

PRECLINICAL EVALUATION OF ZILOVERTAMAB-BASED ANTI-ROR1 CHIMERIC ANTIGEN RECEPTORS IN NKAND T CELLS

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INTRODUCTION

The Wnt5a receptor ROR1 (receptor tyrosine-kinase-like orphan receptor 1) is a promising target for immunotherapies of many cancers, including mantle cell lymphoma, diffuse large B-cell lymphoma, B-cell acute lymphocytic leukemia and chronic lymphocytic leukemia¹. Normally only expressed during embryonic development and only minimally, if at all, in adult normal tissues, ROR1 is overexpressed on malignant cells.

Zilovertamab is a monoclonal antibody binding ROR1 that is currently in Phase 1/2 clinical trials.² The antibody blocks Wnt5a-mediated survival signals.³

Because of the specific ROR1 expression on malignant cells, the antibody could also be used for targeted immunotherapy. Chimeric antigen receptors (CARs) containing the antigen binding domain of zilovertamab could enable NK or T cells to cellular cytotoxicity against ROR1-expressing cancer cells.

OBJECTIVES

This study aimed to evaluate the potency of ROR1 CAR-NK and CAR-T cells using *in vitro* cytotoxicity and cytokine release assays as well as study the antitumour activity in a lymphoma xenograft mouse model.

METHODS

The anti-ROR1 CAR (Fig. 1) was expressed in the NK cell line NK92. CAR NK cells were co-incubated with the ROR1-expressing cell lines CACO-2 (colon carcinoma) and Raji (Burkitt's lymphoma). Degranulation and interferon- γ (IFN- γ) production were analyzed.

In the *in vivo* study, immunodeficient NSG mice were injected with luciferase expressing JeKo-1 cells (mantle cell lymphoma line). After the tumor cells had engrafted, the mice were treated with different doses of anti-ROR1 CAR-T cells and cancer cell growth was monitored using *in vivo* imaging (Fig. 2)



The anti-ROR1 CAR design tested contained the scFv derived from the clinical stage monoclonal antibody zilovertamab, a spacer, a transmembrane domain and the signalling domains of 4-1BB and the CD3 ζ chain.

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CONCLUSIONS

The zilovertamab-based CARs tested in this study enabled NK cells to recognise ROR1-expressing cancer cells and respond with degranulation and cytokine production.

Anti-ROR1 CAR-expressing T cells could control the fast-growing mantle cell lymphoma cell line JeKo-1 in vivo. All three dose levels of CAR-T cells tested led to a reduction of the cancer cell burden initially. While cancer cell growth rebounded in mice treated with the lowest dose, the intermediate and the high dose of anti-ROR1 CAR-T cells were able to stabilize the cancer cell burden at a low level.

Our findings demonstrate the potential of anti-ROR1 cell therapies for treatment of ROR1-positive haematological malignancies. The use of an anti-ROR1 scFv derived from a clinically-utilized monoclonal antibody might further derisk our ROR1 cell therapies.

We are currently preparing a clinical study to test anti-ROR1-CARbased cellular therapies for the treatment of ROR1-positive haematological cancers.

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REFERENCES

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