



A CXCR4-Targeted Site-Specific Antibody–Drug Conjugate**

Sumith A. Kularatne, Vishal Deshmukh, Jennifer Ma, Virginie Tardif, Reyna K. V. Lim, Holly M. Pugh, Ying Sun, Anthony Manibusan, Aaron J. Sellers, Richard S. Barnett, Shailaja Srinagesh, Jane S. Forsyth, Wolf Hassenpflug, Feng Tian, Tsotne Javahishvili, Brunhilde Felding-Habermann, Brian R. Lawson, Stephanie A. Kazane,* and Peter G. Schultz*

Abstract: A chemically defined anti-CXCR4–auristatin antibody–drug conjugate (ADC) was synthesized that selectively eliminates tumor cells overexpressing the CXCR4 receptor. The unnatural amino acid *p*-acetylphenylalanine (pAcF) was site-specifically incorporated into an anti-CXCR4 immunoglobulin G (IgG) and conjugated to an auristatin through a stable, non-cleavable oxime linkage to afford a chemically homogeneous ADC. The full-length anti-CXCR4 ADC was selectively cytotoxic to CXCR4⁺ cancer cells in vitro (half maximal effective concentration (EC₅₀) ≈ 80–100 pM). Moreover, the anti-CXCR4 ADC eliminated pulmonary lesions from human osteosarcoma cells in a lung-seeding tumor model in mice. No significant overt toxicity was observed but there was a modest decrease in the bone-marrow-derived CXCR4⁺ cell population. Because CXCR4 is highly expressed in a majority of metastatic cancers, a CXCR4–auristatin ADC may be useful for the treatment of a variety of metastatic malignancies.

Although increasingly effective drugs are being developed for the treatment of primary tumors, the treatment options for metastatic cancer are relatively limited and, consequently, the prognosis is poor. This is especially the case when multiple

sites and tissues are affected, which further complicates the elimination of malignant versus normal cells. Recently, antibody–drug conjugates (ADCs) have emerged as a new class of immunotherapeutic agents that can deliver drugs selectively to tumors by binding to antigens highly expressed on cancers cells, followed by internalization and intracellular drug release.^[1] In this work, we have applied this strategy to cell-surface antigens that are preferentially expressed on metastatic tumor cells. A number of studies have indicated that CXCR4 (CD184), a G-protein-coupled receptor, is expressed in a variety of cancers, including breast, prostate, pancreatic, ovarian, and brain cancers, melanoma, and many forms of hematological malignancies.^[2] Although CXCR4 expression levels are highly variable between these cancers, CXCR4 is known to be overexpressed in almost all metastatic tumor cells and, consequently, increased expression of CXCR4 is a negative predictor of survival and a strong predictor of tumor relapse in patients.^[3] It has also been established that SDF1α (also known as CXCL12), the cognate ligand of CXCR4, is highly expressed in local, regional, and distant metastatic sites, such as lymph nodes, bone marrow, lung, and liver, thus suggesting that the CXCR4/SDF1α axis plays a major role in regulating the destination of most tumor cell metastases.^[4] Moreover, CXCR4 is known to be overexpressed in cancer stem cells (CSCs) and it induces them to undergo chemotaxis and migrate below marrow stromal cells, thereby allowing these cells access to cellular niches.^[2c–e]

Consequently, considerable effort has been dedicated to the development of CXCR4-targeted therapeutic agents for metastatic cancers. AMD3100 (Plerixafor, a low-molecular-weight CXCR4 antagonist), T140 and T22 (polypeptide CXCR4 antagonists), BKT140 (a 14-residue synthetic peptide), and CTCE-9908 (an analogue of SDF1) lead to reduced metastasis in various animal models and also show activity in non-small-cell lung cancer (NSCLC), acute myeloid leukemia (AML), and multiple myeloma xenograft models.^[5] Moreover, pretreatment of primary human leukemic cells with neutralizing CXCR4 antibodies blocked the entrance of homing leukemic cells into the bone marrow and spleen of transplanted NOD SCID mice.^[6] A number of these CXCR4 antagonists, including AMD3100 and BKT140, as well as anti-CXCR4 antibodies (e.g., BMS-936564) are currently in clinical trials for the treatment of AML and multiple myeloma. However, it may be possible to further increase the efficacy of these agents by combining them with a selectively delivered cytotoxic agent. The ADC strategy is especially attractive since the CXCR4 receptor and its ligands have been shown to be efficiently internalized.^[7] While

[*] Dr. S. A. Kularatne,^[†] Dr. V. Deshmukh,^[†] Prof. P. G. Schultz
Department of Chemistry and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 N. Torrey Pines Rd, La Jolla, CA 92037 (USA)
E-mail: schultz@scripps.edu

Dr. J. Ma, Dr. R. K. V. Lim, H. M. Pugh, Prof. B. R. Lawson,
Dr. S. A. Kazane, Prof. P. G. Schultz
California Institute for Biomedical Research (Calibr)
11119 N. Torrey Pines Road, La Jolla, CA 92037 (USA)
E-mail: spinkerton@calibr.org

Dr. V. Tardif, Prof. B. R. Lawson
Department of Immunology and Microbial Science
The Scripps Research Institute, La Jolla, CA 92037 (USA)

Dr. Y. Sun, A. Manibusan, A. J. Sellers, Dr. R. S. Barnett,
S. Srinagesh, Dr. F. Tian, Dr. T. Javahishvili
Ambrx, Inc., 10975 N. Torrey Pines Rd, La Jolla, CA 92037 (USA)
J. S. Forsyth, Dr. W. Hassenpflug, Prof. B. Felding-Habermann
Department of Molecular and Experimental Medicine
The Scripps Research Institute, La Jolla, CA 92037 (USA)

[†] These authors contributed equally to this work.

[**] This is manuscript No. 28020 from the Scripps Research Institute.
This work was supported by NIH grant R01 GM097206 (P.G.S.), R01
CA170737 (B.F.H.), and R01 CA170140 (B.F.H.).



Supporting information for this article is available on the WWW
under <http://dx.doi.org/10.1002/ange.201408103>.

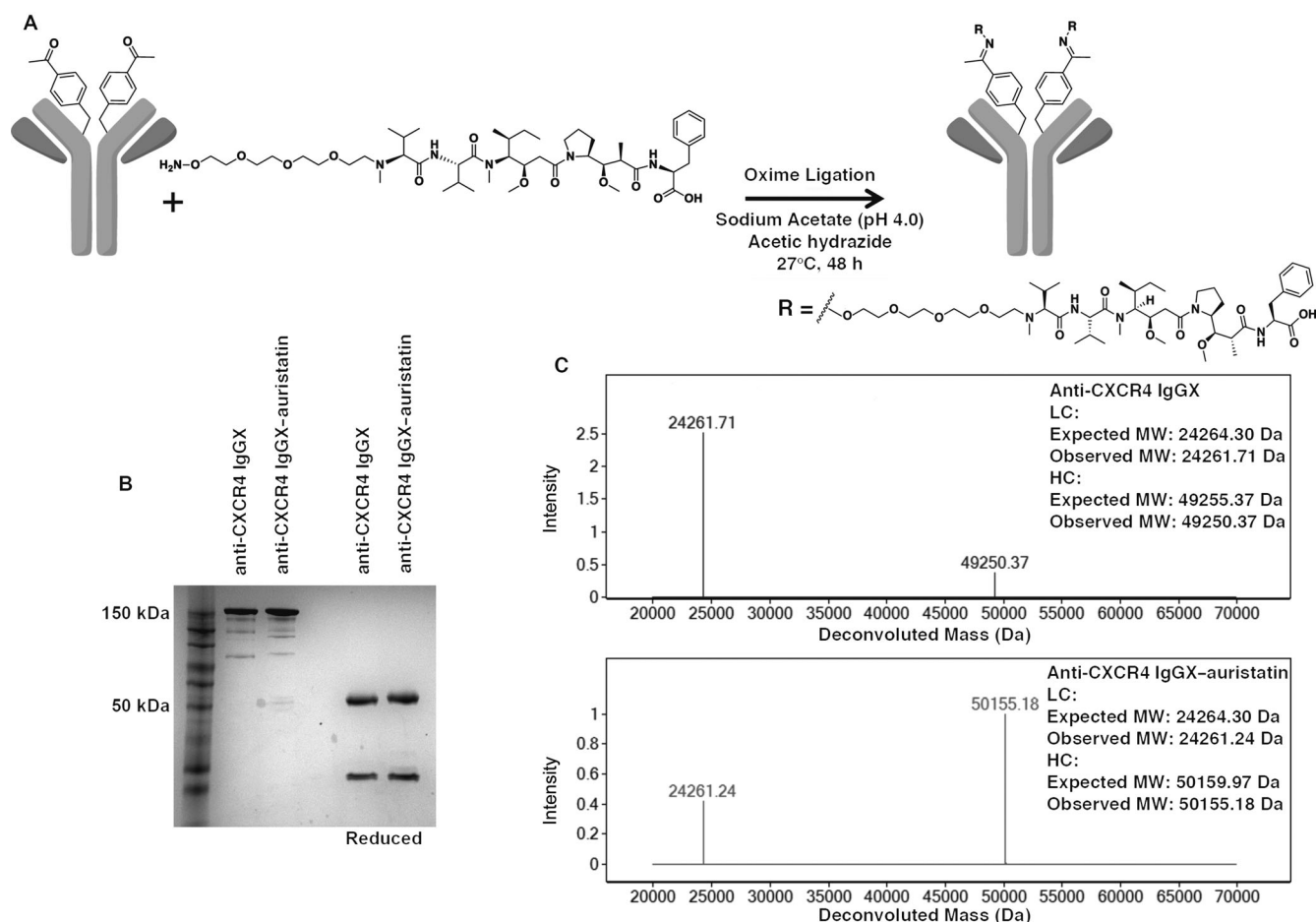


Figure 1. Site-specific conjugation of aminooxy auristatin to anti-CXCR4A122pAcF IgG (anti-CXCR4 IgGX). **A)** Monomethyl auristatin F (MMAF) derivatized with a terminal alkoxy amine group is coupled by oxime ligation to anti-CXCR4A122pAcF IgG through a pAcF residue. **B)** SDS-PAGE of anti-CXCR4 IgGX before and after coupling to auristatin. The mutant IgG includes natural heterogeneous N-linked glycosylation; the band at around 150 kDa corresponds to the full-length IgG; those around 25 kDa and 50 kDa correspond to the reduced light chain and heavy chain, respectively. The 4–20% Tris-glycine gel has a prestained protein ladder in the first lane and was stained with Coomassie Blue. Tris = tris(hydroxymethyl)aminomethane. **C)** ESI-MS analysis of anti-CXCR4 IgGX before and after conjugation to auristatin. Mutant IgG spectra show the heavy and light chains after the removal of glycans with PNGase F (Promega, PBS pH 7.4, 37°C, 12 h) and reduction with 10 mM DTT. All masses are as expected with an approximately 905 Da difference between the conjugated and unconjugated antibodies that corresponds to conjugation of one auristatin per heavy chain. No unreacted antibody was observed by SDS-PAGE or ESI-MS, thus suggesting more than 95% coupling efficiency.

CXCR4-targeted antibody–drug conjugates (ADCs) have not yet been reported for the treatment of cancer, several types of highly cytotoxic agents, including calicheamicin, maytansinoid, and auristatin, have been conjugated to monoclonal antibodies such as anti-Her2, anti-PSMA, and anti-CD30.^[8] Herein, we describe the development of an anti-CXCR4 immunoglobulin G (IgG)–auristatin ADC that demonstrates excellent in vitro efficacy against metastatic SJSA-1-met-luc cells (a human osteosarcoma cell line implanted in the tibia of a mouse and then derived as metastasized cells from the lung). Importantly, this ADC is capable of eliminating tumors in vivo in mice in a lung-seeding tumor model with SJSA-1-met-luc cells.

Auristatin, an antimetabolic agent that prevents tubulin polymerization, has previously been conjugated to a number of antibodies, including anti-CD30, anti-PSMA, and anti-Her2 antibodies, and it has been shown to be highly cytotoxic

to tumors.^[8–9] Moreover, because auristatin kills rapidly proliferating cells by interfering with microtubule function, it might be expected to be less cytotoxic to nonreplicating hematopoietic stem cells that also express CXCR4. Additionally, we chose to conjugate monomethyl auristatin F (MMAF) since it is minimally cell permeable owing to its C-terminal carboxylic acid.^[10] To conjugate MMAF site-specifically to surface-exposed sites in full-length anti-CXCR4 IgG, we produced recombinant antibodies containing *p*-acetyl phenylalanine (pAcF) by using unnatural amino acid (UAA) mutagenesis technology. An orthogonal tRNA/aminocyl-tRNA synthetase (aaRS) pair specific for pAcF was coexpressed with an anti-CXCR4 gene containing a TAG codon at residue A122 in the heavy chain.^[11] The tRNA/aaRS pair and mutant CXCR4 IgG genes were transiently transfected into CHO-S cells (Life Technologies) supplemented with 1.3 mM pAcF. After 10 days at 32°C, the supernatant was

harvested and anti-CXCR4A122pAcF IgG (referred to herein as anti-CXCR4 IgGX) was purified by using protein A affinity chromatography, which gave a yield of ca. 1 mg L⁻¹.

We used a stable, non-cleavable hydrophilic linker containing ethylene glycol units (for increased solubility) to ensure delivery of the intact ADC to the tumor followed by rapid release of the drug in the lysosome.^[12] An aminooxy moiety was incorporated at the distal end of the PEG linker to selectively conjugate the drug to the mutant anti-CXCR4 IgG variant through a stable oxime bond. The pAcF mutant IgG was conjugated to MMAF-PEG₂-aminooxy in sodium acetate buffer (pH 4.0) in the presence of acetic hydrazide at 28 °C for 48 h, and purified by repeated washing with PBS buffer (pH 7.4) using an Amicon concentrator with 10 kDa MWCO (Figure 1 A). Denaturing gel electrophoresis (SDS-PAGE) demonstrated that the mutant anti-CXCR4A122pAcF IgG (anti-CXCR4 IgGX) and anti-CXCR4A122pAcF IgG–auristatin (anti-CXCR4 IgGX–auristatin) were > 90 % pure and resolved into bands of ca. 150 kDa (nonreducing conditions, full-length IgG) and ca. 50 and 25 kDa (reducing conditions, heavy and light chain, respectively; Figure 1 B). Electrospray-ionization mass spectrometry (ESI-MS) analysis indicated that the reduced heavy chain of anti-CXCR4 IgGX has an expected mass of 49250 Da (Figure 1 C and Figure S1 in the Supporting Information). Furthermore, ESI-MS analysis revealed a mass of 50155 Da for the reduced heavy chain of anti-CXCR4 IgGX–auristatin (Figure 1 C and Figure S1); the increase in mass of ca. 905 Da upon conjugation corresponds the mass of two auristatin derivatives per IgG. We did not observe any unconjugated IgG or degraded products, thus indicating a more than 95 % coupling efficiency. We were thus able to produce chemically defined, homogeneous ADCs with a drug to antibody ratio (DAR) of 2.

We next evaluated the specificity, affinity, and internalization of the mutant antibody by conjugating it to Alexa Fluor 488 (AF488), followed by analysis of receptor binding and endocytosis by using flow cytometry and confocal microscopy. Purified anti-CXCR4 IgGX was conjugated site-specifically to an aminooxy-modified AF488. SJSA-1 parental human osteosarcoma cells are known to express high levels of CXCR4.^[13] From these cells, a metastatic variant was derived after intratibial injection of the parental cells into SCID mice and collection of spontaneously metastasized tumor cells from lung lesions. These cells were stably transduced with firefly luciferase (SJSA-1-met-luc), express high levels of CXCR4, and metastasize very efficiently to the lungs from the blood stream in a SCID mouse model. SJSA-1-met-luc cells were used to confirm binding of the mutant anti-CXCR4 IgG. The anti-CXCR4 IgGX–AF488 conjugate bound to SJSA-1-met-luc cells with high affinity (Figure 2 A and Figure S2; half maximal effective concentra-

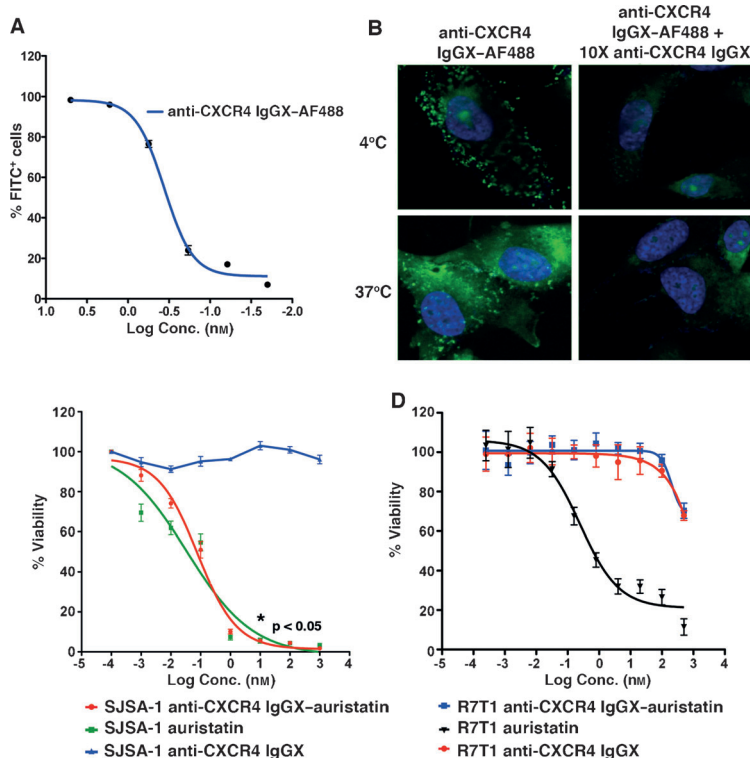


Figure 2. In vitro activity of the conjugated anti-CXCR4 IgGX. A) Binding of anti-CXCR4 IgGX–AF488 to SJSA-1-met-luc cells. Cells were incubated with increasing concentrations of the conjugate at 4 °C for 30 mins and binding was analyzed by flow cytometry. B) SJSA-1-met-luc cells were plated at 80 % confluence and treated with 50 nM anti-CXCR4 IgGX–AF488 in the presence or absence of 500 nM unconjugated anti-CXCR4 IgGX. Cells were incubated at either 4 °C or 37 °C for 30 mins, fixed and imaged with a Zeiss confocal microscope. Dose-dependent in vitro cytotoxicity of anti-CXCR4 IgGX–auristatin with C) SJSA-1-met-luc (CXCR4⁺) and D) R7T1 (CXCR4[−]) cells. Cells were treated with increasing concentrations of anti-CXCR4 IgGX, anti-CXCR4 IgGX–auristatin, or unconjugated auristatin for 72 h at 37 °C, 5 % CO₂, and viability was measured using CellTiter Glo (Promega). The percentage viability was normalized to untreated controls (*n* = 3, mean and standard deviation (SD) determined, error bars represent SD; **p* < 0.05, calculated from the EC₅₀ between anti-CXCR4 IgGX and anti-CXCR4 IgGX–auristatin).

tion (EC₅₀) = 0.48 nM) as measured by flow cytometry; binding was inhibited by a 10-fold excess of unconjugated antibody (determined by confocal microscopy; Figure 2 B). Furthermore, anti-CXCR4 IgGX–AF488 was observed in the cytoplasm within 30 min at 37 °C, thus indicating efficient internalization, which was also blocked by a 10-fold excess of unconjugated antibody (Figure 2 B). Since CXCR4 is expressed on hematopoietic cells, such as HSCs, lymphocytes, monocytes, and NK cells, we further demonstrated binding of the anti-CXCR4 IgGX to CXCR4⁺ Jurkat cells. We also showed that the mutant antibody binds to CHO cells transfected with either human or mouse CXCR4 and to mouse bone marrow cells. Minimal to no binding was observed with nontransfected CHO cells and R7T1 cells (mouse CXCR4[−]; Figure S2 and Figure S3).

To determine the in vitro efficacy of the ADC, SJSA-1-met-luc cells were grown to 80 % confluence and treated with either anti-CXCR4 IgGX–auristatin, unconjugated mutant IgG, or monomethyl auristatin E (MMAE; positive control

for cytotoxicity due to minimal cell permeability of MMAF) for 72 h and cell viability was measured through bioluminescent quantitation of released ATP by using CellTiter Glo. Cell viability significantly decreased ($*p < 0.05$) with increasing concentrations of anti-CXCR4 IgGX–auristatin [EC_{50} of 0.08 ± 0.03 nM (mean $\pm$ SD)] and unconjugated MMAE [EC_{50} of 0.03 ± 0.06 nM (mean $\pm$ SD)], while no significant toxicity was observed with unconjugated mutant antibody at up to 1 μ M concentrations (Figure 2C). Furthermore, no cytotoxicity was observed following the treatment of R7T1 cells (CXCR4⁺) with either anti-CXCR4 IgGX–auristatin or unconjugated anti-CXCR4 IgGX (Figure 2D). Taken together, these data indicate that the observed cell killing by the ADC is due to CXCR4-dependent internalization of auristatin conjugated to the mutant anti-CXCR4 antibody.

We then evaluated the efficacy of the anti-CXCR4 ADC in a mouse tumor xenograft model (lung-seeding model). SJSA-1-met-luc tumor growth rates were determined in vivo by longitudinal noninvasive bioluminescence imaging. The SJSA-1-met-Luc cells (10000/mouse) were injected via the tail vein into NOD/SCID (C. B17) mice (day zero), followed by noninvasive bioluminescence imaging, which showed accumulating signals from tumor cells in the lungs of the mice. Most cells were cleared from the lungs within 2 days, as indicated by a decrease in the observed bioluminescence. By day 10, an increase in bioluminescence was observed in the lungs, thus indicating that the cells that remained in the lungs had seeded and formed tumors. Treatment was initiated when pulmonary tumor lesions reached approximately 1×10^6 photons $cm^{-2} s^{-1}$ (around day 10; Figure 3, Figure S4, and Figure S5). Mice were randomized into three groups and injected intravenously with vehicle (PBS), anti-CXCR4 IgGX–auristatin (3 doses; 2.5 mg kg^{-1} once every 5 days, on the basis of previous pharmacokinetic and efficacy experiments with a similarly generated anti-Her2 IgG–auristatin conjugate),^[8a] or unconjugated anti-CXCR4 IgGX (3 doses; 2.5 mg kg^{-1} once every 5 days). In mice treated with the anti-CXCR4 IgGX–auristatin, initial pulmonary tumor lesions became barely detectable within 8 days of treatment, in contrast to the tumor burden in PBS-treated mice, which displayed lesions that grew continuously at a steady rate (Figure 3A,C and Figure S4, $n = 6$ mice/group; mean and SEM determined, $*p < 0.05$). Mice treated with anti-CXCR4 IgGX–auristatin showed a complete absence of tumor burden even 15 days after the final treatment dose (Figure 3A,C and Figure S4), thus demonstrating excellent efficacy for this site-specific ADC with two drug molecules per antibody. As anticipated, no significant differences were observed between mice treated with either PBS or unconjugated or anti-CXCR4 IgGX (Figure 3B,C and Figure S5, $n = 6$ mice/group, mean and SEM determined, $p > 0.05$). Since the unconjugated mutant antibodies did not exhibit in vitro cytotoxicity toward SJSA-1-met-luc cells and SCID mice do not have adaptive immune systems, a significant treatment effect of unconjugated antibodies either by cell toxicity or antibody-dependent cell-mediated cytotoxicity (ADCC) was not expected. Importantly, the total body weights of the mice treated with the ADC remained unchanged throughout the duration of the

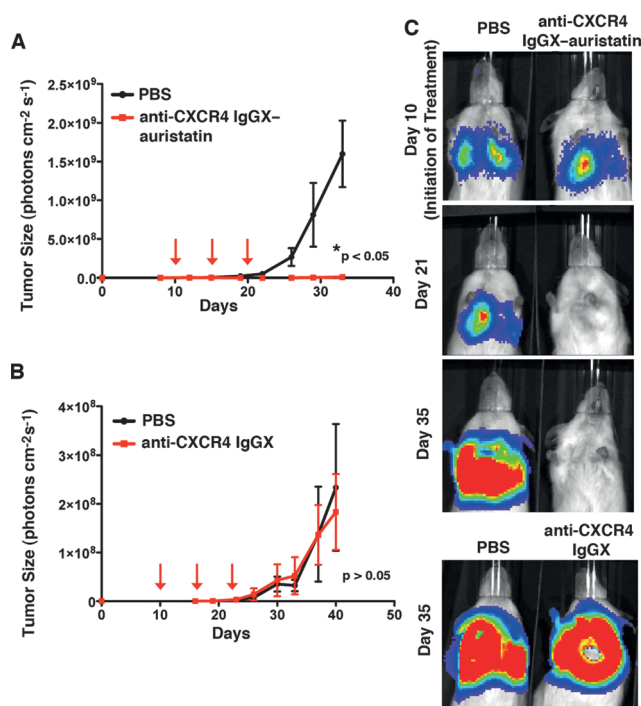


Figure 3. The in vivo efficacy of anti-CXCR4 IgGX–auristatin. Lung tumors were allowed to develop in NOD/SCID (C.B17) mice for 10 days after intravenous (i.v.) injection of 10^4 SJSA-1-met-luc cells. Lesion development and response to antibody treatment was monitored by longitudinal noninvasive bioluminescence imaging (IVIS 200). A) Mice injected i.v. with either anti-CXCR4 IgGX–auristatin (three doses; 2.5 mg kg^{-1} once every 5 days) or PBS [$n = 6$ mice/group; mean and standard error of mean (SEM) determined, error bars represent SEM; $*p < 0.05$, calculated from tumor size between treatment and vehicle groups on day 22 (end of study)]. B) Mice injected with either anti-CXCR4 IgGX (three doses; 2.5 mg kg^{-1} once every 5 days) or PBS [$n = 6$ mice/group, mean and SEM determined, error bars represent SEM; $p > 0.05$, calculated from tumor size between treatment and vehicle groups on day 22 (end of study)]. C) Representative images from IVIS imaging showing bioluminescence corresponding to tumor size on various days during the study.

study (Figure S6), thus suggesting that the therapy is not grossly toxic to the animals.

Hematopoietic stem cells (HSCs) and various cells of the hematopoietic lineage express CXCR4 and may be potential targets for anti-CXCR4 ADCs.^[14] Depletion of these cell types could pose a significant clinical concern for the translation of an anti-CXCR4 ADC to the clinic. However, HSCs in the bone marrow are known to be mostly quiescent and, as such, may not be affected by a microtubule binder like auristatin that primarily targets rapidly proliferating cells.^[14a,15] In order to test the potential effects of the anti-CXCR4 ADC on HSCs in vivo, we injected BALB/c mice with either PBS or anti-CXCR4 IgGX–auristatin (2 doses; 2.5 mg kg^{-1} intravenously once every 5 days). We then isolated bone marrow from these mice and analyzed the HSCs in the bone marrow by flow cytometry (Figure S7A).^[16] HSCs were identified as the Lin[−]/c-kit⁺/sca1⁺ (LSK) populations and were further classified as multipotent progenitor cells (MPP; Lin[−]/c-kit⁺/sca1⁺/CD48⁺/CD150⁺), short-term HSCs (ST-HSCs; Lin[−]/c-kit⁺/sca1⁺/CD34⁺/CD135[−]), and

long-term HSCs (LT-HSCs; Lin⁻/c-kit⁺/sca1⁺/CD34⁻/CD135⁻; Figure S7B and Table S1 in the Supporting Information). Consistent with the nonproliferating nature of endogenous HSCs, a modest decrease (24%) in HSC populations was observed in mice treated with the ADC compared to PBS-treated mice (Figure S7C).

In conclusion, we generated a site-specific anti-CXCR4 IgGX-auristatin conjugate with a DAR of 2 that was able to selectively target and eliminate CXCR4⁺ metastatic cancer cells, both in vitro and in vivo. This approach also significantly spared CXCR4⁺ hematopoietic cells in vivo, thus suggesting that it may represent a promising new approach to the treatment of metastatic cancer.

Received: August 8, 2014

Published online: September 11, 2014

Keywords: antibody engineering · antibody–drug conjugates · cancer · immunotherapy · unnatural amino acids

- [1] P. D. Senter, *Curr. Opin. Chem. Biol.* **2009**, *13*, 235–244.
- [2] a) U. M. Domanska, R. C. Kruizinga, W. B. Nagengast, H. Timmer-Bosscha, G. Huls, E. G. de Vries, A. M. Walenkamp, *Eur. J. Cancer* **2013**, *49*, 219–230; b) X. Sun, G. Cheng, M. Hao, J. Zheng, X. Zhou, J. Zhang, R. S. Taichman, K. J. Pienta, J. Wang, *Cancer Metastasis Rev.* **2010**, *29*, 709–722; c) P. Gassmann, J. Haier, K. Schluter, B. Domikowsky, C. Wendel, U. Wiesner, R. Kubitz, R. Engers, S. W. Schneider, B. Homey, A. Muller, *Neoplasia* **2009**, *11*, 651–661; d) M. Kim, Y. J. Koh, K. E. Kim, B. I. Koh, D. H. Nam, K. Alitalo, I. Kim, G. Y. Koh, *Cancer Res.* **2010**, *70*, 10411–10421; e) T. Sobolik, Y. J. Su, S. Wells, G. D. Ayers, R. S. Cook, A. Richmond, *Mol. Biol. Cell* **2014**, *25*, 566–582.
- [3] M. Darash-Yahana, E. Pikarsky, R. Abramovitch, E. Zeira, B. Pal, R. Karplus, K. Beider, S. Avniel, S. Kasem, E. Galun, A. Peled, *FASEB J.* **2004**, *18*, 1240–1242.
- [4] a) S. Gelmini, M. Mangoni, M. Serio, P. Romagnani, E. Lazzeri, *J. Endocrinol. Invest.* **2008**, *31*, 809–819; b) J. Libura, J. Drukala, M. Majka, O. Tomescu, J. M. Navenot, M. Kucia, L. Marquez, S. C. Peiper, F. G. Barr, A. Janowska-Wieczorek, M. Z. Ratajczak, *Blood* **2002**, *100*, 2597–2606.
- [5] a) D. H. McDermott, J. Lopez, F. Deng, Q. Liu, T. Ojode, H. Chen, J. Ulrick, N. Kwatema, C. Kelly, S. Anaya-O'Brien, M. Garofalo, M. Marquesen, D. Hilligoss, R. DeCastro, H. L. Malech, P. M. Murphy, *J. Cell. Mol. Med.* **2011**, *15*, 2071–2081; b) A. Peled, M. Abraham, I. Avivi, J. M. Rowe, K. Beider, H. Wald, L. Tiomkin, L. Ribakovsky, Y. Ribak, Y. Ramati, S. Aviel, E. Galun, H. L. Shaw, O. Eizenberg, I. Hardan, A. Shimon, A. Nagler, *Clin. Cancer Res.* **2014**, *20*, 469–479; c) M. M. Richert, K. S. Vaidya, C. N. Mills, D. Wong, W. Korz, D. R. Hurst, D. R. Welch, *Oncol. Rep.* **2009**, *21*, 761–767; d) H. Tamamura, A. Hori, N. Kanzaki, K. Hiramatsu, M. Mizumoto, H. Nakashima, N. Yamamoto, A. Otake, N. Fujii, *FEBS Lett.* **2003**, *550*, 79–83; e) H. Tamamura, Y. Xu, T. Hattori, X. Zhang, R. Arakaki, K. Kanbara, A. Omagari, A. Otake, T. Ibuka, N. Yamamoto, H. Nakashima, N. Fujii, *Biochem. Biophys. Res. Commun.* **1998**, *253*, 877–882; f) M. Yu, E. J. Gang, R. Parameswaran, S. Stoddart, F. Fei, S. Schmidhuber, E. Park, Y. T. Hsieh, A. S. Yang, J. Groffen, N. Heisterkamp, Y. M. Kim, *Blood Cancer J.* **2011**, *1*, e14.
- [6] S. Tavor, I. Petit, S. Porozov, A. Avigdor, A. Dar, L. Leider-Trejo, N. Shemtov, V. Deutsch, E. Naparstek, A. Nagler, T. Lapidot, *Cancer Res.* **2004**, *64*, 2817–2824.
- [7] a) J. Roland, B. J. Murphy, B. Ahr, V. Robert-Hebmann, V. Delauzun, K. E. Nye, C. Devaux, M. Biard-Piechaczyk, *Blood* **2003**, *101*, 399–406; b) N. Signoret, J. Oldridge, A. Pelchen-Matthews, P. J. Klasse, T. Tran, L. F. Brass, M. M. Rosenkilde, T. W. Schwartz, W. Holmes, W. Dallas, M. A. Luther, T. N. Wells, J. A. Hoxie, M. Marsh, *J. Cell Biol.* **1997**, *139*, 651–664; c) N. Signoret, M. M. Rosenkilde, P. J. Klasse, T. W. Schwartz, M. H. Malim, J. A. Hoxie, M. Marsh, *J. Cell Sci.* **1998**, *111*, 2819–2830.
- [8] a) J. Y. Axup, K. M. Bajjuri, M. Ritland, B. M. Hutchins, C. H. Kim, S. A. Kazane, R. Halder, J. S. Forsyth, A. F. Santidrian, K. Stafin, Y. Lu, H. Tran, A. J. Seller, S. L. Biroc, A. Szydluk, J. K. Pinkstaff, F. Tian, S. C. Sinha, B. Felding-Habermann, V. V. Smider, P. G. Schultz, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 16101–16106; b) J. A. Francisco, C. G. Cerveny, D. L. Meyer, B. J. Mixan, K. Klussman, D. F. Chace, S. X. Rejniak, K. A. Gordon, R. DeBlanc, B. E. Toki, C. L. Law, S. O. Doronina, C. B. Siegall, P. D. Senter, A. F. Wahl, *Blood* **2003**, *102*, 1458–1465; c) D. Ma, C. E. Hopf, A. D. Malewicz, G. P. Donovan, P. D. Senter, W. F. Goeckeler, P. J. Maddon, W. C. Olson, *Clin. Cancer Res.* **2006**, *12*, 2591–2596.
- [9] S. O. Doronina, B. E. Toki, M. Y. Torgov, B. A. Mendelsohn, C. G. Cerveny, D. F. Chace, R. L. DeBlanc, R. P. Gearing, T. D. Bovee, C. B. Siegall, J. A. Francisco, A. F. Wahl, D. L. Meyer, P. D. Senter, *Nat. Biotechnol.* **2003**, *21*, 778–784.
- [10] a) M. S. Sutherland, R. J. Sanderson, K. A. Gordon, J. Andreyka, C. G. Cerveny, C. Yu, T. S. Lewis, D. L. Meyer, R. F. Zabinski, S. O. Doronina, P. D. Senter, C. L. Law, A. F. Wahl, *J. Biol. Chem.* **2006**, *281*, 10540–10547; b) D. Jackson, J. Gooya, S. Mao, K. Kinneer, L. Xu, M. Camara, C. Fazenbaker, R. Fleming, S. Swamynathan, D. Meyer, P. D. Senter, C. Gao, H. Wu, M. Kinch, S. Coats, P. A. Kiener, D. A. Tice, *Cancer Res.* **2008**, *68*, 9367–9374.
- [11] C. T. Dickerson, D. M. Marquis, V. Obungu, S. B. Peng, P. E. Vaillancourt, Google Patents, **2009**.
- [12] a) N. M. Okeley, J. B. Miyamoto, X. Zhang, R. J. Sanderson, D. R. Benjamin, E. L. Sievers, P. D. Senter, S. C. Alley, *Clin. Cancer Res.* **2010**, *16*, 888–897; b) Y. V. Kovtun, V. S. Goldmacher, *Cancer Lett.* **2007**, *255*, 232–240.
- [13] E. Perissinotto, G. Cavalloni, F. Leone, V. Fonsato, S. Mitola, G. Grignani, N. Surrenti, D. Sangiolo, F. Bussolino, W. Piacibello, M. Aglietta, *Clin. Cancer Res.* **2005**, *11*, 490–497.
- [14] a) M. P. Rettig, G. Ansstas, J. F. DiPersio, *Leukemia* **2012**, *26*, 34–53; b) M. Rosu-Myles, L. Gallacher, B. Murdoch, D. A. Hess, M. Keeney, D. Kelvin, L. Dale, S. S. Ferguson, D. Wu, F. Fellows, M. Bhatia, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 14626–14631.
- [15] A. Wilson, A. Trumpp, *Nat. Rev. Immunol.* **2006**, *6*, 93–106.
- [16] a) G. J. Spangrude, S. Heimfeld, I. L. Weissman, *Science* **1988**, *241*, 58–62; b) G. A. Challen, N. Boles, K. K. Lin, M. A. Goodell, *Cytometry Part A* **2009**, *75*, 14–24.