MCLA-117, A CLEC12AXCD3 BISPECIFIC IGG TARGETING A LEUKEMIC STEM CELL ANTIGEN, INDUCES T CELL MEDIATED AML BLAST LYSIS

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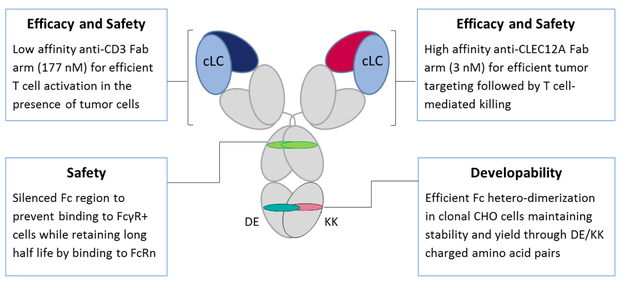
Introduction

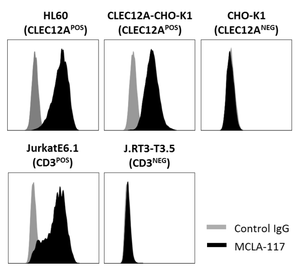
- Patients with acute myeloid leukemia (AML) have a dismal prognosis despite improvements in chemotherapy and supportive care. Novel, more effective therapies are needed for these patients.
- CLEC12A is a myeloid differentiation antigen that is expressed on 90-95% of newly diagnosed and relapsed AML. Moreover, CLEC12A is selectively expressed on leukemic stem cells (LSCs) but not on normal early hematopoietic progenitors, including hematopoietic stem cells (HSCs) *(Van Rhenen et al., 2007)*. This is in contrast to other AML targets like CD33 and CD123, which are more widely expressed on normal CD34^{POS} progenitors, including the pluripotent HSCs *(Taussig et al., 2005)*.
- Because of its more restricted expression profile, T cell-mediated CLEC12A targeting has the potential to selectively eradicate leukemic (stem) cells without affecting normal HSCs allowing the subsequent re-establishment of normal hematopoiesis.
- We report the characterization of MCLA-117, a novel T cell redirecting bispecific antibody for the treatment of AML that targets CLEC12A on leukemic cells and CD3 on T cells.

MCLA-117 bispecific antibody

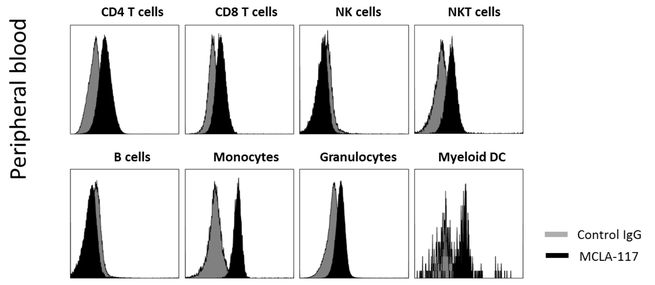
- MCLA-117 is a human full length IgG1 bispecific antibody that uses an IGKV1-39/IGKJ1 common light chain (cLC) together with anti-CLEC12A-specific VH and an anti-CD3-specific VH.
- The Fc portion of MCLA-117 was engineered to selectively facilitate heavy chain heterodimerization and to abrogate Fcγ receptor and C1q-mediated effector function.
- The MCLA-117 affinity for CLEC12A is 60-fold greater than for CD3, 3 nM vs 177 nM respectively, which is predicted to facilitate the preferential opsonization of the AML blasts with MCLA-117.



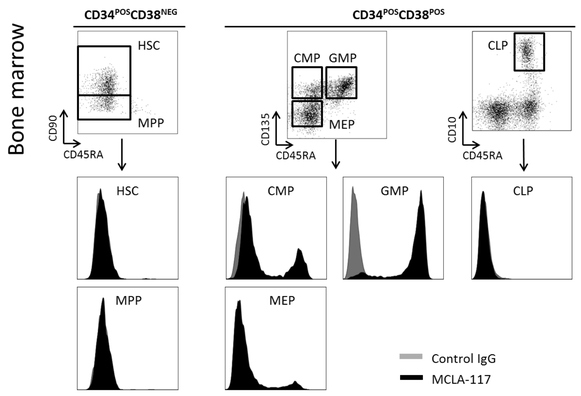
- Flow cytometry using tumor cell lines illustrating CLEC12A and CD3 target antigen binding specificity of MCLA-117.



Restricted MCLA-117 binding profile



- MCLA-117 binding profiling in normal peripheral blood confirms that MCLA-117 specifically binds to CD3 expressing T cells and CLEC12A expressing myeloid cells, including monocytes and granulocytes.

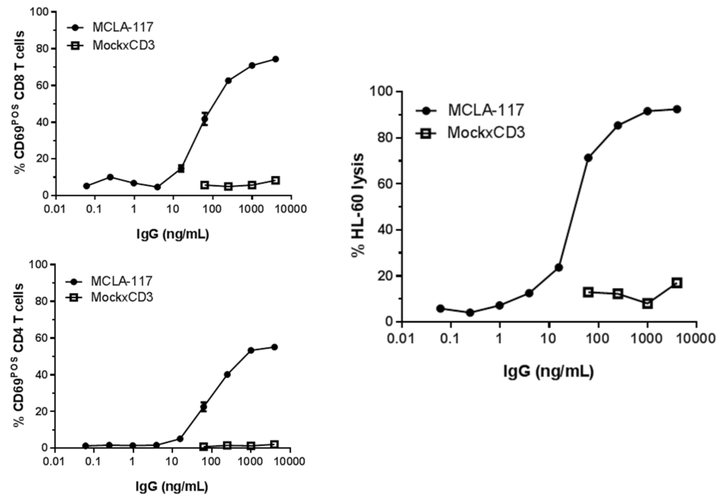


HSC: hematopoietic stem cell; MPP: multipotent progenitor; CMP: common myeloid progenitor; GMP: granulocyte-macrophage progenitor; MEP: megakaryocyte-erythroid progenitor; CLP: common lymphoid progenitor.

- Analysis of the normal bone marrow demonstrates that the MCLA-117 binding profile within the CD34^{POS} progenitor compartment resembles the described CLEC12A expression profile and is primarily restricted to the granulocyte-macrophage progenitor (GMP). Most importantly, MCLA-117-binding is not observed within the CD34^{POS}D38^{NEG} compartment, which includes the HSC.

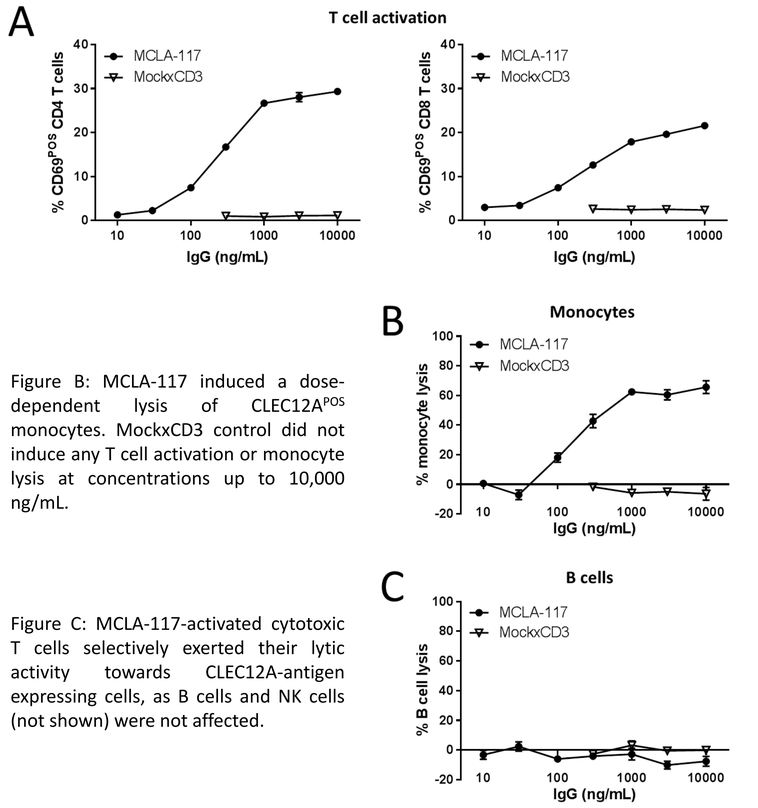
MCLA-117 induces T cell-mediated target cell lysis

- The capacity of MCLA-117 to induce CLEC12A antigen-dependent cytotoxicity was first investigated using healthy donor-derived resting T effector cells and CLEC12A^{POS} HL-60 target cells.
- Purified T cells were co-cultured for 48 hours with HL-60 cells at an effector to target (E:T) ratio of 5:1 in the presence of MCLA-117 or the MockxCD3 control antibody (identical Fc silenced format as MCLA-117).
- These experiments revealed that MCLA-117 efficiently induced CLEC12A antigen dependent T cell activation (EC_{50} of 44 ng/mL) and tumor target cell lysis (EC_{50} of 66±37 ng/mL) (n=6 donors).



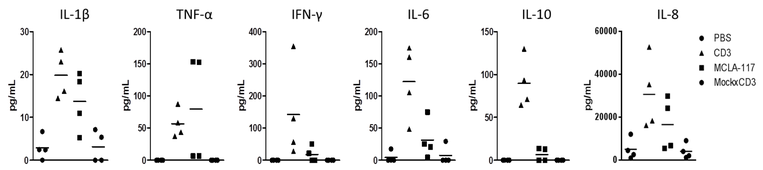
MCLA-117 selectively induces lysis of CLEC12A^{POS} monocytes

- To assess the capacity of MCLA-117 to redirect and activate autologous T cells to induce specific lysis of primary monocytes (expressing CLEC12A) a PBMC-based cytotoxicity assay was performed.
- Primary PBMC samples, at naturally occurring E:T ratios, were incubated for 48 hours with a concentration range of MCLA-117 or MockxCD3 control antibody.
- Figure A: MCLA-117 induced a dose-dependent activation of CD4 T cells and CD8 T cells .
- Figure B: MCLA-117 induced a dose-dependent lysis of CLEC12A^{POS} monocytes. MockxCD3 control did not induce any T cell activation or monocyte lysis at concentrations up to 10,000 ng/mL.
- Figure C: MCLA-117-activated cytotoxic T cells selectively exerted their lytic activity towards CLEC12A-antigen expressing cells, as B cells and NK cells (not shown) were not affected.

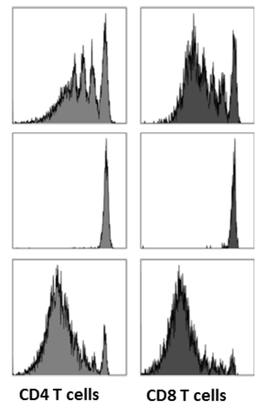


MCLA-117 induces CLEC12A antigen-dependent cytokine release

- Next, cytokine analysis was performed in these PBMC cultures after 48 hours of MCLA-117 exposure at 1000 ng/mL.
- MCLA-117 induced CLEC12A-dependent cytokine release. In general, MCLA-117-induced cytokine levels were lower compared to those obtained after stimulation with the bivalent anti-CD3 positive control antibody.



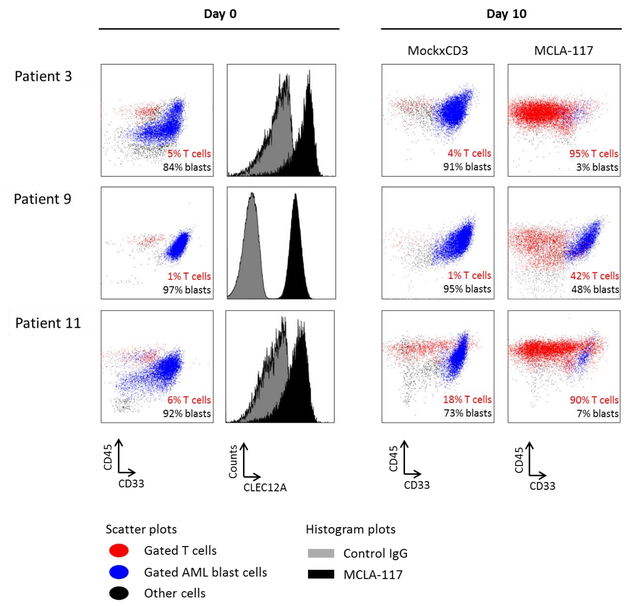
MCLA-117-induces T cell proliferation



- Purified healthy donor derived monocytes were co-cultured with CFSE-labelled resting autologous T cells at an E:T ratio of 5:1 with MCLA-117 or MockxCD3 control antibody at 1000 ng/mL.
- T cell proliferation was demonstrated by the reduction of CFSE positive T cells: at each cell division the amount of cell dye is split over the two daughter cells.
- MCLA-117 efficiently induced CLEC12A-antigen specific proliferation of both CD4 and CD8 T cells at 100 and 1,000 ng/mL, while the MockxCD3 control did not induce any proliferation.

MCLA-117 efficiently induces T cell mediated lysis of AML blasts

- The efficacy of MCLA-117 to induce lysis of AML blasts by autologous T cells in primary AML bone marrow samples with inherently low T cell to AML blast ratios was examined in an *ex vivo* culture system.
- In the primary AML samples tested (listed in Table below), T cells were relatively scarce compared to the AML blasts resulting in low effective E:T ratios (1:3-1:97). FACS analysis revealed patient variance in CLEC12A expression levels.
- The primary AML samples were cultured for 10 days in the presence of a cytokine cocktail to support AML blast and T cell survival during the assay.



- MCLA-117 efficiently induced CLEC12A-mediated T cell expansion (range 7-226-fold) at a concentration of 200 ng/mL. More importantly, MCLA-117-induced AML blast lysis was observed at day 7 and day 10 (phenotypic analysis of the cultures at day 10 shown in Figure above). At day 10, MCLA-117 efficiently induced AML blast lysis (31-99%) in 9/11 patient samples (Table below).
- MCLA-117 has the capacity to induce T cell mediated lysis of primary AML cells, even in AML samples with very low effector to target ratios.

Table: MCLA-117-induced AML blast lysis and T cell expansion in primary AML samples

Patient no.	FAB classification	Risk classification	% CLEC12A positive *	E:T ratio	Recovery in control condition	% Blast killing	Fold T cell expansion
1	M1	Good	39%	1:45	240%	95%	20
2	M2	Good	91%	1:3	52%	99%	39
3	M2	Poor	96%	1:17	609%	87%	226
4	M4	Very poor	88%	1:13	225%	91%	64
5	M4	Poor	99%	1:94	155%	0%	7
6	M4/M5	Very poor	79%	1:32	373%	31%	157
7	M4/M5	Intermediate	58%	1:49	41%	39%	25
8	M4/M5	Poor	99%	1:46	1131%	38%	23
9	M4/M5	Poor	100%	1:97	234%	39%	55
10	M4/M5	Intermediate	74%	1:31	426%	0%	9
11	M4/M5	Intermediate	94%	1:15	57%	67%	130

**Percentage CLEC12A positive AML blasts in sample. Threshold for CLEC12A expression was set based on CLEC12A negative lymphocytes fraction. Patient no.: patient number.*

Conclusion

- **MCLA-117 binds specifically to CD3 and CLEC12A expressing cells within the normal hematopoietic compartment, but not to early myeloid progenitors or hematopoietic stem cells.**
- **MCLA-117 efficiently induces CLEC12A-antigen specific T cell activation, T cell proliferation and redirects T cells to lyse CLEC12A^{POS} target cells.**
- **MCLA-117 efficiently induces CLEC12A-mediated lysis of AML blasts by T cells present in bone marrow samples, even at very low E:T ratios, and in parallel results in robust T cell proliferation.**
- **MCLA-117 is currently being investigated in a Phase I clinical study (MCLA-117-CL01) to evaluate the safety, tolerability and preliminary efficacy of MCLA-117 in adult AML patients.**