Attenuation of Cartilage Pathogenesis in Post-Traumatic Osteoarthritis (PTOA) in Mice by Blocking the Stromal Derived Factor 1 Receptor (CXCR4) With the Specific Inhibitor, AMD3100

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ABSTRACT: SDF-1 was found to infiltrate cartilage, decrease proteoglycan content, and increase MMP-13 activity after joint trauma. In this study, we tested the hypothesis that interference of the SDF-1/CXCR4 signaling pathway via AMD3100 can attenuate pathogenesis in a mouse model of PTOA. We also tested the predictive and confirmatory power of fluorescence molecular tomography (FMT) for cartilage assessment. AMD3100 was continuously delivered via mini-osmotic pumps. The extent of cartilage damage after AMD3100 or PBS treatment was assessed by histological analysis 2 months after PTOA was induced by surgical destabilization of the medial meniscus (DMM). Biochemical markers of PTOA were assessed via immunohistochemistry and in vivo fluorescence molecular tomography (FMT). Regression analysis was used to validate the predictive power of FMT measurements. Safranin-O staining revealed significant PTOA damage in the DMM/PBS mice, while the DMM/AMD3100 treated mice showed a significantly reduced response with minimal pathology. Immunohistochemistry showed that AMD3100 treatment markedly reduced typical PTOA marker expression in chondrocytes. FMT measurements showed decreased cathepsins and MMP activity in knee joints after treatment. The results demonstrate that AMD3100 treatment attenuates PTOA. AMD3100 may provide a viable and expedient option for PTOA therapy given the drug's FDA approval and well-known safety profile. © 2015 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 33:1071–1078, 2015.

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It has been reported that aging, trauma, excessive mechanical load, and genetic factors are correlated with osteoarthritis (OA) development, while the exact mechanisms remain elusive.[1–3] In particular, the treatment and prevention of post-traumatic osteoarthritis (PTOA) is essential as joint-related trauma has been highly correlated with PTOA development.[4–10] Even in light of increasing research into reconstructive strategies and novel developments, joint reconstruction has not been shown to attenuate PTOA development.[11–14]

Chemokines and their receptors have garnered much attention given their role in immune cell function, importance for the regulation of cancer cell invasion, and role in the migration of stem cells. Specific to this study, CXCR4, is a G-protein coupled receptor that promotes activation of intracellular signaling cascades and release of MMP1 and VEGF.[15,16] Originally isolated from a bone marrow stromal line, CXCR4's ligand is the 8 kDa chemokine SDF-1.[17] Even though the mechanism of release remains unknown, overproduction of SDF-1 has been related to inflammatory cytokines including IL-1β and TNF-α.[18,19] SDF-1 has been shown to have a variety of targets, activating primary cells by binding to the CXCR4 receptor, which in turn, stimulates chondrocyte proliferation, differentiation, and apoptosis.[20,21]

Recent evidence would also indicate that SDF-1/CXCR4 signaling plays an important role in PTOA progression. Looking at the distribution of SDF-1 and CXCR4 in human joints, research has shown that SDF-1 is produced in synovial membrane cells while it is receptor is primarily located in articular chondrocytes.[22] Briefly, SDF-1 activates the calcium, Erk and p38 MAP kinase signaling pathways, leading to the release of MMPs and other proteins.[23–25] Clinical data related to synovectomies, a procedure that effectively relieves the pain associated with OA, shows a reduction in the serum SDF-1 level, decreasing intra-articular MMP release.[26] The increase of SDF-1 was also found in the PTOA animal model.[22,23,26,27] These results would point to the SDF-1/CXCR4 as a key pathway in regulating PTOA related cartilage degeneration.[22,23,26,27] Studies have also shown SDF-1/CXCR4 signaling to play an important role in growth plate development. Through the mediation of type X collagen and MMP-13, key markers of hypertrophic chondrocyte differentiation, SDF-1/CXCR4 interaction stimulates chondrocyte hypertrophy at the chondro-osseous junction during bone formation.[28]

Given the alternative role of the SDF-1/CXCR4 in HIV pathways, the development of chemical agents related to pathway attenuation has been greatly accelerated. AMD3100, a specific inhibitor of the SDF-1/CXCR4 pathway, presents an ideal candidate for consideration. A bicyclam with a high specificity for CXCR4, AMD3100 has been approved for human use in HIV and cancer therapy.[29,30] Specific to chondrosar-

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coma, AMD3100 has previously been used to inhibit the expression of MMP1 and cell invasion in vitro.[31] Specific to this study, Wei et al.[32] found that SDF-1/CXCR4 binding induces OA cartilage degeneration and disruption of the pathway via siRNA attenuated the effects of SDF-1 treatment in a primary guinea pig model of natural OA.

In this study, we tested the hypothesis that trauma-associated, SDF-1 mediated cartilage degradation can be prevented by blocking the interaction between SDF-1 and the CXCR4 receptor on articular chondrocytes via continuous infusion of a specific inhibitor, AMD3100, in a mouse model of PTOA. We also tested the predictive and confirmatory power of in vivo fluorescence molecular tomography (FMT), a non-invasive imaging technique that can provide a quantitative measure of catabolic enzymes using specific probes.

METHODS

Animals

Twenty-eight male C57Black6/J mice (2-month-old) were obtained at 8 weeks of age (Charles River, Cambridge, MA). Mice were randomized into three groups: Group 1 ($n=8$) animals underwent destabilization of the medial meniscus (DMM) on the right knee and were treated with AMD3100 via constant infusion osmotic mini-pump; Group 2 ($n=8$) animals underwent DMM on the right knee and were treated with PBS via constant infusion osmotic mini-pump; and Group 3 ($n=5$) animals underwent sham surgery on right knee and received empty pumps at 8 weeks. All animals were euthanized 2 months after surgery. An additional group, which underwent neither surgery nor pump implantation, was included as an additional control ($n=7$). Right hind limbs were harvested immediately after euthanasia. Approval was obtained *via* the Institutional Animal Care and Use Committee (IACUC) at Rhode Island Hospital.

Surgery

To induce PTOA in the destabilization of the medial meniscus (DMM) subgroups, the right medial meniscotibial ligament was cut using a surgical microscope and microsurgical technique to destabilize the medial meniscus (DMM) as previously described by Glasson et al.[33] Attention was paid not to injure the articular cartilage during the procedure. The right knee joints of mice in the Sham subgroups were sham-operated through the same approach without medial meniscotibial ligament injury. Post-operative animals were allowed unrestricted activity, food, and water and housed under standard conditions.

Delivery and Dosing of AMD3100

A 1.5 cm transverse skin incision was made over the dorsal thorax, and a subcutaneous pocket created via blunt dissection. The loaded Alzet osmotic minipumps (model 1004, 0.11 μL/h Alza, Palo Alto, CA) were inserted and the fascia and skin closed with 8-0 nylon, while the skin was closed with surgical staples. AMD3100 (Mozobil; Genzyme, Framingham, MA) was administered systemically. AMD3100 dosing was virtually identical to that used to successfully inhibit autoimmune joint inflammation in IFN-gamma receptor-deficient mice.[34] AMD3100 was delivered at a rate of 180 μg/day, which corresponds to steady serum level of 0.3 μg/ml.[35] Given the maximum duration of the Alzet osmotic pump is 4 weeks, the pumps were exchanged once. After 2 months of treatment the animals were euthanized and the knee joints removed.

Histology

The knee joints of right hind limbs were harvested and immersed in 10% (v/v) formalin for 72 h. The specimens were decalcified in 20% (v/v) EDTA solution (pH 7.2) and dissected in the sagittal plane. They were processed in a Tissue-Tek VIP 1000 tissue processor (Miles, Elkhart, IN) and embedded in a single block of Paraplast X-tra (Thermo-Fisher, Hampton, NH). The slices were cut into 6-μm sections and mounted on slides. Safranin-O staining was performed and the severity of cartilage damage was then assessed using the OARSI osteoarthritis cartilage histopathology assessment system (OOCHAS) grading system (PTOA score = Grade × Stage, total 0–24) by three independent and blinded observers, before the scores were averaged for each joint.[36]

Immunohistochemistry

To determine the expression of inflammatory and catabolic factors immunohistochemistry was performed. To detect the distribution of PTOA markers: MMP-13, type 2 $3/4C_{short}$ (C1, C2) and type X collagen in articular cartilage, 6-μm sections were collected on positively charged glass slides (Thermo-Fisher). Immunohistochemistry was carried out using the DAB Histostain-SP Kit (Zymed-Invitrogen, Carlsbad, CA). Sections were prepared via standard methods. The sections were incubated with specific antibodies against MMP-13 (Santa Cruz, Santa Cruz, CA), type 2 $3/4C_{short}$ (C1,C2), which detects fragments of both type I and type II collagen produced by the action of collagenase (IBEX, Montreal, Quebec), and type X collagen (Santa Cruz) respectively at 4°C overnight. Following staining, slides were qualitatively analyzed for the expression of markers. Photography was performed with a Nikon E800 microscope (Nikon, Melville, NY).

Fluorescence Molecular Tomography (FMT)

Using in vivo FMT imaging methods, real-time information was gained about biological processes using probes and deep tissue imaging.[37–39] ProSense and MMPSense, both protease-activated near-infrared (NIR) fluorescence imaging probes, detect cathepsin and MMP activity respectively. We used FMT imaging at three months to confirm that AMD3100 treatment reduced the presence of inflammatory reactions associated with PTOA pathology. We also used FMT imaging to confirm histological scores and immunohistochemical data.

Similar to the methodology used by Zhou et al.[40] at 2 months, mice were injected with single dose of ProSense 750EX and MMPSense 680 fluorescent agents (PerkinElmer, Waltham, MA) 24 h before scanning. After being anesthetized using an intraperitoneal injection of ketamine (75 mg/kg) and medetomidine (1 mg/kg), mice were placed in an upright position in the imaging chamber and then imaged with the FMT system (ViSen, Waltham, MA). A NIR laser diode emitting continuous wave radiation at wavelengths of 670 or 746 nm transilluminated the lower body of animal from posterior to anterior, and both excitation and emission signals were detected by a charge-coupled device (CCD) camera and appropriate band pass filters. ProSense detects cathepsin B, L, S, and plasmin. MMPSense detects MMP-2, 3, 9, and 13 activities. The DMM/PBS group ($N=5$), DMM/AMD3100 group ($N=6$), and Sham group ($N=6$), were

imaged. Concentrations of the probes in the knee joint were determined using Region of Interest (ROI) analysis, by restricting the area of measurement to the mid-femur to mid-tibia in order to isolate the joint space.

Statistical Analysis

The OOCHAS score in different groups were analyzed by one-way ANOVA with multiple pair-wise comparisons made by the Student–Newman–Keuls method (three comparisons or more) at a rejection level of 5% unless otherwise noted. Mixed linear models were used to compare cartilage inflammation measurements via in vivo FMT measurements. Residual estimates of maximum likelihood were used to fit the models to provide unbiased estimates for missing data due to the small sample size of FMT scans. Post hoc paired comparisons among the three experimental groups were carried out with orthogonal contrasts using the Spearman's rank test to maintain alpha at 0.05. Adjusted p values are reported to account for multiple comparisons. All data are presented as means and p values of the (operative limb treated with AMD3100-control sham limb). The relationships between the Mankin score versus the FMT quantifications of inflammation were assessed with regression analysis. All statistical analyses were done in STATA SE 12.1 (StataCorp, College Station, TX).

RESULTS

Histology

Cartilage histology revealed a safranin-O positive articular cartilage surface in the DMM/AMD3100

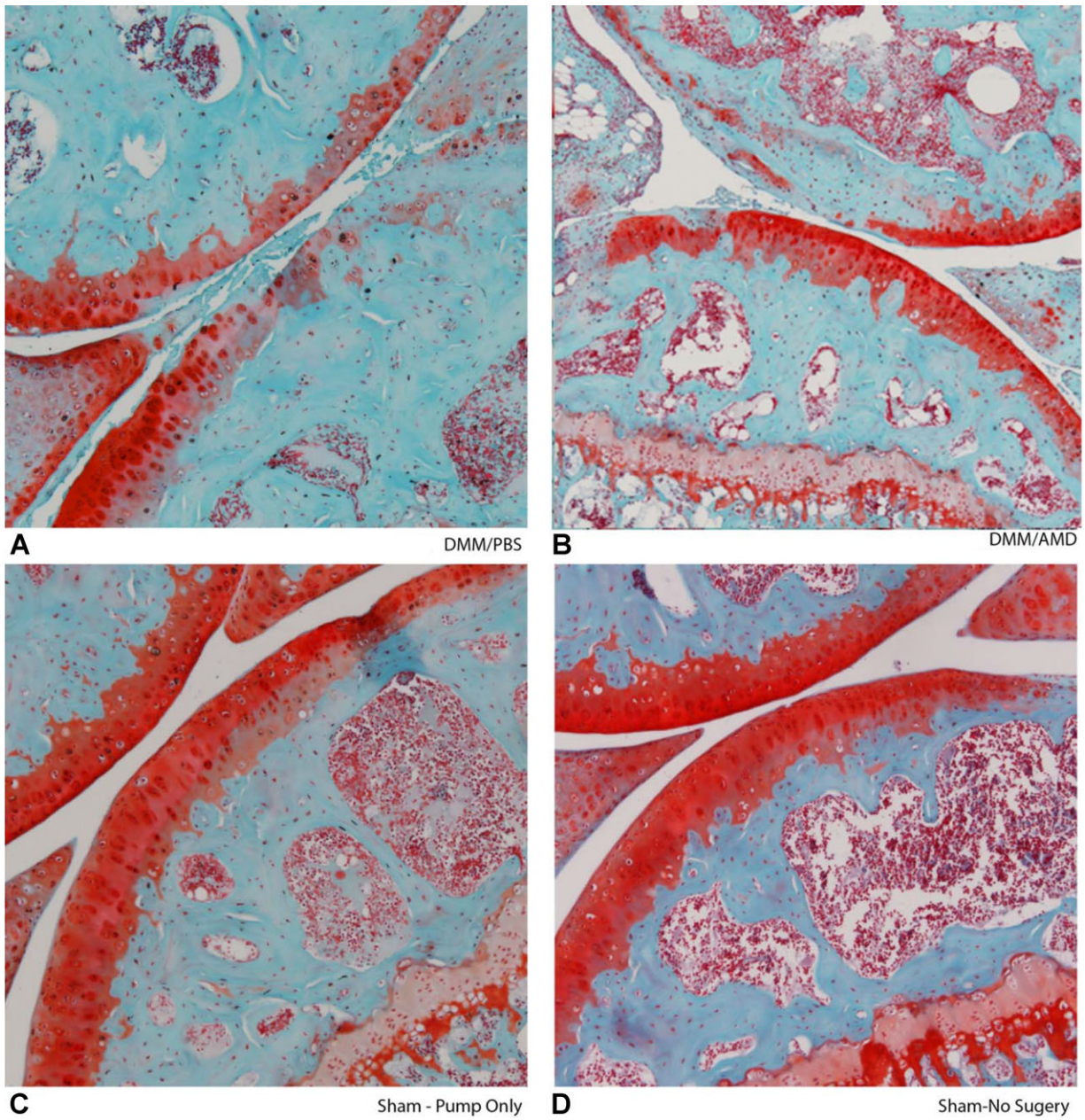

Figure 1. AMD3100 treatment prevents OA cartilage damage. Safranin-O staining shows the changes in proteoglycan and cartilage structure in representative (median OOCHAS scoring) joints. (A) DMM/PBS, (B) DMM/AMD3100, (C) Sham control, (D) Sham—No surgery control.

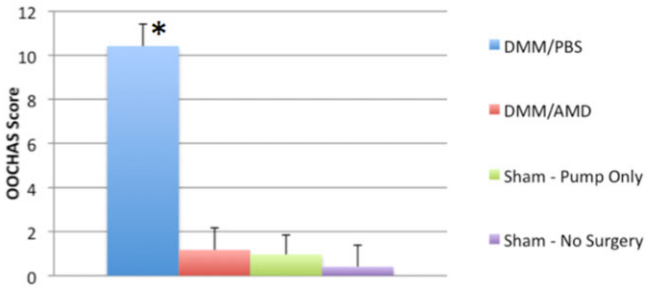

Figure 2. Blockage of SDF-1/CXCR4 by AMD3100 attenuated OA. OOCHAS scores are shown. Mean ± SD. DMM/PBS, $N = 8$. DMM/AMD3100, $N = 8$. Sham—pump only, $N = 5$. Sham—no surgery, $N = 7$. There were no significant differences between the two control groups, but there was as significant difference ($p < 0.001$) between the DMM/PBS group and the DMM/AMD3100 group.

treatment group with a preserved and intact cartilage surface similar to the Sham control (Fig. 1). As expected, the DMM/PBS group showed an extensive reduction in proteoglycan content and severe cartilage damage with matrix erosion at the surface.

Using the OOCHAS score, the extent of OA damage was quantified by blinded observers. Severe OA damage was quantified in the DMM/PBS group, while the DMM/AMD3100 treatment group displayed significantly lower scores ($p < 0.001$) not significantly different from either the sham or No-surgery control groups ($p = 0.704$, 0.169, respectively) (Fig. 2). Related to the safranin-O staining, and in line with the literature, the OOCHAS scores of the sham with pump and no surgery groups were not significantly different from zero ($p < 0.001$) as they showed minimal PTOA damage (Fig. 1).

Immunohistochemistry

In the DMM/PBS group, expression of MMP-13 was elevated compared to the DMM/AMD3100 and Sham groups (Fig. 3). Expression of Col 2 $3/4_{short}$ (C1-2C) and Col X were markedly reduced in the AMD3100 treatment and sham groups, where less PTOA damage was observed, compared to the PBS control, where extensive PTOA damage was observed. Staining was uniform across cartilage surfaces.

Fluorescence Molecular Tomography (FMT)

The MMPSense signal was significantly reduced from 0.55 pmols in the DMM/PBS group to 0.29 pmols in the DMM/AMD3100 group ($p < 0.05$). Similarly, a significant positive correlation was ($p < 0.001$; $N = 17$) was found between the MMPSense signals and the OOCHAS scores across all the study groups ($r^2 = 0.899$). Similarly, AMD3100 treatment significantly reduced the ProSense signal from 1.42 pmols in the DMM/PBS group to 0.61 pmols in the DMM/AMD3100 group ($p < 0.05$; Fig. 4). A significant positive correlation was ($p < 0.001$; $N = 17$) also found between the ProSense signals and the OOCHAS scores ($r^2 = 0.923$; Fig. 5).

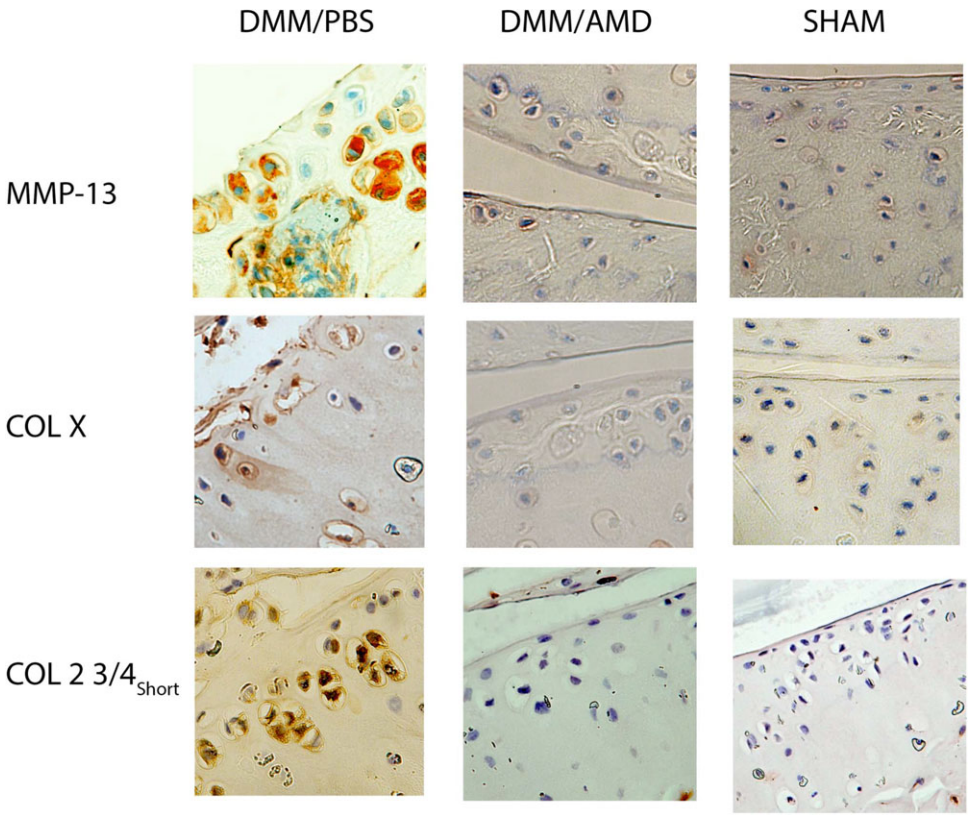

Figure 3. MMP-13, Col X, and Col 2 $3/4_{short}$ expression are reduced in AMD3100 treated mice. Immunohistochemical staining was done on cartilage, and representative images are presented. Positive signal is indicated by brown/red staining.

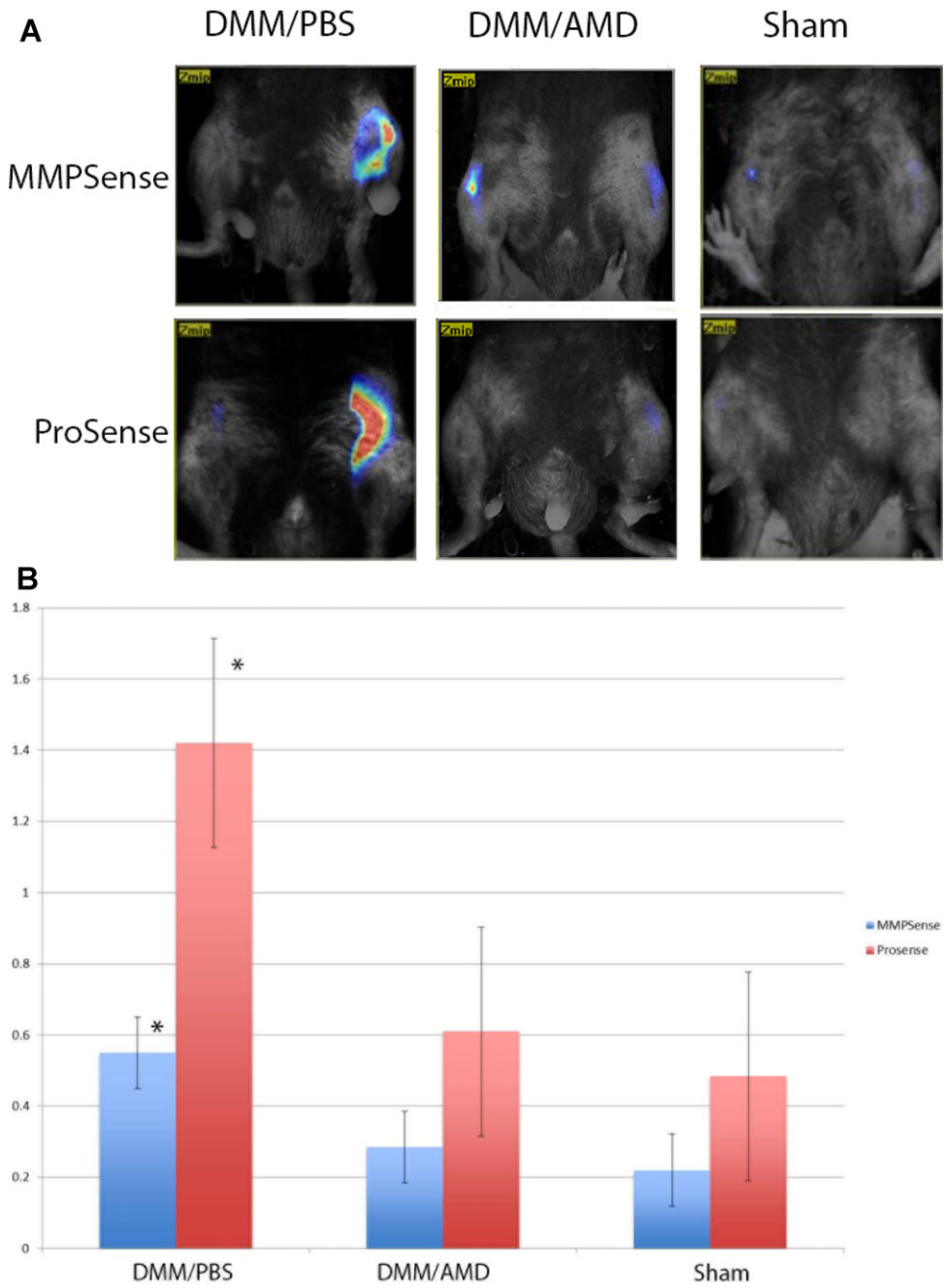

Figure 4. AMD3100 treatment reduces inflammation signals determined by FMT. Cathepsin and MMP activity are decreased in AMD3100 treated joints at 3 months after DMM surgery. (A) Representative MMPSense and ProSense images and (B) summary data means are shown. DMM/PBS, $N = 5$; DMM/AMD3100, $N = 6$; Sham, $N = 6$.

DISCUSSION

Our data suggests that PTOA associated articular cartilage pathogenesis can be prevented by continuous infusion of a specific CXCR4 antagonist, AMD3100, further confirming the role of the SDF-1/CXCR4 pathway in PTOA development. This study also highlights the predictive power of in vivo fluorescence molecular tomography for estimation of cartilage degradation.

While extensive cartilage degeneration was observed in the control (DMM/PBS), minimal damage was observed in the treatment group (DMM/AMD3100) indicating that AMD3100 effectively prevented PTOA associated articular cartilage structural damage (Fig. 1). The structural damage detected using histology was also supported by both the biochemical and in vivo FMT inflammation data, which demonstrate that AMD3100 treatment significantly reduces inflammatory signals associated with the production of proteases after DMM, associated with decreased OA pathology in the same treated mice at both a gross and biochemical level. It is well known that increased

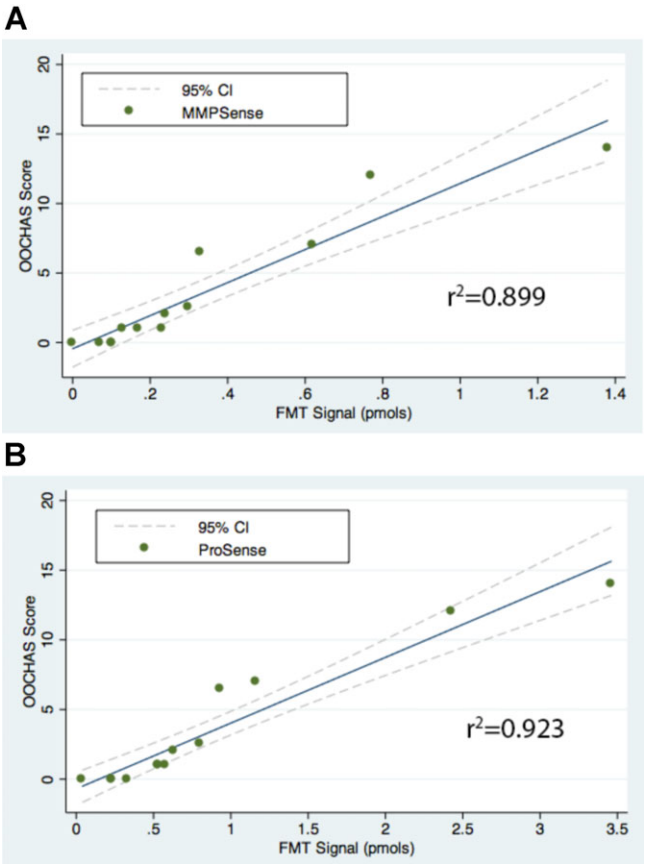

Figure 5. FMT signals are correlated to cartilage pathology process determined by OOCHAS score. (A) A significant positive correlation was ($p < 0.001$; $n = 17$) was found between the MMPSense signal and the OOCHAS score ($r^2 = 0.899$). (B) A significant positive correlation ($p < 0.001$; $n = 17$) was also observed between the ProSense signal and the OOCHAS score ($r^2 = 0.923$).

MMP-13 expression leads to matrix degradation and knockdown of MMP-13 reduces OA associated cartilage damage in mice.[41] Downregulation of C1-2C confirms reduced cleavage of both type II and type I collagen by MMPs in both bone and cartilage, respectively.[42] Work by Shen et al.,[43] which examined the effects of hMeSPC injection on articular protection, has shown the importance of SDF-1 signaling on meniscus repair in PTOA. Although these two studies focus on the same pathway, the present work tests the natural role of the SDF-1/CXCR4 pathway in PTOA progression, rather than the enhancing effect of SDF-1 treatment on hMeSPC migration and morphology for meniscus repair.

We have also demonstrated that in vivo FMT measurements are not only highly correlated with morphological and immunohistochemical data, but that, FMT measurements have the potential to predict PTOA cartilage development (Fig. 2). FMT has previously been used in a variety of fields as a non-invasive tool to measure inflammatory diseases.[37–39,44] FMT represents an optimal technique for non-invasive, longitudinal inflammation measurements and continuous monitoring of disease progression, as measurements have been used to sensitively predict disease development in rheumatoid arthritis.[44] This study has shown both ProSense and MMPSense probes may be correlated with morphological results in PTOA pathogenesis in the murine model.

For patients with a known risk of developing PTOA, this study is particularly relevant. Given a reported window of PTOA onset and disease progression, successful AMD3100 dosing may have the ability to attenuate the biochemical progression of PTOA-associated articular cartilage degradation following reconstructive or corrective surgery. Further research will be required to evaluate the long-term efficiency and pharmacokinetic profile of AMD3100, but the present results are encouraging. Additional research will also be required to evaluate the potential of AMD3100 to attenuate global PTOA progression after the initial onset of disease.

A potential limitation to this study is that surgical DMM may not be as traumatic as an ACL injury sustained during physical activity. Bone bruises and chondral lesions frequently occur in the latter, and these concomitant injuries may also play a role in the development of PTOA. Recognizing the role of SDF-1 signaling in the recruitment of reparative cells, it will be important to demonstrate that use of this drug neither increases the time for general wound healing nor restoration of subchondral cystic changes that often occur in association with traumatic joint injury. Research by Shen et al. demonstrated that AMD3100, in clinical situations involving surgical repair of the meniscus, may be contraindicated as the SDF-1 axis has been implicated in meniscus repair. Nonetheless, the animal ACLT model has been frequently used to study PTOA, and mimics human OA both macroscopically and biochemically.[33] Minimizing joint innate immunity inflammation until ACL reconstruction is performed may be an important preventative measure against the long-term development of PTOA.

In summary, the results of this study demonstrate that pharmacologic inhibition of SDF-1 signaling within the joint may provide protection against cartilage destruction in a mouse model of PTOA and highlights the potential of AMD3100, an FDA approved drug, as an effective attenuator of the SDF-1/CXCR4 pathway. Future clinical studies should explore orthopedic opportunities for pharmacologic inhibition of the SDF-1/CXCR4 signaling pathway, given AMD3100 provides as a promising agent for therapeutic use given its high specificity and well known safety profile in humans.[30,45]

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REFERENCES

1. Wei L, Svensson O, Hjerpe A. Correlation of morphologic and biochemical changes in the natural history of spontaneous osteoarthrosis in guinea pigs. Arthritis Rheum 1997;40:2075–2083 (Internet, 1997/11/19 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/9365098
2. Wei L, de Bri E, Lundberg A, et al. Mechanical load and primary guinea pig osteoarthrosis. Acta Orthop Scand 1998;69:351–357 (Internet, 1998/11/03 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/9798441
3. Wei L, Hjerpe A, Brismar BH, et al. Effect of load on articular cartilage matrix and the development of guinea-pig osteoarthritis. Osteoarthr Cartil 2001;9:447–453 (Internet, 2001/07/27 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/11467893
4. Heard BJ, Solbak NM, Achari Y, et al. Changes of early post-traumatic osteoarthritis in an ovine model of simulated ACL reconstruction are associated with transient acute post-injury synovial inflammation and tissue catabolism. Osteoarthritis Cartilage 21:1–8 (Internet, Elsevier Ltd; 2013 Sep 5 cited 2013 Nov 24). Available from: http://www.ncbi.nlm.nih.gov/pubmed/24012772
5. Haslauer CM, Elsaid K, Fleming BC, et al. Loss of extracellular matrix from articular cartilage is mediated by the synovium and ligament after anterior cruciate ligament injury. Osteoarthritis Cartilage 21:1950–1957. 1–8 (Internet, Elsevier Ltd; 2013 Sep 13; cited 2013 Nov 12). Available from: http://www.ncbi.nlm.nih.gov/pubmed/24036379
6. Anderson DD, Marsh JL, Brown TD. The pathomechanical etiology of post-traumatic osteoarthritis following intraarticular fractures. Iowa Orthop J 2011;31:1–20 (Internet, 2011/11/19 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/22096414
7. Lotz MK, Kraus VB. New developments in osteoarthritis. Posttraumatic osteoarthritis: pathogenesis and pharmacological treatment options. Arthritis Res Ther 2010;12:211 (Internet, 2010/07/07 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/20602810
8. Gelber AC, Hochberg MC, Mead LA, et al. Joint injury in young adults and risk for subsequent knee and hip osteoarthritis. Ann Intern Med 2000;133:321–328 (Internet, 2000/09/09 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/10979876
9. Muthuri SG, McWilliams DF, Doherty M, et al. History of knee injuries and knee osteoarthritis: a meta-analysis of observational studies. Osteoarthr Cartil 2011;19:1286–1293 (Internet, 2011/09/03 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/21884811
10. Lohmander LS, Ostenberg A, Englund M, et al. High prevalence of knee osteoarthritis, pain, and functional limitations in female soccer players twelve years after anterior cruciate ligament injury. Arthritis Rheum 2004;50:3145–3152 (Internet, 2004/10/12 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/15476248
11. Frobell RB, Roos HP, Roos EM, et al. Republished research: treatment for acute anterior cruciate ligament tear: five year outcome of randomised trial. Br J Sport Med 2013;47:373 (Internet). Available from: http://bjsm.bmj.com/content/47/6/373.short
12. Delincé P, Ghafil D. Anterior cruciate ligament tears: conservative or surgical treatment? Knee Surg Sport Traumatol Arthrosc 2013;21:1706–1707 (Internet, Springer-Verlag). Available from: http://dx.doi.org/10.1007/s00167-012-2134-z
13. Claes S, Hermie L, Verdonk R, et al. Is osteoarthritis an inevitable consequence of anterior cruciate ligament reconstruction? A meta-analysis. Knee Surg Sport Traumatol Arthrosc 2013;21:1967–1976 (Internet, Springer Berlin Heidelberg). Available from: http://dx.doi.org/10.1007/s00167-012-2251-8
14. Streich N, Zimmermann D, Bode G, et al. Reconstructive versus non-reconstructive treatment of anterior cruciate ligament insufficiency. A retrospective matched-pair long-term follow-up. Int Orthop 2011;35:607–613 (Internet, Springer-Verlag). Available from: http://dx.doi.org/10.1007/s00264-010-1174-6
15. Wang J, Sun Y, Song W, et al. Diverse signaling pathways through the SDF-1/CXCR4 chemokine axis in prostate cancer cell lines leads to altered patterns of cytokine secretion and angiogenesis. Cell Signal 2005;17:1578–1592 (Internet, 2005/07/12 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/16005185
16. Liang Z, Brooks J, Willard M, et al. CXCR4/CXCL12 axis promotes VEGF-mediated tumor angiogenesis through Akt signaling pathway. Biochem Biophys Res Commun 2007;359:716–722 (Internet, 2007/06/15 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/17559806
17. Jo DY, Rafii S, Hamada T, et al. Chemotaxis of primitive hematopoietic cells in response to stromal cell-derived factor-1. J Clin Invest 2000;105:101–111 (Internet, 2000/01/05 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/10619866
18. Loh SA, Chang EI, Galvez MG, et al. SDF-1 alpha expression during wound healing in the aged is HIF dependent. Plast Reconstr Surg 2009 123:65S–75S (Internet, 2009/02/21 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/19182665
19. Han Y, He T, Huang DR, et al. TNF-alpha mediates SDF-1 alpha-induced NF-kappa B activation and cytotoxic effects in primary astrocytes. J Clin Invest 2001;108:425–435 (Internet, 2001/08/08 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/11489936
20. Kortesidis A, Zannettino A, Isenmann S, et al. Stromal-derived factor-1 promotes the growth, survival, and development of human bone marrow stromal stem cells. Blood 2005;105:3793–3801 (Internet, 2005/01/29 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/15677562
21. Jo DY, Hwang JH, Kim JM, et al. Human bone marrow endothelial cells elaborate non-stromal-cell-derived factor-1 (SDF-1)-dependent chemoattraction and SDF-1-dependent transmigration of haematopoietic progenitors. Br J Haematol 2003;121:649–652 (Internet, 2003/05/20 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/12752108
22. Kanbe K, Takagishi K, Chen Q. Stimulation of matrix metalloprotease 3 release from human chondrocytes by the interaction of stromal cell-derived factor 1 and CXC chemokine receptor 4. Arthritis Rheum 2002;46:130–137 (Internet, 2002/01/31 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/11817585
23. Wei L, Sun X, Kanbe K, et al. Chondrocyte death induced by pathological concentration of chemokine stromal cell-derived factor-1. J Rheumatol 2006;33:1818–1826 (Internet, 2006/09/09 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/16960943
24. Henschler R, Piiper A, Bistrian R, et al. SDF-1alpha-induced intracellular calcium transient involves Rho GTPase signalling and is required for migration of hematopoietic progenitor cells. Biochem Biophys Res Commun 2003;311:1067–1071 (Internet, 2003/11/19 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/14623290
25. Chernock RD, Cherla RP, Ganju RK. SHP2 and cbl participate in alpha-chemokine receptor CXCR4-mediated

signaling pathways. Blood 2001;97:608–615 (Internet, 2001/02/07 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/11157475
26. Kanbe K, Takemura T, Takeuchi K, et al. Synovectomy reduces stromal-cell-derived factor-1 (SDF-1) which is involved in the destruction of cartilage in osteoarthritis and rheumatoid arthritis. J Bone Jt Surg Br 2004;86:296–300 (Internet, 2004/03/30 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/15046450
27. Wei L, Fleming BC, Sun X, et al. Comparison of differential biomarkers of osteoarthritis with and without posttraumatic injury in the Hartley guinea pig model. J Orthop Res 2010;28:900–906. (Internet, 2010/01/29 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/20108346
28. Wei L, Kanbe K, Lee M, et al. Stimulation of chondrocyte hypertrophy by chemokine stromal cell-derived factor 1 in the chondro-osseous junction during endochondral bone formation. Dev Biol 2010;341:236–245 (Internet, 2010/03/09 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/20206617
29. Hendrix CW, Flexner C, MacFarland RT, et al. Pharmacokinetics and safety of AMD-3100, a novel antagonist of the CXCR-4 chemokine receptor, in human volunteers. Antimicrob Agents Chemother 2000;44:1667–1673 (Internet) Available from: <Go to ISI>://000087156100041.
30. De Clercq E. The AMD3100 story: the path to the discovery of a stem cell mobilizer (Mozobil). Biochem Pharmacol 2009;77:1655–1664 (Internet, 2009/01/24 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/19161986
31. Sun X, Wei L, Chen Q, et al. CXCR4/SDF1 mediate hypoxia induced chondrosarcoma cell invasion through ERK signaling and increased MMP1 expression. Mol Cancer 2010;9:17 (Internet, 2010/01/28 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/20102637
32. Wei F, Moore DC, Li Y, et al. Attenuation of osteoarthritis via blockade of the SDF-1/CXCR4 signaling pathway. Arthritis Res Ther 2012;14:R177 (Internet, 2012/08/02 ed. BioMed Central Ltd; Jul 31 [cited 2013 Apr 26]). Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3580571&tool=pmcentrez&rendertype=abstract
33. Glasson SS, Blanchet TJ, Morris EA. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. Osteoarthritis Cartilage 2007;15:1061–1069.
34. Matthys P, Hatse S, Vermeire K, et al. AMD3100, a potent and specific antagonist of the stromal cell-derived factor-1 chemokine receptor CXCR4, inhibits autoimmune joint inflammation in IFN-gamma receptor-deficient mice. J Immunol 2001;167:4686–4692 (Internet, 2001/10/10 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/11591799
35. Datema R, Rabin L, Hincenbergs M, et al. Antiviral efficacy in vivo of the anti-human immunodeficiency virus bicyclam SDZ SID 791 (JM 3100) an inhibitor of infectious cell entry. Antiviral efficacy in vivo of the anti-human immunodeficiency virus Bicyclam SDZ SID 791 (JM 3100) an inhibito. Antimicrob Agents Chemother 1996;40:750–754.
36. Pritzker KPH, Gay S, Jimenez S, et al. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006;14:13–29 (Internet, 2005/10/26 ed.; Jan [cited 2012 Nov 6]). Available from: http://www.ncbi.nlm.nih.gov/pubmed/16242352
37. Weissleder R, Ntziachristos V. Shedding light onto live molecular targets. Nat Med 2003;9:123–128 (Internet, 2003/01/07 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/12514725
38. Ntziachristos V, Bremer C, Weissleder R. Fluorescence imaging with near-infrared light: new technological advances that enable in vivo molecular imaging. Eur Radiol 2003;13:195–208 (Internet, 2003/01/24 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/12541130
39. Vinegoni C, Razansky D, Pitsouli C, Perrimon N, Ntziachristos V, Weissleder R. Mesoscopic Fluorescence Tomography for In-vivo Imaging of Developing Drosophila. Journal of Visualized Experiments: JoVE. 2009;30:1510. doi: 10.3791/1510.
40. Zhou J, Chen Q, Lanske B, et al. Disrupting the Indian hedgehog signaling pathway in vivo attenuates surgically induced osteoarthritis progression in Col2a1-creert2; Ihhfl/fl mice. Arthritis Res Ther 2014;16:R11 (Internet). Available from: http://www.ncbi.nlm.nih.gov/pubmed/24428864
41. Little CB, Barai A, Burkhardt D, et al. Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. Arthritis Rheum 2009;60:3723–3733 (Internet, 2009/12/02 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/19950295
42. Bakker MF, Verstappen SM, Welsing PM, et al. The relation between cartilage biomarkers (C2C, C1,2C, CS846, and CPII) and the long-term outcome of rheumatoid arthritis patients within the CAMERA trial. Arthritis Res Ther 2011;13:R70.
43. Shen W, Chen J, Zhu T, et al. Intra-articular injection of human meniscus stem/progenitor cells promotes meniscus regeneration and ameliorates osteoarthritis through stromal cell-derived factor-1/CXCR4-mediated homing. Stem Cells Transl Med 2014;3:387–394 (Internet). Available from: http://www.ncbi.nlm.nih.gov/pubmed/24448516
44. Peterson JD, Labranche TP, Vasquez KO, et al. Optical tomographic imaging discriminates between disease-modifying anti-rheumatic drug (DMARD) and non-DMARD efficacy in collagen antibody-induced arthritis. Arthritis Res Ther 2010;12:R105 (Internet, 2010/06/01 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/20509880
45. Pusic I, DiPersio JF. Update on clinical experience with AMD3100, an SDF-1/CXCL12-CXCR4 inhibitor, in mobilization of hematopoietic stem and progenitor cells. Curr Opin Hematol 2010;17:319–326 (Internet, 2010/05/18 ed). Available from: http://www.ncbi.nlm.nih.gov/pubmed/20473162