JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

An Anti-B Cell Maturation Antigen Bispecific Antibody for Multiple Myeloma

Nitya S. Ramadoss,[†] Andrew D. Schulman,[†] Sei-hyun Choi,[‡] David T. Rodgers,[†] Stephanie A. Kazane,[†] Chan Hyuk Kim,[†] Brian R. Lawson,[§] and Travis S. Young*[,†]

[†]California Institute for Biomedical Research, 11119 North Torrey Pines Road, Suite 100, La Jolla, California 92037, United States
[‡]Department of Chemistry and the Skaggs Institute for Chemical Biology and [§]Department of Immunology and Microbial Science, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States

Supporting Information

ABSTRACT: The development of immunotherapies for multiple myeloma is critical to provide new treatment strategies to combat drug resistance. We report a bispecific antibody against B cell maturation antigen (BiFab-BCMA), which potently and specifically redirects T cells to lyse malignant multiple myeloma cells. BiFab-BCMA lysed target BCMA-positive cell lines up to 20-fold more potently than a CS1-targeting bispecific antibody (BiFab-CS1) developed in an analogous fashion. Further, BiFab-BCMA robustly activated T cells in vitro and mediated rapid tumor regression in an orthotopic xenograft model of multiple myeloma. The in vitro and in vivo activities of BiFab-BCMA are comparable to those of anti-BCMA chimeric antigen receptor T cell therapy (CAR-T-BCMA), for which two clinical trials have recently been initiated. A BCMA-targeted bispecific antibody presents a promising treatment option for multiple myeloma.

Multiple myeloma (MM) is an incurable plasmacytoma that proliferates uncontrollably in the bone marrow, interfering with the normal production of blood cells and causing painful bone lesions.[1] Although most patients respond to initial chemotherapy cocktails, nearly all patients ultimately relapse because of drug resistance. Cases of MM are estimated to grow by 57% from 2010 to 2030, making curative therapies for MM a major unmet medical need.[2,3]

Immunotherapy is emerging as an attractive alternative to chemotherapy, as the method of tumor clearance circumvents many drug resistance mechanisms. Correspondingly, there is great interest in developing immunotherapies to treat MM; however, canonical markers such as CD38 and CD138 are not restricted to neoplastic cells and can also be found on lymphoid, myeloid, and epithelial cells, which poses a risk of "on target, off tumor" toxicities when targeted for therapeutic purposes.[1,4] Immunotherapies targeting CD19, a marker for B cell leukemia and lymphoma, have seen remarkable results in clinical trials; however, CD19 is lost during maturation of B cells to plasma cells and consequently is detectable in less than 5% of MM patients.[5]

Recently, B cell maturation antigen (BCMA, CD269) and the cell surface glycoprotein CD2 subset 1 (CS1, CD319) have emerged as promising antigens for MM immunotherapy.[6−8] BCMA is involved in tumor proliferation via the delivery of pro-survival signals, and the role of CS1 in myeloproliferation and tumorigenicity has been established.[1] BCMA and CS1 are ideal targets for immunotherapy, as they are nearly ubiquitously expressed on MM cells and have low expression on normal tissues and no expression on $CD34^+$ hematopoietic cells.[6,9] Preclinical and early-stage clinical studies targeting CS1 and BCMA have shown encouraging results.[8,10]

Bispecific antibodies are a particularly promising form of immunotherapy that uses heterobivalent binding through two separate antigen recognition domains—one that recognizes a tumor antigen and the other that targets CD3 on T cells—to recruit and activate T cells to eliminate malignant cells. Bispecific antibodies may be useful for the treatment of MM, as they can target quiescent cancer stem cells as well as cells with low numbers of tumor-associated antigens and are not affected by chemotherapy resistance mechanisms such as efflux pumps.[11] However, there has been little development of bispecific antibodies for MM, in part because of the lack of tumor-restricted antigen targets.[12] Notably, there have been limited or no reports in the academic literature regarding bispecific antibodies targeting BCMA or CS1.

Toward this end, we developed anti-BCMA and anti-CS1 bispecific antibodies using a modular semisynthetic method in which two antigen binding fragments (Fabs) are site-specifically conjugated via unnatural amino acids.[13,14] This methodology enables control over the geometry, valency, and size of the bispecific antibody. The resulting bispecific Fab (BiFab) is homogeneous and stable, which may be advantageous over scFv-based formats.[15] Here we report an improved chemical conjugation strategy for the development of BiFabs.

Previously we and others reported the conjugation of BiFabs through the site-specific modification of Fabs with paired heterobifunctional linkers containing an azide (AZ) or bicyclononyne (BCN).[13,14] These linkers undergo [3 + 2]

Table 1. List of Abbreviations

MM	multiple myeloma	CAR	chimeric antigen receptor
BCMA	B cell maturation antigen	AZ	azide
CS1	CD2 subset 1, SLAMF7	BCN	bicyclononyne
Fab	antibody fragment	TET	tetrazine
BiFab	bispecific antibody	*p*AcF	*p*-acetylphenylalanine
OPM-2	$BCMA^+$ $CS1^+$ cell line	RS4;11	$BCMA^-$ $CS1^-$ cell line

Received: February 19, 2015
Published: March 31, 2015

DOI: 10.1021/jacs.5b01876
J. Am. Chem. Soc. 2015, 137, 5288−5291

Huisgen cycloaddition to provide conjugation without the requirement of a catalyst (Figure 1a). To improve the rate of

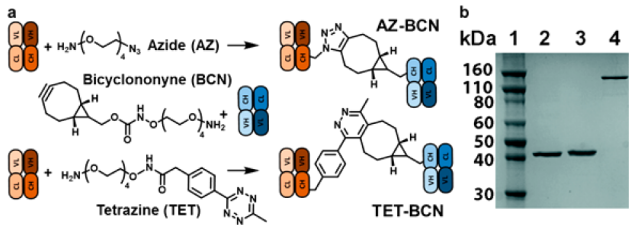

Figure 1. Synthesis and characterization of BiFabs. (a) Structures of linkers ligated to Fabs (red or blue) for BiFab conjugation: (top) previous strategy using AZ- and BCN-based linkers; (bottom) strategy in this work using TET- and BCN-based linkers. (b) Characterization of monomers and conjugates by nonreducing gel electrophoresis: (1) protein ladder; (2) αBCMA Fab; (3) αCD3 Fab; (4) BiFab-(TET-BCN)-BCMA.

the conjugation, we developed a new strategy in which tetrazine (TET) replaces AZ of one of the linkers. Tetrazines undergo an inverse-electron-demand Diels–Alder reaction with BCN that is up to 10^4-fold faster than strain-promoted alkyne–azide cycloadditions (AZ–BCN).[16] Toward this end, the AZ, BCN, and TET linkers were synthesized to compare the conjugation rates in the context of BiFab generation (Supplemental Figure 1). Each linker contained an aminooxy functionality to enable oxime ligation to the ketone of the unnatural amino acid *p*-acetylphenylalanine (*p*AcF) (Figure 1a). Briefly, to incorporate *p*AcF into the Fab, residue K129 (Kabat numbering) was mutated to the TAG codon. Coexpression of the mutant Fab in *Escherichia coli* with the *Methanocaldococcus jannaschii* amino-acyl-tRNA/$tRNA_{CUA}$ pair specific for *p*AcF provided site-specific, homogeneous incorporation of *p*AcF at residue 129 (Supplemental Figure 2). To compare the rates of BiFab conjugation, Fabs harboring *p*AcF were separately ligated with AZ, BCN, and TET linkers.[17] Purified Fab-BCN was then conjugated with Fab-AZ or Fab-TET to produce the BiFab. The reaction as monitored by gel electrophoresis demonstrated that the TET-BCN strategy increased the rate of conjugation, providing 33% BiFab in only 1 min at 37 °C (Supplemental Figure 3). By means of this strategy, BiFab-BCMA and BiFab-CS1 were created using the Fabs of humanized αBCMA clone BCMA-98 and humanized αCS1 clone HuLuc63 (from the monoclonal antibody elotuzumab),[18,19] respectively. Targeting antibodies harboring K129*p*AcF were individually ligated to TET and used to create the corresponding heterodimeric BiFabs by conjugation to αCD3-BCN (clone UCHT1). Each ligation proceeded to ≥95% as determined by high-resolution mass spectrometry (Supplemental Figure 2a). The conjugates were purified by size-exclusion chromatography and confirmed by gel electrophoresis (Figure 1b) and liquid chromatography/mass spectrometry (Supplemental Figure 2b).

In agreement with previous reports of BiFab generation, conjugation of the Fabs did not affect the binding or specificity of either Fab, as demonstrated by flow cytometry against three CS1/BCMA-positive cell lines (Supplemental Figure 4). The thermal stabilities determined by protein melt demonstrated BiFab-BCMA and BiFab-CS1 to be approximately equally as stable as their monomer Fabs (T_m = 88.5 BiFab-BCMA and 89.9 °C for BiFab-CS1) (Supplemental Figure 5).

To assess the activity of BiFab-BCMA and BiFab-CS1, we measured the cytotoxicity of expanded healthy donor-derived T cells against BCMA/CS1-positive OPM-2 MM cells. Both BiFab-BCMA and BiFab-CS1 potently lysed OPM-2 cells but had insignificant toxicity against BCMA/CS1-negative RS4;11 leukemia cells, even at concentrations up to 10^3-fold higher than their EC_{50} (Figure 2a). BiFab-BCMA was 7-, 10-, and 19-fold

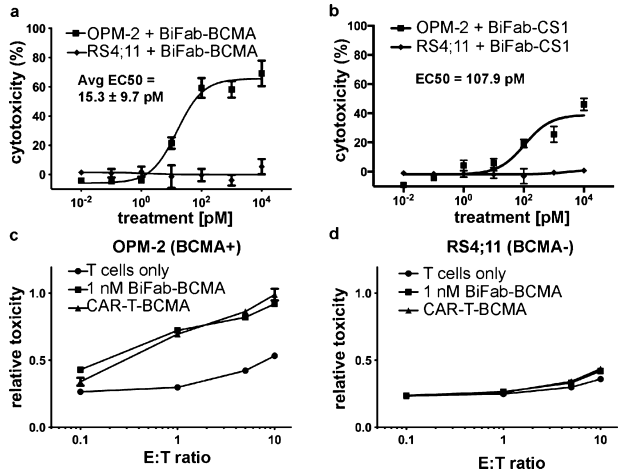

Figure 2. In vitro cytotoxicity of BiFab-BCMA and CAR-T-BCMA. (a, b) Cytotoxicities of (a) BiFab-BCMA and (b) BiFab-CS1 against OPM-2 (■) and RS4;11(◆) cells, as measured by LDH release after 24 h, at an E:T ratio of 10:1. Apoptotic OPM-2 cells were confirmed by Annexin V staining (Supplemental Figure 7). (c, d) Relative killing by CAR-T-BCMA and 1 nM BiFab-BCMA at varying E:T ratios (10:1, 5:1, 1:1, and 1:10) against (c) OPM-2 and (d) RS4;11 cells, as measured by LDH release after 24 h. Data points are averages of three replicates, and error bars show standard deviations.

more potent than BiFab-CS1 against OPM-2, MM.1S, and RPMI8226, respectively (Figure 2b and Supplemental Figure 6), and was therefore selected for further studies. To confirm its potency, the activity of BiFab-BCMA against OPM-2 cells was tested across five independent donor-derived T cells, which yielded an average EC_{50} of 15.3 ± 9.7 pM (Supplemental Figure 7).

A mixture of unconjugated αCD3 and αBCMA Fabs failed to induce cytotoxicity, indicating the requirement for cross-linking of the target and effector cells (Supplemental Figure 8). To further confirm that BiFab-BCMA provided cross-linking of T cells and target cells, we monitored ternary complex formation by flow cytometry in the presence of BiFab-BCMA. Complex formation was negligible in the absence of BiFab and increased in a dose-dependent manner, indicating that T cell recruitment was mediated specifically by BiFab-BCMA (Supplemental Figure 9).

To compare the activity of BiFab-BCMA with that of an anti-BCMA chimeric antigen receptor (CAR) T cell therapy (CAR-T-BCMA) that recently entered clinical trials, we engineered healthy donor-derived T cells with a second-generation CAR harboring the same anti-BCMA single-chain antibody scFv (clone C11D5.3) as reported by Kochenderfer and colleagues (Supplemental Figure 10a).[6,20] These cells activate by cross-linking of the scFv to a BCMA-positive cell. This delivers activation signals through the CD3ζ intracellular domain of the chimeric receptor, resulting in target cell lysis.[21,22] To create CAR-T-BCMA, lentivirus expressing the chimeric receptor was produced in HEK cells and used to transduce freshly isolated, activated human peripheral blood monocytes (Supplemental Figure 10b).[6,20] For comparison with BiFab-BCMA, T cells from

DOI: 10.1021/jacs.5b01876
J. Am. Chem. Soc. 2015, 137, 5288–5291

the same donor were expanded without transduction in parallel (see Supplemental methods).

We next assessed the in vitro cytotoxicity of BiFab-BCMA and CAR-T-BCMA. Cytotoxicity against OPM-2 cells showed similar killing by CAR-T-BCMA and 1 nM BiFab-BCMA (saturating value in cytotoxicity assays) even at low E:T ratios, indicating that both can engage in serial killing of OPM-2 cells (Figure 2c).[23] CAR-T-BCMA and 1 nM BiFab-BCMA demonstrated similar maximum cell lysis at an E:T ratio of 10:1 against two other BCMA-positive cell lines (Supplemental Figure 11). Neither had significant toxicity against RS4;11 cells, demonstrating the specificity of both therapies (Figure 2d).

Because MM cells rarely express ligands for costimulatory receptors and often present an immunosuppressive environment, strong intrinsic T cell activation is required for an effective therapy. To examine the magnitude of T cell activation, we assessed cytokine release, degranulation, and expression of activation markers in CAR-T-BCMA- and BiFab-BCMA-activated T cells. Both the CAR-T and 1 nM BiFab treatments resulted in robust multicytokine production in the presence of OPM-2 cells. CAR-T-BCMA released significantly higher levels of cytokines (Figure 3a), likely because of the presence of the CAR costimulatory domain 4-1BB. This is in agreement with previous reports showing enhanced cytokine production of CARs engineered with 4-1BB costimulatory domains.[20]

To further explore the magnitude of T cell activation with BiFab-BCMA and CAR-T-BCMA, we assessed T cell activation via degranulation and expression of early and late markers of activation (CD69 and CD25). BiFab-BCMA led to a dose-dependent increase in the percentage of activated cells only in the presence of OPM-2 cells and not in the presence of RS4;11 cells. BiFab-BCMA (1 nM) and CAR-T-BCMA led to significant degranulation in response to OPM-2 cells, as determined by cell surface CD107a expression. In both cases, BiFab-BCMA activated T cells in similar fashion to CAR-T-BCMA in the presence of OPM-2 and exhibited good selectivity by sparing RS4;11 cells (Figure 3b–d and Supplemental Figure 12). These assays indicated that BiFab-BCMA is capable of robust activation of T-cells in response to BCMA-positive MM cells.

Having established the potency and selectivity in vitro, we next determined whether BiFab-BCMA could mediate tumor regression in vivo. For this purpose we used an orthotopic xenograft model using luciferized OPM-2 cells in immunocompromised NOD/SCID/IL-2Rγ$^{-/-}$(NSG) mice.[24] Previous studies have used solid tumor models to evaluate the efficacy of a similar CAR-T-BCMA therapy.[6] However, the cutaneous microenvironment is very different from the bone marrow, which promotes MM survival and resistance.[25] For this reason, we used the OPM-2 model, which infiltrates the bone marrow and better recapitulates the pathogenesis of the human disease.[24,26,27] In this model, mice without treatment succumb to disease with hind-leg paralysis and cachexia within 30 days of OPM-2 inoculation. To monitor the tumor burden, OPM-2 cells were luciferized with firefly luciferase (Supplemental Methods). Mice from the treatment groups received two doses of 15 million T cells (either CAR-T-BCMA or expanded T cells) on days 14 and 18. BiFab-BCMA treatment was dosed intravenously at 1 mg/kg every day for 10 days. BiFab-BCMA-treated mice were able to eliminate the tumor burden after just 8 days of treatment (Figure 4). The kinetics of regression were similar to that for CAR-T-BCMA, demonstrating the potent and rapid response of BCMA-targeted T cell recruitment strategies for MM.

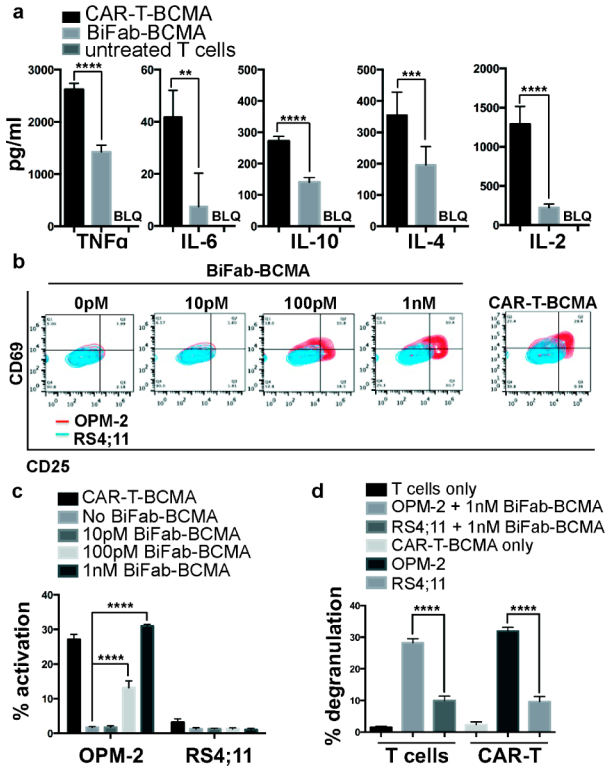

Figure 3. BCMA-dependent T cell activation. (a) Cytokine profile of CAR-T-BCMA or nontransduced T cells with 1 nM or 0 nM BiFab-BCMA (untreated T cells) with OPM-2 cells at E:T = 1:1. The result for 0 nM was below the limit of quantification (BLQ). Quantification was done by flow cytometry using a cytokine bead array (CBA) assay. (b) Expression of activation markers CD69 and CD25 on CAR-T-BCMA and varying concentrations of BiFab-BCMA-treated T cells cocultured for 24 h with either OPM-2 (red) or RS4;11 (blue) at E:T = 1:1. Expression was determined by flow cytometry using FITC-conjugated anti-CD69 and PE-conjugated anti-CD25 antibodies. (c) Quantification of CD69+ CD25+ T cells shown in (b). (d) Degranulation of CAR-T-BCMA and T cells treated with 1 nM BiFab-BCMA when cocultured with OPM-2 or RS4;11 cells for 4 h, as determined by monitoring CD107a expression on the cell surface, at E:T = 1:1. All experiments represent $n = 3$ and standard deviation error bars. Significance by one-way ANOVA (****, $p \leq 0.0001$; ***, $p = 0.0006$; **, $p = 0.0038$).

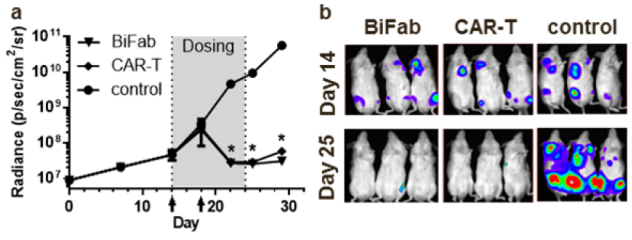

Figure 4. In vivo efficacy of BiFab-BCMA and CAR-T-BCMA. (a) Kinetics of tumor growth monitored by the luciferase signal from an OPM-2 tumor xenograft. Luciferized OPM-2 cells (0.6×10^6 cells) were injected on day 0, followed by CAR-T-BCMA or nontransduced T cells (BiFab-BCMA group) on days 14 and 18 (arrows). BiFab dosing was 1 mg/kg q.d. for 10 days starting on day 14 (shaded area). $n = 3$; *, $p \leq 0.001$ vs untreated control by one-way ANOVA. (b) Representative luminescence images at the start of treatment (day 14) and the day following the final BiFab treatment (day 25).

In summary, we have developed and compared the in vitro efficacies of two antimyeloma bispecific antibodies targeting BCMA and CS1. Although BCMA targeting was more potent

DOI: 10.1021/jacs.5b01876
J. Am. Chem. Soc. 2015, 137, 5288–5291

than CS1 targeting in vitro, differences in the affinities and epitope locations of the specific BCMA-98 and HuLuc63 clones may be determining factors.[28] Alternative α-CS1 antibodies for BiFab-CS1 are currently being explored. BiFab-BCMA exhibited in vitro and in vivo efficacies comparable to those of CAR-T-BCMA in a dose-titratable manner. This may be relevant in clinical applications to control the activation of T cells in vivo. Cytokine storm (high levels of cytokine release) is a serious complication of excessive T cell activation in the clinic, and thus, the ability to titrate a bispecific antibody therapy may be important. Furthermore, there is evidence that BCMA is essential for the long-term survival of plasma cells in the bone marrow,[29] and the effects of sustained BCMA depletion from CAR-T cell therapy are unknown. Indeed, long-term B cell aplasia is a consequence of CAR-T therapy targeting CD19 in the clinic.[30] Thus, BiFab-BCMA may be an important treatment modality for MM therapy in that it can eradicate disease while allowing healthy cells to repopulate in the bone marrow after treatment is complete.

■ ASSOCIATED CONTENT

Supporting Information

Supplemental methods and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*tyoung@calibr.org

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Peter G. Schultz for helpful discussions.

■ REFERENCES

(1) Garfall, A. L.; Fraietta, J. A.; Maus, M. V. *Discovery Med.* **2014**, *17*, 37.

(2) Orlowski, R. Z. *Cancer Cell* **2013**, *24*, 275.

(3) Smith, B. D.; Smith, G. L.; Hurria, A.; Hortobagyi, G. N.; Buchholz, T. A. *J. Clin. Oncol.* **2009**, *27*, 2758.

(4) Stevenson, G. T. *Mol. Med.* **2006**, *12*, 345.

(5) Mateo, G.; Montalban, M. A.; Vidriales, M. B.; Lahuerta, J. J.; Mateos, M. V.; Gutierrez, N.; Rosinol, L.; Montejano, L.; Blade, J.; Martinez, R.; de la Rubia, J.; Diaz-Mediavilla, J.; Sureda, A.; Ribera, J. M.; Ojanguren, J. M.; de Arriba, F.; Palomera, L.; Terol, M. J.; Orfao, A.; San Miguel, J. F.; Group, P. S.; Group, G. E. M. S. *J. Clin. Oncol.* **2008**, *26*, 2737.

(6) Carpenter, R. O.; Evbuomwan, M. O.; Pittaluga, S.; Rose, J. J.; Raffeld, M.; Yang, S.; Gress, R. E.; Hakim, F. T.; Kochenderfer, J. N. *Clin. Cancer Res.* **2013**, *19*, 2048.

(7) Tai, Y. T.; Mayes, P. A.; Acharya, C.; Zhong, M. Y.; Cea, M.; Cagnetta, A.; Craigen, J.; Yates, J.; Gliddon, L.; Fieles, W.; Hoang, B.; Tunstead, J.; Christie, A. L.; Kung, A. L.; Richardson, P.; Munshi, N. C.; Anderson, K. C. *Blood* **2014**, *123*, 3128.

(8) Lonial, S.; Kaufman, J.; Laubach, J.; Richardson, P. *Expert Opin. Biol. Ther.* **2013**, *13*, 1731.

(9) Hsi, E. D.; Steinle, R.; Balasa, B.; Szmania, S.; Draksharapu, A.; Shum, B. P.; Huseni, M.; Powers, D.; Nanisetti, A.; Zhang, Y.; Rice, A. G.; van Abbema, A.; Wong, M.; Liu, G.; Zhan, F.; Dillon, M.; Chen, S.; Rhodes, S.; Fuh, F.; Tsurushita, N.; Kumar, S.; Vexler, V.; Shaughnessy, J. D., Jr.; Barlogie, B.; van Rhee, F.; Hussein, M.; Afar, D. E.; Williams, M. B. *Clin. Cancer Res.* **2008**, *14*, 2775.

(10) Ryan, M. C.; Hering, M.; Peckham, D.; McDonagh, C. F.; Brown, L.; Kim, K. M.; Meyer, D. L.; Zabinski, R. F.; Grewal, I. S.; Carter, P. J. *Mol. Cancer Ther.* **2007**, *6*, 3009.

(11) Amin, M. L. *Drug Target Insights* **2013**, *7*, 27.

(12) Honemann, D.; Kufer, P.; Rimpler, M. M.; Chatterjee, M.; Friedl, S.; Riecher, F.; Bommert, K.; Dorken, B.; Bargou, R. C. *Leukemia* **2004**, *18*, 636.

(13) Kim, C. H.; Axup, J. Y.; Dubrovska, A.; Kazane, S. A.; Hutchins, B. A.; Wold, E. D.; Smider, V. V.; Schultz, P. G. *J. Am. Chem. Soc.* **2012**, *134*, 9918.

(14) Lu, H.; Zhou, Q.; Deshmukh, V.; Phull, H.; Ma, J.; Tardif, V.; Naik, R. R.; Bouvard, C.; Zhang, Y.; Choi, S.; Lawson, B. R.; Zhu, S.; Kim, C. H.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2014**, *53*, 9841.

(15) Quintero-Hernandez, V.; Juarez-Gonzalez, V. R.; Ortiz-Leon, M.; Sanchez, R.; Possani, L. D.; Becerril, B. *Mol. Immunol.* **2007**, *44*, 1307.

(16) Lang, K.; Chin, J. W. *ACS Chem. Biol.* **2014**, *9*, 16.

(17) Zhu, Z.; Carter, P. *J. Immunol.* **1995**, *155*, 1903.

(18) Kufer, P.; Raum, T.; Hoffmann, P.; Kischel, R.; Lutterbuese, R.; Rau, D.; Adam, P.; Borges, E.; Hebeis, B.; Hipp, S. Google Patents, 2013.

(19) Tai, Y. T.; Dillon, M.; Song, W.; Leiba, M.; Li, X. F.; Burger, P.; Lee, A. I.; Podar, K.; Hideshima, T.; Rice, A. G.; van Abbema, A.; Jesaitis, L.; Caras, I.; Law, D.; Weller, E.; Xie, W.; Richardson, P.; Munshi, N. C.; Mathiot, C.; Avet-Loiseau, H.; Afar, D. E.; Anderson, K. C. *Blood* **2008**, *112*, 1329.

(20) Kochenderfer, J. N.; Feldman, S. A.; Zhao, Y.; Xu, H.; Black, M. A.; Morgan, R. A.; Wilson, W. H.; Rosenberg, S. A. *J. Immunother.* **2009**, *32*, 689.

(21) Curran, K. J.; Pegram, H. J.; Brentjens, R. J. *J. Gene Med.* **2012**, *14*, 405.

(22) Barrett, D. M.; Singh, N.; Porter, D. L.; Grupp, S. A.; June, C. H. *Annu. Rev. Med.* **2014**, *65*, 333.

(23) Hoffmann, P.; Hofmeister, R.; Brischwein, K.; Brandl, C.; Crommer, S.; Bargou, R.; Itin, C.; Prang, N.; Baeuerle, P. A. *Int. J. Cancer* **2005**, *115*, 98.

(24) Garg, T. K.; Szmania, S. M.; Khan, J. A.; Hoering, A.; Malbrough, P. A.; Moreno-Bost, A.; Greenway, A. D.; Lingo, J. D.; Li, X.; Yaccoby, S.; Suva, L. J.; Storrie, B.; Tricot, G.; Campana, D.; Shaughnessy, J. D., Jr.; Nair, B. P.; Bellamy, W. T.; Epstein, J.; Barlogie, B.; van Rhee, F. *Haematologica* **2012**, *97*, 1348.

(25) Mitsiades, C. S.; Mitsiades, N. S.; Bronson, R. T.; Chauhan, D.; Munshi, N.; Treon, S. P.; Maxwell, C. A.; Pilarski, L.; Hideshima, T.; Hoffman, R. M.; Anderson, K. C. *Cancer Res.* **2003**, *63*, 6689.

(26) Veitonmaki, N.; Hansson, M.; Zhan, F.; Sundberg, A.; Lofstedt, T.; Ljungars, A.; Li, Z. C.; Martinsson-Niskanen, T.; Zeng, M.; Yang, Y.; Danielsson, L.; Kovacek, M.; Lundqvist, A.; Martensson, L.; Teige, I.; Tricot, G.; Frendeus, B. *Cancer Cell* **2013**, *23*, 502.

(27) Ishii, T.; Seike, T.; Nakashima, T.; Juliger, S.; Maharaj, L.; Soga, S.; Akinaga, S.; Cavenagh, J.; Joel, S.; Shiotsu, Y. *Blood Cancer J.* **2012**, *2*, e68.

(28) Bluemel, C.; Hausmann, S.; Fluhr, P.; Sriskandarajah, M.; Stallcup, W. B.; Baeuerle, P. A.; Kufer, P. *Cancer Immunol. Immunother.* **2010**, *59*, 1197.

(29) O'Connor, B. P.; Raman, V. S.; Erickson, L. D.; Cook, W. J.; Weaver, L. K.; Ahonen, C.; Lin, L. L.; Mantchev, G. T.; Bram, R. J.; Noelle, R. J. *J. Exp. Med.* **2004**, *199*, 91.

(30) Kalos, M.; Levine, B. L.; Porter, D. L.; Katz, S.; Grupp, S. A.; Bagg, A.; June, C. H. *Sci. Transl. Med.* **2011**, *3*, 9573.

DOI: 10.1021/jacs.5b01876
J. Am. Chem. Soc. 2015, 137, 5288−5291