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


Macrogenics, Inc., Rockville, MD and South San Francisco, CA

Presented at the 2016 American Association for Cancer Research Annual Meeting, April 16–20, 2016, New Orleans, Louisiana

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 monoclonal 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 for ROR1 and CD3 antigens, and retained the Fc-bearing ROR1 x CD3 bispecific DART protein has been engineered, expressed, and purified to homogeneity.

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, ovarian, pancreatic, and certain sarcomas
- ROR1 has limited adult tissue expression and is absent in normal immune cells

Results:

- Humanized anti-ROR1 and anti-CD3 variable domains were assembled through cell-free display technology to form the DART protein
- A modified Fc domain lacking effector function was introduced for half-life extension in human T cells
- DART molecule was expressed in human CHO cells and purified to homogeneity
- No detectable activation or cytokine release with human effector cells
- Strict dependency on target cell engagement for T-cell activation

Conclusions:

- ROR1 is overexpressed in a range of liquid and solid tumors
- ROR1 shows limited adult tissue expression and is absent in normal 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:
  - Tumor cell- and target-engaged 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
  - Inhibition of ROR1-positive lymphoma, medulloblastoma, as well as lung and prostate cancer tumor xenografts in vivo in both Tumor71/CLL cotransplantation mouse models and established tumor models in human T-cell reconstructed mice
  - Extended circulating half-life (~2.7 days) in human FcRn transgenic mice (data not shown)

- Humanized ROR1 x CD3 DART molecule as a potential candidate for the treatment of ROR1-expressing liquid and solid tumors