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Design, Synthesis and Evaluation of Anti-CD38 Antibody Drug Conjugate Based on Daratumumab and Maytansinoid

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Abstract

Daratumumab, an FDA approved antibody drug, displays specific targeting ability to abnormal white blood cells overexpressing CD38 and provides efficacious therapy for multiple myeloma. Here, in order to achieve enhanced remission of multiple myeloma, we designed **Dara-DM4**,

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Declarations of interest

The authors declare no competing financial interest.

antibody drug conjugates (ADCs) by conjugating Daratumumab and DM4 via a disulfide linker. **Dara-DM4** showed significantly higher cellular uptake and inhibitory efficacy on MM1S cells that overexpressing CD38 with an IC_{50} of 0.88 $\mu\text{g/mL}$ post 72 h treatment. These results support a promising ADCs strategy for multiple myeloma treatment.

Graphical Abstract

Keywords

CD38; Maytansinoid; Multiple Myeloma; Antibody Drug Conjugate

1. Introduction

Multiple myeloma is a malignancy of plasma cells.¹ It affected 449.3 thousands people and resulted in 107.1 thousands deaths from all over the world in 2017.² Without treatment, typical survival is only seven months.¹ Conventionally, steroids, chemotherapy, thalidomide or lenalidomide, and stem cell transplant are used for the remission of this cancer.^{1, 3-5} However, these types of therapeutics lack targeting specificity to the cancerous plasma cells in patients.^{3, 5}

Antibody drug conjugates (ADCs) represented an effective approach for tumor targeting and chemotherapy drugs delivery.⁶⁻¹¹ For example, anti-CD46 ADCs were reported to inhibit proliferation of myeloma cells.¹² The mechanisms of action of ADCs include direct binding between the antibody and its targeted cell surface antigen, and intracellular release of cytotoxic drugs.⁹ In 2015, Daratumumab, a CD38 antibody, was approved by the U.S. Food and Drug Administration (FDA) for the treatment of multiple myeloma.^{13, 14} Daratumumab binds to CD38, which is overexpressed on the surface of multiple myeloma cells, and consequently induces the tumor cell death by complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and other mechanisms directly associated with the functional change of surface antigen after binding.^{13, 14} In this study, we designed Daratumumab derived antibody drug conjugates (**Dara-DM4**) to enable specific delivery of DM4 to CD38 overexpressed cancer cells. Scheme 1 showed the synthetic route to **Dara-DM4**. Daratumumab is able to target CD38 overexpressed cells^{13, 14}; DM4, a maytansinoid derivative, is an effective chemotherapeutic drug¹⁵; and the disulfide linker is cleavable towards intracellular glutathione (GSH).¹⁶ Hence, **Dara-DM4** is designed to targeting CD38 overexpressed cells. After taken by tumor cells via receptor mediated endocytosis process, the ADCs will be cleaved inside cells. The released DM4 from **Dara-DM4** leads to tumor cell death.

2. Results and discussion

2.1 Design and synthesis of Dara-DM4

Dara-DM4 is synthesized through a two-step reaction (Scheme 1). First, Daratumumab was reacted with *N*-succinimidyl 3-(2-pyridyldithio) propionate (SPDP), a crosslinker and afforded the intermediate **Dara-SPDP**. The reaction mixture was purified using desalting columns through centrifugation to remove excess SPDP. Then, **Dara-SPDP** was conjugated with DM4 and purified using the same desalting columns to yield the **Dara-DM4**. In order to quantify cellular uptake, Daratumumab and IgG, a control antibody, were conjugated to Cy7, a fluorophore and produced **Dara-Cy7** and **IgG-Cy7** (Scheme S1).

2.2 Characterization of Dara-DM4

We then characterized **Dara-DM4** using MALDI-TOF-MS. As shown in Figure 1, the molecular weight of Daratumumab and **Dara-DM4** conjugate were 148379 and 150307, respectively. Based on the molecular weight of DM4 and SPDP, the drug to antibody ratio (DAR) was calculated to be 2.47, which is a preferred ratio for antibody drug conjugates as reported previously.⁷ Next, we studied the effects of GSH on the **Dara-DM4** conjugate. The results showed that DM4 was detected after 4 hr incubation in GSH solution (Figure S3A), suggesting that GSH cleaved the disulfide bond and released DM4 (Figure S3B).¹⁶

2.3 Cellular Uptake of Dara-Cy7 and IgG-Cy7

In order to study the cellular uptake of **Dara-Cy7** and **IgG-Cy7**, we incubated MM1S (a CD38 positive cell line) and U266 (a CD38 negative cell line) with these two conjugates and measured the fluorescence intensity through the fluorescence-activated cell sorting (FACS) analysis. The fluorescence intensity was then normalized by the intensity of control group (**IgG-Cy7**) in both cell lines. In MM1S cells (Figure 2A), **Dara-Cy7** group displayed significantly higher fluorescence intensity than that in the **IgG-Cy7** group, which indicated more uptake of **Dara-Cy7** than **IgG-Cy7**. On the contrary, as in the CD38 negative cell line (Figure 2B), no significant difference of fluorescence intensity was observed in U266 cells, which reveals equal uptake of **IgG-Cy7** and **Dara-Cy7**. Two groups of FACS assay indicate that conjugates based on Daratumumab show enhanced cellular uptake towards CD38 positive cell line.

2.4 Cytotoxicity of Dara-DM4

Lastly, to evaluate the cytotoxicity of **Dara-DM4** to MM1S cells, we treated these cells with **Dara-DM4** for different time. As shown in Figure 3, **Dara-DM4** showed stronger cytotoxicity than DM4 and antibody at all three time points (24, 48, and 72 h). Specifically, **Dara-DM4** induced around 88% of MM1S cell death in 72 h at the concentration of 2.8 $\mu\text{g/mL}$ with an IC_{50} value of 0.88 $\mu\text{g/mL}$. However, DM4 showed approximate 22% of cell death at the same concentration. Therefore, **Dara-DM4** demonstrated stronger cytotoxicity towards MM1S cells in comparison with free DM4. In order to evaluate the potential side effect of **Dara-DM4** in myeloma therapy, we tested the cytotoxicity of **Dara-DM4** to macrophages, CD38 negative cells.¹⁷ As shown in Figure S4, the results showed that no apparent cytotoxicity was found in **Dara-DM4** treated group at the concentrations tested.

3. Conclusion

In summary, we designed and synthesized a new anti-CD38 antibody drug conjugate, **Dara-DM4**, by conjugating Daratumumab and DM4 through a cleavable crosslinker SPDP. FACS assays showed that Daratumumab enhanced cellular uptake in CD38 positive cells. More importantly, **Dara-DM4** exhibited potent cytotoxicity in MM1S cells with an IC₅₀ of 0.88 µg/mL post 72hr treatment. Therefore, the newly designed anti-CD38 antibody drug conjugates provides a potential therapeutic strategy for treating multiple myeloma.

4. Experimental procedure

4.1 Materials

DM4 was purchase from BroadPharm (CA, USA). SPDP was purchased from Sigma-Aldrich (MO, USA). Zeba desalt column was purchased from Sigma-Aldrich (MO, USA). Other chemical agents were purchased form Sigma-Aldrich (MO, USA) or Alfa Aesar (MA, USA). RPMI-1640 Medium and Eagle's Minimum Essential Medium (EMEM) were purchased from Corning Incorporated (NY, USA).

4.2 Synthesis of Dara-DM4

Dara-DM4 was synthesized according to the reported method.^{18,19} Firstly, DM4 and SPDP were dissolved in DMSO to obtain 5 mM stock solution, respectively. Then, 40 µL of Daratumumab (20 mg/mL), 5.6 µL SPDP stock solution (5mM), 280 µL PBS (pH 7.40) and 80 µL NaFICO₃ (1 M) were mixed and incubated at room temperature for 1 h. After purification through a Zeba column, the intermediate was mixed with 5.6 µL DM4 and incubated at 4 °C for 18 h. The resulted mixture was purified through the Zeba column to yield Dara-DM4 (400 µL, 2 mg/mL).

4.3 Synthesis of Dara-Cy7 and IgG-Cy7

Cy7 was synthesized according to the reported method.²⁰ Firstly, Cy7 was dissolved in DMSO to obtain 5 mM stock solution. Then, 10 µL Daratumumab or IgG (20 mg/mL), 1.4 µL Cy7, 70 µL PBS (pH 7.40) and 20 µL NaHCO₃ (1 M) were mixed and incubated at room temperature for 2 h. The resulted mixture was purified through the Zeba column and yielded **Dara-Cy7** or **IgG-Cy7** (100 µL, 2 mg/mL).

4.4 Characterization of the conjugates and GSH-mediated drug release

Dara-DM4, **Dara-Cy7** and **IgG-Cy7** were characterized on the Bruker Ultraflextreme MALDI TOF instrument using sinapic acid (SA) as the matrix and BSA as the standard (Figure S1, 2). The DM4 (Cy7)/Daratumumab ratio was calculated according to the molecular weight (MW). A solution was prepared by dissolving 18 mM GSH in DPBS.¹⁶ After incubating **Dara-DM4** in the solution for 4 hr, the sample was then tested by mass spectrometry.

4.5 Cellular uptake of Dara-Cy7 and IgG-Cy7

MM1S and U266 cells (ATCC) were cultured in RPMI-1640 medium with 10% FBS. U266 cell line (ATCC) was cultured in Eagle's Minimum Essential medium with 10% FBS. All cells are maintained in 37 °C with 5% CO₂.

Cellular uptake of **Dara-Cy7** or **IgG-Cy7** were examined in MM1S and U266 cells by the fluorescence-activated cell sorting (FACS). Cells were seeded in a 24-well plate at the density of 2×10^5 cells/well. After overnight incubation at 37 °C, 2 µl of **Dara-Cy7** or **IgG-Cy7** (antibody protein concentration 2 mg/ml) were added to MM1S and U266 cells. After incubating for three hours, cells were washed and re-suspended in PBS before measuring the fluorescence intensity on a BD LSR II flow cytometer (BD Biosciences, San Jose, CA).

4.6 Cytotoxicity of Dara-DM4

Cytotoxicity of **Dara-DM4** was examined in MM1S cells and macrophages by MTT assays as reported previously.¹⁸ Macrophages were cultured as described in the literature.²¹ Cell viability of **Dara-DM4** was normalized by untreated cells. Generally, cells were grown in 96-well plates 24 h prior to treatment. For 24 hr cytotoxicity assay, cells were prepared at a density of 8×10^3 cells/well. For 48 and 72 hr cytotoxicity assay, cells were prepared at a density of 5×10^3 cells/well. The absorbance at a wavelength of 570 nm was measured on a SpectraMax M5 microplate reader (San Jose, CA). Absolute IC₅₀ was then calculated by GraphPad Prism 6 (La Jolla, CA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
MALDI-TOF mass spectrum of Daratumumab and **Dara-DM4**.

Figure 2. Cellular uptake of **Dara-Cy7** and **IgG-Cy7** in MM1S (a CD38 positive cell line) and U266 (a CD38 negative cell line). ***, $p < 0.001$; n.s., no significant difference; t test, double-tailed.

Figure 3. Cell viability of MM1S cells after treated with **Dara-DM4** for (A) 24hr, (B) 48 hr and (C) 72 hr.

Scheme 1.

Synthetic route to **Dara-DM4** antibody drug conjugates.