RXR agonist IRX4204 improves BCMA CAR-T cell functionality by suppressing ferroptosis via CHAC1 downregulation and promoting mitophagy

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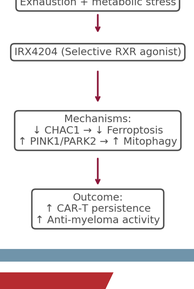
INTRODUCTION

Multiple myeloma (MM) remains incurable, and although BCMA CAR-T therapy induces deep responses, most patients relapse due to T-cell exhaustion, limited persistence, and metabolic stress in the tumor microenvironment. Activated T cells are vulnerable to ferroptosis driven by glutathione depletion, oxidative stress, and CHAC1 activity. Enhancing redox resilience may improve CAR-T durability. IRX4204, a selective RXR agonist, may protect CAR-T cells from ferroptosis stress to enhance persistence and antitumor function.

AIM

To investigate whether the selective RXR agonist IRX4204 enhances BCMA CAR-T persistence and antitumor function by protecting against ferroptosis and promoting mitochondrial fitness through CHAC1 suppression and PINK1/PARK2-mediated mitophagy.



METHOD

- **Patient samples:** PBMCs from RRMM patients treated with commercial BCMA CAR-T.
- **CAR-T generation:** Healthy donor T cells activated, lentivirally transduced, and expanded in IL-7/IL-15.
- **IRX4204 treatment:** CAR-T exposed to IRX4204 (4 μM); ferroptosis modulated with RSL3/Fer-1.
- **In-vitro assays:** CAR-T cytotoxicity, cytokines, exhaustion markers, lipid-ROS/Fe^{2+}, and metabolic profiling (Seahorse, JC-1).
- **Mechanistic edits:** CHAC1 and PPARα CRISPR knockdown/overexpression.
- **In-vivo model:** NSG mice with MM1.S-luc tumors given BCMA CAR-T ± IRX4204; tumor burden and T-cell persistence monitored.

RESULTS

1. Baseline RXR expression correlates with T-cell profiles and clinical outcomes in MM patients receiving CAR-T therapy.

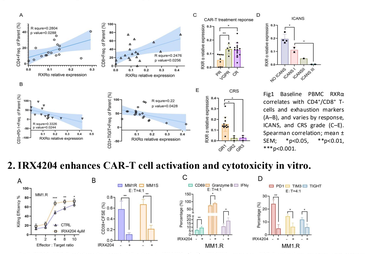


Fig1 Baseline PBMC RXRα correlates with CD4+/CD8+ T-cells and exhaustion markers (A–B), and varies by response, ICANS, and CRS grade (C–E). Spearman correlation; mean ± SEM; *p<0.05, **p<0.01, ***p<0.001.

2. IRX4204 enhances CAR-T cell activation and cytotoxicity in vitro.

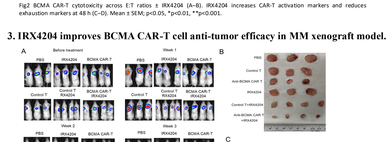


Fig2 BCMA CAR-T cytotoxicity across E:T ratios ± IRX4204 (A–B). IRX4204 increases CAR-T activation markers and reduces exhaustion markers at 48 h (C–D). Mean ± SEM; p<0.05, *p<0.01, **p<0.001.

3. IRX4204 improves BCMA CAR-T cell anti-tumor efficacy in MM xenograft model.

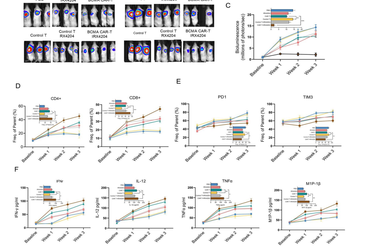


Fig3 NSG mice with subcutaneous myeloma tumors received PBS, IRX4204, control T cells, BCMA CAR-T, or combinations. (A–C) IRX4204 enhances CAR-T tumor control by bioluminescence and tumor mass. (D–E) IRX4204 increases human CD4+/CD8+ T-cell expansion and reduces exhaustion markers. (F) Increased plasma cytokines. Mean ± SEM; p<0.05, *p<0.01, **p<0.001.

4. IRX4204 protects CAR-T cells from ferroptosis-induced dysfunction.

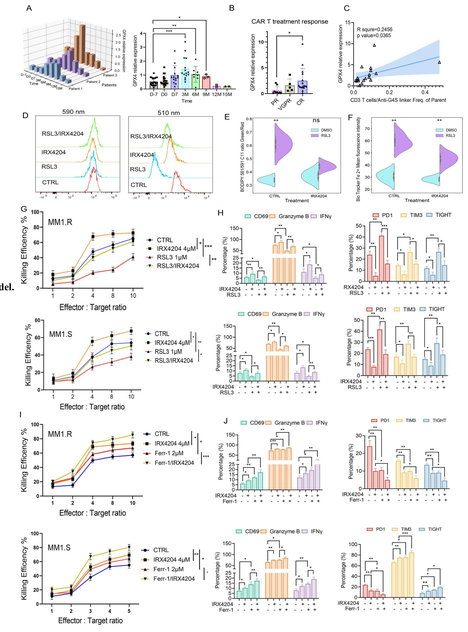


Fig4 GPX4 expression across timepoints and response groups, and correlation with a CAR-T persistence marker (A–C). IRX4204 decreases lipid peroxidation and intracellular Fe^{2+} in CAR-T cells, including under RSL3 treatment (E–F). IRX4204 improves cytotoxicity and activation and reduces exhaustion in CAR-T cells co-cultured with MM1.S ± RSL3 or Fer-1 (G–J).Mean ± SEM; p<0.05, p<0.01, p<0.001.

5. IRX4204 downregulates CHAC1 via RXR/PPARα-mediated transcriptional repression.

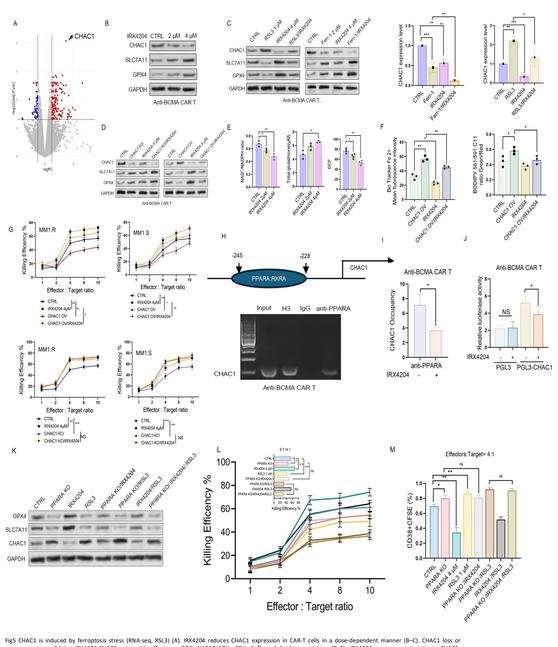


Fig5 CHAC1 is induced by ferroptosis stress (RNA-seq, RSL3) (A). IRX4204 reduces CHAC1 expression in CAR-T cells in a dose-dependent manner (B–C). CHAC1 loss or overexpression validates IRX4204-CHAC1 axis, with effects on ROS, NAD+/NADH, GSH, Fe^{2+}, and lipid peroxidation (D–F). IRX4204 rescues cytotoxicity in CHAC1-overexpressing CAR-T cells (G). PPARα binds the CHAC1 promoter and is suppressed by IRX4204 (H–I). PPARα modulation alters IRX4204 activity, affecting CHAC1 and CAR-T cytotoxicity against MM1.S (K–M).Mean ± SEM; p<0.05, *p<0.01, **p<0.001.

6. IRX4204 enhances mitophagy and prevents ferroptosis in CAR-T cells.

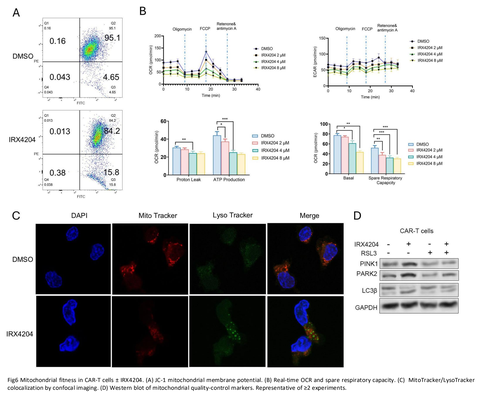


Fig6 Mitochondrial fitness in CAR-T cells ± IRX4204. (A) JC-1 mitochondrial membrane potential. (B) Real-time OCR and spare respiratory capacity. (C) MitoTracker/LysoTracker colocalization by confocal imaging. (D) Western blot of mitochondrial quality-control markers. Representative of ≥2 experiments.

7. Proposed mechanistic model of IRX4204-enhanced BCMA CAR-T cell function.

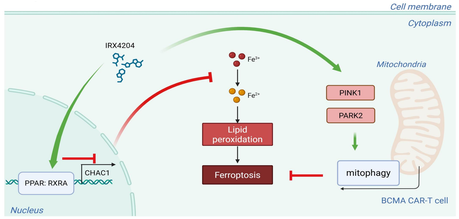


Fig7 IRX4204 enhances BCMA CAR-T persistence and antitumor function by suppressing CHAC1-mediated ferroptosis and promoting PINK1/PARK2-driven mitochondrial quality control.

CONCLUSIONS

- IRX4204 significantly enhances BCMA CAR-T activation, cytotoxicity, and persistence.
- RXR activation attenuates T-cell exhaustion and strengthens metabolic fitness under tumor stress.
- Mechanistically, IRX4204 suppresses CHAC1-driven ferroptosis and activates PINK1/PARK2-mediated mitophagy, preserving mitochondrial integrity.
- In vivo, IRX4204 improves tumor control and prolongs CAR-T persistence in MM xenograft models.

IRX4204 represents a promising adjunct strategy to improve durability and efficacy of BCMA CAR-T therapy in multiple myeloma.

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