Development of a Humanized ROR1 x CD3 Bispecific DART® Molecule for the Treatment of Solid and Liquid Tumors

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Abstract

Introduction: The receptor tyrosine kinase-like orphan receptor 1 (ROR1) is overexpressed in chronic lymphocytic leukemia (CLL) and a subset of solid tumors, including lung, breast, ovarian, colon, and pancreatic cancers, as well as certain sarcomas. Limited adult tissue expression and its absence in normal leukocytes makes ROR1 a promising cancer therapeutic target. We have developed a Dual-Affinity Re-Targeting (DART®) protein for redirecting T lymphocytes to lyse tumor cells via monovalent recognition of ROR1 on tumor and CD3 on T cells. ROR1 x CD3 DART protein was engineered for improved half-life with the incorporation of a modified Fc domain, lacking effector function.

Methods: The ROR1 x CD3 DART protein was stably expressed in CHO cells and purified to homogeneity by a standard antibody platform. Bispecific binding was evaluated by ELISA and surface plasmon resonance (SPR) analysis. In vitro functional studies were performed with lymphoma and solid tumor cell lines in the presence of primary human T cells. Tumor growth inhibition was evaluated in NOD/SCID/IL-2 gamma chain KO (NOG) mice co-implanted with human T cells and either mantle cell lymphoma (MCL) or lung cancer cell lines (1:5 effector:target [E:T] cell ratio) followed by treatment with ROR1 x CD3 DART protein by intravenous (IV) administration. In vivo activity was also evaluated in human peripheral blood mononuclear cell (PBMC)-reconstituted NOG/B2m deficient mice bearing established intradermal tumor xenografts following IV treatment with ROR1 x CD3 DART molecule. Pharmacokinetic (PK) analysis of the DART molecule was performed in human neonatal Fc receptor (hFcRn) transgenic mice.

Results: The ROR1 x CD3 DART protein displayed the expected bispecific binding for ROR1 and CD3 antigens, and retained the affinity and specificity of the parent mAbs. The DART mediated dose-dependent lysis of ROR1-positive MCL and solid tumor (breast, lung, and osteosarcoma) cell lines through recruitment of human T cells. DART molecule-mediated killing of ROR1-expressing target cells was accompanied by target-dependent T-cell activation and cytokine release; however, no activity was observed in the absence of target or effector cells and no cytokine release was observed with human PBMCs alone. The ROR1 x CD3 DART protein displayed extended circulating half-life after administration to hFcRn-transgenic mice. In mouse efficacy studies, the growth of HBL-2 (MCL), HOP-92 (lung cancer), or NCI-H1975 (lung cancer line resistant to erlotinib) cells co-implanted with human T cells in mice was inhibited by treatment with the ROR1 x CD3 DART protein at doses in the µg/kg range. The ROR1 x CD3 DART molecule also demonstrated antitumor activity with high complete response rates in human PBMC-reconstituted mice bearing established HBL-2 cell xenografts.

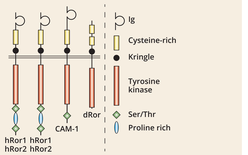
Conclusion: The promising in vitro and in vivo activity of the Fc-bearing ROR1 x CD3 DART molecule supports further investigation as a potential candidate for cancer treatment.

Introduction

ROR1: Receptor Tyrosine Kinase-like Orphan Receptor 1

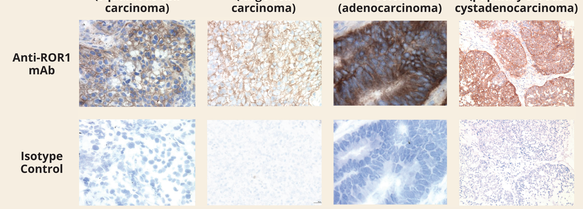
- Glycosylated type I transmembrane protein of ~104 kDa of the ROR family of Wnt receptors
- Pseudokinase that lacks catalytic activity and may interact with non-canonical Wnt signaling pathway
- High level expression during embryonic development
- Absent in adult tissues, except for B-cell precursors and low levels of transcripts in adipocytes, pancreas, and lung

Structure of ROR Receptors



Green, et al. Trends Cell Biol. 2008.

Expression of ROR1 in Cancer

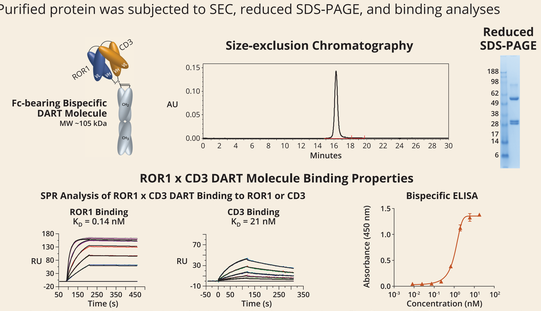


- ROR1 protein is overexpressed in CLL and certain lymphomas, as well as in a range of solid tumors including lung, breast, colon, ovarian, pancreatic cancers, and certain sarcomas
- ROR1 has limited adult tissue expression and is absent in normal immune cells

Results

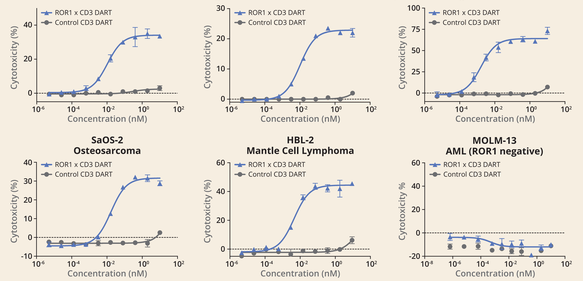
ROR1 x CD3 Fc-bearing DART Protein

- Humanized anti-ROR1 and anti-CD3 variable domains were assembled through disulfide-linked heterodimer to form the DART protein
- A modified Fc domain lacking effector function was introduced for half-life extension
- DART protein was expressed in CHO cells
- Purified protein was subjected to SEC, reduced SDS-PAGE, and binding analyses



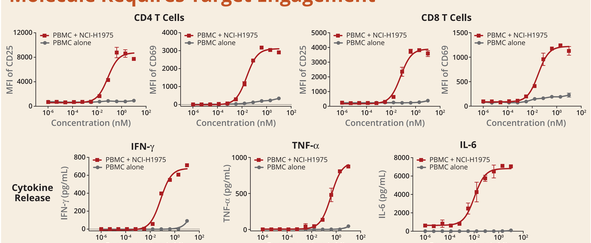
Simultaneous binding to both antigens in a bispecific ELISA: ROR1, capture antigen; CD3, detection reagent

ROR1 x CD3 DART Molecule Mediates Redirected Killing of ROR1-positive Solid and Liquid Tumor Lines



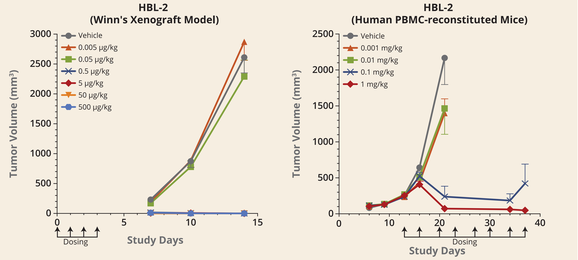
Target cells were incubated with increasing concentrations of DART molecules in the presence of human T cells at an E:T cell ratio of 10:1 for 18-24 h. Cytotoxicity was determined by LDH release assay.

T-cell Activation and Cytokine Release by ROR1 x CD3 DART Molecule Requires Target Engagement



Human PBMCs were incubated for 18-24 h with increasing DART molecule concentrations in the absence or presence of NCI-H1975 target cells (E:T = 10:1) as indicated. At the end of incubation, expression of CD25 and CD69 on CD4+ and CD8+ gated T cells was detected by flow cytometry (upper panels) and cytokine release in supernatants was measured by a cytokine bead array assay (lower panels). No T-cell activation or cytokine release was observed in absence of ROR1+ target cells.

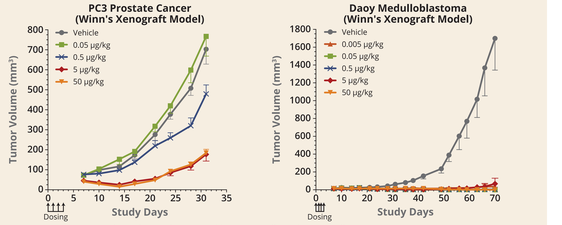
In Vivo Antitumor Activity of ROR1 x CD3 DART Molecule Against a Mantle Cell Lymphoma Line



NOG mice (N = 8/group) were implanted SC with activated human T cells and target cells (E:T ratio = 1:5) on Day 0. ROR1 x CD3 DART was administered IV once daily for 4 days starting on Day 0 as indicated by arrows.

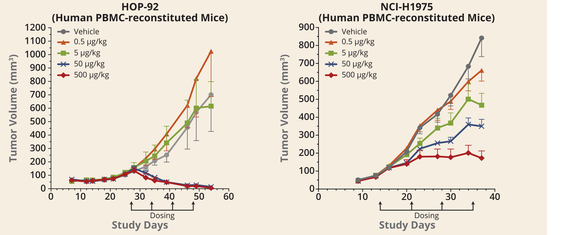
NOG/B2m-/- mice (N = 7/group) were implanted with human PBMCs (10 x 10⁶) IP and HBL-2 cells (5 x 10⁶) SC on Day 0. Following randomization, mice were first treated on Day 13 and at 3 to 4 day intervals thereafter (as indicated by arrows). In the group receiving 0.1 mg/kg ROR1 x CD3 DART, there was 5 out of 7 complete responses.

In Vivo Antitumor Activity of ROR1 x CD3 DART Molecule Against Prostate and Medulloblastoma Cancer Cell Lines



NOG mice (N = 8/group) were implanted SC with activated human T cells and target cells (E:T ratio = 1:5) on Day 0. ROR1 x CD3 DART was administered IV once daily for 4 days starting on Day 0 as indicated by arrows.

In Vivo Antitumor Activity of ROR1 x CD3 DART Molecule Against Lung Cancer Cell Lines



NOG/MHCI-/- mice were implanted with human PBMCs (10 x 10⁶) IP and target cells (5 x 10⁶) SC on Day 0. Following randomization for HOP-92 model, mice (N = 7/group) were first treated on Day 27 and at weekly intervals thereafter for a total of 4 doses as indicated by arrows. For NCI-H1975 model, mice (N = 6/group) were first treated on Day 14 and at weekly intervals thereafter for a total of 4 doses as indicated by arrows.

Conclusions

- ROR1 is overexpressed in a range of liquid and solid tumors
  - ROR1 shows limited adult tissue expression and is absent in circulating leukocytes
- An Fc-bearing ROR1 x CD3 bispecific DART protein has been engineered, expressed, and purified to homogeneity
- ROR1 x CD3 DART protein exhibited:
  - Dose-dependent redirected cell killing against a wide range of solid and liquid tumor cell lines in vitro
  - Strict dependency on target cell engagement for T–cell activation and cytokine release
  - No detectable activation or cytokine release with human effector cells alone
  - Inhibition of ROR1-positive lymphoma, medulloblastoma, as well as lung and prostate cancer tumor xenografts in vivo in both tumor/T-cell co-implantation models and in established tumor models in human T-cell reconstituted mice
  - Extended circulating half-life (~2.7 days) in human FcRn transgenic mice (data not shown)

These data support further investigation of ROR1 x CD3 DART molecule as a potential candidate for the treatment of ROR1-expressing liquid and solid tumors

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