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616.ACUTE MYELOID LEUKEMIAS: INVESTIGATIONAL THERAPIES, EXCLUDING TRANSPLANTATION AND CELLULAR IMMUNOTHERAPIES

Monoclonal Antibodies Targeting LIGHT Block Ltbr Signalling and Eliminate Acute Myeloid Leukemia Stem Cells

Sabine Hoepner, PhD[1], *Tim Delahaye*[2], *Carsten Riether, PhD*[1], *Adrian Ochsenbein, MD*[3]

[1] University and University Hospital of Bern, Bern, Switzerland
[2] ArgenX, Gent, BEL
[3] University Hospital of Bern, Bern, Switzerland

Introduction
Acute myeloid leukemia (AML) is a molecular heterogeneous disease that originates from the malignant transformation of a hematogenic stem- or progenitor cells resulting in a leukemia stem cell (LSC). LSCs are a main cause of resistance to therapy and relapse. Despite recent advances in targeted therapy and immunotherapy, prognosis for elderly patients not fit do undergo intensive therapy remains poor. We recently documented that targeting CD70, the ligand of the tumor necrosis factor receptor (TNFR) CD27, by the antibody-dependent cell-mediated cytotoxicity (ADCC)-enhanced monoclonal antibody (mAb) cusatuzumab eliminates LSC. We now studied the role of the related TNFR lymphotoxin b receptor (LTbR) with its ligand LIGHT in the pathogenesis of AML in mice and humans and developed a LIGHT targeting mAb to specifically eliminate LSCs.
Experimental design
We investigated the impact of LIGHT/LTbR signalling in AML development by using the retrovirally-induced MLL-AF9 syngeneic AML mouse model. *Light*-, *Ltbr* -deficient and control MLL-AF9 transduced LSCs were injected intravenously into non-irradiated BL/6 recipient mice. AML development was evaluated by analysing MLL-AF9-GFP-positive leukemic cells in blood, spleen, and bone marrow (BM). BM LSC were phenotypically characterized by FACS and functionally by performing colony forming unit (CFU) assays *in vitro* and by secondary transplantations of LSCs including limited dilution assays (LDA) *in vivo*.
Correlative survival analysis for *LIGHT* and *LTbR* expression was performed on a publicly available gene dataset (GSE6891). LIGHT and LTBR protein expression was analysed on primary AML CD34 $^{+}$ leukemic stem and progenitor cells (LSPCs) and control human CD34 $^{+}$ hematopoietic stem cell and progenitor cells (HSCPs). LIGHT/LTbR signalling was blocked by either siRNA treatment or by treatment with novel LIGHT-targeting mAb. The effect of blocking LIGHT/LTbR-signalling was analysed *in vitro* by CFU assays, RNA-sequencing and xenotransplant experiments *in vivo*.
LIGHT-targeting mAbs were generated by immunization of llamas and phage display selections followed by cloning of FABs into a human backbone (hIgG1). Clones were selected based on their blocking potency of receptor/ligand interaction using competition ELISA and FACS-based cell binding affinity assays. ADCC-enhanced anti-LIGHT mAbs were generated via POTELLIGENT® technology. Anti-LIGHT mAbs that specifically block the interaction of LIGHT/LTBR with deficiency in effector functions (Fc-death) were produced by amino acid substitutions in the CH2 domain of the parental clone.
Results
LTbR was expressed by HSCs and AML LSCs. In contrast, LIGHT was only expressed on murine and human LSCs but not normal HSCs. The absence of LIGHT or its receptor LTbR on LSCs resulted in significantly prolonged survival and decreased numbers of LSC in BM of AML mice. *Light* $^{-/-}$ and *Ltbr* $^{-/-}$ LSCs had a reduced CFU capacity compared to control LSCs. Similarly, inhibition of LIGHT-signalling on LSCs via LTbR-Ig significantly reduced colony formation. Moreover, secondary transplantation of *Light* $^{-/-}$LSCs resulted in prolonged survival compared to *Light*-proficient LSCs. An LDA analysis revealed a 11-fold reduction of the LSC frequency in BM in the absence of LIGHT or LTBR.
Similarly, inhibiting LIGHT signalling by siLIGHT RNA in human AML LSPCs resulted in reduced CFU capacity compared to control siRNA treatment. In addition, a GEO analysis revealed that low *LIGHT* or *LTbR* mRNA levels correlated with improved overall survival in AML patients.
After immunizations of llamas and phage-display selection three humanized anti-LIGHT mAbs with high blocking and high binding efficiency were selected (mAb10F11 and 12C2 specific for human LIGHT and the cross-reactive murine/human 9F6 mAB). Incubation of LSCs with the blocking mAbs significantly reduced CFU capacity. HSPCs from healthy donors remained

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unaffected by the treatment. A gene expression analysis revealed that blocking LIGHT/LTbR-signalling in LSCs reduced stemness and promoted differentiation. In addition, ADCC-enhanced mAbs activated NK cells to eliminate LIGHT-expressing LSCs. Our findings reveal that LIGHT/LTbR-signalling is crucial for the pathogenesis of AML and especially for the maintenance and expansion of LSCs. We developed and validated novel LIGHT targeting mAbs in vitro and in vivo for further development in clinical trials.

Disclosures Delahaye: *ArgenX:* Current Employment.

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