therapy is unknown but modulating the cytokine milieu of the tumor microenvironment may contribute to the therapeutic efficacy of the antibody. In addition, the antibody induces antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) of AML cell lines *in vitro* (Sutherland et al., Mabs 1:5, 481-490, 2009). ADCC is considered to be a decisive mechanism for anti-tumor activity of antibodies in hematological malignancies. Data from clinical trials with the CD20-specific monoclonal antibody Rituximab have demonstrated the significance of effector cell mediated mechanisms for treatment of B-cell malignancies with respect to response to antibody treatment (Weng and Levy, J Clin Oncol. 21 (21): 3940-7, 2003).

In conclusion, it has been shown that CD33 antigen is expressed on normal cells of the myelomonocytic lineage and frequently expressed on tumor cells in myeloid leukemias. In a phase I trial with an antibody against CD33 (lintuzumab) first signs of efficacy were observed without severe adverse events. However, clinical development of lintuzumab was discontinued after results from a phase II trial in combination with chemotherapy did not yield the expected improvement in efficacy. Therefore, there is a clear need for the development of improved CD33-targeting treatment modalities.

In view of the prior art there is a need for providing a further improved therapy for myeloid cell malignancies and MDS, particularly for acute myeloid leukemia.

In particular there is a need for providing further improved antagonistic binding agents to CD33 for treating cancer, in particular AML.

Summary ~~of the invention~~

The present ~~invention~~disclosure provides novel CD33 binding agents binding to human CD33 and that are defined by

a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from SeqID No:1-14,

CDR2 has an amino acid sequence selected from SeqID No:14-28, CDR3 has an amino acid sequence selected from SeqID No:29-42, CDR4 has an amino acid sequence selected from SeqID No:43-56, CDR5 has an amino acid sequence selected from SeqID No:57-70, CDR6 has an amino acid sequence selected from SeqID No:71-84, or

b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (SeqID No: 141) of human CD33.

In a first aspect, there is provided a CD33 binding agent selected from

an antibody comprising a CDR1 of SeqID No: 1, a CDR2 of SeqID No: 15, a CDR3 of SeqID No: 29, a CDR4 of SeqID No: 43, a CDR5 of SeqID No: 57 and a CDR6 of SeqID No: 71,

an antibody comprising a CDR1 of SeqID No: 2, a CDR2 of SeqID No: 16, a CDR3 of SeqID No: 30, a CDR4 of SeqID No: 44, a CDR5 of SeqID No: 58 and a CDR6 of SeqID No: 72,

an antibody comprising a CDR1 of SeqID No: 3, a CDR2 of SeqID No: 17, a CDR3 of SeqID No: 31, a CDR4 of SeqID No: 45, a CDR5 of SeqID No: 59 and a CDR6 of SeqID No: 73,

an antibody comprising a CDR1 of SeqID No: 4, a CDR2 of SeqID No: 18, a CDR3 of SeqID No: 32, a CDR4 of SeqID No: 46, a CDR5 of SeqID No: 60 and a CDR6 of SeqID No: 74,

an antibody comprising a CDR1 of SeqID No: 5, a CDR2 of SeqID No: 19, a CDR3 of SeqID No: 33, a CDR4 of SeqID No: 47, a CDR5 of SeqID No: 61 and a CDR6 of SeqID No: 75,

an antibody comprising a CDR1 of SeqID No: 6, a CDR2 of SeqID No: 20, a CDR3 of SeqID No: 34, a CDR4 of SeqID No: 48, a CDR5 of SeqID No: 62 and a CDR6 of SeqID No: 76,

an antibody comprising a CDR1 of SeqID No: 7, a CDR2 of SeqID No: 21, a CDR3 of SeqID No: 35, a CDR4 of SeqID No: 49, a CDR5 of SeqID No: 63 and a CDR6 of SeqID No: 77,

an antibody comprising a CDR1 of SeqID No: 8, a CDR2 of SeqID No: 22, a CDR3 of SeqID No: 36, a CDR4 of SeqID No: 50, a CDR5 of SeqID No: 64 and a CDR6 of SeqID No: 78,

an antibody comprising a CDR1 of SeqID No: 9, a CDR2 of SeqID No: 23, a CDR3 of SeqID No: 37, a CDR4 of SeqID No: 51, a CDR5 of SeqID No: 65 and a CDR6 of SeqID No: 79,

an antibody comprising a CDR1 of SeqID No: 10, a CDR2 of SeqID No: 24, a CDR3 of SeqID No: 38, a CDR4 of SeqID No: 52, a CDR5 of SeqID No: 66 and a CDR6 of SeqID No: 80,

an antibody comprising a CDR1 of SeqID No: 11, a CDR2 of SeqID No: 25, a CDR3 of SeqID No: 39, a CDR4 of SeqID No: 53, a CDR5 of SeqID No: 67 and a CDR6 of SeqID No: 81,

an antibody comprising a CDR1 of SeqID No: 12, a CDR2 of SeqID No: 26, a CDR3 of SeqID No: 40, a CDR4 of SeqID No: 54, a CDR5 of SeqID No: 68 and a CDR6 of SeqID No: 82,
an antibody comprising a CDR1 of SeqID No: 13, a CDR2 of SeqID No: 27, a CDR3 of SeqID No: 41, a CDR4 of SeqID No: 55, a CDR5 of SeqID No: 69 and a CDR6 of SeqID No: 83,
an antibody comprising a CDR1 of SeqID No: 14, a CDR2 of SeqID No: 28, a CDR3 of SeqID No: 42, a CDR4 of SeqID No: 56, a CDR5 of SeqID No: 70 and a CDR6 of SeqID No: 84.

The present ~~invention~~disclosure further provides CD33 binding agents wherein the internalization kinetics of the CD33 binding agents are such that at least 30%, preferably 40%, of the initial amount of the antibody remain on the cell surface of HL60 cells at a time of 4 hours after incubation.

The present ~~invention~~disclosure further provides CD33 binding agents, wherein the heavy chain variable region comprises an amino acid sequence selected from SeqID No:85-98 and the light chain variable region comprises an amino acid sequence selected from SeqID No:99-112.

In a second aspect, there is provided a CD33 binding agent selected from
an antibody comprising a heavy chain variable region of SeqID No: 85 and a light chain variable region of SeqID No: 99,
an antibody comprising a heavy chain variable region of SeqID No: 86 and a light chain variable region of SeqID No: 100,
an antibody comprising a heavy chain variable region of SeqID No: 87 and a light chain variable region of SeqID No: 101,
an antibody comprising a heavy chain variable region of SeqID No: 88 and a light chain variable region of SeqID No: 102,
an antibody comprising a heavy chain variable region of SeqID No: 89 and a light chain variable region of SeqID No: 103,
an antibody comprising a heavy chain variable region of SeqID No: 90 and a light chain variable region of SeqID No: 104,

an antibody comprising a heavy chain variable region of SeqID No: 91 and a light chain variable region of SeqID No: 105,
an antibody comprising a heavy chain variable region of SeqID No: 92 and a light chain variable region of SeqID No: 106,
an antibody comprising a heavy chain variable region of SeqID No: 93 and a light chain variable region of SeqID No: 107,
an antibody comprising a heavy chain variable region of SeqID No: 94 and a light chain variable region of SeqID No: 108,
an antibody comprising a heavy chain variable region of SeqID No: 95 and a light chain variable region of SeqID No: 109,
an antibody comprising a heavy chain variable region of SeqID No: 96 and a light chain variable region of SeqID No: 110,
an antibody comprising a heavy chain variable region of SeqID No: 97 and a light chain variable region of SeqID No: 111,
an antibody comprising a heavy chain variable region of SeqID No: 98 and a light chain variable region of SeqID No: 112.

The present ~~invention~~disclosure further provides CD33 binding agents, wherein the heavy chain having an amino acid sequence selected from SeqID No:113-126 and the light chain having an amino acid sequence selected from SeqID No: 127-140.

In a third aspect, there is provided a CD33 binding agent selected from
an antibody comprising a heavy chain of SeqID No: 113 and a light chain of SeqID No: 127,
an antibody comprising a heavy chain of SeqID No: 114 and a light chain of SeqID No: 128,
an antibody comprising a heavy chain of SeqID No: 115 and a light chain of SeqID No: 129,
an antibody comprising a heavy chain of SeqID No: 116 and a light chain of SeqID No: 130,
an antibody comprising a heavy chain of SeqID No: 117 and a light chain of SeqID No: 131,
an antibody comprising a heavy chain of SeqID No: 118 and a light chain of SeqID No: 132,
an antibody comprising a heavy chain of SeqID No: 119 and a light chain of SeqID No: 133,
an antibody comprising a heavy chain of SeqID No: 120 and a light chain of SeqID No: 134,
an antibody comprising a heavy chain of SeqID No: 121 and a light chain of SeqID No: 135,
an antibody comprising a heavy chain of SeqID No: 122 and a light chain of SeqID No: 136,
an antibody comprising a heavy chain of SeqID No: 123 and a light chain of SeqID No: 137,
an antibody comprising a heavy chain of SeqID No: 124 and a light chain of SeqID No: 138,

an antibody comprising a heavy chain of SeqID No: 125 and a light chain of SeqID No: 139,
an antibody comprising a heavy chain of SeqID No: 126 and a light chain of SeqID No: 140.

The present ~~invention~~disclosure further provides CD33 binding agents having mutations in the F_c domain that increase ADCC.

Further preferred embodiments are outlined in the following specification and in the claims.

The CD33 binding agents according to the present ~~invention~~disclosure have been found to have high affinity to human CD33 and further to have favourable internalization kinetics, which is characterized by a long presence of the CD33 binding agents when bound to CD33 on the surface of the target cells, which translates into

favourable ADCC activity.

The inventors have also found that the CD33 binding agents according to the present ~~invention~~disclosure bind to a different epitope of the extracellular domain of CD33 compared to Lintuzumab. Without wishing to be bound by any particular theory this is believed to be the reason for the different internalization kinetics of the CD33 binding agents according to the present ~~invention~~disclosure and Lintuzumab.

In a fourth aspect, there is provided a DNA molecule comprising a region encoding the heavy chain variable region of a CD33 binding agent as defined above.

In a fifth aspect, there is provided a DNA molecule comprising a region encoding the light chain variable region of a CD33 binding agent as defined above.

In a sixth aspect, there is provided an expression vector containing a DNA molecule as defined above.

In a seventh aspect, there is provided a host cell carrying a DNA molecule as defined above and a DNA molecule as defined above.

In an eighth aspect, there is provided a method for producing a CD33 binding agent as defined above, comprising transfecting a host cell with one or more vectors as defined above, culturing the host cell and recovering and purifying the antibody molecule.

In a ninth aspect, there is provided a pharmaceutical composition comprising, as the active ingredient, one or more CD33 binding agents as defined above, and at least a physiologically acceptable carrier.

In a tenth aspect, there is provided the pharmaceutical composition as defined above for depleting cells that express CD33 on their surface.

In an eleventh aspect, there is provided the pharmaceutical composition as defined above for the treatment of myeloid cell malignancies and myelodysplastic syndrome (MDS).

In a twelfth aspect, there is provided the pharmaceutical composition as defined above for the treatment of autoimmune and inflammatory diseases that involve myeloid cells in their pathology.

In a thirteenth aspect, there is provided the use of one or more CD33 binding agents as defined above or a pharmaceutical composition as defined above in the manufacture of a medicament for the depletion of CD33 expressing myeloid cells in a patient.

Description of the Figures

Figures 1-3 show the internalization of exemplary CD33 binding agents according to the invention in comparison to lintuzumab on HL60 cells.
Figure 4 shows the internalization rate on HL60 cells of two exemplary antibodies according to the invention in comparison to lintuzumab.
Figure 5 shows the ADCC performance on HL60 cells of two exemplary antibodies according to the invention in comparison to lintuzumab.

Detailed description of the invention

CD33 Binding Agents

The term "binding agent" as used herein means a protein or peptide that specifically binds to a target antigen. A binding agent can be, for example, an antibody, a derivative of such an antibody, or other agent that specifically binds to the target antigen. A binding agent can also be a protein comprising an Fv region or a portion thereof (e.g., a V_H or V_L or a CDR(s) of an antibody that specifically binds to the target antigen). In a preferred embodiment herein the binding agent is

an antibody.

The term "CD33 binding agent" as used herein refers to a binding agent that specifically binds to CD33, typically a portion of the extracellular domain of human CD33.