Expression of putative targets of immunotherapy in acute myeloid leukemia and healthy tissues

Citation
Goswami, M., N. Hensel, B. D. Smith, G. T. Prince, L. Qin, H. I. Levitsky, S. A. Strickland, et al. 2014. “Expression of putative targets of immunotherapy in acute myeloid leukemia and healthy tissues.” Leukemia 28 (5): 1167-1170. doi:10.1038/leu.2014.14. http://dx.doi.org/10.1038/leu.2014.14.

Published Version
doi:10.1038/leu.2014.14

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:12407050

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
inhibitor (ibrutinib) or PI3Kδ inhibitor (CAL-101) results in increased resistance to antitumor activity of anti-CD20 mAbs. Current study demonstrates that BCR inhibitors strongly down-regulate CD20 expression in tumor cells, leading to decreased binding of anti-CD20 mAbs to the surface of tumor cells and impairment of CDC and ADCC mechanisms that mediate antitumor effects of anti-CD20 mAbs in vivo. Our observations strongly imply that before investigating novel therapeutic combinations in cancer patients, extensive preclinical studies should be carried out to evaluate possible interactions between drugs at the molecular level.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGEMENTS
This work was supported by the National Science Center grant 2012/07/B/NZ6/03498 (MW), the Polish Ministry of Science and Higher Education grant IP2011 060271 (MW), the European Commission 7th Framework Programme: FP7-REGPOT-2012-CT2012-316254-BASTION (JG), and the Medical University of Warsaw grant 1M19/PMT12D/13 (KB).

K Bojarczuk¹, M Siemicka¹, M Dwojak¹, M Bobrowicz¹, B Pyrzynska¹, B Gaj¹, M Karp², K Giannopoulos², DG Efremov³, C Fauriat³, J Golab³ and M Winiarska³

¹Department of Immunology, Center for Biostucture Research, Medical University of Warsaw, Warsaw, Poland;
²Department of Experimental Hematooncology, Medical University of Lublin, Lublin, Poland;
³International Centre for Genetic Engineering and Biotechnology, Molecular Hematology Group, Campus A. Buzzatti-Traverso, Rome, Italy;
⁴IBISA Cancer Immunomonitoring Platform, Institut Paoli Calmettes, UM 105, Aix-Marseille Université, Marseille, France and
⁵Department 3, Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland
E-mail: jakub.golab@wum.edu.pl or mwiniarska@wum.edu.pl

Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)

OPEN

Expression of putative targets of immunotherapy in acute myeloid leukemia and healthy tissues

Leukemia (2014) 28, 1167–1170; doi:10.1038/leu.2014.14

The ability to target myeloid malignancies using immunotherapy through means other than allogeneic transplantation depends on the capability to target leukemic clones while sparing normal tissues. It is now possible to generate clinical grade ex-vivo expanded T cells specific for leukemia-associated antigens (LAAs) for use in adoptive cell therapy. Although a variety of putative LAAs in acute myeloid leukemia (AML) have been identified for use as potential targets for immunotherapy²–⁸ and consensus panels have attempted to prioritize generic cancer antigens,⁹ a comprehensive evidence-based list of AML antigen targets has not yet been established. As a first step toward this goal, we therefore analyzed, using quantitative real-time PCR, the gene expression of 65 potential LAAs (Supplementary Table S1) in de-identified, clinically annotated samples from 48 newly diagnosed untreated AML patients that were collected under institutional review board-approved protocols from three NCCN cancer centers. A total of 52 samples (30 peripheral blood (PB) and 22 bone marrow aspirate (BM) samples) from 48 AML patients were analyzed, which included 4 patients for whom both PB and BM samples were available. The average age of the patients was 52 years (range 24–86); 52% of the patients were women. A total of 7 patients had favorable cytogenetics, whereas 11 were classified as adverse, 24–86); 52% of the patients were women. A total of 7 patients had favorable cytogenetics, whereas 11 were classified as adverse,

REFERENCES

1. Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature 2010; 463: 88–92.
2. Duhren-von Minden M, Uebelhoer R, Schneider D, Wossning T, Bach MP, Buchner M et al. Chronic lymphocytic leukaemia is driven by antigen-independent cell-autonomous signalling. Nature 2012; 489: 309–312.
3. Gobesi S, Laurenti L, Longo PG, Carsetti L, Berno V, Sica S et al. Inhibition of constitutive and BCR-induced Syk activation downregulates Mcl-1 and induces apoptosis in chronic lymphocytic leukemia B cells. Leukemia 2009; 23: 686–697.
4. Buchner M, Baer C, Prinz G, Dieters C, Burger M, Zenz T et al. Spleen tyrosine kinase inhibition prevents chemokine- and integrin-mediated stromal protective effects in chronic lymphocytic leukemia. Blood 2010; 115: 4497–4506.
5. Herman SE, Barr PM, McAuley EM, Liu D, Wiestra A, Friedberg JW. Fostamatinib inhibits B-cell receptor signaling, cellular activation and tumor proliferation in patients with relapsed and refractory chronic lymphocytic leukemia. Leukemia 2013; 27: 1769–1773.
6. Byrd JC, Furman RR, Coupé SE, Flinn IW, Burger JA, Blum KA et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med 2013; 369: 32–42.
7. Cheng S, Ma J, Guo A, Lu P, Leonard JP, Coleman M et al. BTK inhibition targets in vivo CLL proliferation through its effects on B-cell receptor signaling activity. Leukemia 2013; e-pub ahead of print 25 November 2013; doi:10.1038/leu.2013.358.
8. Hoellenriegel J, Meadows SA, Sivina M, Wierda WG, Kantarjian H, Keating MJ et al. The phosphoinositide 3′-kinase delta inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. Blood 2011; 118: 3603–3612.
9. Kharfan-Dabaja MA, Wierda WG, Cooper LNJ. Immunotherapy for chronic lymphocytic leukemia in the era of BTK inhibitors. Leukemia 2013; e-pub ahead of print 25 October 2013; doi:10.1038/leu.2013.311.
10. Winiarska M, Gledowska-Mrowka E, Bil J, Golab J. Moleculare mechanisms of the antitumor effects of anti-CD20 antibodies. Front Biosci (Landmark Ed) 2011; 16: 277–306.
11. Herman SE, Gordon AL, Wagner AJ, Heerema NA, Zhao W, Flynn JM et al. Phosphatidylinositol 3-kinase-delta inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. Blood 2010; 116: 2078–2088.
12. Chen L, Monti S, Juszczynski P, Ouyang J, Chapuy B, Neuberg D et al. SYK inhibition modulates distinct PI3K/AKT- dependent survival pathways and cholesterol biosynthesis in diffuse large B cell lymphomas. Cancer Cell 2013; 23: 826–838.
13. Srivivasan S, Sasaki Y, Calado DP, Zhang B, Paik JH, DePinho RA et al. PI3 kinase signals BCR-dependent mature B cell survival. Cell 2009; 139: 573–586.
13 patients had FLT3 mutations (including 8 patients with FLT3-ITD) and 9 patients had mutations in NPM1 (Supplementary Tables S2 and S3). RNA and DNA were isolated from the ficoll-purified PB and BM samples using AllPrep Mini Kits (Qiagen, Valencia, CA, USA), and the quantity, quality and integrity of isolated RNA were assessed using a Nanodrop 1000 Spectrophotometer (Wilmington, DE, USA) and Agilent RNA 6000 Nano Kit and 2100 Bioanalyzer (Santa Clara, CA, USA). Only RNA with an RNA Integrity Number (RIN) of 7.0 or greater was used for subsequent analysis (Supplementary Table S4). An amount of 400 ng high-quality, total RNA was reverse-transcribed into cDNA using RT2 First Strand Kit (Qiagen). Custom RT2 Profiler PCR array plates (SABiosciences, Qiagen) were used for PCRs performed using RT2 SYBR Green ROX qPCR Mastermix (SABiosciences) according to the manufacturer’s instructions on an ABI 7900 thermal cycler (Applied Biosystems, Foster City, CA, USA) with a program of 10 min at 95°C, followed by 40 cycles at 95°C for 15 s and at 60°C for 1 min. Controls for human genomic DNA contamination, reverse transcription and PCR efficacy were included. Fold-change expression values were calculated according to the comparative C(t) method. D_C(t) was calculated as the C(t) of target gene 'X' minus the geometric mean C(t) of reference genes HPRT1, PPIH and TFRC in a sample. D_C(t) for each target gene 'X' in healthy donor samples was also computed in this manner. To calculate DDC(t), median D_C(t) of gene 'X' in healthy donor blood or BM (depending on the source of the AML sample) was subtracted from the D_C(t) of X in the AML sample (D_C(t) of X in healthy donor samples was also computed in this manner. To calculate DDC(t), median D_C(t) of gene 'X' in healthy donor blood or BM (depending on the source of the AML sample) was subtracted from the D_C(t) of X in the AML sample (D_C(t) of X in

Figure 1. Expression of proposed leukemia associated antigens in acute myeloid leukemia (AML) patient samples and healthy tissues. (a) No single antigen was expressed in all cases of AML and many proposed antigen candidates are not frequently overexpressed in AML. BM, bone marrow; PB, peripheral blood. Fold change OE compared with median expression in healthy donors where light red indicates OE of 5–50×, red indicates OE of 50–500×, bright red indicates OE >500×. Black indicates no detectable expression; white indicates expression values seen in similar range as healthy donors. First 30 AML samples listed were from PB and are therefore compared with healthy donor PB, the remaining 18 are from BM and are compared with expression in healthy donor BM. (b) Antigen expression in various human tissue types. Compared with median expression in healthy donors using same heat-map schema same as above.
Similarly, overexpressed in AML, often to a high level (Figures 1a and 2). The ideal targetable leukemia antigen would have high tumor-specific expression but no expression in healthy tissues. We observed considerable heterogeneity in levels of RNA overexpression (OE) of putative LAAs compared with healthy donor tissues (Figure 1). Every AML sample had at least one LAA overexpressed, but no antigen was overexpressed in any of the AML samples. Surprisingly, the hemoglobin gamma globin gene HBG2, ordinarily expressed in the fetal liver, spleen and BM but not usually in adulthood, which was recently identified as a leukemia antigen in a study of induced immune responses to GVAX/K562 vaccination in chronic myeloid leukemia (CML),11 was found to be frequently overexpressed in AML, often to a high level (Figures 1a and 2). Similarly, CCNA1, WT1, BAALC, PR3 and PRAME were also highly overexpressed in multiple AML samples (Figure 2). We were able to confirm the previously reported1213 association between FLT3-ITD-mutated AML and OE of WT1 (Supplementary Figure S2) but not of the other antigens. Finally, consistent with the fact that much of the existing evidence for myeloid LAA OE has been derived from the study of induced immune responses to GVAX/KS62 vaccination in chronic myeloid leukemia (CML),11 we were able to detect LAA OE of that gene, average levels of OE and OE in sorted lineage negative, CD34-positive and CD38-negative cells and normal donor tissues (see Supplementary Information for additional details).

AML sample – median ΔC(t) of X in healthy donor samples). Statistical analysis was performed using GraphPad Prism (La Jolla, CA, USA).

We observed considerable heterogeneity in levels of RNA overexpression (OE) of putative LAAs compared with healthy donor tissues (Figure 1). Every AML sample had at least one LAA overexpressed, but no antigen was overexpressed in any of the AML samples. Surprisingly, the hemoglobin gamma globin gene HBG2, ordinarily expressed in the fetal liver, spleen and BM but not usually in adulthood, which was recently identified as a leukemia antigen in a study of induced immune responses to GVAX/K562 vaccination in chronic myeloid leukemia (CML),11 was found to be frequently overexpressed in AML, often to a high level (Figures 1a and 2). Similarly, CCNA1, WT1, BAALC, PR3 and PRAME were also highly overexpressed in multiple AML samples (Figure 2). We were able to confirm the previously reported1213 association between FLT3-ITD-mutated AML and OE of WT1 (Supplementary Figure S2) but not of the other antigens. Finally, consistent with the fact that much of the existing evidence for myeloid LAA OE has been derived from the study of induced immune responses to GVAX/K562 vaccination in chronic myeloid leukemia (CML),11 we were able to detect LAA OE of that gene, average levels of OE and OE in sorted lineage negative, CD34-positive and CD38-negative cells and normal donor tissues (see Supplementary Information for additional details).

Several technical features are worthy of note. We found that samples with RIN scores of less than 7.0 resulted in higher than expected Ct outputs, which correspond to lower gene expression when compared with samples with higher RIN scores (Supplementary Figure S3) and were therefore excluded from analysis. Our array platform was highly sensitive and reproducible (Supplementary Figure S1A), allowing for the reliable medium throughput analysis performed here. In most cases, antigen expression profiles from presentation blood and marrow samples from the same patient correlated closely (Supplementary Figure S4). Gene expression of LAA in phenotypically identified AML blast populations sorted through flow cytometry did not markedly differ from the gene expression seen in the presentation PB sample from which they were isolated (Supplementary Methods; Supplementary Table S5). Finally, we were able to detect LAA OE across multiple samples from the same patient including unsorted PB samples, sorted AML blasts and a sorted (that is lineage negative, CD34 positive and CD38 negative) PB population enriched for stem cells (Supplementary Methods; Supplementary Table S6).

This work has several obvious limitations. We performed this work on ‘real world’ first-presentation primary samples from three different leukemia centers in an attempt to limit bias introduced...
Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)

by presentation and referral patterns. Nevertheless, all institutions are highly specialized tertiary academic medical centers located in the northeastern and southeastern United States. Although we do have patient demographics on age and gender (Supplementary Table S2) and all these samples were from the first diagnosis before initiation of treatment for AML, we unfortunately do not have any information on race or ethnic background, medical history including details on antecedent hematological conditions, prior/concurrent malignancies or current medications with epigenetic or immune activity. We quantified total RNA expression levels (necessary but not sufficient for a targetable LAAs) but did not provide information on protein expression or epitope processing and presentation by major histocompatibility complex; these factors will be addressed in future work now that the list of candidate AML LAAs has been substantially refined to exclude those not overexpressed in AML. Neo-antigens (that is, those generated by somatic mutations including single nucleotide variations, insertions, deletions and splice variants) are an important potential class of AML LAAs that were not investigated in this work, although extensive data on these AML-specific sequence changes are now available and immune responses to epitopes created by these mutations have recently been described.15

The ideal AML LAA would be expressed in most or all cases of AML but not in healthy tissues. Using a novel, highly sensitive and reproducible, real-time reverse transcription–PCR array testing only high-quality RNA we show in this work that the majority of proposed ‘LAAs’ are expressed in the leukemia cell line K562 but often not in primary samples from AML patients. Although we identified no antigen that was universally overexpressed in all AML samples, every patient did have at least one potentially targetable antigen overexpressed. We also noted significant healthy organ-specific tissue expression of many LAAs, highlighting the possibility of ‘off-target’ effects, a finding not evident from the study of expression levels in PB and BM alone. This list of genes overexpressed in AML, together with information regarding expression in a wide range of healthy tissue types, may be of use in AML as a reference for the selection of antigenic targets in adoptive T-cell therapy and may also have use in the PCR-based detection of minimal residual disease.15

CONFLICT OF INTEREST
HIL is an employee of Roche and a part-time faculty member at Johns Hopkins University. ATF serves on advisory boards for Seattle Genetics and Agios and has investigator initiated clinical trials currently funded by Elexelis, Seattle Genetics and Millennium. The remaining authors declare no conflict of interest.

ACKNOWLEDGEMENTS
We thank Heidi Sardon, Ann Williams, Pradeep Dagur and J Phillip McCoy of the Flow Cytometry Core Facility, National Heart, Lung, and Blood Institute, National Institutes of Health, Alan Hoofring of the NIH Medical Arts Service and the Johns Hopkins University Specimen Accessioning Core Lab for their assistance with this study. This work was supported by the Intramural Research Program of the National Heart, Lung, and Blood Institute of the National Institutes of Health.

AUTHOR CONTRIBUTIONS
MG and CSH designed and performed the research, analyzed and interpreted the data, and wrote the manuscript. TR and MJL performed the research. NH, BDS, GTP, SAS, MJ, BNS, JWF, HS, LQ, ATF and HIL collected data. SI, NAJ, MB and AJB interpreted data.

REFERENCES
1 Weber G, Gerdemann U, Caruana I, Savoldo B, Hensel NF, Rabin KR et al. Generation of multi-antigen antigen-specific T cells to enhance the graft-versus-leukemia effect after allogeneic stem cell transplant. Leukemia 2013; 27: 1538–1547.
2 Greiner J, Schneider V, Schmitt M, Goetz M, Dohner H, Dohner K, Wiesneth M et al. Leukemia-associated antigens and their relevance to the immunotherapy of acute myeloid leukemia. Leukemia 2012; 26: 2186–2196.
3 el-Shami K, Smith BD. Immunotherapy for myeloid leukemias: current status and future directions. Leukemia 2008; 22: 1658–1664.
4 Greiner J, Bullinger L, Guinn B-a, Doehner H, Schmitt M. Leukemia-associated antigens are critical for the proliferation of acute myeloid leukemia cells. Clin Cancer Res 2008; 14: 7161–7166.
5 Hourigan CS, Levitsky HI. Evaluation of current cancer immunotherapy: hematologic malignancies. Cancer J 2011; 17: 309–324.
6 Atanackovic D, Luetkens T, Kloth B, Fuchs G, Cao Y, Hildebrandt Y et al. Generation of multi-leukemia antigen-specific T cells to enhance the graft-versus-leukemia effect after allogeneic stem cell transplant. Leukemia 2013; 27: 1538–1547.
7 Ochsenreither S, Majeti R, Schmitt T, Stirewalt D, Keilholz U, Loeb KR et al. Cancer-testis antigen expression and its epigenetic modulation in acute myeloid leukemia. Am J Hematol 2011; 86: 918–922.
8 Ochsenreither S, Majeti R, Schmitt T, Stirewalt D, Keilholz U, Loeb KR et al. Cyclin-A1 represents a new immunogenic targetable antigen expressed in acute myeloid leukemia stem cells with characteristics of a cancer-testis antigen. Blood 2012; 119: 5492–5501.
9 Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res 2009; 15: 5323–5337.
10 Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008; 3: 1101–1108.
11 Qiu L, Smith BD, Tsai HL, Yagihi N, Neele PH, Moake M et al. Induction of high-titer IgG antibodies against multiple leukemia-associated antigens in CML patients with clinical responses to KS62/GVAX immunotherapy. Blood Cancer J 2013; 3: e145.
12 Brackertz B, Conrad H, Daniel J, Kast B, Kronig H, Busch DH et al. FLI3-regulated antigens as targets for leukemia-reactive cytotoxic T lymphocytes. Blood Cancer J 2011; 1: e1.
13 Greiner J, Schmitt M, Li L, Giannopoulos K, Bosch K, Schmitt A et al. Expression of tumor-associated antigens in acute myeloid leukemia: implications for specific immunotherapeutic approaches. Blood 2006; 108: 4109–4117.
14 Greiner J, Schneider V, Schmitt M, Goitz M, Dohner K, Wiesenth et al. Immune responses against the mutated region of cytoplasmatic NPM1 might contribute to the favorable clinical outcome of AML patients with NPM1 mutations (NPM1mut). Blood 2013; 122: 1087–1088.
15 Hourigan CS, Karp JE. Minimal residual disease in acute myeloid leukemia. Nat Rev Clin Oncol 2013; 10: 460–471.