[0013] FIG. 9: CD8+ T cell compartment of AML patients compared to healthy donors.

[0014] FIG. 10: Specific CD3xCLEC12A DM-Fc induced T cell activation and HL60 tumor cell lysis by AML patient T cells.

[0015] FIG. 11: Specific lysis of AML blasts by autologous AML patient T cells.

[0016] FIG. 12: Specific monocyte lysis by patient T cells.

[0017] FIG. 13: Fc silencing significantly eliminates bystander cell cytokine release.

[0018] FIG. 14A: FACS staining anti-CD3 antibodies on HPB-ALL cells

[0019] FIG. 14B: Plate bound IgG, T cells labeled with CFSE, read out at day 5 by FACS

[0020] FIG 15:HL60 cytotoxicity assay

[0021] FIG 16: FACS staining anti-CLEC12A antibodies on HL60 cells

[0022] FIG. 17: HL60 cytotoxicity assay

[0023] FIG 18: FACS analysis

[0024] FIG 19: HL60 cytotoxicity assay

[0025] FIG 20: VH sequences of CD3-specific and CLEC12A-specific Fab arms. VL sequence of O12 common light chain. CDR sequences are bold and underlined.

SUMMARY

[0026] The present disclosure describes a fully human IgG bispecific full length antibody for the treatment of AML. One arm of the antibody binds an epitope on immune effector cells, preferably CD3, whilst the other arm targets CLEC12A, a myeloid cell specific surface target that is expressed in 90-95% of de novo and relapsed AML patients. CLEC12A is expressed on AML leukemic stem cells, but not on normal haematopoietic cells. Unlike CD33, CLEC12A is not expressed on erythroid precursors or megakaryocytes, so the CD3xCLEC12A bispecific IgG1 antibody of the present disclosure should not induce platelet or red blood cell depletion.Experiments with bone marrow cell colonies have shown that depletion of CLEC12A+ cells in normal bone marrow does not affect the myeloid lineages that give rise to platelets and red blood cells. A CD3xCLEC12A bispecific IgG antibody according to the present disclosure in a preferred example contains a modified Fc region so as to reduce non-specific immune activation resulting from engagement of T cells and FcγR expressing cells within PBMC. Based

on data described for the triomAb bispecific antibody in the prior art it was highly doubted that a CD3xTAA bispecific IgG of a fully human IgG1 format would be able to induce lytic anti-tumor activity in resting peripheral blood lymphocytes without the need for pre-activation of T cells. In addition, the available data for the BiTE® format suggested that a full length IgG molecule would be too large to create effective cytolytic synapses between tumor cells and effector cells. Surprisingly, we demonstrated that a fully human CD3xCLEC12A bispecific full length IgG1 was able to induce very efficient T cell mediated lysis of CLEC12A-positive HL60 AML tumor cells in vitro. In fact, effective lysis was mediated by resting T lymphocytes purified from PBMC without the need of prior activation of the T cells. Furthermore, we demonstrated that this lytic activity is not necessarily dependent on interactions with FcγR present on HL60 cells as this lytic activity was not affected by the presence of excess human IgG when the assay was performed in human serum containing media. This is the first time that a full length human IgG1 bispecific T cell engager antibody exerts efficient tumor cell lysis without the need of pre-activation of T cells or the need of active FcγR interactions. Effective lysis is achieved despite the relatively large size of the IgG1 when compared to BiTE® molecules. Remarkably, when CH2/lower hinge mutations were introduced in the CD3xCLEC12A bispecific IgG1 molecule to further decrease Fc receptor interactions, this still resulted in efficient tumor cell lysis by immune effector cells. A bispecific human IgG1 T cell engager antibody has advantages over current IgG that make use of the hybrid subclass combination mouse IgG2a/rat IgG2b, since a human IgG1 will be less immunogenic and can thus be applied for repeated therapy. In addition, a full length bispecific human IgG1 T cell engager antibody has advantages over immunoglobulin/like molecules such as DART™, TandAb® or BiTE® as the full length human IgG1 is not rapidly cleared from the circulation and dosing of patients will thus not require continuous infusion, which is more beneficial to patients.

[0026a] In one aspect, the present disclosure refers to a bispecific immunoglobulin, wherein said bispecific immunoglobulin comprises one arm that specifically recognizes CLEC12A, or CLL 1 or DCAL 2 or MICL or KLRL1, and a second arm that specifically recognizes an antigen on immune effector cells capable of recruiting such cells to an aberrant cell expressing CLEC12A or CLL 1 or DCAL 2 or MICL or KLRL1, wherein both arms comprise a common light chain.

[0026b] In another aspect, the present disclosure refers to a method for producing a bispecific immunoglobulin as disclosed herein from a single cell, wherein said bispecific immunoglobulin comprises two CH3 domains that are capable of forming an interface, said method comprising providing:

- a cell comprising a) a first nucleic acid sequence encoding an immunoglobulin heavy chain that specifically recognizes CLEC12A and that comprises a 1st CH3 domain, and b) a second nucleic acid sequence encoding an immunoglobulin heavy chain that specifically recognizes an antigen on immune effector cells, preferably CD3, and that comprises a 2nd CH3 domain, wherein said nucleic acid sequences are provided with amino acid substitutions in the 1st and 2nd CH3 domains for preferential pairing of said 1st and 2nd CH3 domains, said method further comprising the step of culturing said cell and allowing for expression of said two nucleic acid sequences and harvesting said bispecific immunoglobulin from the culture.

[0026c] In another aspect, the present disclosure refers to a pharmaceutical composition comprising a bispecific immunoglobulin as disclosed herein and a pharmaceutically acceptable carrier.

[0026d] In another aspect, the present disclosure refers to a bispecific immunoglobulin as disclosed herein for use as a pharmaceutical in the treatment of myelodysplastic syndrome (MDS), chronic myelogenous leukemia (CML) or preferably acute myeloid leukemia (AML).

[0026e] In another aspect, the present disclosure refers to use of a bispecific immunoglobulin as disclosed herein in the preparation of a medicament for the treatment or prevention of myelodysplastic syndrome (MDS), chronic myelogenous leukemia (CML) or preferably acute myeloid leukemia (AML).

EMBODIMENTS

[0027] The invention provides a bispecific IgG antibody, wherein said bispecific IgG antibody comprises one arm that specifically recognizes CLEC12A or a functional equivalent thereof, and a second arm that specifically recognizes an antigen on immune effector cells capable of recruiting such cells to an aberrant cell expressing CLEC12A or said functional equivalent.

CLAIMS

1. A bispecific immunoglobulin, wherein said bispecific immunoglobulin comprises one arm that specifically recognizes CLEC12A, or CLL 1 or DCAL 2 or MICL or KLRL1, and a second arm that specifically recognizes an antigen on immune effector cells capable of recruiting such cells to an aberrant cell expressing CLEC12A or CLL 1 or DCAL 2 or MICL or KLRL1, wherein both arms comprise a common light chain.

2. The bispecific immunoglobulin according to claim 1, wherein said immune effector cells comprise T cells.

3. The bispecific immunoglobulin according to claim 1 or 2, wherein said antigen on said immune effector cells is CD3.

4. The bispecific immunoglobulin according to claim 3, wherein said antibody specifically recognizes CD3ε.

5. The bispecific immunoglobulin according to any one of claims 1-4, wherein said common light chain is a germline light chain, preferably the rearranged germline human kappa light chain IgVκ1-39*01/IGJκ1*01.

6. The bispecific immunoglobulin according to any one of claims 1-5, wherein said bispecific immunoglobulin is a human IgG antibody, preferably a human IgG1.

7. The bispecific immunoglobulin according to any one of claims 1-6, wherein the arm that specifically recognizes CLEC12A, or CLL 1 or DCAL 2 or MICL or KLRL1, comprises a heavy chain CDR1 sequence consisting of a sequence that is at least 90% identical to SGYTFTSY and a heavy chain CDR2 sequence consisting of a sequence that is at least 90% identical to IINPSGGS
and a heavy chain CDR3 sequence consisting of a sequence that is at least 90% identical to GTTGDWFDY.

8. The bispecific immunoglobulin according to claim 7, wherein the arm that specifically recognizes CLEC12A, or CLL 1 or DCAL 2 or MICL or KLRL1, comprises a variable heavy chain sequence consisting of a sequence that is at least 90% identical to QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGS TSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAKGTTGDWFDYWGQGTLV TVSS.

9. The bispecific immunoglobulin according to any one of claims 1-6, wherein the arm that specifically recognizes CLEC12A, or CLL 1 or DCAL 2 or MICL or KLRL1, comprises a heavy chain CDR1 sequence consisting of a sequence that is at least 90% identical to SGYTFTSY and a heavy chain CDR2 sequence consisting of a sequence that is at least 90% identical to IINPSGGS and a heavy chain CDR3 sequence consisting of a sequence that is at least 90% identical to GNYGDEFDY.

10. The bispecific immunoglobulin according to claim 9, wherein the arm that specifically recognizes CLEC12A, or CLL 1 or DCAL 2 or MICL or KLRL1, comprises a variable heavy chain sequence consisting of a sequence that is at least 90% identical to EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGS TSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGNYGDEFDYWGQGTLV TVSS.

11. The bispecific immunoglobulin according to any one of claims 1-6, wherein the arm that specifically recognizes CLEC12A, or CLL 1 or DCAL 2 or MICL or KLRL1, comprises a heavy chain CDR1 sequence consisting of a sequence that is at least 90% identical to SGYTFTGY and a heavy chain CDR2 sequence consisting of a sequence that is at least 90% identical to WINPNSGG and a heavy chain CDR3 sequence consisting of a sequence that is at least 90% identical to DGYFADAFDY.

12. The bispecific immunoglobulin according to claim 11, wherein the arm that specifically recognizes CLEC12A, or CLL 1 or DCAL 2 or MICL or KLRL1, comprises a variable heavy

chain sequence consisting of a sequence that is at least 90% identical to QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSG GTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDGYFADAFDYWGQGT LVTVSS.

13. The bispecific immunoglobulin according to any one of claims 3-12, wherein the second arm that specifically recognizes CD3 comprises a heavy chain CDR1 sequence consisting of the sequence SYGMH and a heavy chain CDR2 sequence consisting of the sequence IIWYSGSKKNYADSVKG and a heavy chain CDR3 sequence consisting of the sequence GTGYNWFDP.

14. The bispecific immunoglobulin according to claim 13, wherein the second arm that specifically recognizes CD3 comprises a variable heavy chain sequence consisting of the sequence QVQLVESGGGVVQPGRSLRLSCAASGFTFRSYGMHWVRQAPGKGLEWVAIIWYSGSK KNYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGTGYNWFDPWGQGTLV TVSS.

15. The bispecific immunoglobulin according to any one of claims 1-14, wherein the first and the second arms further comprise a light chain CDR1 sequence consisting of a sequence that is at least 90% identical to RASQSISSYLN and a light chain CDR2 sequence consisting of a sequence that is at least 90% identical to AASSLQS and a light chain CDR3 sequence consisting of a sequence that is at least 90% identical to QQSYSTPPT.

16. The bispecific immunoglobulin according to claim 15, wherein the first and the second arms comprise a variable light chain sequence consisting of a sequence that is at least 90% identical to DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIK.

17. The bispecific immunoglobulin according to any one of claims 1-16, wherein said bispecific immunoglobulin comprises mutated CH2 and/or lower hinge domains such that interaction of said bispecific immunoglobulin with Fcγ receptors is significantly reduced.

18. The bispecific immunoglobulin according to claim 17, wherein said mutated CH2 and/or lower hinge domains comprise at least one substitution at amino acids position 235 and/or 236.

19. The bispecific immunoglobulin according to claim 17 or 18, wherein said mutated CH2 and/or lower hinge domains comprise substitution L235G and/or G236R, preferably L235G and G236R.

20. A method for producing a bispecific immunoglobulin according to any one of claims 1-19 from a single cell, wherein said bispecific immunoglobulin comprises two CH3 domains that are capable of forming an interface, said method comprising providing:
- a cell comprising a) a first nucleic acid sequence encoding an immunoglobulin heavy chain that specifically recognizes CLEC12A and that comprises a 1st CH3 domain, and b) a second nucleic acid sequence encoding an immunoglobulin heavy chain that specifically recognizes an antigen on immune effector cells, preferably CD3, and that comprises a 2nd CH3 domain, wherein said nucleic acid sequences are provided with amino acid substitutions in the 1st and 2nd CH3 domains for preferential pairing of said 1st and 2nd CH3 domains, said method further comprising the step of culturing said cell and allowing for expression of said two nucleic acid sequences and harvesting said bispecific immunoglobulin from the culture.

21. A method according to claim 20, wherein said cell has a third nucleic acid sequence encoding a common light chain, preferably the rearranged germline human kappa light chain IgVκ1-39*01/IGJκ1*01.

22. A method according to claim 20 or 21 for producing a bispecific immunoglobulin, wherein said first CH3 domain comprises the amino acid substitutions L351K and T366K and wherein said second CH3 domain comprises the amino acid substitutions L351D and L368E,

said method further comprising the step of culturing said cell and allowing for expression of said nucleic acid sequences and harvesting said bispecific immunoglobulin from the culture.

23. A pharmaceutical composition comprising a bispecific immunoglobulin of any one of claims 1-19 and a pharmaceutically acceptable carrier.

24. A bispecific immunoglobulin of any one of claims 1-19 for use as a pharmaceutical in the treatment of myelodysplastic syndrome (MDS), chronic myelogenous leukemia (CML) or preferably acute myeloid leukemia (AML).

25. Use of a bispecific immunoglobulin according to any one of claims 1-19 in the preparation of a medicament for the treatment or prevention of myelodysplastic syndrome (MDS), chronic myelogenous leukemia (CML) or preferably acute myeloid leukemia (AML).