

ALK CHIMERIC ANTIGEN RECEPTOR T CELLS COOPERATE WITH ALK INHIBITORS TO TARGET NEUROBLASTOMA CELLS WITH LOW TARGET DENSITY

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Background Neuroblastoma is the most common extracranial solid tumor of childhood¹ and accounts for 12-15% of cancer-related deaths in children.² The survival of patients with refractory or relapsed neuroblastoma remains dismal.³ In neuroblastoma, chimeric antigen receptor (CAR) T cells against GD2 have shown encouraging clinical results, but relapses are associated with loss of antigen expression. The selection of the best target is critical for the therapeutic success of CAR-T cells in hematologic malignancies and solid tumors. The Anaplastic Lymphoma Kinase (ALK) receptor is expressed by most neuroblastoma while virtually absent in the majority of normal tissues. It is an oncogenic driver in neuroblastoma and ALK inhibitors show promising clinical activity. All these features render ALK a great candidate for CAR-T therapy.

Methods We generated seven ALK.CAR constructs using the single-chain variable fragment derived from different anti-ALK monoclonal antibodies that recognize the ALK extracellular domain into a CAR construct that included the CD28 costimulatory endodomain. Their ability to target and kill was tested in vitro and in vivo against neuroblastoma cells expressing different intensities of ALK. The activity of ALK.CAR-T cells was compared to GD2.CAR-T cells.

Results ALK.CAR-T cells showed potent activity without on-target or off-target toxicity against neuroblastoma with high ALK expression. Combination with ALK inhibitors specifically potentiated the activity of ALK.CAR-T cells, but not GD2.CAR-T cell, against neuroblastoma with low ALK expression in cell lines and in a patient-derived xenograft (PDX), where the combination of ALK inhibitors with ALK.CAR-Ts significantly reduced tumor growth and extended mice survival. Mechanistically, ALK inhibitors impaired tumor growth and upregulated the expression of ALK, thereby improving the targeting of neuroblastoma tumors by ALK.CAR-T cells.

Conclusions These data indicate that ALK.CAR-T cells are effective and safe as monotherapy against neuroblastoma with high ALK expression. Furthermore, treatment with ALK inhibitors increases the efficacy of ALK.CAR-T cells by enhancing ALK targeting.

REFERENCES

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Ethics Approval All mouse experiments were performed under protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Boston Children's Hospital (Protocol 00001530).

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