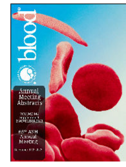
Check for updates

Blood 142 (2023) 1415–1417



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

AML Immunotherapy Using a Novel Tcrm-Based Bispecific Antibody That Targets a Leader Sequence Peptide Derived from Cathepsin G

Chunhua Shi[1], Ze Tian[1], Jun Yan[1], Mao Zhang[2], Pariya Sukhumalchandra[2], Edward Chang[1], Guojun Yang[1], Junping You[1], Meng Cui[1], Qing Shi[1], Anne Philips[2], Na Qiao[2], Anna Sergeeva, PhD[3], Lisa St. John[2], Hong He, MD PhD[3], Dongxing Zha[4], Jeffrey J. Molldrem, MD[5], Gheath Alatrash, PhDDO[2]

[1] Oncology Research for Biologics and Immunotherapy Translation (ORBIT), University of Texas MD Anderson Cancer Center, Houston
[2] Department of Hematopoietic Biology and Malignancy, University of Texas MD Anderson Cancer Center, Houston
[3] Department of Hematopoietic Biology and Malignancy, University of Texas M. D. Anderson Cancer Center, Houston, TX
[4] Alloy Therapeutics, Boston
[5] Department of Hematopoietic Biology and Malignancy, The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: Myeloid azurophil granules provide a rich source of intracellular leukemia antigens. Cathepsin G (CG) is a serine protease that has higher expression in AML blasts in comparison to normal myeloid progenitors. Based on the unique biology of HLA-A*0201 (HLA-A2), in which presentation of leader sequence (LS)-derived peptides is favored, we focused on the LS-CG-derived peptide CG1 (FLLPTGAEA). LS peptides are naturally processed and loaded on HLA-A2, providing an abundant source of surface peptide/HLA (pHLA) targets. We eluted CG1 from the surface of primary HLA-A2 $^{+}$ AML blasts and AML cell lines, and demonstrated CG1-targeting immunity in leukemia patients following allogeneic stem cell transplant, hence highlighting the potential for CG1 to be a promising immunotherapeutic target in AML.
Numerous platforms have been developed to target cell surface pHLA complexes. T cell receptor (TCR) mimic (m) antibodies are immunotherapeutic antibodies that target pHLA, which are the natural ligands for the TCR. TCRm antibodies have been a breakthrough in immunotherapy, as they: (1) target *intracellular* antigens expressed on HLA; (2) are engineered to have high affinity for HLA; (3) can elicit their cytotoxic effect independent of the TCR; and (4) can be produced in large quantities providing an off-the shelf-product for patient use. Here we report on the engineering, preclinical efficacy, and safety evaluation of a novel CG1-targeting, T cell-engager, bispecific antibody (CG1xCD3e), which incorporates a novel TCRm construct that recognizes cell surface CG1/HLA-A2 complexes.
Methods: To generate the CG1/HLA-A2-binding monoclonal antibodies, H2L2 human transgenic mice were immunized with CG1/HLA-A2 monomers subcutaneously. The resulting paired heavy and light chains were cloned from the antigen-sorted memory B cells into the single pcDNA3.1(+) vector with both human (h) immunoglobulin (Ig)G1 and hKappa for subsequent ExpiCHO high-throughput transient expression. Antibody binding was assessed by Bio-Layer Interferometry (BLI) and T2 cellular binding assays. Xencor anti-CD3e scFv and Knobs-in-holes technology was applied for the bispecific antibody engineering for xMab format. *In vitro* activity of CG1xCD3e and T cell cytokine secretion was confirmed using flow cytometry-based T cell-dependent cellular cytotoxicity (TDCC).
The specificity of CG1xCD3e for leukemia was assessed using colony forming unit assays. *In vivo* efficacy was evaluated in NSG mice engrafted with AML cell lines and treated with CG1xCD3e with normal donor peripheral blood mononuclear cells (ND-PBMC).
Results: Using BLI, binding avidity of the CG1xCD3e to CD3-Fc, KD= 7.52 x 10-10 M, and affinity to CG1/HLA-A2, KD= 1.28 x 10-10 M were calculated. Using flow cytometry, we confirmed high affinity binding of CG1xCD3e to CG1/HLA on the CG1-expressing EM2 HLA-A2 $^{+}$ cell line, and also demonstrated binding of CG1xCD3e to CD3 using jurkat T cell line.
After co-culturing GFP/Luciferase $^{+}$ HLA-A2 $^{+}$ AML (U937-A2 and ML-2) or CML (EM2) cell lines with ND-PBMC and different concentrations of CG1xCD3e bispecific antibody for 24 or 48 hours, flow cytometry analysis demonstrated highly specific CG1/HLA-A2 dependent killing of AML cells by T cells cultured with CG1xCD3e bispecific antibody, in comparison with control bispecific antibody with a disabled CG1 binding arm. This correlated with both tumor- and bispecific antibody- dependent T cell activation and cytokine secretion. To study the *in vivo* anti-leukemia activity of CG1xCD3e, we engrafted NSG mice with the HLA-A2 $^{+}$ ML-2 and U937-A2 AML cell lines, and treated mice after tumor engraftment with ND-PBMC (1 x 107 cells)

© 2023 by The American Society of Hematology

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/1415/2198026/blood-8018-main.pdf by guest on 17 May 2024

and weekly intraperitoneal injections of CG1xCD3e bispecific antibody (0.01 mg/Kg, 0.05 mg/Kg, and 0.1 mg/Kg) or PBS. Bioluminescence imaging demonstrated that the mice treated with ND-PBMC + CG1xCD3e had significantly lower levels of leukemia burden compared to the mice treated with ND-PBMC alone (Figure 1). Lastly CFU assays using HLA-A2 $^{+}$ normal donor marrow in a semi-solid matrix of methylcellulose in the presence of T cells and either CG1xCD3e bispecific antibody or isotype antibody, confirmed specificity of CG1xCD3e bispecific antibody for leukemia.

Conclusion: Our study provides strong pre-clinical evidence supporting the targeting of LS- derived peptides, specifically CG1, in the setting of AML using a novel TCRm-based bispecific antibody.

Disclosures Zha: *Alloy Therapeutics:* Current Employment.

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/1415/2198026/blood-8018-main.pdf by guest on 17 May 2024

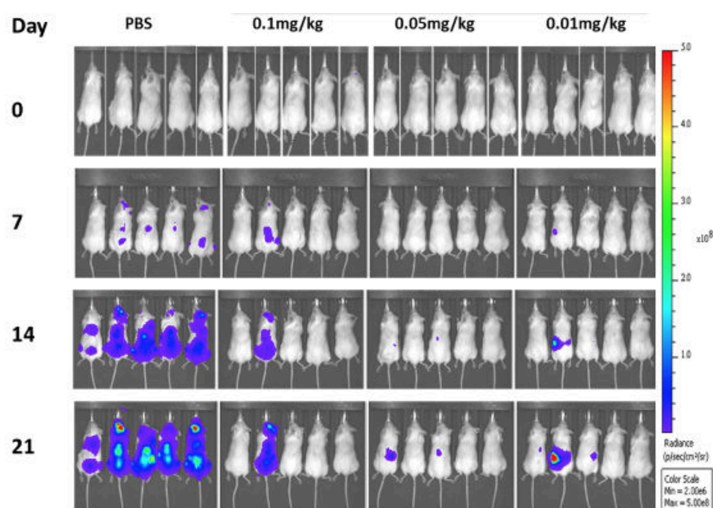


Figure 1. Anti-leukemia activity of CG1xCD3e bispecific antibody. NSG mice were engrafted with GFP/Luciferase-transduced HLA-A2+ ML-2 AML cells. Mice were then treated with normal donor PBMC and either CG1xCD3e bispecific antibody at increasing doses or PBS. Bioluminescence imaging of mice was performed. Data show activity of CG1xCD3e bispecific antibody, in comparison with PBS.

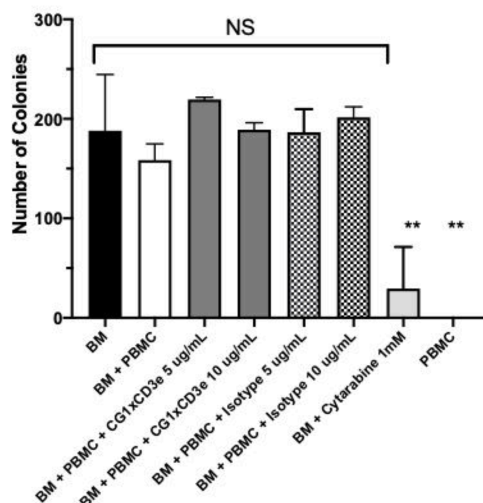


Figure 2. CG1xCD3e does not inhibit normal hematopoiesis. HLA-A2+ normal donor bone marrow (BM) was co-cultured with normal donor peripheral blood mononuclear cells (PBMC) and either CG1xCD3e bispecific antibody (Ab) or isotype Ab. Data show that CG1xCD3e bispecific Ab does not inhibit normal hematopoiesis. Cultures were grown in Mammocult medium in a 6-well plate for 14 days before CFU counts were taken. Results show CFU on day 14. BM co-cultured with cytarabine (1 mM) served as a positive killing control. Statistical testing was performed using ANOVA. NS indicates not significant. **$p<0.001$

Figure 1

https://doi.org/10.1182/blood-2023-189514

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/1415/2198026/blood-8018-main.pdf by guest on 17 May 2024