Changes in peripheral monocyte populations 48-72 hours after subcutaneous denosumab administration in women with osteoporosis

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Introduction

Medication related osteonecrosis of the jaw (MRONJ) is a complication associated with the use of bone antiresorptive agents1, mainly bisphosphonates (BPs) that are widely used for the management of osteoporosis, bone metastasis and other bone-loss related disorders1-4. It has been suggested that the development of MRONJ is more frequently reported with the use of high doses of IV BPs, for the treatment or prevention of skeletal related events (SREs) in patients with advanced cancer and bone metastasis compared to standard doses used for the treatment of osteoporosis5. Nonetheless, recent good quality of evidence suggests that MRONJ is also seen among patients receiving BPs, per os or intravenous for non-malignant indications6-8. The hallmark of MRONJ development is the finding of necrotic exposed bone in the oral cavity1. In the majority of cases, the precipitating event appears to be a dental extraction or other dental invasive procedures1, and use of dentures1,9,10. However, 40% of MRONJ cases appear to occur spontaneously and to be unrelated to dental treatment11,12.

Abstract

Objectives: To examine the effect of denosumab administration in the peripheral blood white cell population, to further elucidate a plausible pathophysiological link between denosumab and osteonecrosis of the jaw. Methods: Thirty women with osteoporosis, after denosumab treatment were included. Peripheral blood samples were obtained prior to and 48-72 hours following denosumab administration. Flow cytometry gated at the monocyte population for CD14/CD23/CD123/CD16 stainings were performed. Results: We were able to record a number of changes in the monocyte populations between baseline and after denosumab administration. Most importantly, in the monocyte populations we were able to detect statistically significant increased populations of CD14+/CD23+ (p=0.044), CD14-/CD23+ (p=0.044), CD14+/CD123+ (p=0.011), CD14+/CD123- (p=0.011) and CD14-/CD16+ (p=0.028). In contrast, statistically significant decreased populations of CD14-/CD123+ (p=0.034), CD14+/CD16+ (p=0.037) and CD14+/CD16- (p=0.014) were detected. Conclusions: Our results provide evidence supporting the hypothesis that denosumab administration modifies the monocyte mediated immune response in a manner similar to that of bisphosphonates. This may partly explain the trivial immunity changes recorded with denosumab.

Keywords: Bisphosphonates, Denosumab, Macrophages, Osteoclasts, Osteonecrosis Of The Jaws

References

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oral cavity has also been implicated in the pathogenesis. Denosumab (Dmab), is a fully human monoclonal antibody, which binds to receptor activator of nuclear factor kappa beta ligand (RANKL), and is a potent anti-resorptive agent used for the management of osteoporosis and the prevention of SREs in cancer patients, showing favorable results in terms of efficacy and safety. Due to its unique pharmacokinetics Dmab exerts a maximal suppression of bone turnover during treatment, but unlike BPs that are embedded in the bone matrix, Dmab-induced suppression is reversed after treatment discontinuation.

Since osteoclasts and macrophages stem from a common progenitor cell lineage, it has been proposed that a plausible compromised local defense due to insufficient numbers or reduced functional capacity of macrophages, when combined with the impaired oral mucosa that has been reported in patients receiving BPs, could allow oral pathogens to reach the bone surface of the jaws. What is more, given the more discrete RANKL pathway inhibition by Dmab, this agent might be a more appropriate target to examine RANKL inhibition effects on the immune system. We have previously reported an increase of CD14+ peripheral blood monocyte (PBMC) populations along with a decrease of CD14- PBMC populations in breast cancer women receiving intravenous Zolendronic Acid (ZA).

To shed more light in the role of anti-resorptive agents in the aetiopathogenesis of MRONJ through a possible modification of the immune system we designed this prospective study in order to examine the effect of subcutaneous administration of Dmab in postmenopausal women with osteoporosis using an immune phenotype quantified sampling profile for B-cells, T helper cells, T cytotoxic cells, Natural Killer (NK) cells, NK-like cells, Monocytes, Polymorphonuclear leukocytes (PMN) and Eosinophil granulocytes.

Patients and methods

Sample

Female patients diagnosed with postmenopausal osteoporosis and treated with denosumab for at least one year that were under regular follow up at the endocrinology outpatient clinic were candidates for enrollment. Exclusion criteria were: i) secondary osteoporosis, ii) renal and or liver insufficiency iii) medical history of cancer, iv) untreated hypo or hyperthyroidism, v) metabolic bone diseases other than osteoporosis, vi) medical history of osteonecrosis of the jaw, vii) history of previous Zolendronic acid use for the treatment of osteoporosis.

All patients gave their informed consent for participation in the study and the study was approved by the Institutional Review Board of the Faculty of Dentistry (IRB protocol 51/06-06-2019) of Aristotle University of Thessaloniki.

Anthropometric and demographic data (age, sex, place of residence, social security type, marital status) and disease status (initial diagnosis, history of treatments received, current treatment regimes) were recorded for each patient.

Study protocol

After an overnight fast, blood sample was drawn at the hospital, prior to Dmab administration. Then, a second visit was planned within 48-72 h after Dmab administration, in the hospital, for a second sample.

Flow cytometry

Immunostaining and subsequent flow cytometry were performed according to standard protocol prior to Dmab administration and 48-72 hours after, on peripheral blood samples. The antibodies used were CD45 (PerCP), CD14 (FITC), CD 23 (PE), CD 123 (PE), CD 4(FITC)/ CD 8(PE)/ CD 3(PerCP), CD 3(FITC)/ CD 16+56(PE)/ CD 45(PerCP)/ CD 19(APC)/ CD16 (PE) (BD Bioscience), as previously described. Briefly, 100 μl of whole fresh blood were stained with the appropriate antibodies as instructed by the manufactures for 30 min at RT. 2 ml of BD lysis buffer was added in order to lyse the erythrocytes and the samples were incubated for 10 min at RT. The samples were centrifuged at 500 xg and the supernatants were discarded. Pellets were washed once with serum-free PBS and centrifuged at 500 xg for 5 min. The final pellet was re-suspended in 0.5 ml serum-free PBS and the samples were immediately analyzed using FACs Calibur and Cell Quest software. 50,000 events were collected for each staining. The percentage of positive cells for each antibody was determined. The gating for each cell population has been previously described.

Statistical analyses

Normality explorations were performed on all variables. Non-parametric tests were used where normality assumptions were not met. Descriptives and absolute and relative frequencies for all variables were obtained. Pearson’s r or Spearman’s rho correlation coefficients were used, following normality explorations. Paired t-test was used for paired sample comparisons. Bootstrapping was used for internal validation. Alpha level was set at 0.05. An alpha value smaller than 0.10 was considered a trend. Statistical analyses were performed using the IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY: IBM Corp.

Results

Patients

Thirty postmenopausal osteoporotic women under treatment with denosumab were finally enrolled in the study. The patient’s anthropomorphic, clinical and biochemical characteristics are depicted in Table 1.

Six patients (20%) had sustained at least one vertebral fracture and 4 had a history of a non-vertebral fracture (13%) (Table 1) before initiation of denosumab treatment.

No history of new or worsening vertebral fractures, hip fractures or other non-vertebral fractures were reported during treatment with denosumab.
CD14-CD123+ population was decreased and a decrease in the CD14+CD16+ population.

Approximately 2-9% of the peripheral human blood leukocytes are peripheral blood monocytes (PBMC), but only 40% of the available monocytes circulate while 60% migrate. CD14 (55 kDa) is a glycoprotein released by monocytes and macrophages in humans, which is located on the cellular membrane. Normal mature osteoclasts and human monocytes have been reported to express high levels of CD14. In our sample, PBMC CD14, CD21, CD23, CD56 populations have been found to be markedly increased following Dmab administration. In this regard, we have previously reported similar findings in PBMC of breast cancer patients treated with ZA. The latter finding is in agreement with previous in vivo and experimental studies demonstrating an increase in CD14+ expression after zolendronic acid exposure which was documented in vitro from human PBMC derived cultures and subsequently ex-vivo from human jaw tissues. Further, Dmab administration increased the population of CD14-/CD23+ monocytes, 48 hours after the infusion. CD 23 is a marker of activated macrophages associated with B-cell activation.

CD 123 antigen is present in blood dendritic cells and it is lost when monocytes are transformed in macrophages in which CD68 and CD168 predominate. CD123 is a molecule currently under intensive research as a potential therapeutic target for haematologic malignancies. We were able to detect a subset of CD14+ that were CD123+ probably reflecting the blood dendritic cell population. Notably, the increase in this cell population following Dmab administration was similar to the increase in the original CD14+ population. In contrast, we found the CD14-CD123+ population to be decreased, a fact probably attributed to a generic decrease of CD14-PBMC following Dmab administration.

To examine the patients’ monocyte population, gating at the monocyte area with the CD14/CD123, CD14/CD23 and CD14/CD16 stainings were performed (Table 1, Figure 1). The instrument was set in order to position the cells appropriately in the dot plots by using isotype controls, voltage, and compensation tools. A dot plot of FSC versus SSC was established and the region of interest was selected excluding any other cell type and cellular debris. Each staining was performed twice for each patient, one prior and on 48-72 hours post treatment administration (Table 2). Statistically significant increase was found in CD14+/CD23+, CD14+/CD23+, CD14+/CD123+, CD14+/CD123- and CD14+/CD16+ populations. Decrease was found in CD14-/CD123+, CD14+/CD16+, CD14+/CD16- populations. No statistically significant difference was found for CD14+/CD23+, CD14+/CD23-, CD14+/CD23-, CD14-/CD123-, CD14-/CD16-monocyte populations (Table 3).

**Discussion**

In our sample of thirty postmenopausal osteoporotic women under treatment with denosumab we were able to record a shift towards CD23+ expression in the monocyte population, an increase in the CD14+CD123+ population while CD14-CD123+ population was decreased and a decrease in the CD14+CD16+ population.

| Parameters | Values |
|------------|--------|
| Age (yrs) | 67.8 ± 9 |
| Age at menopause (yrs) | 46.2 ± 4.9 |
| Drug – naive patients (n, %) | 11.36% |
| History of gastroesophageal reflux disease and/or peptic ulcer (n, %) | 9.30% |
| Duration of previous treatment (yrs) | 5.1 ± 4 |
| Duration of treatment with denosumab | 3.1 ± 1.6 |
| Patients with a history of VF (n, %) | 6.20% |
| Patients with a history of NVF (n, %) | 4.13% |
| Serum calcium (NR: 8.7-10.3 mg/dl) | 9.5 ± 0.5 |
| Serum phosphate (NR: 2.5-4.5 mg/dl) | 3.3 ± 0.7 |
| Serum creatinin (NR: 0.7-1.2 mg/dl) | 0.7 ± 0.14 |
| Serum PTH (NR: 11-54 pg/ml) | 47.4 ± 12.8 |
| Serum osteocalcin (NR: 9-42 ng/ml) | 10.6 ± 4.8 |
| BMD LS (gr/cm²) | 0.921 ± 0.12 |
| T-score LS | -2.03 ± 0.96 |
| BMD LFN (gr/cm²) | 0.743 ± 0.06 |
| T-score LFN | -2.02 ± 0.66 |
| BMD LTH (gr/cm²) | 0.808 ± 0.08 |
| T-score LTH | -1.54 ± 0.71 |
Figure 1. Representative flow cytometry analysis of a patient prior (left Column) and 48-hours following (right Column) denosumab administration. FACS plot of Forward scatter (FSC) vs side scatter (SSC) is presented, indicative of the experiments. The dot blots represent the percentages of single or double positive cells for the indicated markers (CD14/CD23, CD14/CD123 and CD14/CD16) from gated monocyte population. (A: Gating all populations; R1: Lymphocytes, R2: Monocytes, R3: Granulocytes. B: Increased CD14-/CD23+ - CD14-/CD23+ and increased CD14+/CD23+ - CD14+/CD23+. Left: Before; Right: After Denosumab administration. CD14+/CD23+ - CD14+/CD23+ increased and CD14-/CD23- - CD14-/CD23- decreased in case image but not statistically significant in total sample of patients. C: Increased CD14+/CD123+ - CD14+/CD123+ and CD14+/CD123- - CD14+/CD123 and decreased CD14-/CD123+ - CD14-/CD123+. Left: Before; Right: After Denosumab administration. CD14-/CD123- - CD14-/CD123- decreased in case image but not statistically significant in total sample of patients. D: Decreased CD14+/CD16+ - CD14+/CD16+ and decreased CD14+/CD16- - CD14+/CD16, along with increased CD14-/CD16+ - CD14-/CD16+. Left: Before; Right: After Denosumab administration. CD14-/CD16- - CD14-/CD16- decreased in case image but not statistically significant in total sample of patients.)
conditioned media (MSC-CM) which contained various cytokines (to facilitate the recruitment of cells during osteogenesis, angiogenesis and cell proliferation), showed function maintenance in osteoclasts despite the presence of RANKL inhibitors. Kambayashi et al reported augmented matrix metalloproteases expression and tumor associated proliferation following RANKL treatment in CD14+ cells isolated from PBMCs of healthy donors. Thus the RANK/RANKL pathway may further contribute to the development and maintenance of the immunosuppressive tumor microenvironment and denosumab may even be a promising adjuvant therapy for targeting tumor associated macrophages (TAMs) in other cancers. Dmab has already shown favorable results for the treatment of Giant Cell Tumor of Bone, however, neoplastic cells with certain mutations survive denosumab treatment and undergo dramatic histological changes in response to this agent. Still, because high RANKL mRNA expression has been reported in patients with aneurysmal bone cyst, fibrous dysplasia, osteosarcoma, chondrosarcoma and enchondroma, primary bone tumors present new therapeutic targets for denosumab, particularly those tumors expressing RANKL and those involving bone resorption by osteoclasts.

Table 2. Descriptives of antigen expression prior and 48-72 hours following subcutaneous denosumab administration. CD14/C23/CD123/CD16 stainings. Thirty postmenopausal osteoporotic women under treatment with denosumab.

| Staining          | 1st measurement (Baseline) | 2nd measurement | 95% Confidence Interval of the Difference | p-value |
|-------------------|-----------------------------|-----------------|------------------------------------------|---------|
|                   | Mean | Std. Deviation | Median | IQR | Lower | Upper |
| CD14+/CD23+       | 2,6546 | 3,81430 | 9,9927 | 20,78235 |
| CD14+/CD23-       | 59,0086 | 30,06067 | 69,8865 | 23,04598 |
| CD14-/CD123+      | .9821 | 1,00949 | 1,6569 | 1,42718 |
| CD14-/CD123-      | 25,9381 | 22,39671 | 23,2454 | 19,53608 |
| CD14+/CD123+      | 9,0567 | 11,86702 | 17,3707 | 17,27212 |
| CD14+/CD123-      | 62,2593 | 19,89991 | 73,0904 | 20,50811 |
| CD14-/CD123+      | 6,5161 | 4,14880 | 3,9878 | 4,56302 |
| CD14-/CD123-      | 14,0196 | 11,19632 | 13,6048 | 12,44166 |
| CD14+/CD16+       | 15,6682 | 18,63325 | 8,2926 | 4,60213 |
| CD14+/CD16-       | 65,1189 | 22,28524 | 56,0948 | 24,47873 |
| CD14-/CD16+       | 10,0671 | 6,55533 | 15,4348 | 13,83929 |
| CD14-/CD16-       | 9,1775 | 10,43060 | 6,4259 | 6,26783 |

Table 3. Mean differences of antigen expression prior and 48-72 hours following subcutaneous denosumab administration. CD14/C23/CD123/CD16 stainings. Thirty postmenopausal osteoporotic women under treatment with denosumab.

| Staining          | Paired Differences | 95% Confidence Interval of the Difference | p-value |
|-------------------|-------------------|------------------------------------------|---------|
|                   | Mean | Std. Deviation | Lower | Upper | Lower | Upper |            |         |
| CD14+/CD23+       | 7,193 | 17,25673 | 0,223 | 14,163 | .044 |
| CD14+/CD23-       | 13,358 | 37,36448 | -1,733 | 28,450 | .080 |
| CD14-/CD123+      | .605 | 1,45269 | .018 | 1,911 | .044 |
| CD14-/CD123-      | -1,511 | 26,79658 | -9,312 | 12,334 | .776 |
| CD14+/CD123+      | 8,461 | 16,00697 | 2,128 | 14,793 | .011 |
| CD14+/CD123-      | 10,161 | 19,03547 | 2,631 | 17,691 | .010 |
| CD14-/CD123+      | -2,273 | 5,28709 | -4,364 | -1,181 | .034 |
| CD14-/CD123-      | -699 | 15,71201 | -6,914 | 5,516 | .819 |
| CD14+/CD16+       | -7,502 | 17,76660 | -14,531 | -.474 | .037 |
| CD14+/CD16-       | -8,773 | 17,32542 | -15,627 | -1,919 | .014 |
| CD14-/CD16+ - CD14-/CD16+ | 5,394 | 12,06589 | .620 | 10,167 | .028 |
| CD14-/CD16- - CD14-/CD16- | -2,902 | 10,83524 | -7,188 | 1,384 | .176 |

Statistical significance typed in bold. Increase (positive difference) typed in green. Decrease (negative difference) typed in red.
From an epidemiological perspective, MRONJ presents only in a very small percentage of osteoporotic patients receiving Dmab. Furthermore, 40% of MRONJ cases appear spontaneously with no previous documented mucosal injury. It has been reported that the initiating event in MRONJ is likely the infection, instead of the low bone turnover. In this regard, sterile inflammation alone in the soft tissues surrounding the jaw was not found to be enough to induce ONJ. Thus the presence of bacterial populations is also a requisite for MRONJ. The pathogenesis of MRONJ could be a series of events initiating from infection, followed by inflammation which might also be augmented by the use or bone antiresorptive agents. It has been reported that the presence and function of macrophages and monocytes could be crucial in the development of local infection. MRONJ has been reported to be associated with various bacterial pathogens populations, the numbers of whom do not decrease despite antimicrobial chemotherapy. These might be the reasons for the differential response to monocyte impairment in patients receiving antiresorptive agents. Differences in the populations of macrophages but also differences in the oral flora might explain the occurrence of MRONJ only in some patients, of whom some even develop MRONJ without mucosal injury.

A second significant side effect of antiresorptives is the occurrence of atypical femoral fractures. We have previously reported that altered microdamage repair and microfractures accumulation, “fatigue” could be implicated in the pathogenesis of ONJ. In this regard an experimental study showed that treatment with granulocyte colony-stimulating factor (G-CSF) result in increased bone healing along with upregulation of monocytes, granulocytes and macrophages. Other experimental studies suggested that CD34+ and CD31+ cells isolated from peripheral blood might be potential therapeutic autologous treatments to augment fracture healing. Interestingly, monocytes appear to express both CD34 and CD31. A study of the potential changes in those monocyte subpopulations would be required to explore the possible aetiopathogenetic link between Dmab and altered microdamage repair.

Through this study we were able to document changes in the peripheral blood monocyte population, 48-72 hours following subcutaneous Dmab administration. Denosumab has long been identified as a cause for MRONJ and this is – to the best of our knowledge – the first clinical report to document an increase in the peripheral blood monocyte CD14+ population and a decrease in the CD14+ PBMC population in female patients with osteoporosis, following Dmab administration. This finding is in accordance with currently existing evidence and creates further research queries that need to be addressed by future studies.

References

1. Khan AA, Morrison A, Hanley DA, et al. Diagnosis and management of osteonecrosis of the jaw: a systematic review and international consensus. J Bone Miner Res 2015;30:3-23.
2. Kyrgidis A, Triaridis S, Antoniades K. Effects of bisphosphonates on keratinocytes and fibroblasts may have a role in the development of osteonecrosis of the jaw. Bioscience Hypotheses 2009;2:153-9.
3. Kyrgidis A, Triaridis S, Vahtsevanos K, Antoniades K. Osteonecrosis of the jaw and bisphosphonate use in breast cancer patients. Expert Review of Anticancer Therapy 2009;9:1125-34.
4. Kyrgidis A, Yavropoulou M, Tilaveridis I, Andreadis C, Antoniades K, Kouvelas D. A Systematic Review of Bone Anti-Resorptive Treatment Toxicity in Innate and Adaptive Immunity Cells: Osteonecrosis of the Jaws and Future Implications. The Journal of Dentists 2015;3:50-9.
5. Kyrgidis A, Tzellos T-G, Toulis K, Antoniades K. The Facial Skeleton in Patients with Osteoporosis: A Field for Disease Signs and Treatment Complications. Journal of Osteoporosis 2011;2011:Article ID 147689, 10 pages, doi:10.4061/2011/.
6. Conwell LS, Chang AB. Bisphosphonates for osteoporosis in people with cystic fibrosis. The Cochrane database of systematic reviews 2014;3:CD002010.
7. Sharma A, Einstein AJ, Vallakati A, et al. Risk of Atrial Fibrillation With Use of Oral and Intravenous Bisphosphonates. The American Journal of Cardiology 2014;113:1815-21.
8. Lee SH, Chang SS, Lee M, Chan RC, Lee CC. Risk of osteonecrosis in patients taking bisphosphonates for prevention of osteoporosis: a systematic review and meta-analysis. Osteoporosis International 2013;25:1131-9.
9. Kyrgidis A, Vahtsevanos K, Koloutsos G, et al. Bisphosphonate related osteonecrosis of the jaws: risk factors in breast cancer patients. A case control study. J Clin Oncol 2008;26:4634-8.
10. Vahtsevanos K, Kyrgidis A, Verrou E, et al. Longitudinal Cohort Study of Risk Factors in Cancer Patients of Bisphosphonate-Related Osteonecrosis of the Jaw. J Clin Oncol 2009;27:5356-62.
11. Hess LM, Jeter JM, Benham-Hutchins M, Alberts DS. Factors associated with osteonecrosis of the jaw among bisphosphonate users. Am J Med 2008;121:475-83 e3.
12. Badros A, Terpos E, Katodritou E, et al. Natural History of Osteonecrosis of the Jaw in Patients With Multiple Myeloma. Journal of Clinical Oncology 2008;26:5904-9.
13. Kyrgidis A. Novel hypotheses in the etiopathogenesis of bisphosphonate-related osteonecrosis of the jaws. J Oral Maxillofac Surg 2009;67:2554.
14. Katsarelis H, Shah NP, Dharwal DK, Pazianas M. Infection and Medication-related Osteonecrosis of the Jaw. J Dent Res 2015;94:534-9.
15. Pazianas M. Osteonecrosis of the Jaw and the Role of Macrophages J Nati Cancer Inst 2011;103:232-40.
16. Bone HG, Wagman RB, Brandi ML, et al. 10 years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomised FREEDOM trial and open-label extension. The lancet Diabetes & endocrinology 2017;5:513-23.
17. Cummings SR, Martin JS, McClung MR, et al. Denosumab for Prevention of Fractures in Postmenopausal Women with Osteoporosis. N Engl J Med 2009;361:756-65.
18. Papapoulos S, Chapurlat R, Libanati C, et al. Five years of denosumab exposure in women with postmenopausal osteoporosis: results from the first two years of the FREEDOM extension. J Bone Miner Res 2012;27:694-701.
19. Kyrgidis A, Toulis KA. Denosumab-Related Osteonecrosis of The Jaws. Osteoporos Int 2010;DOI 10.1007/s00198-010-1177-6.
20. Toulis K, Anastasilakis A. Increased risk of serious infections in women with osteopenia or osteoporosis treated with denosumab. Osteoporosis International 2010;21:1963-4.
21. Kyrgidis A, Yavropoulou MP, Lagoudaki R, Andreadis C, Antoniades K, Kouvelas D. Increased CD14+ and decreased CD14- populations of monocytes 48 h after zolendronic acid infusion in breast cancer patients. Osteoporos Int 2017;28:991-9.
22. Reuter S, Lang D. Life span of monocytes and platelets: importance of interactions. Frontiers in bioscience (Landmark edition) 2009;14:2432-47.
23. Ziegler-Heitbrock HW, Ulevitch RJ. CD14: cell surface receptor and differentiation marker. Immunology today 1993;14:121-5.
24. Jersmann HPA. Time to abandon dogma: CD14 is expressed by non-myeloid lineage cells. Immunol Cell Biol 2005;83:462-7.
25. Zamani F, Zare Shahneh F, Aghebati-Maleki L, Baradaran B. Induction of CD14 Expression and Differentiation to Monocytes or Mature Macrophages in Promyelocytic Cell Lines: New Approach. Advanced Pharmaceutical Bulletin 2013;3:329-32.
26. Hoefert S, Schmitz I, Weichert F, Gaspar M, Eufinger H. Macrophages and bisphosphonate-related osteonecrosis of the jaw (BRONJ): evidence of local immunosuppression of macrophages in contrast to other infectious jaw diseases. Clin Oral Investig 2015;19:497-508.
27. Marcu-Malina V, Balbir-Gurman A, Dardik R, Braun-Moscovici Y, Segel M, Bank I. A novel prothrombotic pathway in systemic sclerosis patients: possible role of bisphosphonate activated γδ T cells. Frontiers in Immunology 2014;5.
28. Biosciences B. Human and Mouse CD Marker Handbook. San Jose, CA 95131: Becton, Dickinson and Company; 2010.
29. Lecooanet-Henchoz S, Gauchat J-F, Aubry J-P, et al. CD23 Regulates monocyte activation through a novel interaction with the adhesion molecules CD11b-CD18 and CD11c-CD18. Immunity 1995;3:119-25.
30. Armant M, Rubio M, Delespesse G, Sarfati M. Soluble CD23 directly activates monocytes to contribute to the antigen-independent stimulation of resting T cells. The Journal of Immunology 1995;155:4868-75.
31. Bigley V, Hanifia M, Doulatov S, et al. The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. The Journal of experimental medicine 2011;208:227-34.
32. Roberts AW, He S, Ritchie D, Hertzberg MS, Kerridge I. A phase I study of anti-CD123 monoclonal antibody (CD123) CSL360 targeting leukemia stem cells (LSC) in AML. J Clin Oncol 2010;28.
33. Novak N, Allam JP, Hagemann T, et al. Characterization of FCepsilonRI-bearing CD123 blood dendritic cell antigen-2 plasmacytoid dendritic cells in atopic dermatitis. The Journal of allergy and clinical immunology 2004;114:364-70.
34. Ueda Y, Hagiwara M, Okamoto A, et al. Frequencies of dendritic cells (myeloid DC and plasmacytoid DC) and their ratio reduced in pregnant women: comparison with umbilical cord blood and normal healthy adults. Human immunology 2003;64:1144-51.
35. Gill S, Tasiwn S, Ruella M, et al. Anti-CD123 chimeric antigen receptor T cells (CART-123) provide a novel myeloablative conditioning regimen that eradicates human acute myeloid leukemia in preclinical models. Blood 2013;122.
36. Mardiros A, Dos Santos C, McDonald T, et al. T cells expressing CD123-specific cytolytic effector functions and anti-tumor effects against human acute myeloid leukemia. In: Blood; 2013.
37. Skrzeczynska-Moncznik J, Bzowska M, Loseke S, Grage-Griebenow E, Zembala M, Pryjma J. Peripheral blood CD14high CD16+ monocytes are main producers of IL-10. Scandinavian journal of immunology 2008;67:152-9.
38. Anastasilakis AD, Toulis KA, Goulis DG, et al. Efficacy and safety of denosumab in postmenopausal women with osteopenia or osteoporosis: a systematic review and a meta-analysis. Horm Metab Res 2009;41:721-9.
39. Chen JH, Lin CY, Chen YM, Tian WT, Chu HM, Chang TW. Bispecific Antibody Binding To RANKL and Osteonectin with Enhanced Localization to the Bone. Molecular pharmaceutics 2017;14:4113-20.
40. Ogata K, Katagiri W, Hibi H. Secretomes from mesenchymal stem cells participate in the regulation
of osteoclastogenesis in vitro. Clin Oral Investig 2017;21:1979-88.
41. Kambayashi Y, Fujimura T, Furudate S, et al. The Expression of Matrix Metalloproteinases in Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL)-expressing Cancer of Apocrine Origin. Anticancer research 2018;38:113-20.
42. Kyrgidis A, Toulis, K. Safety and efficacy of denosumab in giant-cell tumour of bone. Lancet Oncol 2010;11:513-4.
43. Katol, Furuya M, Matsuo K, Kawabata Y, Tanaka R, Ohashi K. Giant cell tumours of bone treated with denosumab: histological, immunohistochemical and H3F3A mutation analyses. Histopathology 2018;72:914-22.
44. Yamagishi T, Kawashima H, Ogose A, et al. Receptor-Activator of Nuclear KappaB Ligand Expression as a New Therapeutic Target in Primary Bone Tumors. PloS one 2016;11:e0154680.
45. Tsurushima H, Kokuryo S, Sakaguchi O, Tanaka J, Tominaka K. Bacterial promotion of bisphosphonate-induced osteonecrosis in Wistar rats. International Journal of Oral and Maxillofacial Surgery 2013;42:1481-7.
46. Muratsu D, Yoshiga D, Taketomi T, et al. Zoledronic acid enhances lipopolysaccharide-stimulated proinflammatory reactions through controlled expression of SOCS1 in macrophages. PloS one 2013;8:e67906.
47. Ji X, Pushalkar S, Li Y, Glickman R, Fleisher K, Saxena D. Antibiotic effects on bacterial profile in osteonecrosis of the jaw. Oral Dis 2012;18:85-95.
48. De Bruyn L, Coropciuc R, Coucke W, Politis C. Microbial population changes in patients with medication-related osteonecrosis of the jaw treated with systemic antibiotics. Oral surgery, oral medicine, oral pathology and oral radiology 2018;125:268-75.
49. Kyrgidis A, Verrou E. Fatigue in bone: A novel phenomenon attributable to bisphosphonate use. Bone 2010;46:556.
50. Kyrgidis A, Vahtsevanos K. “Fatigue” having a role in the pathogenesis of osteonecrosis of the jaws. Clin Oral Investig 2009;13:479-80.
51. Herrmann M, Zeiter S, Eberli U, et al. Five Days Granulocyte Colony-Stimulating Factor Treatment Increases Bone Formation and Reduces Gap Size of a Rat Segmental Bone Defect: A Pilot Study. Frontiers in bioengineering and biotechnology 2018;6:5.
52. Kuroda R, Matsumoto T, Niikura T, et al. Local transplantation of granulocyte colony stimulating factor-mobilized CD34+ cells for patients with femoral and tibial nonunion: pilot clinical trial. Stem cells translational medicine 2014;3:128-34.
53. Joly P, Schaus T, Sass A, et al. Biophysical induction of cell release for minimally manipulative cell enrichment strategies. PloS one 2017;12:e0180568.
54. Eto H, Ishimine H, Kinoshita K, et al. Characterization of human adipose tissue-resident hematopoietic cell populations reveals a novel macrophage subpopulation with CD34 expression and mesenchymal multipotency. Stem cells and development 2013;22:985-97.
55. Barnett FH, Rosenfeld M, Wood M, et al. Macrophages form functional vascular mimicry channels in vivo. Scientific Reports 2016;6:36659.
56. Whitelaw DM, Bell M. The Intravascular Lifespan of Monocytes. Blood 1966;28:455-64.