NEOPLASIA
www.neoplasia.com

Volume 20 Number xx Month 2018 pp. 1–11 1

Gemtuzumab Ozogamicin (GO) Inclusion to Induction Chemotherapy Eliminates Leukemic Initiating Cells and Significantly Improves Survival in Mouse Models of Acute Myeloid Leukemia

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Abstract

Gemtuzumab ozogamicin (GO) is an anti-CD33 antibody-drug conjugate for the treatment of acute myeloid leukemia (AML). Although GO shows a narrow therapeutic window in early clinical studies, recent reports detailing a modified dosing regimen of GO can be safely combined with induction chemotherapy, and the combination provides significant survival benefits in AML patients. Here we tested whether the survival benefits seen with the combination arise from the enhanced reduction of chemoresidual disease and leukemic initiating cells (LICs). Herein, we use cell line and patient-derived xenograft (PDX) AML models to evaluate the combination of GO with daunorubicin and cytarabine (DA) induction chemotherapy on AML blast growth and animal survival. DA chemotherapy and GO as separate treatments reduced AML burden but left significant chemoresidual disease in multiple AML models. The combination of GO and DA chemotherapy eliminated nearly all AML burden and extended overall survival. In two small subsets of AML models, chemoresidual disease following DA chemotherapy displayed hallmark markers of leukemic LICs (CLL1 and CD34). *In vivo*, the two chemoresistant subpopulations (CLL1+/CD117− and CD34+/CD38+) showed higher ability to self-renewal than their counterpart subpopulations, respectively. CD33 was coexpressed in these functional LIC subpopulations. We demonstrate that the GO and DA induction chemotherapy combination more effectively eliminates LICs in AML PDX models than either single agent alone. These data suggest that the survival benefit seen by the combination of GO and induction chemotherapy, nonclinically and clinically, may be attributed to the enhanced reduction of LICs.

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Introduction

Acute myeloid leukemia (AML) is the most common acute leukemia in adults. Treatment options for AML are limited, with standard first-line therapy consisting of a chemotherapy combination including cytarabine and an anthracycline (daunorubicin most commonly used). Despite favorable complete remission rates in young and old patients, early relapsed disease is common, and the prognosis of relapsed AML patients is poor as 5-year survival rates are low (30% in patients <60 years, 5%-10% in patients ≥60 years).

Gemtuzumab ozogamicin (GO) is a CD33-antibody-drug conjugate (ADC) composed of a humanized monoclonal antibody linked to the potent cytotoxic agent calicheamicin via a hydrazone linker. GO binds

Abbreviations: GO, gemtuzumab ozogamicin; AML, acute myeloid leukemia; DA, daunorubicin/cytarabine; NSG, nonobese diabetic severe combined immunodeficiency gamma; PDX, patient-derived xenograft; LIC, leukemic-initiating cell; PB, peripheral blood; BM, bone marrow; IV, intravenous

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Primary Scientific Category: Myeloid Neoplasia.
Received 26 September 2017; Revised 29 October 2017; Accepted 30 October 2017

1476-5586
https://doi.org/10.1016/j.neo.2017.10.008

the AML antigen CD33 and, upon internalization, releases its DNA-damaging warhead to kill the target AML cells [1]. CD33 is ubiquitously expressed on AML myeloblasts including those responsible for relapsed disease [2]. Mylotarg received FDA approval in 2000 for CD33-positive AML patients but was voluntary withdrawn in 2010 due to safety concerns. However, four other large trials were pursued simultaneously evaluating the combination of lower-dose GO with induction chemotherapy [3]. In a collective meta-analysis of individual patient data from these trials, the addition of GO to induction chemotherapy did not affect complete remission rates; however, it significantly reduced the risk of relapse and improved the relapse-free survival and overall survival of adult AML patients with favorable cytogenetics [3]. These data suggest that the inclusion of GO to induction chemotherapy results in more durable remissions in AML patients treated with the combination.

The poor long-term prognosis of standard induction (DA) therapy correlated with the levels of minimal residual disease (MRD) [4]. A high level of MRD was found to correlate with a higher percentage of chemoresistant leukemic stem cells, which are characterized by their inherent ability for self-renewal, in the bone marrow (BM) of AML patients treated with induction therapy [5,6]. The resistance to standard of care chemotherapy and the ability of leukemic-initiating cells (LICs) to initiate AML have been extensively studied. LICs were found to be a significant predictor of clinical outcome and patient survival in AML [7,8].

Herein, we hypothesize that the combination of low-dose GO and induction chemotherapy achieves more durable remissions by more effectively clearing minimal residual disease, including LICs, than either component separately. We utilize *in vivo* cell-line xenograft and patient-derived xenograft (PDX) AML models to demonstrate the impact on overall efficacy and duration of response that the combination of low-dose GO and induction chemotherapy provides. In so doing, we provide evidence that this combination can target cells responsible for chemorefractory disease.

Materials and Methods

GO (Mylotarg, Pfizer, New York, NY) was diluted in deionized water, and cytarabine (Pfizer, New York, NY) and daunorubicin (Teva Parenteral Medicines) were diluted with phosphate-buffered saline and stored at 4°C before usage. 8.8 ADC is a nonbinding antibody conjugated to N-Ac-γ-calicheamicin DMH (Pfizer, New York, NY). AC220 was purchased from Selleck Chemicals (Houston, TX) and prepared as previously described [9].

Flow Cytometry

Cell surface marker staining procedures were performed according to the antibody manufacturer's instructions. Freshly prepared cells were analyzed on a BD FACSCalibur Flow Cytometer equipped with BD CellQuest Pro (BD Bioscience, San Jose, CA). Quadrant markers were set relative to negative immunoglobulin isotype controls. The percent of human AML engraftment was defined as the percentage of the human CD33+/CD45+ cells relative to the total number of BM cells. AML engraftment was also monitored by quantifying the population of human CD33+/CD45+ cells in peripheral blood (PB). Individual subpopulations were gated on live human CD45+ cells. The antibodies used were anti–hCD33-APC and anti–hCD117-APC (Thermo Fisher Scientific, San Diego, CA), anti–hCD45-FITC and anti–hCD38-FITC (BD Biosciences, San Jose, CA), anti–hCD34-PE (Miltenyi Biotec Inc., San Diego, CA), and anti–hCLL1-PE (Biolegend, San Diego, CA).

AML Models

Female NSG (NOD/SCID IL2Rγ$^{-/-}$, Jackson Laboratories, Bar Harbor, ME) mice (7-8 weeks old) were used in all studies in this report. All experimental animal procedures complied with the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 2011) and were approved by the Institutional Animal Care and Use Committee (IACUC).

MV4-11 cells were purchased from ATCC (Manassas, VA) and stably transfected with luciferase (Luc) before implantation to generate the MV4-11-Luc cell line. For the MV4-11-Luc model, 1×10^6 cells were implanted intravenously (IV) into the tail vein of nonirradiated mice. Leukemic disease progression was monitored as previously described [10] on an IVIS200 system (PerkinElmer, Waltham, MA). Treatment was initiated when the mean value of bioluminescence intensity (BLI) in each group reached approximately 5×10^7 photons/sec.

To establish the BM0407 (BM120407L) and BM2407 (BM012407L) PDX models, the xenografted AML mice with P1 passage were purchased from Jackson Laboratory (Farmington, CT) and served as donors for PDX expansion. BM0407 and BM2407 were derived from FLT3-ITD–positive patients with the FAB-M2 subtype. BM0407 also harbors *NPM1* mutation (exon 12 insertion). Once engrafted in mice, BM AML blasts were deep sequenced with a cancer gene panel using multiplex amplicon technologies [11] to confirm the molecular profiles. One to 2×10^6 viable AML cells were collected from the BM of donor mice and were injected via the tail vein after the NSG mice were sublethally irradiated (150 cGy) using the X-Rad 225Cx (Precision X-Ray Inc., North Branford, CT). Engraftment was tracked periodically by quantifying the population of human CD45+ AML cells in the PB with flow cytometry. When the mean engraftment levels reached 1% to 5% CD33+/CD45$^+$ cells in the PB, leukemic mice were randomly assigned to receive treatments.

In Vivo Treatment and Antileukemic Efficacy Assessment

The maximum tolerated dose of GO in AML mice is 0.3 mg/kg (IV). The plasma level in this dose is within the range of the clinical exposure of high-GO dosing regimen (9 mg/m^2). To mimic the clinical strategy of fractionated GO regimen, we used low dose levels of GO (0.01 mg or 0.06 mg) for combination studies. The exact dose level for each model was preoptimized to avoid the nonspecific activity by control ADC. For each treatment cycle of the daunorubicin and cytarabine (DA) doublet arm, mice received daunorubicin intravenously on days 1, 3, and 5 and cytarabine subcutaneously on days 1 to 5. MV4-11-Luc and other models received one DA treatment cycle. BM2407 and BM0407 received two cycles of DA treatment in the combination test. AC220 was orally administered daily for 12 days at 10 mg/kg, which is a biologically effective dose for inhibiting the target (phospho-FLT3) [9]. The antileukemic efficacy was evaluated in the BM for the presence of human CD33+/CD45+ cells 5 days after the last treatment.

Assessment of Self-Renewal Ability of the Residual Cells After Induction Therapy

Bone marrow cells were collected from engrafted BM2407-diseased mice, sorted into CD34+ and CD34– subpopulations, and injected intravenously into sublethally irradiated recipient mice (n = 5). Similar methods were used for the self-renewal analysis