CRISPR-CAS-9 TARGETING OF CXCR4 IN WHIM SYNDROME AS A STRATEGY TO OVERCOME MICROENVIRONMENTAL RESISTANCE IN B-CELL MALIGNANCIES

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INTRODUCTION

CXCR4–CXCL12 signaling anchors malignant B cells within protective marrow and nodal niches, driving immune evasion and drug resistance.

WHIM syndrome (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis) provides a human proof-of-concept that CXCR4 hyperactivation is disease-defining and reversible.

Activating **CXCR4 mutations** (WHIM-like truncations) in **~30–40%** of WM cases; associated with inferior BTK inhibitor responses.

AIM

Can CRISPR-Cas9 disruption of CXCR4 — informed by WHIM syndrome biology — overcome microenvironmental resistance in WM and DLBCL?

STRATEGIC APPROACH

Establish CXCR4 as a tractable driver via gene editing

Model combination strategies with BTK inhibitors and CAR T

Translate WHIM paradigm to oncology

METHOD

- **Public Datasets:** TCGA-DLBC and CCLE assessing CXCR4 expression and outcomes.
- **Preclinical Models:** WM and DLBCL models of CXCR4 dependence.
- **Intervention Modeling:** CXCR4 disruption combined with BTK inhibitors and CD19-directed CAR T cells.

RESULTS

Preclinical Synergy

CAR T INFILTRATION

Preclinical data support **improved CAR T-cell infiltration and function** with CXCR4 blockade.

BTK INHIBITOR COMBINATION

BTKi

Synergy predicted between **CXCR4 disruption** and **BTK inhibition** via increased tumor accessibility.

TWO-PRONGED STRATEGY

1. **Dislodge tumor cells** from protective niches.
2. **Exploit accessibility** with CAR T or BTKi.

Together, these findings support a **two-pronged strategy** to overcome microenvironmental resistance and enhance therapeutic efficacy.

TCGA-DLBC Survival Data

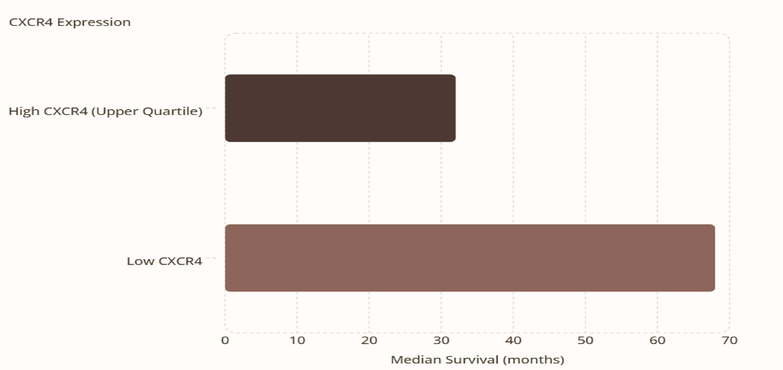

Figure 1. High CXCR4 expression predicts inferior survival in TCGA-DLBC (~32 vs 68 months; HR ~1.5–1.7), while consistent expression across CCLE DLBCL cell lines supports a lineage-associated dependency.

CONCLUSIONS

- **CXCR4** is a central mediator of tumor–microenvironment interactions that promote immune evasion and therapeutic resistance in B-cell malignancies.
- **CRISPR-Cas9** correction of **CXCR4 mutations** in **WHIM** syndrome provides compelling proof-of-concept that targeting aberrant CXCR4 signaling can reverse pathogenic biology.
- **CXCR4 disruption** as a novel strategy to mobilize malignant cells, reduce stromal protection, and enhance the efficacy of **BTK inhibitors** and **CAR T-cell therapy**.
- These findings establish a strong biologic rationale for integrating **CXCR4-directed** approaches into future precision immunotherapy strategies.

FUTURE DIRECTIONS

- **Evaluate CXCR4 antagonists** in combination with BTK inhibitors in WM/DLBCL models.
- **Define patient selection criteria** based on CXCR4 mutation/expression status for precision trials.

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