MAPK/ERK pathway, we examined how 10 nM CuEt affects phosphorylation of key proteins and tumor cell susceptibility to $CD8^+$ T-cell cytotoxicity.

Methods

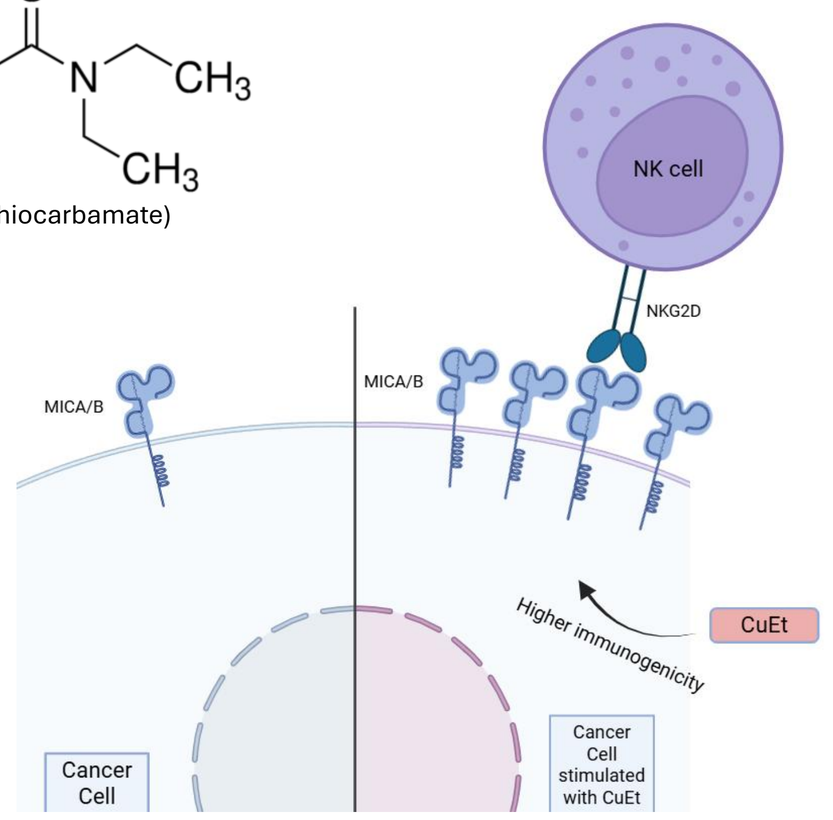
Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats by density-gradient centrifugation using Ficoll-Hypaque. PBMCs were plated in tissue-culture flasks and allowed to adhere for 24 h to remove monocytes and adherent cells. The non-adherent lymphocyte fraction was collected and used for isolation of $CD8^+$ T cells and NK cells via magnetic bead-based negative selection.Purified cells were stimulated under multiple conditions, including CuEt, Dasatinib, their combination, and phosphatase inhibitors TP-I (inhibitor 1) and PTB-IB (inhibitor2). Following stimulation, cells were lysed and analyzed by Western blot to assess phosphorylation of key TCR signaling proteins. Treated cells were also put up against tumour cell lines in an E:T ratio of 5:1 and cytotoxicity was measured using PI.



Copper bis(N,N-diethyldithiocarbamate)



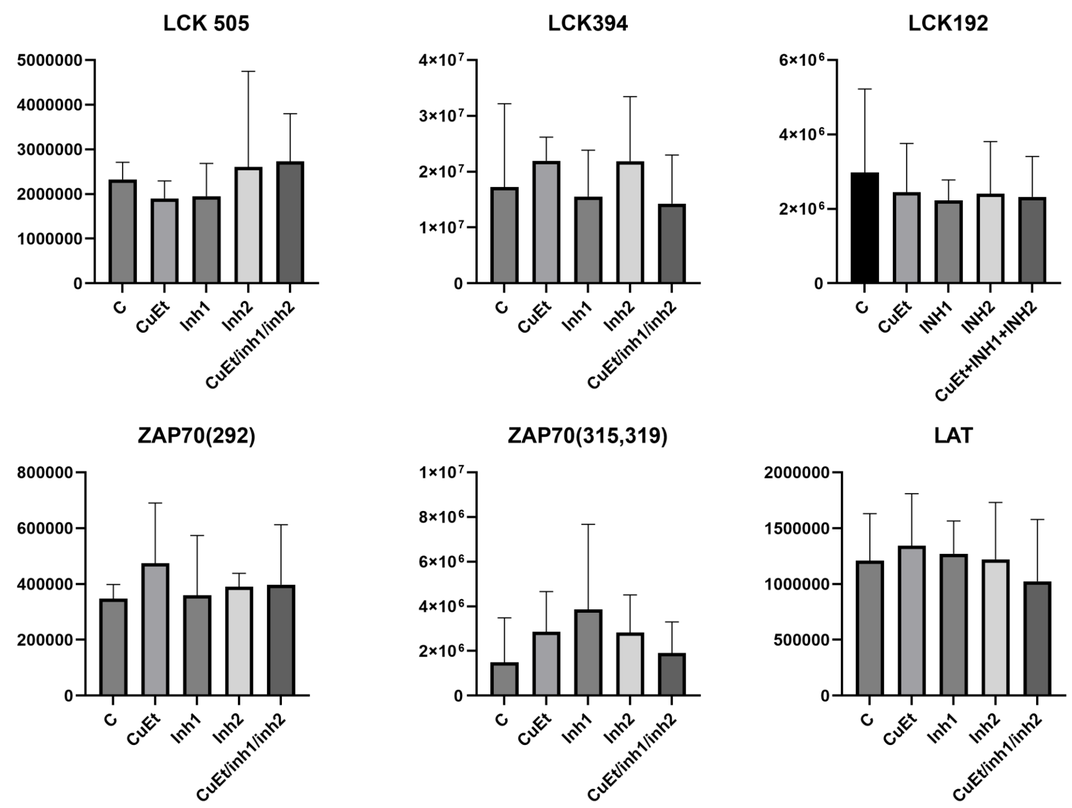
Proposed Mechanism of CuEt-Mediated Modulation of TCR Signaling



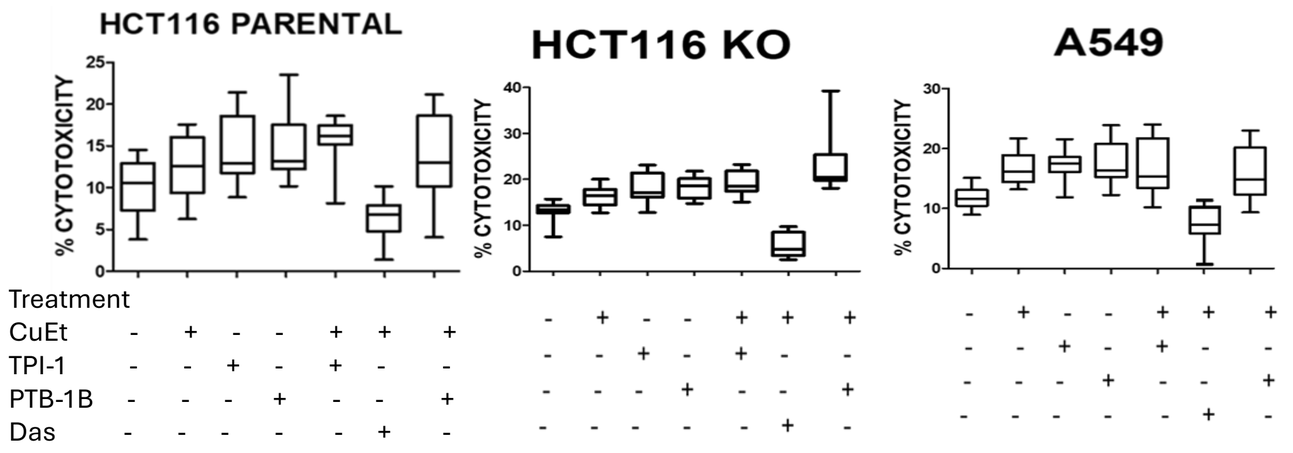
Possible Mechanism of CuEt-Mediated Enhancement of Immune Recognition

Results

Western Blot results for phosphorylation on different tyrosines on LCK, ZAP70 and LAT in CD8+ T-cells



Cytotoxicity in stimulated cells



Conclusion

CuEt increases cytotoxicity; however, phosphatase inhibitors do not potentiate the effect of CuEt. More data quantity is needed to be able to exclude donor heterogeneity.
Phosphatase inhibitors have no major effect on Lck. Dasatinib blocks the cytotoxic effect of CuEt.

References

Dumut DC, Hajduch M, et al. Diethyldithiocarbamate-copper complex ignites the tumor microenvironment through NKG2D-NKG2DL axis. Front Immunol. 2025 Feb 12;16:1491450.

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Aknowledgement

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