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CD38 as a PET Imaging Target in Lung Cancer

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Abstract

Daratumumab (Darzalex, Janssen Biotech) is a clinically approved antibody targeting CD38 for the treatment of multiple myeloma. However, CD38 is also expressed by other cancer cell types, including lung cancer, where its expression or absence may offer prognostic value. We therefore developed a PET tracer based upon daratumumab for tracking CD38 expression, utilizing murine models of non-small cell lung cancer to verify its specificity. Daratumumab was prepared for radiolabeling with ⁸⁹Zr (*t*_{1/2} = 78.4 h) through conjugation with desferrioxamine (Df). Western blot, flow cytometry, and saturation binding assays were utilized to characterize CD38 expression and binding of daratumumab to three non-small cell lung cancer cell lines: A549, H460, and H358. Murine xenograft models of the cell lines were also generated for further *in vivo* studies. Longitudinal PET imaging was performed following injection of ⁸⁹Zr-Df-daratumumab out to 120 h postinjection, and nonspecific uptake was also evaluated through the injection of a radiolabeled control IgG antibody in A549 mice, ⁸⁹Zr-Df-IgG. *Ex vivo* biodistribution and histological analyses were also performed after the terminal imaging time point at 120 h postinjection. Through cellular studies, A549 cells were found to express higher levels of CD38 than the H460 or H358 cell lines. PET imaging and *ex vivo* biodistribution studies verified *in vitro* trends, with A549 tumor uptake peaking at 8.1 ± 1.2%ID/g at 120 h postinjection according to PET analysis, and H460 and H358 at lower levels at the same time point (6.7 ± 0.7%ID/g and 5.1 ± 0.4%ID/g, respectively; *n* = 3 or 4). Injection of a nonspecific radiolabeled IgG into A549 tumor-bearing mice also demonstrated lower tracer uptake of 4.4 ± 1.3%ID/g at 120 h. Immunofluorescent staining of tumor tissues showed higher staining levels present in A549 tissues over H460 and H358. Thus, ⁸⁹Zr-Df-daratumumab is able to image CD38-expressing tissues *in vivo* using PET, as verified through the exploration of non-small cell lung cancer models in this study. This agent

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Notes

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therefore holds potential to image CD38 in other malignancies and aid in patient stratification and elucidation of the biodistribution of CD38.

Graphical abstract

Keywords

CD38; positron emission tomography (PET); lung cancer; zirconium-89 (^{89}Zr); daratumumab

INTRODUCTION

CD38 is an enzyme critically involved in the transport of calcium ions through the catalysis of cyclic ADP ribose and the Ca^{2+} -mobilizing secondary messenger nicotinic acid adenine dinucleotide phosphate.^{1–3} Additionally, as CD38 is also a cell surface receptor, it may be easily targeted through a number of therapeutic avenues through this function.^{4,5} Activation of CD38 leads to proliferation under physiological conditions; however, misregulation of this receptor has been detected in cancerous phenotypes, in conjunction with characteristic overproliferation and increased metastasis.⁶ While CD38 has been extensively studied in hematological malignancies such as leukemia and multiple myeloma,^{6,7} as well as in autoimmune disorders,^{8,9} recent studies have indicated a link between CD38 expression and lung cancer-initiating cells,^{10,11} as well as resistance to immune checkpoint blockade treatments.¹²

Among CD38-targeted therapies, daratumumab (Darzalex, Janssen Biotech, Inc.) has demonstrated clinical benefit in combination with standard-of-care chemotherapies in multiple myeloma treatments and is the sole clinically approved antibody targeting this receptor.¹³ Standard-of-care chemotherapy for multiple myeloma involves combinations of either bortezomib or lenalidomide with dexamethasone, resulting in overall response rates on the order of 60%. However, phase III trials employing daratumumab in combination with bortezomib and dexamethasone provided significant benefit with overall response rates of 83% for multiple myeloma patients.¹⁴ While daratumumab has not been clinically tested in solid cancers, CD38 is also expressed by other malignant cell types, including lung cancer, where its expression or absence may offer prognostic value.^{10,15,16} As the most commonly diagnosed cancer in the world, lung cancer treatments can greatly benefit from additional patient stratification,^{17,18} an area in which molecular imaging holds unmatched potential.

Imaging of overexpressed biomarkers in cancer such as CD38 is of great interest clinically, as a greater understanding of their dynamic expression can provide critical insight into disease progression and therapeutic interventions.¹⁹ However, to date no studies have evaluated the *in vivo* expression of CD38 using molecular imaging techniques.²⁰

Correlations have been drawn between traditional positron emission tomography (PET) imaging agents (e.g., ^{18}F -fluorodeoxyglucose) and single-photon emission computed tomography (SPECT) agents (e.g., $^{99\text{m}}\text{Tc}$ -methoxyisobutylisonitrile) and CD38 levels as determined through *ex vivo* analysis,^{21,22} but these studies still require invasive biopsy procedures. Employing antibody-based tracers for PET provides unparalleled sensitivity for imaging specific biomarkers noninvasively and longitudinally.²³

We therefore present a PET tracer based upon daratumumab for imaging CD38 expression noninvasively in many diseases, including the lung cancer herein, as well as lymphatic and autoimmune diseases. Targeting of CD38 for noninvasive imaging will allow unparalleled insight into mechanisms of these malignancies, and will enable visualization of the dynamic expression of CD38 over the course of therapies. Using murine models of non-small cell lung cancer, we have verified the specificity of our tracer, ^{89}Zr -Df-daratumumab, and demonstrated its potential as a powerful tool toward personalized medicine in oncology.

METHODS AND MATERIALS

Cell Culture

A549, H460, and H358 cells were obtained from the American Type Culture Collection (ATCC). Both H460 and H358 cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium, while A549 cells were cultured in F-12K medium. All media were supplemented with 10% fetal bovine serum. Cells were maintained in a humidified incubator at 5% CO_2 and 37 °C.

Western Blot

Cells were harvested and lysed in RIPA buffer supplemented with protease inhibitor cocktail (Thermo-Fisher Scientific). Centrifugation was performed at 12,000 rpm for 10 min at 4 °C to remove cellular debris. Total protein concentration was measured using the Pierce Coomassie protein assay kit (ThermoFisher Scientific). 40 μg of total protein was loaded into each well of a 4–12% Bolt Bis-Tris Plus gel (ThermoFisher Scientific). Electrophoresis was performed at 100 mV for 75 min at 4 °C. After proteins were transferred to a nitrocellulose membrane using the iBlot 2 (ThermoFisher Scientific), the membrane was blocked with Odyssey blocking buffer (LI-COR Biosciences), and incubated with anti-CD38 (1:1500) and anti- α -tubulin (1:2000) primary antibodies from Novus Biologicals overnight at 4 °C. The membrane was washed three times with PBST (phosphate buffered saline with 0.1% Tween 20), and incubated with the secondary antibodies donkey-anti-mouse DyLight 800 and donkey-anti-rabbit Dy-Light 680 (LI-COR Biosciences). The membrane was washed and then scanned using the LI-COR Odyssey infrared imaging system.

Flow Cytometry

Flow cytometry was employed to verify the varying levels of CD38 expression by the lung cancer cell lines. Daratumumab served as the primary antibody, at a concentration of 20 μg of antibody per 1 mL of solution, while goat anti-human AlexaFluor488 secondary antibody was utilized. Proper controls of cells alone, primary antibody alone, secondary antibody alone, and a nonspecific IgG antibody were employed. Staining and flow cytometry analysis

followed standard protocols.²⁴ Analysis was performed using the MACSQuant cytometer and FlowJo (V.10) software.

Preparation of Radiolabeled Daratumumab

Daratumumab was obtained in its clinically available iv injection form, buffer-exchanged to PBS, and prepared for radiolabeling through the conjugation of desferrioxamine (Df) at a 1:10 ratio following previously described procedures.²⁵ Using a PETTrace cyclotron (GE Healthcare), ⁸⁹Zr ($t_{1/2}$ = 78.4 h) was produced via proton irradiation of natural yttrium foils.²⁶ Following conjugation with Df and purification, Df–daratumumab was prepared for PET imaging through incubation with ⁸⁹Zr-oxalate and purified as previously described.²⁷ A control tracer, ⁸⁹Zr-Df–IgG, was prepared using similar methods and a nonspecific human IgG antibody. Radiolabeling yields for both tracers were consistently above 70%.

Receptor Density Assay

In order to determine the daratumumab binding affinity for A549 cells, a receptor binding assay was carried out using radiolabeled ⁸⁹Zr-Df–daratumumab. To perform the assay, approximately 1×10^5 A549 cells were seeded to the wells of a 96-well filter plate (Corning, Sigma-Aldrich). Varying concentrations of ⁸⁹Zr-Df–daratumumab (ranging from 0.01 to 33 nM) were added to the wells and allowed to incubate with gentle shaking for 2 h at room temperature. The plate was then rinsed three times with 0.1% bovine serum albumin in PBS, and the filter paper was blow-dried. Filters were then collected and counted with a PerkinElmer automated gamma counter. Analysis of data was performed in GraphPad Prism in order to obtain approximate receptor density values for A549 cells.

Animal Models

The University of Wisconsin—Madison Institutional Animal Care and Use Committee approved all animal studies. Lung cancer cells were detached from flasks using Accutase (Innovative Cell Technologies) once they reached 60–70% confluency and mixed in a 1:1 ratio of cells and Matrigel Matrix Basement Membrane (Corning). A 100 μ L sample of this mixture ($\sim 1 \times 10^6$ cells) was then subcutaneously injected into the lower right flank of 4- to 7-week-old female athymic nude mice (CrI: NU(NCr)-Fox1nu; Envigo), and tumors were allowed to grow until they reached 5 to 7 mm in diameter, at which point mice were used for imaging and biodistribution studies.

Longitudinal PET Imaging and Biodistribution Studies

For PET studies, mice bearing lung cancer xenografts ($n = 4$ or 5 per group) were intravenously injected with 5–10 MBq (5–15 μ g of antibody) of ⁸⁹Zr-Df–daratumumab. Static scans of 40 million coincidence events were acquired at regular time intervals from 6 h postinjection to 120 h postinjection using the small animal Inveon PET/CT (Siemens). Following the terminal imaging time point, mice were euthanized, various organs were harvested and wet-weighed, and gamma counting was performed to determine their radioactive content using a WIZARD2 automatic gamma counter (PerkinElmer). All uptake values from PET region-of-interest (ROI) analysis (quantified using the Inveon Research Workspace) and *ex vivo* biodistribution studies are presented as percentage of the injected

dose per gram (%ID/g). Additionally, one group of mice ($n = 4$) bearing A549 xenografts were injected with 5–10 MBq of ^{89}Zr -Df-IgG, a nonspecific human monoclonal antibody, to map the distribution of nonspecific binding. PET ROI analysis and biodistribution studies were similarly performed for this study group.

Immunofluorescent Staining

Immunofluorescent staining was performed to visualize the distribution of CD38 on lung cancer tissues excised from mice 120 h postinjection of ^{89}Zr -Df-daratumumab using standard procedures.²⁸ Primary mouse anti-human CD38 antibody (Novus Biologicals) and secondary goat anti-mouse AlexaFluor488 were employed for staining, as well as DAPI-containing hard mount solution (Vector Laboratories). Confocal imaging of slides was then performed using a Nikon A1RS microscope. Fluorescent intensities were analyzed using ImageJ FIJI software.

Statistical Analysis

All data are presented as mean $\pm$ standard deviation. Comparisons between groups (such as from PET ROI analysis) were made using the Student *t*-test, wherein *p*-values less than 0.05 were considered statistically significant. GraphPad Prism was used to analyze receptor binding assay data.

RESULTS

In Vitro Analysis Shows Varying CD38 Expression in Lung Cancer Cell Lines

Western blot and flow cytometry analysis both demonstrated differential expression of CD38 by the studied non-small cell lung cancer lines (A549, H460, and H358). Flow cytometry showed the highest level of CD38 staining in A549 cells, while H460 and H358 cells demonstrated similarly low binding (Figure 1A), with minimal binding of the nonspecific IgG antibody to any tissues. Western blot analysis of the cell lines further verified the presence of CD38 (MW: ~45 kDa) expression by A549 cells (Figure 1B). To further explore the interaction of CD38 and daratumumab, a receptor binding assay was conducted in CD38-expressing A549 cells. Specific binding of daratumumab to A549 cells was demonstrated (Figure 1C), with approximately 50,000 receptors per cell calculated through analysis of the binding curve.

PET Imaging Distinguishes CD38-Expressing Tissues

PET imaging studies demonstrated the ability of ^{89}Zr -Df-daratumumab to differentiate tissues based upon their CD38 expression (Figures 2 and 3). A549 tumors, with the highest levels of CD38 determined through *in vitro* studies, displayed the highest uptake at the last imaging time point (120 h postinjection) with $8.1 \pm 1.2\%$ ID/g. H460 and H358 tumors had lower uptakes of $6.7 \pm 0.7\%$ ID/g and $5.1 \pm 0.4\%$ ID/g, respectively, at 120 h postinjection of ^{89}Zr -Df-daratumumab. Injection of nonspecific ^{89}Zr -Df-IgG into A549 tumor-bearing mice provided tumor uptake of $4.4 \pm 1.3\%$ ID/g at the same time point. Statistically higher uptake was observed in A549 mice injected with ^{89}Zr -Df-daratumumab at all time points after 12 h postinjection over the nonspecific tracer ($p < 0.05$, $n = 3$ or 4). A549 tumors also had significantly higher tracer uptake than H358 at both 72 and 120 h postinjection.

At the terminal imaging time point, sizable ^{89}Zr -Df–daratumumab uptake was observed in the liver (approximately 7%ID/g across all groups), consistent with the biological clearance pattern of antibody-based tracers. Other off-target organs displayed minimal uptake, and the tracer cleared out of circulation over time. Notably, ^{89}Zr -Df–IgG accumulated to a higher extent than the specific tracer in the liver, while other organs had similar uptake of the two tracers. ^{89}Zr -Df–daratumumab displayed good *in vivo* stability, as minimal bone accumulation of radioactivity was observed.

Ex Vivo Analysis Confirms PET Results

Ex vivo biodistribution studies verified the trends observed through PET ROI analysis (Figure 4). A549 tumors again were found to have the highest uptake at $9.8 \pm 1.1\%$ ID/g, followed by H460, H358, and the nonspecific tracer ($7.2 \pm 0.1\%$ ID/g, $5.5 \pm 1.0\%$ ID/g, and $5.0 \pm 1.7\%$ ID/g, respectively). In all groups injected with ^{89}Zr -Df–daratumumab, uptake in off-target organs was similar. Injection of the nonspecific tracer ^{89}Zr -Df–IgG resulted in increased liver uptake ($11.6 \pm 0.9\%$ ID/g vs $7.2 \pm 2.0\%$ ID/g, $p < 0.05$), with other organs remaining comparable across groups.

As seen in Figure 5, immunofluorescent staining of tumor tissues once again revealed differential CD38 expression by lung cancer cell lines. The most intense staining was found in A549 tissues, with staining localized to the surface of a subpopulation of cells. H460 and H358 tissues demonstrated near-background levels of staining. A549 tumors were found to have mean CD38 staining levels twice that of both H460 and H358, when normalized to DAPI staining. The absence of staining in H460 and H358 tumors confirmed that uptake in *in vivo* studies was due to the enhanced permeability and retention (EPR) effect, rather than specific binding.

DISCUSSION

Personalized medicine is becoming an ideal goal in all types of cancer treatments, with molecular imaging playing a major role in this revolution.^{29,30} Imaging of cancer-specific targets may enable better selection of patients to particular therapies in clinical settings, as well as greater insight into disease mechanisms and biomarker distributions in preclinical studies. To this end, we herein describe the generation and validation of a PET tracer for imaging of human CD38 in mouse xenograft models of lung cancer.

CD38 is a traditional biomarker for hematological diseases,⁴ where in many cases its expression offers prognostic value.^{15,31,32} High CD38 expression has been associated with favorable prognosis in acute B lymphoblastic leukemia, whereas high expression has conversely been correlated with poor outcomes in hairy cell leukemia and extranodal NK/T cell lymphoma.^{15,31,32} Additionally, expression of CD38 by lung cancer cells has recently been indicated as a marker of resistance to PD-1/PD-L1 immune blockade treatments.¹² Further studies into the impact of CD38 expression are thus warranted to address these contrasting observations. *In vivo* imaging of CD38 will certainly provide invaluable insight for this endeavor.

To date, the only direct molecular imaging of myeloma cells has employed bioluminescent or fluorescent techniques in murine models.^{33–36} These studies, while informative, are limited in translatability, as they monitor the distribution of labeled, injected cells. The use of a molecular imaging agent, such as ⁸⁹Zr-Df–daratumumab, will allow direct visualization of CD38 biodistribution, and may be more easily translated as it is based upon a clinically approved antibody. CD38 protein has a very limited normal tissue biodistribution, mostly in immune and reproductive system tissues,³⁷ making it an attractive imaging target.

The tracer developed in this study demonstrated differential uptake in CD38-expressing xenografts, showing that it is able to clearly delineate CD38-expressing tissues with minimal off-target nonspecific accumulation, as daratumumab does not cross-react with murine CD38.³⁸ This binding occurs even while the positive cell line (A549) only displayed a receptor density of 50,000 per cell, in contrast to the >200,000 receptors per positive cell in many other imaging studies, such as epidermal growth factor receptor (EGFR)-targeting in breast cancer, where nearly 700,000 receptors per cell have been reported.³⁹ Additionally, as evidenced through tissue staining, only a subpopulation of lung cancer cells express CD38. This low expression level makes *in vitro* characterization difficult, and certainly may play a role in the interesting trends observed between *in vitro* expression and *in vivo* tracer accumulation. It is thus expected that drugs which modulate or induce CD38 expression may increase this proportion and lead to differing uptake of ⁸⁹Zr-Df–daratumumab, an area which has yet to be explored.

There appears to be some baseline accumulation of ⁸⁹Zr-Df– daratumumab due to the EPR effect in both H460 and H358 tumors, as evidenced through notable uptake in these CD38-negative tissues, a common phenomenon with large platforms such as antibodies.⁴⁰ Certainly, factors other than just receptor density play a role in tracer accumulation, including vascularization of the tumors and their cellular structure.

CD38 expression in lung cancer may offer prognostic value^{10–12} but requires further exploration with differing treatments. Not only will imaging of this target allow insight into the biodistribution of CD38 in cancerous tissues compared to normal, but as a major function of CD38 involves calcium regulation, imaging of this target will allow greater insight into misregulation of this pathway in cancerous phenotypes. We expect that ⁸⁹Zr-Df–daratumumab and similar molecular imaging tracers will aid in this effort, allowing noninvasive imaging of CD38 and its dynamic expression.

Many exciting options exist for future application of the CD38 tracer, ⁸⁹Zr-Df–daratumumab. A clear extension of this study is the exploration of the tracer in hematological and lymphatic disease models. CD38 expression has been thoroughly investigated through biopsy sampling in these diseases, and correlations between this expression and patient outcomes have been extensively documented.^{6,15,31,32} As we have herein demonstrated that uptake of ⁸⁹Zr-Df–daratumumab corresponds to CD38 expression, the tracer certainly holds potential for stratification of patients based upon CD38 levels, which may be mapped throughout the course of therapy using this tracer. Long circulation half-lives of antibodies such as that herein are certainly of concern; thus, fragments of daratumumab may be found to be more clinically suitable in the future.

Preclinically, this PET tracer may find application in a wide variety of malignancies in which CD38 expression has been correlated with patient outcomes in order to better understand disease progression.^{9,16} Additionally, T-cell expression of CD38 has been shown to be important in a number of diseases,^{41–43} such that imaging of this target in humanized mice⁴⁴ may provide insight into the behavior of T-cells and their homing to tumors.

In conclusion, we have herein demonstrated that ⁸⁹Zr-Df-daratumumab delineates CD38-expressing tissues effectively and noninvasively. Thus, this tracer may provide both researchers and clinicians with invaluable insight into mechanisms of response and patient stratification in CD38-expressing malignancies, preclinically and clinically.

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References

1. Kwong AKY, Chen Z, Zhang H, Leung FP, Lam CMC, Ting KY, Zhang L, Hao Q, Zhang L-H, Lee HC. Catalysis-Based Inhibitors of the Calcium Signaling Function of CD38. *Biochemistry*. 2012; 51(1):555–564. [PubMed: 22142305]
2. Deaglio S, Mehta K, Malavasi F. Human CD38: a (r)evolutionary story of enzymes and receptors. *Leuk Res*. 2001; 25:1. [PubMed: 11137554]
3. Malavasi F, Funaro A, Roggero S, Horenstein A, Calosso L, Mehta K. Human CD38: a glycoprotein in search of a function. *Immunol Today*. 1994; 15:95. [PubMed: 8172650]
4. van de Donk NWJ, Janmaat ML, Mutis T, Lammerts van Bueren JJ, Ahmadi T, Sasser AK, Lokhorst HM, Parren PWH. Monoclonal antibodies targeting CD38 in hematological malignancies and beyond. *Immunol Rev*. 2016; 270(1):95–112. [PubMed: 26864107]
5. Teiluf K, Seidl C, Blechert B, Gaertner FC, Gilbertz K-P, Fernandez V, Bassermann F, Endell J, Boxhammer R, Leclair S, Vallon M, Aichler M, Feuchtinger A, Bruchertseifer F, Morgenstern A, Essler M. α -Radioimmunotherapy with 213 Bi-anti-CD38 immunoconjugates is effective in a mouse model of human multiple myeloma. *Oncotarget*. 2014; 6(7):4692.
6. Deaglio S, Vaisitti T, Serra S, Audrito V, Bologna C, D'Arena G, Laurenti L, Gottardi D, Malavasi F. CD38 in Chronic Lymphocytic Leukemia: From Bench to Bedside? *Mini-Rev Med Chem*. 2011; 11(6):503–507. [PubMed: 21561406]
7. de Weers M, Tai Y-T, van der Veer MS, Bakker JM, Vink T, Jacobs DCH, Oomen LA, Peipp M, Valerius T, Slootstra JW, Mutis T, Bleeker WK, Anderson KC, Lokhorst HM, van de Winkel JGJ, Parren PWH. Daratumumab, a Novel Therapeutic Human CD38 Monoclonal Antibody, Induces Killing of Multiple Myeloma and Other Hematological Tumors. *J Immunol*. 2011; 186(3):1840–1848. [PubMed: 21187443]
8. Antonelli A, Fallahi P, Nesti C, Pupilli C, Marchetti P, Takasawa S, Okamoto H, Ferrannini E. Anti-CD38 autoimmunity in patients with chronic autoimmune thyroiditis or Graves' disease. *Clin Exp Immunol*. 2001; 126(3):426–431. [PubMed: 11737057]
9. Chen J, Chen Y-G, Reifsnnyder PC, Schott WH, Lee C-H, Osborne M, Scheuplein F, Haag F, Koch-Nolte F, Serreze DV, Leiter EH. Targeted Disruption of CD38 Accelerates Autoimmune Diabetes in NOD/Lt Mice by Enhancing Autoimmunity in an ADP-Ribosyltransferase 2-Dependent Fashion. *J Immunol*. 2006; 176(8):4590–4599. [PubMed: 16585549]
10. Karimi-Busheri F, Rasouli-Nia A, Zadorozhny V, Fakhrai H. CD24(+)/CD38(–) as new prognostic marker for non-small cell lung cancer. *Multidiscip Respir Med*. 2013; 8(1):65–65. [PubMed: 24094028]

11. Karimi-Busheri F, Zadorozhny V, Li T, Lin H, Shawler DL, Fakhrai H. Pivotal role of CD38 biomarker in combination with CD24, EpCAM, and ALDH for identification of H460 derived lung cancer stem cells. *J Stem Cells*. 2011; 6(1):9–20. [PubMed: 22997842]
12. Chen, L., Byers, LA., Ullrich, S., Wistuba, II., Qin, XF., Gibbons, DL. CD 38 as a novel immune checkpoint and a mechanism of resistance to the blockade of the PD-1/PD-L1 axis; 2017 ASCO-SITC Clinical Immuno-Oncology Symposium; Orlando, FL. Orlando, FL: 2017.
13. Lokhorst HM, Plesner T, Laubach JP, Nahi H, Gimsing P, Hansson M, Minnema MC, Lassen U, Krejcik J, Palumbo A, van de Donk NWCJ, Ahmadi T, Khan I, Uhlar CM, Wang J, Sasser AK, Losic N, Lisby S, Basse L, Brun N, Richardson PG. Targeting CD38 with Daratumumab Monotherapy in Multiple Myeloma. *N Engl J Med*. 2015; 373(13):1207–1219. [PubMed: 26308596]
14. Sanchez L, Wang Y, Siegel DS, Wang ML. Daratumumab: a first-in-class CD38 monoclonal antibody for the treatment of multiple myeloma. *J Hematol Oncol*. 2016; 9(1):51. [PubMed: 27363983]
15. Jiang Z, Wu D, Lin S, Li P. CD34 and CD38 are prognostic biomarkers for acute B lymphoblastic leukemia. *Biomarker Res*. 2016; 4(1):23.
16. Stone L. Prostate cancer: On the down-low [mdash] low luminal cell CD38 expression is prognostic. *Nat Rev Urol*. 2017; doi: 10.1038/nrurol.2016.269
17. Malik PS, Raina V. Lung cancer: Prevalent trends & emerging concepts. *Indian J Med Res*. 2015; 141(1):5–7. [PubMed: 25857489]
18. Torre, LA., Siegel, RL., Jemal, A. Lung Cancer Statistics. In: Ahmad, A., Gadgeel, S., editors. *Lung Cancer and Personalized Medicine: Current Knowledge and Therapies*. Springer International Publishing; Cham: 2016. p. 1-19.
19. Ehlerding EB, England CG, McNeel DG, Cai W. Molecular Imaging of Immunotherapy Targets in Cancer. *J Nucl Med*. 2016; 57(10):1487–1492. [PubMed: 27469363]
20. Mesguich C, Zanotti-Fregonara P, Hindie E. New Perspectives Offered by Nuclear Medicine for the Imaging and Therapy of Multiple Myeloma. *Theranostics*. 2016; 6(2):287–90. [PubMed: 26877786]
21. AK , Aslan V, Vardareli E, Gülba Z. Tc-99m methoxyisobutylisonitrile bone marrow imaging for predicting the levels of myeloma cells in bone marrow in multiple myeloma: correlation with CD38/CD138 expressing myeloma cells. *Ann Hematol*. 2003; 82(2):88–92. [PubMed: 12601486]
22. Ak , Gulbas Z. F-18 FDG uptake of bone marrow on PET/CT scan: it's correlation with CD38/CD138 expressing myeloma cells in bone marrow of patients with multiple myeloma. *Ann Hematol*. 2011; 90(1):81–87. [PubMed: 20690019]
23. van de Watering FCJ, Rijpkema M, Perk L, Brinkmann U, Oyen WJG, Boerman OC. Zirconium-89 Labeled Antibodies: A New Tool for Molecular Imaging in Cancer Patients. *BioMed Res Int*. 2014; 2014:203601. [PubMed: 24991539]
24. Hernandez R, Sun H, England CG, Valdovinos HF, Ehlerding EB, Barnhart TE, Yang Y, Cai W. CD146-targeted immunoPET and NIRF Imaging of Hepatocellular Carcinoma with a Dual-Labeled Monoclonal Antibody. *Theranostics*. 2016; 6(11):1918–1933. [PubMed: 27570560]
25. Hong H, Severin GW, Yang Y, Engle JW, Zhang Y, Barnhart TE, Liu G, Leigh BR, Nickles RJ, Cai W. Positron emission tomography imaging of CD105 expression with 89Zr-Df-TRC105. *Eur J Nucl Med Mol Imaging*. 2012; 39(1):138–148. [PubMed: 21909753]
26. Nickles RJ, Avila-Rodriguez MA, Nye JA, Houser EN, Selwyn RG, Schueller MJ, Christian BT, Jensen M. Sustainable production of orphan radionuclides at Wisconsin. *Q J Nucl Med Mol Imaging*. 2008; 52(2):134–139. [PubMed: 18043541]
27. Yang Y, Hernandez R, Rao J, Yin L, Qu Y, Wu J, England CG, Graves SA, Lewis CM, Wang P, Meyerand ME, Nickles RJ, Bian X-w, Cai W. Targeting CD146 with a 64Cu-labeled antibody enables in vivo immunoPET imaging of high-grade gliomas. *Proc Natl Acad Sci U S A*. 2015; 112(47):E6525–E6534. [PubMed: 26553993]
28. England CG, Ehlerding EB, Hernandez R, Rekoske BT, Graves SA, Sun H, Liu G, McNeel DG, Barnhart TE, Cai W. Preclinical pharmacokinetics and biodistribution studies of 89Zr-labeled pembrolizumab. *J Nucl Med*. 2017; 58(1):162–168. [PubMed: 27493273]

29. Basu S, Alavi A. PET-Based Personalized Management in Clinical Oncology: An Unavoidable Path for the Foreseeable Future. *PET clinics*. 2016; 11(3):203–7. [PubMed: 27321025]
30. Nunez Miller R, Pozo MA. Non-FDG PET in oncology. *Clin Transl Oncol*. 2011; 13(11):780–6. [PubMed: 22082641]
31. Poret N, Fu Q, Guihard S, Cheok M, Miller K, Zeng G, Quesnel B, Troussard X, Galiègue-Zouitina S, Shelley CS. CD38 in Hairy Cell Leukemia Is a Marker of Poor Prognosis and a New Target for Therapy. *Cancer Res*. 2015; 75(18):3902–3911. [PubMed: 26170397]
32. Wang L, Wang H, Li P-f, Lu Y, Xia Z-j, Huang H-q, Zhang Y-j. CD38 expression predicts poor prognosis and might be a potential therapy target in extranodal NK/T cell lymphoma, nasal type. *Ann Hematol*. 2015; 94(8):1381–1388. [PubMed: 25865943]
33. Rozemuller H, van der Spek E, Bogers-Boer LH, Zwart MC, Verweij V, Emmelot M, Groen RW, Spaapen R, Bloem AC, Lokhorst HM, Mutis T, Martens AC. A bioluminescence imaging based *in vivo* model for preclinical testing of novel cellular immunotherapy strategies to improve the graft-versus-myeloma effect. *Haematologica*. 2008; 93(7):1049–1057. [PubMed: 18492693]
34. Riedel SS, Mottok A, Brede C, Bäuerlein CA, Jordán Garrote A-L, Ritz M, Mattenheimer K, Rosenwald A, Einsele H, Bogen B, Beilhack A. Non-Invasive Imaging Provides Spatiotemporal Information on Disease Progression and Response to Therapy in a Murine Model of Multiple Myeloma. *PLoS One*. 2012; 7(12):e52398. [PubMed: 23300660]
35. Postnov AA, Rozemuller H, Verweij V, Lokhorst H, De Clerck N, Martens AC. Correlation of High-Resolution X-Ray Micro-Computed Tomography with Bioluminescence Imaging of Multiple Myeloma Growth in a Xenograft Mouse Model. *Calcif Tissue Int*. 2009; 85(5):434. [PubMed: 19816649]
36. Mitsiades CS, Mitsiades NS, Bronson RT, Chauhan D, Munshi N, Treon SP, Maxwell CA, Pilarski L, Hideshima T, Hoffman RM, Anderson KC. Fluorescence Imaging of Multiple Myeloma Cells in a Clinically Relevant SCID/NOD in Vivo Model: Biologic and Clinical Implications. *Cancer Res*. 2003; 63(20):6689–6696. [PubMed: 14583463]
37. The Human Protein Atlas. p. CD38 proteinatlas.org
38. European Medicines Agency, Committee for Medicinal Products for Human Use. Assessment report: Darzalex. 2016
39. Fitzpatrick SL, LaChance MP, Schultz GS. Characterization of Epidermal Growth Factor Receptor and Action on Human Breast Cancer Cells in Culture. *Cancer Res*. 1984; 44(8):3442–3447. [PubMed: 6331647]
40. Maeda H. Toward a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity. *Adv Drug Delivery Rev*. 2015; 91:3–6.
41. Abousamra NK, El-Din MS, Azmy E. T-cell CD38 expression in B-chronic lymphocytic leukaemia. *Hematol Oncol*. 2009; 27(2):82–89. [PubMed: 19247976]
42. Pavón EJ, Zumaquero E, Rosal-Vela A, Khoo K-M, Cerezo-Wallis D, García-Rodríguez S, Carrascal M, Abian J, Graeff R, Callejas-Rubio J-L, Ortego-Centeno N, Malavasi F, Zubiaur M, Sancho J. Increased CD38 expression in T cells and circulating anti-CD38 IgG autoantibodies differentially correlate with distinct cytokine profiles and disease activity in systemic lupus erythematosus patients. *Cytokine*. 2013; 62(2):232–243. [PubMed: 23538292]
43. Xu L, Chen D, Lu C, Liu X, Wu G, Zhang Y. Advanced Lung Cancer Is Associated with Decreased Expression of Perforin, CD95, CD38 by Circulating CD3+CD8+ T Lymphocytes. *Ann Clin Lab Sci*. 2015; 45(5):528–532. [PubMed: 26586704]
44. Gonzalez L, Strbo N, Podack ER. Humanized mice: novel model for studying mechanisms of human immune-based therapies. *Immunol Res*. 2013; 57(1):326–334. [PubMed: 24248605]

Figure 1.

In vitro analysis of CD38 expression and binding of daratumumab to lung cancer cells. (A) Flow cytometry demonstrated differential expression of CD38 in the studied lung cancer cell lines. (B) Western blot analysis verified high CD38 expression by A549 cells. (C) A binding assay was performed in A549 cells, demonstrating specific binding of daratumumab to the cell surface.

Figure 2.

Longitudinal PET imaging in mice bearing lung cancer xenografts after injection of ^{89}Zr -Df-daratumumab. A549 tumors displayed the highest uptake at the final imaging time point (120 h), followed by H460 and H358. Injection of a nonspecific tracer (^{89}Zr -Df-IgG) into A549-bearing mice demonstrated significantly decreased uptake as compared to the specific tracer at all time points after 6 h postinjection ($p < 0.05$, $n = 3$ or 4). Tumors are designated by fuchsia circles in the whole body maximum intensity projection (MIP) images.

Figure 3.

Quantification of longitudinal PET imaging after injection of ^{89}Zr -Df-daratumumab and ^{89}Zr -Df-IgG. (A) Uptake of the tracers in lung cancer xenograft tumors. At the final time point, A549 tumors displayed significantly higher uptake than H358 tumors and the nonspecific tracer. (B) Blood pool activity was similar over time in all groups. * $p < 0.05$; ** $p < 0.01$.

Figure 4.

Ex vivo biodistribution studies were conducted after the terminal imaging time point at 120 h postinjection of the tracer. A549 tumors exhibited significantly higher uptake than all other groups. Most off-target organs displayed similar uptake except the liver, where ^{89}Zr -Df-IgG accumulated to a higher level than ^{89}Zr -Df-daratumumab. * $p < 0.05$; ** $p < 0.01$.

Figure 5. Immunofluorescent staining of tumor sections. A549 tumors were found to have the highest level of staining, followed by H460 and H358 with lower levels, all localized to cell surfaces. Scale bar: 10 μm .