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BP1223, a Novel T Cell Engager Targeting CD39 for Potent Antitumor Activity in Acute Myeloid Leukemia

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INTRODUCTION

Acute myeloid leukemia (AML) remains difficult to treat due to the high rate of relapse and poor long-term survival rates. CD39, an ectoenzyme that converts extracellular ATP to adenosine and promotes immunosuppression in the tumor microenvironment, is associated with poor prognosis in AML patients[1]. Moreover, upregulation of CD39 expression in leukemic cells after chemotherapy is linked to chemoresistance[1, 2]. T cell engaging bispecific antibodies targeting CD33 or CD123 have emerged as effective therapeutic agents in AML. Based on CD39 expression pattern and immunosuppressive function, T cell engagers targeting CD39 positive AML blasts could represent a potential therapeutic strategy in AML.

1. *Cancer Discov* (2020) 10 (10): 1544–1565.
2. *Front Oncol* (2023) 13:1244280.

AIM

This study examines whether BP1223, a CD39 x CD3 bispecific antibody, enhances T cell-mediated cytotoxicity against CD39 positive AML blasts.

METHOD

- The cytotoxic effects of BP1223 on AML blasts were evaluated using ex vivo cultures of bone marrow samples from AML patients.
- *In vivo* efficacy was tested using an immunodeficient mouse model engrafted with Kasumi-1 AML cells and human PBMCs, where BP1223 was administered intravenously, and tumor growth was monitored by caliper measurements.
- All human samples were handled under the guidelines of Institutional Review Board (IRB) of National Cancer Center Hospital East (NCCHE) with an approved protocol (#2022-179).

CONCLUSIONS

- CD39 expression in AML blasts correlated with venetoclax resistance.
- BP1223 exhibited anti-tumor activity against both CD39-positive and -negative AML blasts through T cell engagement and bystander effects.
- BP1223 demonstrated selective cytotoxicity against cancer cells with minimal effects on normal bone marrow and endothelial cells.
- In an AML xenograft model, BP1223 administration at 0.1 mg/kg induced complete tumor regression in all treated mice (n=5).

These data indicate that BP1223 represents novel therapeutic approach for AML, including CD39-positive and chemotherapy-resistant cases.

RESULTS

BP1223 is a novel T cell engager targeting CD39

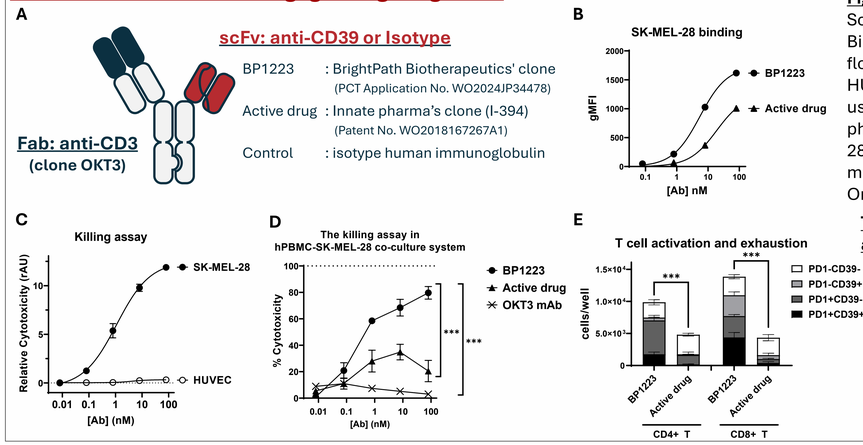


Fig 1. Characteristics of BP1223 as a T cell engager. Schematic representation of the BP1223 molecule (A). Binding of BP1223 to SK-MEL-28 cells, as assessed by flow cytometry (B). Cytotoxicity of BP1223 against HUVEC (C) and SK-MEL-28 (C, D) cells, determined using a PBMC-mediated killing assay. Immuno-phenotypic analysis of the killing assay against SK-MEL-28 cells by flow cytometry (E). Data are presented as mean ± SD. Tukey's multiple comparison following One-way ANOVA were performed(***p < 0.001).

Table 1. Cytotoxicity of BP1223 and CD39 surface antigen counts of various cell lines

	Cytotoxicity (EC_{50})	Antigen-Binding Capacity
SK-MEL-28 (melanoma)	1.1 nM	4.7×10^4
Kasumi-1 (AML)	54.4 pM	1.5×10^4
MOLP-8 (multiple myeloma)	< 64.0 pM	6.8×10^4
HUVEC	no effect	4.3×10^3

High CD39 expression in drug-resistant AML patients

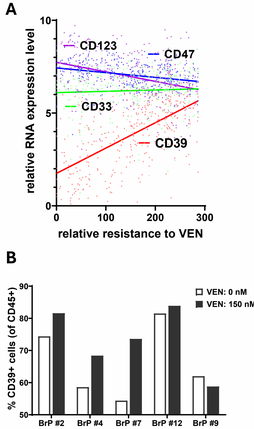


Table 2. The slope of the linear regression equation and the correlation coefficient

	CD39	CD33	CD123	CD47
Venetoclax	**+13.7**	+0.7	-2.5	-5.1
	+0.60	+0.05	-0.29	-0.32
Azacitidine	+9.8	-0.3	-0.8	-3.6
	+0.26	-0.01	-0.45	-0.13
Ara-C	+4.3	-4.4	-1.8	-3.0
	+0.12	-0.19	-0.14	-0.12

(Top: slope ($\times 10^{-3}$), Bottom: correlation coefficient (R))

Fig 2. Sensitivity to chemotherapy as a standard of care for AML. The correlation between the resistance to venetoclax (VEN) and the mRNA expression level of CD33, IL3RA (CD123), CD47, and ENTPD1 (CD39) using RNAseq data (n=942) from Beat AML 2.0 Vizome database (A). The population of $CD39^+$ cells treated by VEN in AML patient-derived cells (PDC) (B).

Less effects on normal BM cells

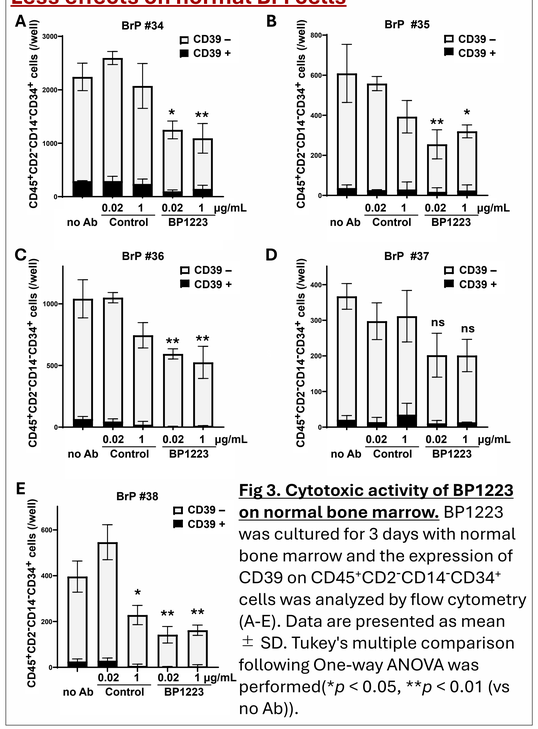


Fig 3. Cytotoxic activity of BP1223 on normal bone marrow. BP1223 was cultured for 3 days with normal bone marrow and the expression of CD39 on $CD45^+CD2^-CD14^-CD34^+$ cells was analyzed by flow cytometry (A-E). Data are presented as mean ± SD. Tukey's multiple comparison following One-way ANOVA was performed(*p < 0.05, **p < 0.01 (vs no Ab)).

Additive effects with VEN/AZA on PDC

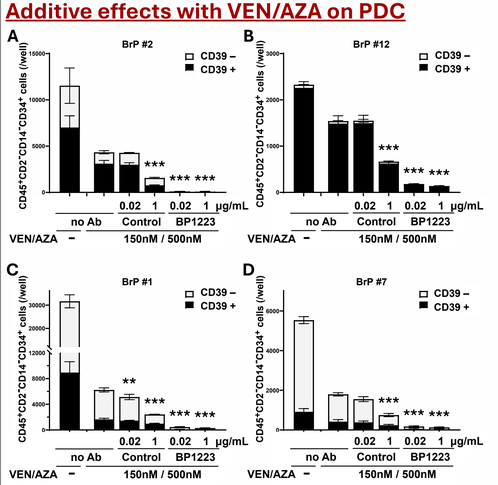


Fig 4. Combined effects of BP1223 and VEN/AZA on cytotoxic activity against PDC using allogenic effector cells. PDC and allogeneic effector cells from healthy donor were co-cultured with/without drugs (VEN and azacitidine (AZA)) and antibody (BP1223 or control) for 3 days. The drug effects were evaluated by analyzing the number of AML blasts ($CD45^+CD2^-CD14^-CD34^+$) and their CD39 expression as target cells using flow cytometry. Four patient specimens (A-D) were analyzed. Data are presented as mean ± SD. Tukey's multiple comparison following One-way ANOVA was performed (**p < 0.01, ***p < 0.001 (vs no Ab)).

Cytotoxic activity on Primary AML blasts

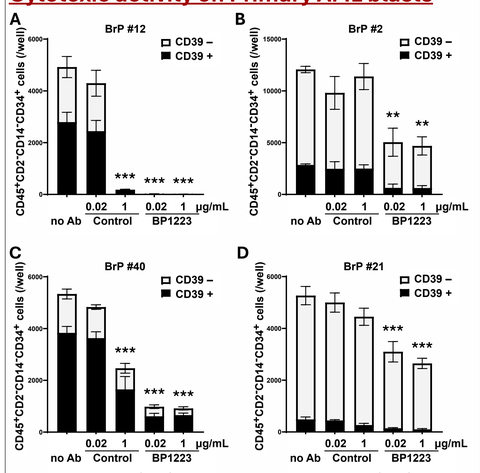
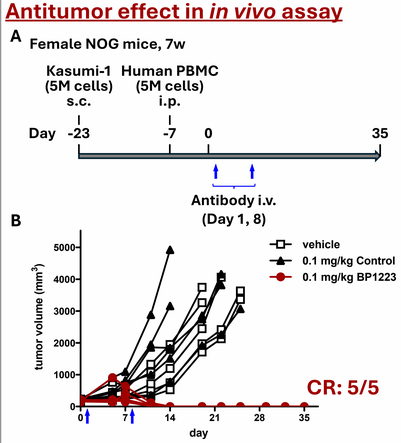


Fig 5. Cytotoxic activity of BP1223 against primary AML blasts mediated by autologous bone marrow effector cells. To investigate the potency of BP1223 against AML blasts with autologous effector cells in bone marrow, primary AML cells from patients were cultured with antibodies (BP1223 or control) for 3 days (A-D). The cytotoxic effects were evaluated by measuring viable AML blasts and CD39 expression using flow cytometry. Data are presented as mean ± SD. One-way ANOVA followed by Tukey's multiple comparison test was used to assess significance ; **p < 0.01, ***p < 0.001 (vs no Ab).

Antitumor effect in *in vivo* assay



No changes were observed in body weight or general conditions in any animals.

Fig 6. *In vivo* antitumor effects of BP1223 in an AML xenograft mouse model. The AML cell Kasumi-1 was transplanted into female NOG mice, and 16 days later, hPBMCs were injected intraperitoneally. One week later, BP1223 or control antibody (0.1 mg/kg, N= 5/group) was administered intravenously twice (A). Tumor diameter was measured with calipers. Data points represent individual mice (B).

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