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Anti-CD33-antibodies labelled with the alpha-emitter Bismuth-213 kill CD33-positive acute myeloid leukaemia cells specifically by activation of caspases and break radio- and chemoresistance by inhibition of the anti-apoptotic proteins X-linked inhibitor of apoptosis protein and B-cell lymphoma-extra large ☆

Claudia Friesen [a,b,*], Mareike Roscher [a,b], Inis Hormann [a,b], Oliver Leib [c], Sebastian Marx [c], Josue Moreno [c], Erich Miltner [a,b]

[a] *Center for Biomedical Research, University Ulm, Helmholtzstraße 8/1, 89081 Ulm, Germany*
[b] *Institute for Legal Medicine, University Ulm, Prittwitzstraße 6, 89075 Ulm, Germany*
[c] *Isotope Technologies Garching GmbH, Lichtenbergstraße 1, 85748 Garching, Germany*

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Abstract ***Aim:*** The emerging interest in radioimmunotherapies employing alpha-emitters for cancer treatment like high risk-leukaemia leads to the question of how these radionuclides exhibit their cytotoxicity. To clarify the molecular mechanisms of cell death induction, we investigated the molecular effects of the alpha-emitter Bismuth-213 (Bi-213) bound to a monoclonal anti-CD33-antibody ([Bi-213]anti-CD33) on the cell cycle and on apoptosis induction in sensitive as well as in beta- and gamma-radiation-resistant CD33-positive acute myeloid leukaemia (AML) cells.

Methods: The cytotoxic potential of the radioimmunoconjugate [Bi-213]anti-CD33 was analysed in the CD33-expressing human AML cell line HL-60 and in radiation- and chemoresistant HL-60-derived cell lines. Cell cycle and apoptosis induction analyses were performed via flow cytometry. Activation of apoptosis pathways was determined by immunodetection.

Results: [Bi-213]anti-CD33 induced apoptotic cell death in CD33-positive AML cells specifically. Molecular analyses revealed that the intrinsic mitochondrial pathway of apoptosis was activated resulting in caspase-9 activation. In the apoptotic executioner cascade caspase-3 was activated and its substrate poly (ADP-ribose) polymerase (PARP) was cleaved. Notably, [Bi-213]anti-CD33 overcame radio- and chemoresistance by reversing deficient activation of

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* *Corresponding author at:* Center for Biomedical Research, University of Ulm, Helmholtzstraße 8/1, D-89081 Ulm, Germany. Tel.: +49 731 500 65080; fax: +49 731 500 65003.
E-mail address: claudia.friesen@uni-ulm.de (C. Friesen).

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apoptosis pathways in resistant CD33-positive AML cells and by the downregulation of inhibitors of apoptosis B-cell lymphoma-extra large (Bcl-x_L) and X-linked inhibitor of apoptosis protein (XIAP) involved in leukaemia resistance.

Conclusion: [Bi-213]anti-CD33 exhibits its cytotoxic effects specifically in CD33-expressing AML cells via induction of the intrinsic, mitochondrial pathway of apoptosis. The abrogation of chemo- and radioresistances and the reactivation of apoptotic pathways seem to be promising for the treatment of patients with so far untreatable resistant AML and underline the importance of this emerging therapeutic approach of targeted alpha-therapies.

1. Introduction

Acute myeloid leukaemia (AML) is a heterogeneous disease with many subtypes classified according to morphology, phenotypes, cytogenetics and clinical behaviour determining the response to treatment.[1,2] Although remission rates in up to 70% of the patients can be achieved, many of these relapse with a poor outcome.[3]

One of the primary causes for therapeutic failure are resistances against conventional treatment modalities like chemotherapeutics and/or radiation caused by alterations in signalling pathways like proliferation or apoptosis.[4–6] During apoptosis either the mitochondrial pathway or the external pathway are induced by the specific binding of death ligands to their cognate receptors depending on the cytotoxic stimuli. The point-of-no-return in apoptosis induction is the activation of the caspase cascade leading to the concerted destruction of the cell.[7]

A quite novel treatment option for AML is the antibody-mediated targeting of the receptor CD33 which can be found on about 90% of myeloid leukaemia blasts and progenitors but not on bone marrow resident haematopoietic stem cells or non-haematopoietic tissues.[8–11] Antibodies against CD33 can be used as vehicles to deliver toxic agents into the cells as the receptor is internalised after antibody binding.[12] Gemtuzumab ozogamizin coupled with calicheamicin improves the outcome of relapsed AML patients[13] but nevertheless resistances against the cytostatic agent can occur. The monoclonal anti-CD33-antibody coupled with the alpha-emitter Bismuth-213 ([Bi-213]anti-CD33) could be shown in different pre-clinical and clinical trials of radioimmunotherapies (RIT) to be safe and efficient in its activity against AML and acute promyeloid leukaemia.[14–17]

As the underlying mechanisms of cell death induction have not been analysed for [Bi-213]anti-CD33, we investigated cytotoxicity and triggered signalling pathways of [Bi-213]anti-CD33 on radio-/chemosensitive as well as beta- and gamma-radiation-resistant CD33-positive AML cells.

2. Material and methods

2.1. Cell culture

The human AML cell line HL-60 ($CD33^{+/+}$, $HER2^{-/-}$) and the human T-cell leukaemia cell line CEM ($CD33^{-/-}$) were obtained from the DMSZ (Braunschweig, Germany). In suspension, all cell lines were grown in RPMI supplemented with 1 mmol/L glutamine, 1% penicillin/streptomycin (Invitrogen, Karlsruhe, Germany), 25 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Biochrom AG, Berlin, Germany) and 10% foetal calf serum (FCS) (Lonza, Verviers, Belgium) at 37 °C, 95% air/5% CO_2. HL-60^{gammaR} ($CD33^{+/+}$, $HER2^{-/-}$) are resistant to 10 Gy absorbed dose of γ-irradiation, HL-60^{betaR} ($CD33^{+/+}$, $HER2^{-/-}$) are resistant to activities of up to 876 kBq/mL of β-irradiation Yttrium-90 (Y-90). HL-60^{gammaR} and HL-60^{betaR} are cross-resistant to different anticancer drugs such as cisplatin, doxorubicin, etoposide and methotrexate. Before treatment, cells were seeded in a density of 1×10^5 cells/mL in 96 well plates or 175 cm^2 flasks. Spontaneous cell death of HL-60, HL-60^{gammaR} and HL-60^{betaR} cells which were untreated (controls) was in a range between 2% and 5%.

2.2. Radioimmunoconjugate

Bi-213 ($t_{1/2}$ 45.6 min) is considered an alpha-emitter due to its branching ratio of 98% to the pure and ultra short-lived alpha-emitter Polonium-213 (Po-213).[18] Activities were applied to the cells labelled to the respective antibody with a specific activity of ~1.0 MBq/μg antibody for the radioimmunoconjugates [Bi-213]anti-CD33 or [Bi-213]anti-HER2. Bi-213 was eluted from the established Actinium-225 (Ac-225)/Bi-213 generator system. After 5 min incubation with the radionuclide the labelled antibody was purified.

24 h, 48 h and 72 h after applying the radioimmunoconjugates [Bi-213]anti-CD33 or [Bi-213]anti-HER2, respectively, using 3.3, 11, 33, 110 and 330 kBq/mL of Bi-213, analyses were performed. The specific activity of ~1.0 MBq/μg antibody was used for all different activity concentrations.