#2234

A novel asymmetrical anti-CLL-1×CD3 bispecific antibody, ABL602, induces potent CLL1-specific antitumor activity with minimized sensitization of pro-inflammatory cytokines

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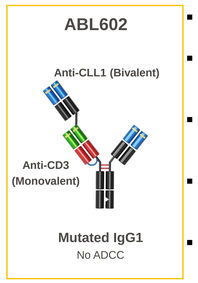
INTRODUCTION

- Acute myeloid leukemia (AML) is a disease with high incidence of relapse that is originated and maintained from leukemia stem cells (LSCs).
- Expression of C-type lectin-like molecule-1 (CLL-1; also known as CLEC12A, c-type lectin domain family 12 member A) is mainly restricted to LSCs but absent in normal hematopoietic stem cells (HSCs).
- This unique CLL-1 expression pattern paves the way to develop therapies that potentially eliminate CLL-1-positive LSC while preserving CLL-1-negative HSC.

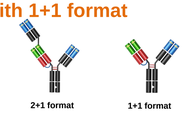
SUMMARY

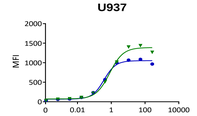


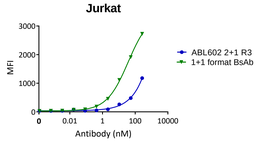
- ABL602 has bivalent binding arms to CLL-1 as a cancer target and a monovalent binding arm to CD3.
- ABL602 exhibited higher binding activity to CLL1-expressing AML cell lines and greater tumor-killing efficacy than 1+1 format BsAb and benchmark antibody MCLA-117.
- ABL602 induced potent cytotoxic activities on CLL1-expressing AML cell lines with concomitant T cell activation and cytokine/granzyme B release.
- ABL602 did not or minimally induced TNF-α and IL-6 in PBMC in the absence of AML cell lines, while MCLA-117 triggered high level of expression of those cytokines.
- In established orthotopic AML mouse model using HL-60 Luc, ABL602 demonstrated statistically significant anti-tumor activity in a dose-dependent manner.

RESULT

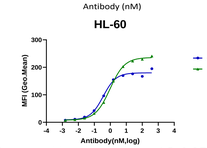
ABL602 2+1 shows distinct target binding properties compared with 1+1 format





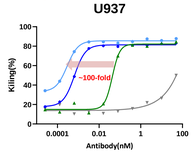


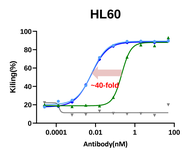
Lowered binding activity of 2+1 ABL602 to CD3



Increased binding activity of 2+1 ABL602 to CLL-1

ABL602 2+1 induces potent cytotoxic activity on AML cell lines compared with 1+1 format

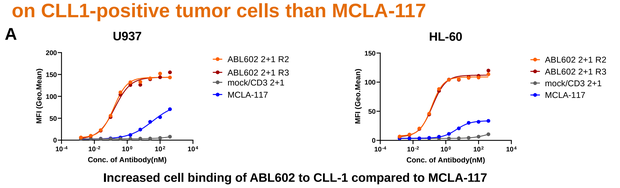




Activity of ABL602 was assessed with T cell-dependent cellular cytotoxicity (TDCC) assays. PBMC were incubated with target cells with ET ratio of 10:1 and ABL602 2+1 or 1+1 format. Cytotoxicity was assessed by flow cytometry after 48 h incubation at 37°C. Two T cell donors were tested per cell line, in 2 independent experiments. R represents G4S linker.

RESULT

ABL602 induces much stronger T cell activation and cytotoxicity on CLL1-positive tumor cells than MCLA-117



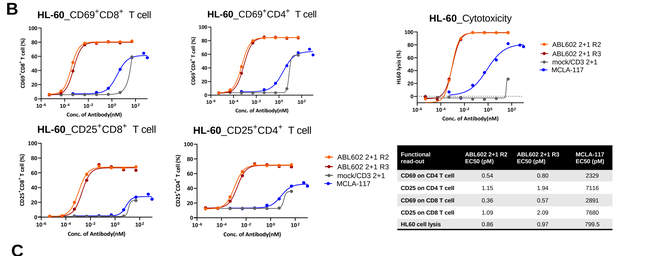
Increased cell binding of ABL602 to CLL-1 compared to MCLA-117

Functional read-out	ABL602 2+1 R2 EC50 (pM)	ABL602 2+1 R3 EC50 (pM)	MCLA-117 EC50 (pM)
CD69 on CD4 T cell	0.54	0.80	2329
CD25 on CD4 T cell	1.15	1.94	7116
CD69 on CD8 T cell	0.36	0.57	2891
CD25 on CD8 T cell	1.09	2.09	7680
HL60 cell lysis	0.86	0.97	799.5

Functional read-out	ABL602 2+1 R2 EC50 (pM)	ABL602 2+1 R3 EC50 (pM)	MCLA-117 EC50 (pM)
CD69 on CD4 T cell	0.21	0.61	1190
CD25 on CD4 T cell	0.39	1.23	2526
CD69 on CD8 T cell	0.14	~0.5	~713
CD25 on CD8 T cell	0.30	1.10	2879
U937 cell lysis	0.05	0.15	127.7

A.U937 and HL-60 cell lines were stained with various concentrations of ABL602, or MCLA 117 to characterize the surface-binding profiles of the bispecific antibody. HL-60 **(B)** or U937**(C)** suspension target cells (2 x 10^4 cells) were seeded onto 96-well U bottom plates. Various concentrations of ABL602, mock/CD3, or MCLA-117 and human purified T cells were added to the plates at an effector:target ratio of 5:1. After 48hrs, T cell activation and the number of remaining CD33 positive target cells were quantified by flow cytometry. T cells were analyzed by flow cytometry for cell surface levels of CD25 and CD69 as a markers of T cell activation.

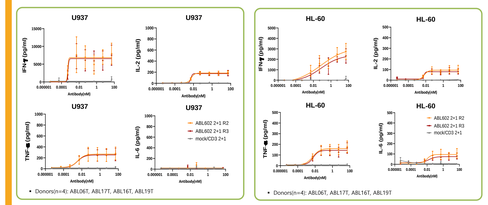
ABL602 induces potent T cell activation and cytotoxicity on CLL1-expressing cell lines but not on CLL1-negative cell lines



Cytotoxicity and T cell activation of ABL602 were assessed by flow cytometry after 48 hrs of incubation. Human purified T cells from 8 different donors were incubated with U937 cell line **(A),** HL-60 cell line **(B)**, or Raji cell line **(C)** with an ET ratio of 5:1 in the presence of serially diluted 2+1 ABL602. EC_{50} standard error of the mean is shown.

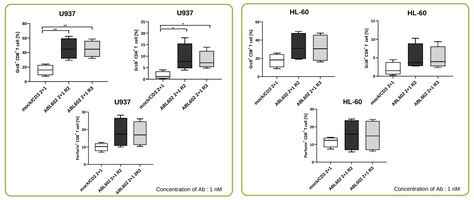
RESULT

ABL602 induces cytokine expression in the presence of U937 and HL-60 cell lines



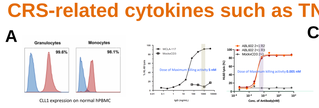
The purified T cells were treated with ABL602 2+1 either with U937 cell line or HL60 cell line for 48 h. The purified T cells were added at a 5:1 effector to target cell ratio. Supernatant was harvested to measure the human cytokine levels using ELISA. Cytokine levels were detectable for the four cytokines shown.

ABL602 induces Granzyme B and Perforin in the presence of U937 and HL-60 cell lines

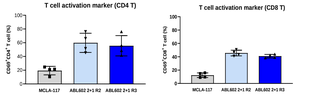


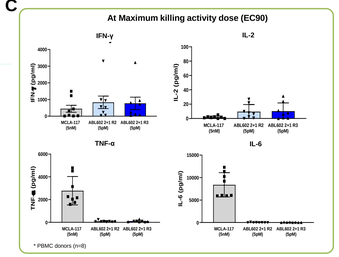
Human T cells are isolated from healthy PBMCs and then incubated with tumor cells and ABL602 2+1 or mock/CD3 2+1 for 24 h. After 5 hours of treatment with Golgi-plug(protein transport inhibitor), granzyme B and perforin protein were measured by flow cytometry.
* : P<0.5, ** : P<0.05

ABL602 strongly induces T cell activation and cytokine expression of IFN-γ and IL-2 but weakly CRS-related cytokines such as TNF-α and IL-6



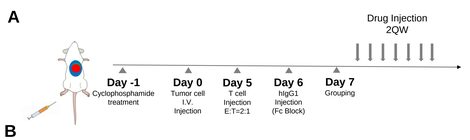
- Maximum killing activity dose of ABL602 (EC90) : 0.005nM
- Maximum killing activity dose of MCLA 117 (EC90) : 5nM

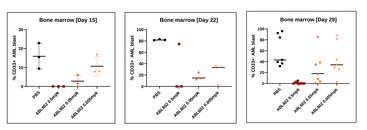


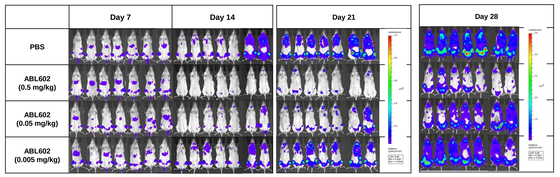


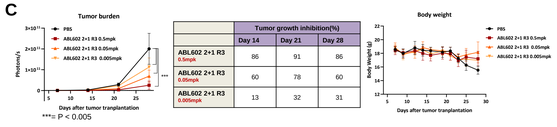
Both granulocytes and monocytes from peripheral blood cells express CLL1 antigen **(A, left)**. Dose curves show cytotoxicity of ABL602 or MCLA 117 in HL-60 cell line **(A, right)**. Healthy donor-derived PBMCs were cultured with ABL602 2+1 or MCLA-117 antibody for 48 hours. T cells were analyzed by flow cytometry for cell surface levels of CD25 and CD69 as a markers of T cell activation **(B)**. Supernatant was harvested to measure the human cytokine levels in these supernatants using ELISA. Cytokine levels were detectable for the four cytokines shown **(C)**.

ABL602 inhibits tumor growth in a dose-dependent manner in HL-60 orthotopic model

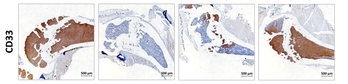








	Tumor growth inhibition(%)		
	Day 14	Day 21	Day 28
ABL602 2+1 R3 0.5mpk	86	91	86
ABL602 2+1 R3 0.05mpk	60	78	60
ABL602 2+1 R3 0.005mpk	13	32	31



To generate systemic luciferase labeled HL60 orthotopic model, 1×10^7 HL60-luc cells were injected IV on day 0. Animals were randomized into groups of 7 on day 6 by bioluminescence intensity. The four groups were PBS control , ABL602 2+1 R3 (0.5 mg/kg), ABL602 2+1 R3 (0.05 mg/kg), and ABL602 2+1 R3 (0.005 mg/kg). Beginning on Day 7, mice were treated IP twice a week with ABL602 2+1 R3 at 0.005, 0.05, and 0.5mg/kg or vehicle for a total of 7 doses **(A)**. ABL602 2+1 R3 at 0.005, 0.05, and 0.5 mg/kg resulted in a dose-dependent reduction of tumor burden in the bone marrow, spine, and hind limb as observed by bioluminescence **(B)**. ABL602 2+1 R3 at 0.005, 0.05, and 0.5 mg/kg significantly inhibited tumor growth as assessed by bioluminescence (91%, 78%, and 32%, respectively) compared with PBS-treated mice on day 21 **(C)**. Tumor cells in the bone marrow was measured by flow cytometric analysis and IHC staining **(D)**.

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