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Anti-FcμR Antibody Drug Conjugate as a Potential Therapeutic Agent for the Treatment of Chronic Lymphocytic Leukemia

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INTRODUCTION

We investigate antibody drug conjugates (ADCs) to specifically target CLL cells. An advantage of ADCs is their functional independence of host immune effector mechanisms which are often compromised in patients with CLL.

We and others have previously reported over expression of the Fc receptor for IgM (FcμR) in CLL B cells compared to other leukocytes and such over expression of FcμR is an independent indicator of shorter survival in patients with CLL. FcμR binds to human IgM Fc fragment (Fcμ) with high avidity and is rapidly shuttled via clathrin-coated endocytic vesicles into lysosomes.

We developed recombinant rabbit/human chimeric mAbs against FcμR in double variable domain (DVD) formats. The DVD mAbs contain an outer VH domain that targets FcμR on the cell surface and an inner VH domain with a uniquely positioned catalytic (or reactive) lysine residue that allows quick, site-specific conjugation of a cytotoxic drug (e. g. MMAF). Here we demonstrate that anti-FcμR DVD mAbs specifically bind to malignant B cell lines and primary CLL cells and rapidly internalize into lysosomes. Anti-FcμR DVD-ADCs selectively and efficiently killed target cells in FcμR-dependent manner and therefore, can be considered as potential therapeutic agents for the treatment of CLL.

AIM

1. To generate and characterize dual variable domain (DVD) mAbs against human FcμR and demonstrate their binding to cell lines and primary CLL cells
2. To demonstrate FcμR-specific cytotoxicity induced by DVD antibody drug conjugates (ADC)

METHODS

Rabbit mAbs (Fvs, in green) that bind to human FcμR were recombinantly grafted on to H38C2 IgG backbone to generate DVD-IgGs in different formats: clone A4 wild type (DVD-IgG) or Fc mutant (DVD-IgG*). HM-7 is a mouse anti-human FcμR mAb.

Cell surface binding: The binding of A4-DVD-IgG and A4-DVD-IgG* to human B cell lines, PBMCs from healthy donors (HD) or from patients with CLL (CLL) was detected by flow cytometry using AlexaFlour 647-conjugated F(ab')2 preparation of GaR Ab and co-staining with CD5/CD19 Abs.

Internalization: Target cells were incubated with anti-FcμR mAbs at 4ºC, washed and kept at 37ºC for various periods of time and then stained with corresponding secondary Ab for subsequent analysis by flow cytometry or processed in chamber slides for evaluation by confocal microscopy.

Cytotoxicity: Parent Mino and NU-DHL-1 cell lines (wt) and their FcμR knock out counterparts (FcμR-KO) were incubated with different concentrations of A4-DVD-ADCs for 3 days and the viability was determined using CellTiter Glo assay (Promega). A4-DVD-ADCs with a non-cleavable linker (β-Lactam) or DBCO (β-Lactam-MMAF and DBCO-MMAF, respectively) or a cleavable linker DBCO-VC- (DBCO-VC-MMAF) were used. H38C2-β-Lactam-MMAF served as a non-targeting control. Cytotoxicity of primary CLL cells with 100 nM A4-DVD-IgG*-DBCO-VC-MMAF was determined by flow cytometry, gating on CD5+/19+ cells and staining with Annexin-V and DAPI. Unconjugated A4-DVD-IgG*, MMAF and MMAE were used as negative and positive controls, respectively. Each color dot represent a patient sample and two independent experiments were performed for each sample.

RESULTS

Figure 1. FcμR expression in cell lines

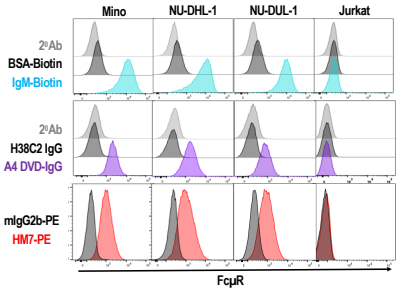


Figure 2. Anti-FcμR DVD mAbs bind selectively to CLL cells

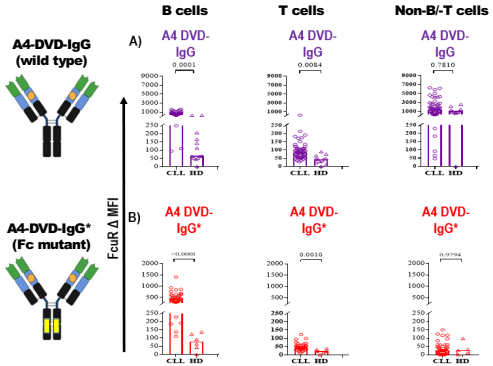


Figure 3. Anti-FcμR DVD mAbs internalize rapidly into lysosomes

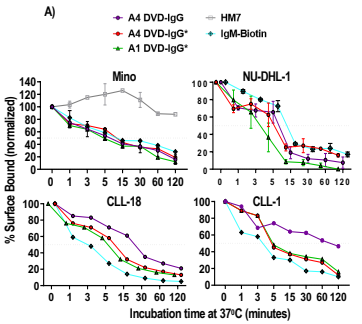


Figure 4. Anti-FcμR DVD-ADCs induced cytotoxicity of cell lines

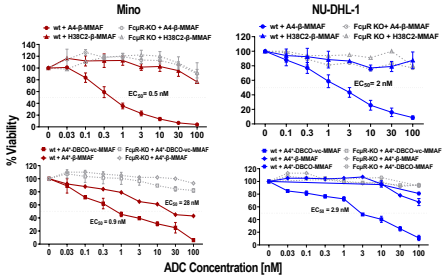
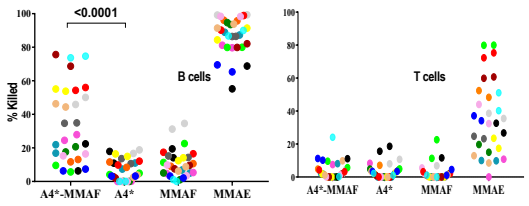
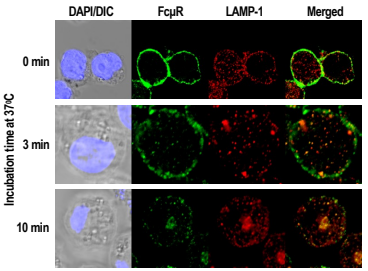


Figure 5. Anti-FcμR DVD-ADC induced cytotoxicity of CLL cells



B) Internalization of A4-DVD-IgG* in Mino cells



SUMMARY AND CONCLUSIONS

- Anti-FcμR DVD mAbs specifically bound to several human lymphoma cell lines and primary CLL cells, rapidly internalized and localized into lysosomes.
- Anti-FcμR-DVD-MMAF induced specific cytotoxicity in lymphoma cell lines and primary CLL cells.
- Together, these results identify FcμR as an actionable, novel target in CLL and DVD-ADCs can be potentially therapeutic.

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