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Potent Antitumor Activity of Duvortuxizumab, a CD19 x CD3 DART® Molecule, in Lymphoma Models

Liat Izhak[1*], Dana E. Cullen[1], Maha Elgawly[1], Leopoldo Luistro[1], Syd Johnson[2], Jaime Bald[1], Kevin Bellew[1], A. Kate Sasser[1], Sriram Balasubramanian[1]

[1]Janssen Research & Development, LLC, Spring House, PA, USA; [2]MacroGenics, Inc., Rockville, MD, USA

*Presenting Author

INTRODUCTION

- Duvortuxizumab, also known as JNJ-64052781 and MGD011, is a bispecific CD19 x CD3 DART® molecule.
- Duvortuxizumab was designed to engage and redirect CD3$^+$ T-cells to eliminate CD19$^+$ B-cells through T-cell-mediated cytotoxicity.[1]
- In lymphoma cell lines and mouse xenograft models, duvortuxizumab exhibited potent single-agent cytolytic activity and tumor regression, respectively.[2]
- Duvortuxizumab is currently in clinical development for the potential treatment of B-cell malignancies.
- Here we examined duvortuxizumab activity alone and in combination with standard chemotherapy regimens used to treat B-cell malignancies in preclinical lymphoma models.

Note: Data have been updated since abstract submission.

METHODS

Cytotoxicity and T-Cell Activation Assays

- Human healthy donor T-cells or total peripheral blood mononuclear cells (PBMCs) at an effector:target cell (E:T) ratio of 5:1 or 10:1, respectively, were used as effector cells for CD19$^+$ DHL-4 and Raji target cells.
- Cells were incubated with duvortuxizumab and bendamustine or cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) for 48 hrs.
- Cytotoxicity was measured using a CFSE assay.
- T-cell activation was measured by flow cytometry of CD4$^+$CD25$^+$ and CD8$^+$CD25$^+$ cells.

Diffuse Large B-Cell Lymphoma (DLBCL) Patient-Derived Xenograft (PDX) Model

- Human DLBCL tumor fragments (~70 mg) were implanted subcutaneously in NOD SCID mice.
- Mice were injected intraperitoneally with human PBMCs and treated with duvortuxizumab (0.5 mg/kg, q3d x 8), a single dose of CHOP regimen, or in combination; CD3 x fluorescein isothiocyanate (FITC) was included as a control DART molecule. Samples were collected from each treatment group; tumor volume was estimated from caliper measurements.

Burkitt's Lymphoma Xenograft Model

- Daudi cells were implanted subcutaneously in female NOD SCID gamma mice followed by inoculation with human PBMCs on day 4 or purified, activated T-cells on day 15 after tumor implantation.
- Mice were treated with duvortuxizumab (0.5 mg/kg, q3d x 8), bendamustine (dosed once at 25 mg/kg), or in combination; CD3 x FITC was included as a control DART molecule.
- Samples were collected from 2 mice from each treatment group for T-cell analysis by flow cytometry.

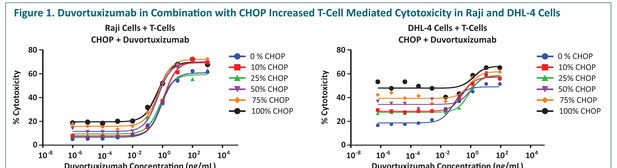
RESULTS

Duvortuxizumab in Combination with CHOP

In Vitro Studies in Lymphoma Cells

- Duvortuxizumab in combination with increasing concentrations of CHOP decreased the viability of Raji and DHL-4 cells (**Figure 1**).

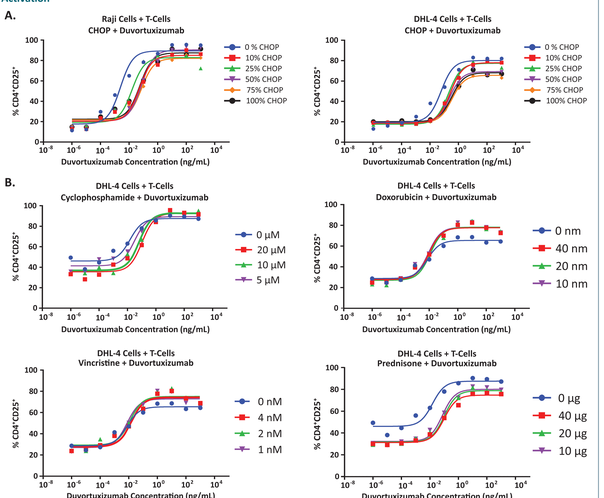
Figure 1. Duvortuxizumab in Combination with CHOP Increased T-Cell Mediated Cytotoxicity in Raji and DHL-4 Cells



CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone.

- A CHOP-dependent decrease in T-cell activation, as measured by CD4$^+$CD25$^+$ (**Figure 2A**) and CD8$^+$CD25$^+$ (data not shown) cells, was observed when duvortuxizumab was combined with CHOP.
  - This decrease was likely due to the inclusion of prednisone, which was the only agent in the CHOP regimen, when tested individually, that caused a decrease in T-cell activation when used in combination with duvortuxizumab (**Figure 2B**).

Figure 2. Increasing Concentrations of (A) CHOP in Raji and DHL-4 Cells and (B) Prednisone in DHL-4 Cells Decreased T-Cell Activation

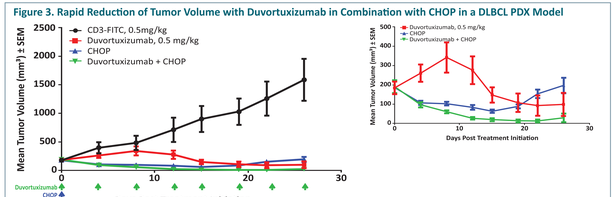


CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone.

In Vivo Studies in a DLBCL PDX Model

- In a DLBCL PDX model, duvortuxizumab in combination with CHOP resulted in rapid tumor regression that was more sustained than CHOP treatment alone (**Figure 3**).

Figure 3. Rapid Reduction of Tumor Volume with Duvortuxizumab in Combination with CHOP in a DLBCL PDX Model



CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; DLBCL = diffuse large B-cell lymphoma; FITC = fluorescein isothiocyanate; PDX = patient-derived xenograft; SEM = standard error of the mean. Inset shows a higher magnification of the lower part of the graph (CD3-FITC control not shown).

Duvortuxizumab in Combination with Bendamustine

In Vitro Studies in Lymphoma Cells

- Duvortuxizumab in combination with bendamustine increased T-cell-mediated cytotoxicity against CD19$^+$ Raji and DHL-4 cells (**Figure 4**).
- Minimal inhibition of T-cell activation was observed with bendamustine concentrations up to 40 µM when combined with duvortuxizumab, as assessed by CD4$^+$CD25$^+$ (**Figure 5A**) and CD8$^+$CD25$^+$ (**Figure 5B**) cells.

Figure 4. Duvortuxizumab in Combination with Bendamustine Increased T-Cell Mediated Cytotoxicity in Raji and DHL-4 Cells

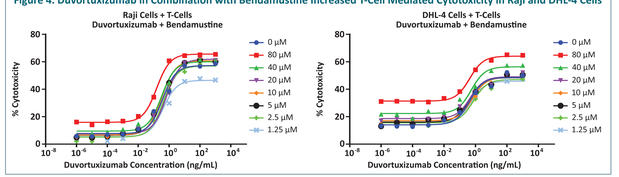
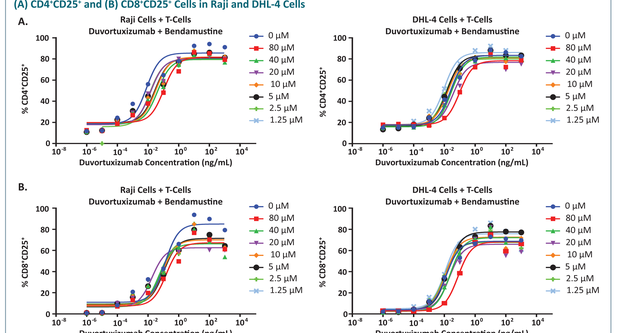


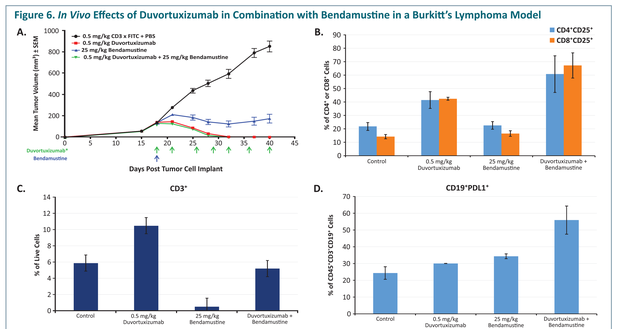
Figure 5. Duvortuxizumab in Combination with Bendamustine Slightly Decreased T-Cell Activation as Measured by (A) CD4$^+$CD25$^+$ and (B) CD8$^+$CD25$^+$ Cells in Raji and DHL-4 Cells



In Vivo Studies in a Burkitt's Lymphoma Model

- In a Burkitt's lymphoma model with PBMCs, bendamustine inhibited tumor growth by 79% (p=0.0002) whereas duvortuxizumab alone resulted in complete tumor regression (**Figure 6A**).
  - The combination of duvortuxizumab and bendamustine resulted in complete and durable tumor regression and elimination.
  - Greater activation of CD4$^+$CD25$^+$ and CD8$^+$CD25$^+$ T-cells was observed in the tumors when duvortuxizumab was combined with bendamustine (**Figure 6B**).
  - Analysis of tumor-infiltrating lymphocytes (TILs) 3 days after the start of treatment showed that bendamustine inhibited CD3$^+$ T-cell infiltration whereas duvortuxizumab increased infiltration (**Figure 6C**).
  - Upregulated expression of programmed death-ligand 1 on tumor cells was observed when duvortuxizumab was combined with bendamustine (**Figure 6D**).

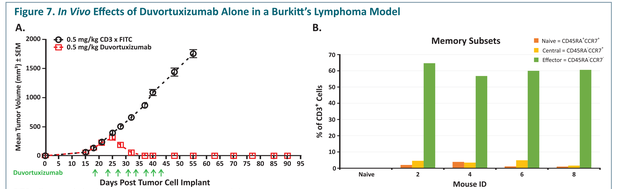
Figure 6. *In Vivo* Effects of Duvortuxizumab in Combination with Bendamustine in a Burkitt's Lymphoma Model



FITC = fluorescein isothiocyanate; PDL1 = programmed death-ligand 1; SEM = standard error of the mean.
*The study was terminated early at 40 days due to development of graft vs. host disease.

- Studies were repeated with duvortuxizumab alone vs. CD3 x FITC control in a Burkitt's lymphoma model with pan T-cells.
  - Duvortuxizumab alone resulted in rapid and prolonged regression of established tumors with no relapse for up to 90 days, although the last dose of duvortuxizumab was administered on day 42 (**Figure 7A**).
  - Analysis of TILs 48 days after the last duvortuxizumab dose revealed a large percentage of CD3$^+$CD45RA$^-$CCR7$^-$ cells, indicative of effector memory T-cells (**Figure 7B**).

Figure 7. *In Vivo* Effects of Duvortuxizumab Alone in a Burkitt's Lymphoma Model



FITC = fluorescein isothiocyanate; SEM = standard error of the mean

CONCLUSIONS

- Duvortuxizumab displayed potent anti-tumor activity as a single agent and in combination with standard chemotherapy in lymphoma preclinical models.
- Duvortuxizumab-mediated tumor killing and T-cell activation were maintained or increased in the presence of multiple chemotherapeutics, suggesting the potential clinical utility of combining duvortuxizumab with standard chemotherapies in the treatment of B-cell malignancies.

REFERENCES

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