

Department of Stomatology<sup>1</sup>, Guangdong Women and Children Hospital; Department of Endodontics<sup>2</sup>, Stomatological Hospital, Southern Medical University, Guangzhou; Department of Stomatology<sup>3</sup>, Shenzhen Maternity & Child Healthcare Hospital, Shenzhen, China

## Immune-related expression profiles of bisphosphonates-related osteonecrosis of the jaw in multiple myeloma

JUNCHENG HE<sup>1</sup>; QIFENG ZHOU<sup>3</sup>, XIANCHAO JIA<sup>3</sup>, PENG ZHOU<sup>1</sup>, LIN CHEN<sup>2</sup>

Received December 24, 2020, accepted January 24, 2021

\*Corresponding author: Lin Chen, Department of Endodontics, Stomatological Hospital, Southern Medical University, 521533 Guangzhou, China  
clstudy0830@126.com

Pharmazie 76: 159-164 (2021)

doi: 10.1691/ph.2021.01013

**Objective:** To investigate the immune cellular and genomic profiles of bisphosphonates-related osteonecrosis (BRONJ) of the jaw and excavate potential small molecule drugs. **Methods and materials:** The genomic profile of a multiple myeloma (MM) patient with BRONJ was downloaded from Gene Expression Omnibus (GEO). The 22 immune cell subsets infiltration in the patient were predicted by cell-type identification by estimating relative subsets of RNA transcripts. In addition, the differently expressed immune-related genes (DEMGs) of BRONJ were identified, followed by Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses for functional annotation. Then, potential drugs for BRONJ treatment were predicted by Connectivity Map (CMAP) based on DEGs. **Results:** High proportions of native CD4+T cells and M0 macrophages were observed while resting mast cells, NK cells, and eosinophils were downregulated in the BRONJ patient ( $P<0.05$ ). Resting dendritic cells and gamma delta T cells were positively correlated ( $r=0.93$ ). Additionally, 36 DEMGs were screened from 336 DEGs in BRONJ expression profiles. GO enrichment analysis revealed that DEMGs were most associated with peptidyl-tyrosine modification, myeloid leukocyte migration, leukocyte chemotaxis and regulation of chemokine production ( $P<0.05$ ). KEGG analysis indicated that DEMGs were mainly related to cytokine-cytokine receptor interaction, IL-17 signaling pathway and NF-Kappa B signaling pathway ( $P<0.05$ ). Besides, 12 small molecule drugs were screened in MM patient with ONJ. **Conclusion:** The discovery of different composition of immune cell types and immune-related transcriptomes in BRONJ helps to explain the onset and development of MRONJ, which provides a novel target for BRONJ therapy.

### 1. Introduction

Bisphosphonates (BPs) are bone-targeted drugs with chemically stable pyrophosphate analogues, which were clinically applied to treat multiple disorders of calcium metabolism, including multiple myeloma (MM), breast cancer, Paget's disease, and osteoporosis (OP) (Gralow et al. 2020; Rosini et al. 2015). MM is a plasma cell malignancy which is characterized by hypercalcemia, renal insufficiency, bone marrow failure and lytic bone lesions, causing severe bone pain and pathological fractures (Chapman et al. 2018; Pozzi and Raje 2011). BP are the cornerstone in the management of patients with MM (Alaranji et al. 2020). Application of bisphosphonates in patients with MM distinctly reduces pathological vertebral fractures, skeletal-related events (SREs), pain (Mhaskar et al. 2017) and lowers mortality (Mhaskar and Djulbegovic 2018). Not only did bisphosphonates prevent osteoclast formation and adhesion to bone, but also promote osteoclasts apoptosis (Criscitiello et al. 2015). Although BP show an excellent safety profile in reducing bone loss and enhancing bone mineral density (BMD) (Oryan and Sahvieh 2020), the extensive application of bisphosphonates increases the risk of osteonecrosis. Bisphosphonates-related osteonecrosis of the jaws (BRONJ), first reported by Marx (2003), is a serious adverse event characterized by necrotic bone exposure in the oral cavity. It was demonstrated that the incidence of BRONJ in patient with MM receiving BP was approximately 1/1000 around the world (Mhaskar et al. 2017). It occurs in patients receiving high-dose and long-term administration of bisphosphonates following dental treatment, including bone trauma (tooth

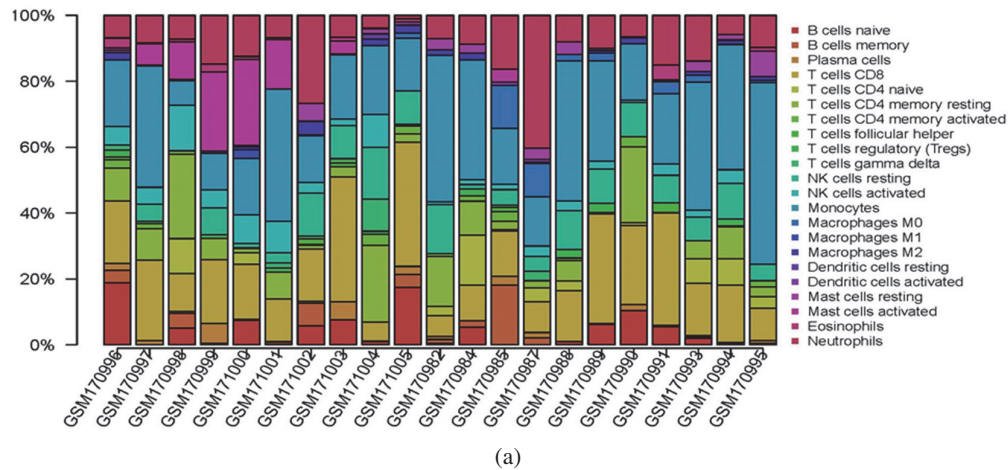
extraction and implant) and dental infection (periodontal diseases). However, the pathogenesis of BRONJ remains unclear.

With the growing knowledge of crosstalk between bone and immune system, the concept of osteoimmunology which focuses on the complex molecular understanding of the link of both systems was proposed (Criscitiello et al. 2015). Both bone and immune dysfunction is frequently observed in MM cancer (Criscitiello et al. 2015). Zoledronic acid (ZOA) and pamidronate, the nitrogen-containing BP, are acting on both bone and immune system and exert antitumor effects (Santini et al. 2009; Takimoto et al. 2020). Hence, we aimed to investigate the immune profiles in an MM patient with ONJ through bioinformatic methods, which might improve the safety of this therapy.

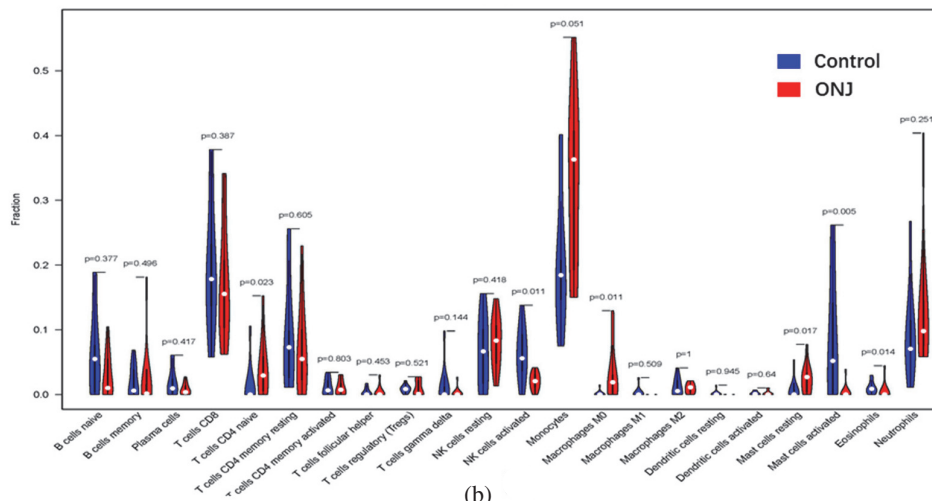
### 2. Investigations and results

#### 2.1. Composition of infiltrated immune cell subpopulations in MM with patient with BRONJ

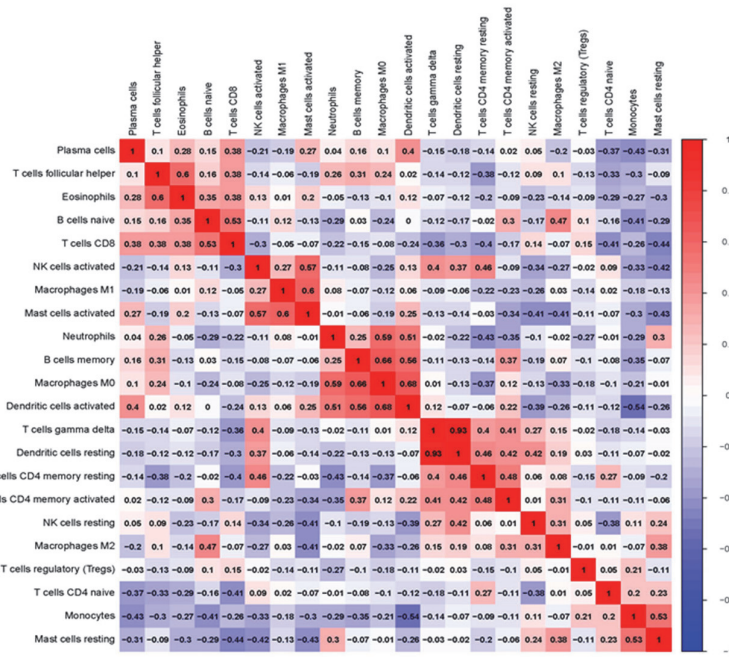
CIBERSORT analysis reveals that PBMCs samples in the patient with MM was mainly infiltrated with monocytes, CD8+ T cells, resting NK cells, neutrophils and resting memory CD4+ T cell. However, by comparison, a high proportion of native CD4+T cells and M0 macrophages were observed in PBMCs of MM patient with BRONJ while resting mast cells, NK cells, and eosinophils were downregulated in the BRONJ patient ( $P<0.05$ ) (Fig.1 a and b). The positive correlation ( $r=0.93$ ) between resting dendritic cells and gamma delta T cells is presented in Fig.1c.



(a)



(b)



(c)

Fig. 1: Composition of infiltrated immune cell subpopulations in MM with patient with BRONJ. (a) Fraction of 22 infiltrated immune cell types determined by CIBERSORT; (b) Comparison of infiltrated immune cell subpopulations in MM patient with or without BRONJ. (c) Correlation Analysis Between Immune Cell types. P means *P* value by Wilcoxon test between control group and ONJ group.

**2.2. Identification of different expression of immune-related genes in MM patient with ONJ**

Only 36 different expressions of immune-related genes (DEMGs) were identified, including 27 downregulated DEMGs and 9 upregulated DEMGs. The first 10 down-and 9 up-regulation DEMGs are listed in Table 1. Upregulated DEMGs included GHRL, CNTF, FGF17, IL17RA, SEMG1, ROBO1, IGF1R, PCSK1 and IL6R. Downregulated were CXCL2, IGHG1, IL1B, CXCL3, EREG, TNF, NFKBIZ, KLRC4, CCL4 and TXK ( $P < 0.05$ ). Interestingly, CXCL2 was the most relevant DEMGs for the onset of BRONJ.

**Table 1: Identification of 36 different expression of immune-related genes**

Gene symbol	logFC	FDR
CXCL2	-3.68919	0.007785
IGHG1	-3.07152	0.022074
IL1B	-2.88048	0.022074
CXCL3	-2.85511	0.0302
EREG	-2.81463	0.036598
TNF	-2.27828	0.028834
NFKBIZ	-2.05531	0.008388
KLRC4	-2.0264	0.043745
CCL4	-1.96048	0.028289
TXK	-1.9528	0.022711
GHRL	1.029456	0.044221
CNTF	1.09701	0.024131
FGF17	1.1121	0.039787
IL17RA	1.241639	0.026966
SEMG1	1.342865	0.023445
ROBO1	1.429953	0.049861
IGF1R	1.592922	0.010685
PCSK1	1.624519	0.044221
IL6R	1.799382	0.012246

FC: fold change; FDR: false discovery rate

**2.3. Immune-related functional enrichment based on GO and KEGG**

To further explore the function and pathway enrichment of DEMGs, GO and KEGG analyses were performed (Fig. 2). GO enrichment analysis revealed that DEMGs were associated with several vital

biological processes, including the increasing expression of peptidyl-tyrosine phosphorylation, peptidyl-tyrosine modification, myeloid leukocyte migration, leukocyte chemotaxis and regulation of chemokine production ( $P < 0.05$ ). In addition, the DEMGs were mainly located at the external side of the plasma membrane (cell component). KEGG analysis indicated that DEMGs were mainly related to the following pathways, such as cytokine-cytokine receptor interaction, MAPK signaling pathway, PI3K-Akt signaling pathway, IL-17 signaling pathway and NF-Kappa B signaling pathway ( $P < 0.05$ ) (Fig. 3).

**2.4. CMAP analysis**

In CMAP database, 12 small molecule drugs were screened based on 336 DEGs in MM patient with or without ONJ. As shown in Table 2, Gly-His-Lys, adiphenine, mometasone, bumetanide, valinomycin, anisomycin, chloropyrazine, strophanthidin, thioridazine, clemastine, PHA-00745360 and prestwick-692 might be the potential drugs for the research on BRONJ drug target treatment.

**Table 2: CMAP and mechanism of actions analysis of samples with different SRGs expression**

Cmap name	Mean	Enrichment	p	Mechanism of actions
Gly-His-Lys	-0.681	-0.943	0.00028	
Adiphenine	-0.652	-0.838	0.0003	Nicotine receptor inhibitors
Mometasone	-0.592	-0.878	0.00052	Glucocorticoid
Bumetanide	-0.678	-0.864	0.00062	Diuretic, NKCC1 inhibitor
Valinomycin	-0.585	-0.857	0.00074	Antibiotic, Potassium carrier
Anisomycin	-0.731	-0.847	0.00101	JNK activator
Chloropyrazine	-0.567	-0.826	0.00173	Dopamine receptor antagonist
Strophanthidin	-0.579	-0.808	0.00263	Cardiotonic
Thioridazine	-0.287	-0.388	0.00327	Dopamine Receptor antagonist
Clemastine	-0.68	-0.876	0.00385	Histamine H1 antagonist
PHA-00745360	-0.398	-0.583	0.00405	
Prestwick-692	-0.532	-0.781	0.00465	

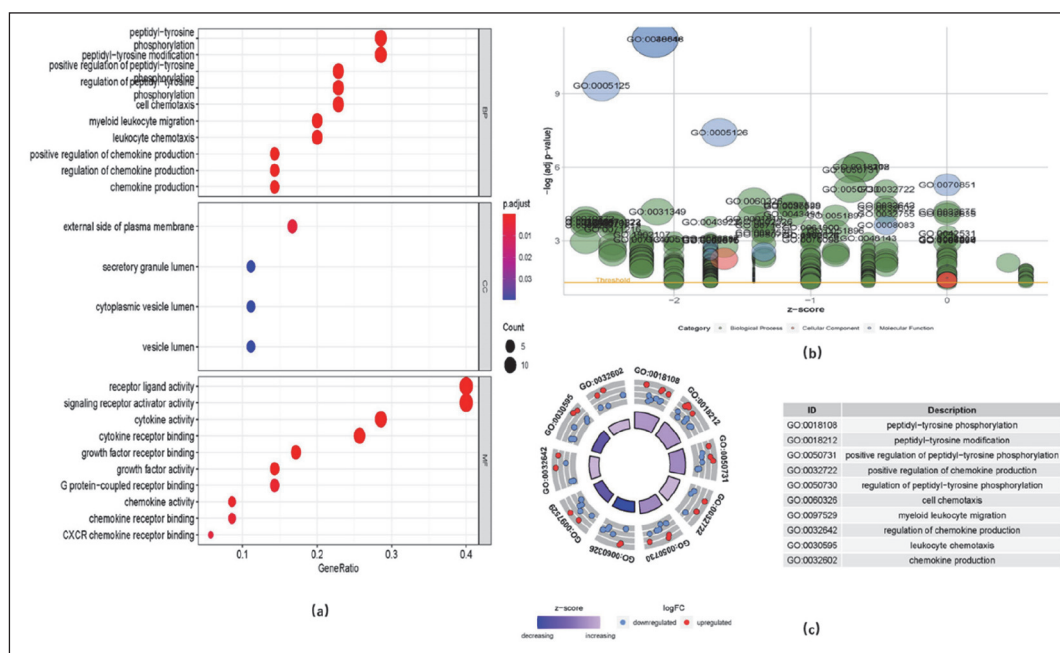


Fig. 2: GO functional enrichment analysis based on DEMGs. (a) Biological processes (BP), Cellular components (CC) and Molecular functions (MF) enriched in the DEMGs; (b) GO Bubble; (c) GO Heat

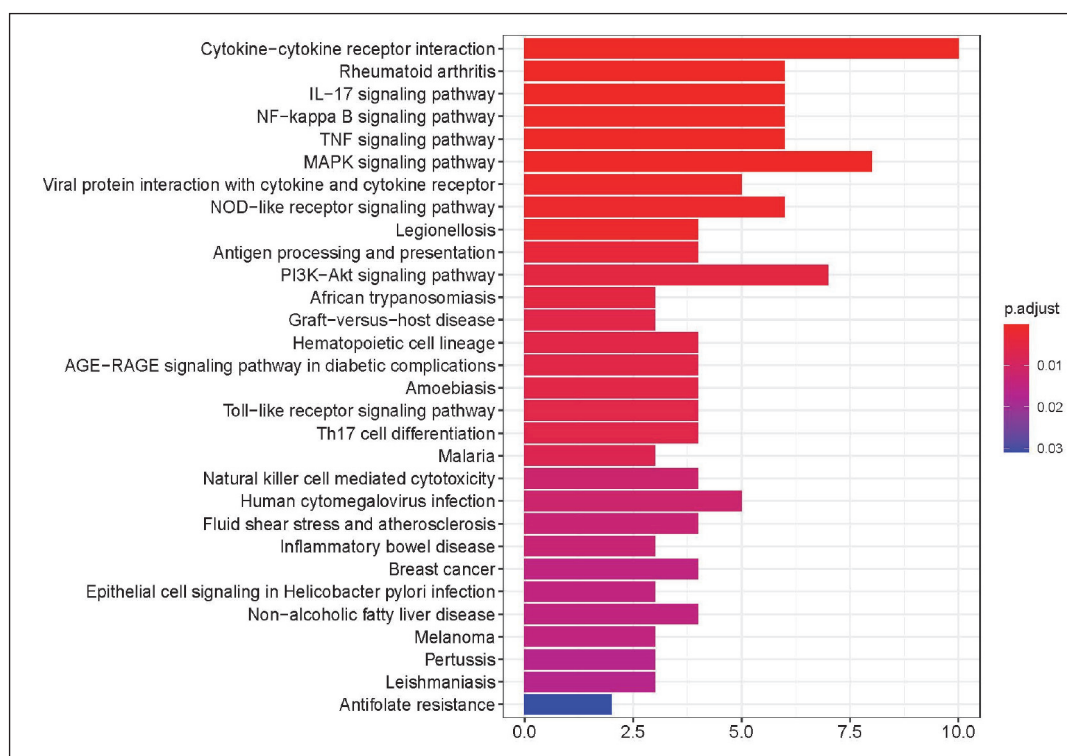


Fig. 3: KEGG pathways enrichment analysis based on DEMGs in BRONJ

### 3. Discussion

Medication-related osteonecrosis of the jaw (MRONJ) is a severe bone damage in the jaw, which is mainly caused by BPs. However, in patients receiving other anticancer compounds, except antiresorptive drugs, including antiangiogenics, tyrosine kinase inhibitors, mammalian target of rapamycin (mTOR) inhibitors, immune checkpoint inhibitors (ICI), BRAF inhibitor and cytotoxic chemotherapy ONJ occurred equally (Nicolatou-Galitis et al. 2019). A recent case report described a patient with osteonecrosis of the jaw (ONJ), who suffered from metastatic melanoma and experienced treatment with anti-PD-1 immunotherapy with nivolumab (Pundole et al. 2020). The above studies revealed an intimate link between immune microenvironment disorder and ONJ in cancer.

Macrophage phenotype alteration plays an important role in regulating innate immunity. M1 macrophages tend to promote inflammation on account of their production of reactive oxygen species (ROS), interleukin (IL)-12 and tumor necrosis factor (TNF)- $\alpha$ ; whereas M2 macrophages incline to play an anti-inflammatory role, featured IL-10 and transforming growth factor (TGF)- $\beta$ , resulting in resolution of inflammation and tissue modeling (Zhang et al. 2013). A recent clinical investigation found that a stage 1 MRONJ patient manifested a high density of M2 macrophages in mucosal tissues bordering necrotic bone, while Stage 2 and 3 MRONJ patients manifested M1 macrophages (Paschalidi et al. 2020). Mechanistically, zoledronate enhanced the expression of IL-17 and accelerated STAT-1 phosphorylation to induce M1 polarization, while inhibiting M2 conversion *via* suppressing STAT-6 activation (Zhang et al. 2013). Zoledronate also promotes M1 macrophage polarization through activating the TLR-4/NF- $\kappa$ B signaling pathway in BRONJ mice (Zhu et al. 2019). Convincingly, histone modifications were involved in the onset of MRONJ. Zoledronate managed to regulate the macrophage through upregulating the expression of histone demethylase (Kdm6a and Kdm6b) (Yang et al. 2020). Taken together, the macrophage phenotype has a pivotal implication on the development of MRONJ *via* multi-signal pathways.

Dendritic cells, the most pivotal antigen presenting cells (APCs), play an important role in maintaining the integrity of oral mucosa and alveolar bone. Zoledronate treatment inhibited the differ-

entiation and migration of dendritic cells (DCs) *in vitro* and *in vivo*. DC-deficient mice displayed a higher risk of osteonecrosis following tooth extraction (Elsayed et al. 2020). Zoledronate accelerated bacteria accumulation and impaired bone regeneration after extraction. Meanwhile, zoledronate hindered DC differentiation and migration from murine bone marrow precursors, impaired antigen presentation and inhibited DC-dependent T-cell activation. In addition, Zoledronate treatment rendered the activation of gamma-delta ( $\gamma\delta$ ) T cells (Santini et al. 2009). However, a significant depletion of V $\gamma$ 9V $\delta$ 2 T cells in patients with osteoporosis receiving BP treatment was observed in other studies (Kalyan et al. 2015). This study might explain a positive correlation between DC and  $\gamma\delta$  T cells in our result.

There were few studies reporting the relationship between MRONJ and eosinophils. Only one retrospective cohort study demonstrated that decreased eosinophil counts had been implicated in delayed wound healing after tooth extraction in patients taking immunosuppressive drugs, which was consistent with our results (Hayashi et al. 2018). A recent study uncovered a new role of eosinophils in promoting healthy ageing by regulating adipose tissue inflammation and sustaining immunological fitness (Brigger et al. 2020).

Osteoclasts are differentiated bone marrow mononuclear cells, which implicates a close relationship between osteoclast and immune system. Natural killer (NK) cells are innate lymphocytes which can rapidly eliminate infected or tumor cells by secreting various cytokines and chemokines. Communication between osteoclasts and NK cells has an implication for the development of BRONJ. A recent study demonstrated that osteoclasts intervened with ZOA inhibited NK cell mediated cytotoxicity which significantly induced the secretion of cytokines and chemokines (Tseng et al. 2015), which is consistent with our results. Then, an immunosuppressive microenvironment was developed in the jaw of tooth sockets. Interestingly, unlike the present study, there was one report demonstrating that no change was found in BRONJ rats (de Barros Silva et al. 2016).

It is known that IL-17 and the NF-kappa B signaling pathway play a vital role in the regulation of MRONJ (Huang et al. 2019). Upregulated IL-17 was usually observed in tooth sockets of BRONJ patients. The elevated IL-17 contributes to the pathogenesis of

BRONJ through altering the M1/M2 macrophage polarization (Zhang et al. 2013). NF- $\kappa$ B signaling is important for osteoclastogenesis and bone remodeling. More importantly, it plays an important role in immune system development and regulation. BPs, an antiresorptive agents, are suppressing the expression of the receptor activator of NF-kappa B ligand (RANK-L) in rat osteoblast cells to reduce the bone loss. However, targeting the RANK pathway for bone remodeling might be relevant to MRONJ, which needs more research to explore the related mechanism. ZOL enabled to suppress the JNK signaling pathway, which may serve as a potential agent for the treatment of BRONJ (Huang et al. 2019). Our CMAP result found that a JNK inhibitor (anisomycin) was available to changeover the expression profile in BRONJ. Interestingly, we firstly found that agents acting at the dopamine and nicotine receptors, and histamine H1 antagonists were selected as potential small molecular drugs for BRONJ. However, the above potential drug for BRONJ and its drug targeting mechanism need requires further examination. Recognizing the clinically significant toxicity of BP on ONJ is critical. The discovery of different compositions of immune cell types and immune-related transcripts in BRONJ helps to explain the onset and development of MRONJ, which provides a novel strategy for BRONJ therapy.

## 4. Experimental

### 4.1. Affymetrix microarray data

Raw data of GSE7116 were downloaded from the Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/geo/>) database based on the platform of the GPL570. This dataset contains 21 samples of peripheral blood mononuclear cells (PBMCs) of MM patient with or without BRONJ when receiving amino-bisphosphonate treatment. All subjects were separated into two groups: control group (n=10) with MM patient and ONJ group (n=11) with MM patient with BRONJ.

### 4.2. Immune cell infiltration with CIBERSORT

Cell-type identification by estimating relative subsets of RNA transcripts (CIBERSORT) (<http://cibersort.stanford.edu/>) (Newman et al. 2015) was an analytical tool to predict the fraction of immune cell types in gene expression profiles (GEP) of PBMCs in MM patients with or without BRONJ. CIBERSORT assesses 22 leukocyte subsets deconvolution based on a linear support vector regression (SVR), a machine learning approach which is highly robust to noise (Scholkopf et al. 2000). The 22 leukocyte subsets included naïve B cells, memory B cells, plasma cells, seven types of T cells, monocytes, resting NK cells, activated NK cells, three types of macrophages, resting dendritic cells, activated dendritic cells, resting mast cells, activated mast cells, eosinophils and neutrophils. Normalized GEP of GSE7116 were input in CIBERSORT for analysis based on a deconvolution algorithm with 100 permutations.

### 4.3. Immune-related gene definition

After normalization and standardization, differentially expressed genes (DEGs) in MM patients with BRONJ was screened. The immune-related gene list was downloaded from the Immunology Database and Analysis Portal (Immport) (<https://www.immport.org>), a immunology database containing 1233 immune-related genes (Bhattacharya et al. 2014). After taking the intersection between differentially expressed genes in GSE7116 and immune-related genes with R, the differentially expressed immune-related genes (DEMGs) and DEGs were filtered when  $|\log_2(\text{FC})| > 1$  and false discovery rate (FDR)  $< 0.05$  were considered as the significant cutoff value.

### 4.4. Immune-related functional annotation and KEGG pathway enrichment analysis

To obtain the functional annotation of DEMGs, Gene ontology (GO, <http://wego.genomics.org.cn>) and The Kyoto Encyclopedia of Genes and Genomes (KEGG, <http://www.genome.jp/kegg>) pathways analysis were conducted. Then, the visualize functional profiles (GO and KEGG) of DEMGs were implemented by R package (ClusterProfiler) (Yu et al. 2012). The enrichment of GO terms includes three independent ontologies: biological process (BP), molecular functions (MFs) and cellular component (CC) (Ashburner et al. 2000). The expression profile of BRONJ for both GO and KEGG analysis are based on over-representation analysis (ORA).

### 4.5. CMAP database

The Connectivity Map (CMAP, <https://portals.broadinstitute.org/cmap/>) is a database connecting drugs, genes and diseases through a collection of genome-wide transcriptional expression data from cultured human cells treated with bioactive small molecules (Lamb 2007; Lamb et al. 2006). It contains the more than 7000 genomic profiles of multiple cancer cell lines treated with 1309 small bioactive molecules DEGs were queried into CMAP and the screened small molecules drugs showing a negative enrichment score were considered as potential target-drug for BRONJ.

### 4.6. Statistical analysis

Comparisons between two groups (control ad ONJ group) were performed by two-side Wilcoxon test. Pearson correlation coefficient was used to measure the linear correlation among immune cells types fraction. Statistical analyses were conducted with the R package. Results with a  $P < 0.05$  were considered statistically significant.

Conflicts of interest: None declared

## References

- Alaraj G, Goyal A, Bansal P (2020) Bisphosphonate toxicity StatPearls. Treasure Island (FL), <https://www.ncbi.nlm.nih.gov/books/NBK562331/>.
- Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G. (2000). Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 25, 25-29.
- Bhattacharya S, Andorf S, Gomes L, Dunn P, Schaefer H, Pontius J, Berger P, Desborough V, Smith T, Campbell J, Thomson E, Monteiro R, Guimaraes P, Walters B, Wiser J, Butte AJ (2014) ImmPort: disseminating data to the public for the future of immunology. *Immunol Res* 58: 234-239.
- Brigger D, Riether C, van Brummelen R, Mosher KI, Shiu A, Ding Z, Zbären N, Gasser P, Guntern P, Yousef H, Castellano JM, Storni F, Graff-Radford N, Britschgi M, Grandgirard D, Hinterbrandner M, Siegrist M, Moullan N, Hofstetter W, Leib SL, Villiger PM, Auwerx J, Villeda SA, Wyss-Coray T, Noti M, Egger A (2020) Eosinophils regulate adipose tissue inflammation and sustain physical and immunological fitness in old age. *Nat Metab* 2: 688-702.
- Chapman MA, Sive J, Ambrose J, Roddie C, Counsell N, Lach A, Abbasian M, Popat R, Cavenagh JD, Oakervee H, Streetly MJ, Schey S, Koh M, Willis F, Virchis AE, Crowe J, Quinn MF, Cook G, Crawley CR, Pratt G, Cook M, Braganza N, Adedayo T, Smith P, Clifton-Hadley L, Owen RG, Sonneveld P, Keats JJ, Herrero J, Yong K (2018) RNA-seq of newly diagnosed patients in the PADIMAC study leads to a bortezomib/lenalidomide decision signature. *Blood* 132: 2154-2165.
- Crisciello C, Viale G, Gelao L, Esposito A, De Laurentis M, De Placido S, Santangelo M, Goldhirsch A, Curigliano G (2015). Crosstalk between bone niche and immune system: osteoimmunology signaling as a potential target for cancer treatment. *Cancer Treat Rev* 41: 61-68.
- de Barros Silva PG, de Oliveira CC, Brizeno L, Wong D, Lima Júnior R, Gonçalves RP, Sousa FB, Mota M, de Albuquerque Ribeiro R, Alves A (2016). Immune cellular profile of bisphosphonate-related osteonecrosis of the jaw. *Oral Dis* 22: 649-657.
- Elsayed R, Kurago Z, Cutler CW, Arce RM, Gerber J, Celis E, Sultan H, Elashiry M, Meghil M, Sun C, Auersvald CM, Awad ME, Zeitoun R, Elsayed R, Eldin M, Elshikh M, Isales C, Elsalty ME (2020) Role of dendritic cell-mediated immune response in oral homeostasis: A new mechanism of osteonecrosis of the jaw. *FASEB J* 34: 2595-2608.
- Gralow JR, Barlow WE, Paterson AHG, M'iao JL, Lew DL, Stopeck AT, Hayes DF, Hershman DL, Schubert MM, Clemons M, Van Poznak CH, Dees EC, Ingle JN, Falkson CI, Elias AD, Messino MJ, Margolis JH, Dakhlil SR, Chew HK, Dammann KZ, Abrams JS, Livingston RB, Hortobagyi GN (2020) Phase III randomized trial of bisphosphonates as adjuvant therapy in breast cancer: S0307. *J Natl Cancer Inst* 112: 698-707.
- Hayashi M, Morimoto Y, Iida T, Tanaka Y, Sugiyama S (2018) Risk of delayed healing of tooth extraction wounds and osteonecrosis of the jaw among patients treated with potential immunosuppressive drugs: a retrospective cohort study. *Tohoku J Exp Med* 246: 257-264.
- Huang XL, Huang LY, Cheng YT, Li F, Zhou Q, Wu C, Shi QH, Guan ZZ, Liao J, Hong W (2019) Zoledronic acid inhibits osteoclast differentiation and function through the regulation of NF-kappaB and JNK signalling pathways. *Int J Mol Med* 44: 582-592.
- Kalyan S, Wang J, Quabius ES, Huck J, Wiltfang J, Baines JF, Kabelitz D (2015). Systemic immunity shapes the oral microbiome and susceptibility to bisphosphonate-associated osteonecrosis of the jaw. *J Transl Med* 13: 212.
- Lamb J (2007) The connectivity map: a new tool for biomedical research. *Nat Rev Cancer* 7, 54-60.
- Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, Lerner J, Brunet JP, Subramanian A, Ross KN, Reich M, Hieronymus H, Wei G, Armstrong SA, Haggarty SJ, Clemons PA, Wei R, Carr SA, Lander ES, Golub TR (2006). The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science* 313: 1929-1935.
- Marx RE (2003) Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. *J Oral Maxillofac Surg* 61: 1115-1117.
- Mhaskar R, Djulbegovic B (2018). Bisphosphonates for patients diagnosed with multiple myeloma. *JAMA* 320: 1483-1484.
- Mhaskar R, Kumar A, Miladinovic B, Djulbegovic B (2017) Bisphosphonates in multiple myeloma: an updated network meta-analysis. *Cochrane Database Syst Rev* 12, CD003188.
- Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA (2015). Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods* 12: 453-457.
- Nicolatou-Galitis O, Kouri M, Papadopoulou E, Vardas E, Galiti D, Epstein JB, Elad S, Campisi G, Tsoukalas N, Bektas-Kayhan K, Tan W, Body JJ, Migliorati C, Lalla RV; MASCC Bone Study Group (2019). Osteonecrosis of the jaw related to non-antiresorptive medications: a systematic review. *Support Care Cancer* 27: 383-394.
- Oryan A, Sahvieh S (2020) Effects of bisphosphonates on osteoporosis: Focus on zoledronate. *Life Sci* 118681.

- Paschalidi P, Gkouveris I, Soundia A, Kalfarentzos E, Vardas E, Georgaki M, Kostakis G, Erovc BM, Tetradis S, Perisanidis C, Nikitakis NG (2020) The role of M1 and M2 macrophage polarization in progression of medication-related osteonecrosis of the jaw. *Clin Oral Investig* doi: 10.1007/s00784-020-03602-z
- Pozzi S, Raje N (2011) The role of bisphosphonates in multiple myeloma: mechanisms, side effects, and the future. *Oncologist* 16: 651–662.
- Pundole X, Jones AL, Tetzlaff MT, Williams MD, Murphy WA Jr, Otun A, Goepfert RP, Davies MA (2020). Osteonecrosis of the jaw induced by treatment with anti-PD-1 immunotherapy: a case report. *Immunotherapy* 12: 1213–1219.
- Rosini S, Rosini S, Bertoldi I, Frediani B.(2015) Understanding bisphosphonates and osteonecrosis of the jaw: uses and risks. *Eur Rev Med Pharmacol Sci* 19: 3309–3317.
- Santini D, Martini F, Fratto ME, Galluzzo S, Vincenzi B, Agrati C, Turchi F, Piacentini P, Rocci L, Manavalan JS, Tonini G, Poccia F (2009) In vivo effects of zoledronic acid on peripheral gammadelta T lymphocytes in early breast cancer patients. *Cancer Immunol Immunother* 58: 31–38.
- Scholkopf B, Smola AJ, Williamson RC, Bartlett PL (2000) New support vector algorithms. *Neural Comput* 12: 1207–1245.
- Takimoto R, Suzawa T, Yamada A, Sasa K, Miyamoto Y, Yoshimura K, Sasama Y, Tanaka M, Kinoshita M, Ikezaki K, Ichikawa M, Yamamoto M, Shiota T, Kamijo R (2020) Zoledronate promotes inflammatory cytokine expression in human CD14-positive monocytes among peripheral mononuclear cells in the presence of gammadelta T cells. *Immunology* doi: 10.1111/imm.13283
- T seng HC, Kanayama K, Kaur K, Park SH, Park S, Kozłowska A, Sun S, McKenna CE, Nishimura I, Jewett A (2015) Bisphosphonate-induced differential modulation of immune cell function in gingiva and bone marrow in vivo: role in osteoclast-mediated NK cell activation. *Oncotarget* 6: 20002–20025.
- Yang X, Xu X, Chen J, Wang Q, Wang G, Ai X, Wang X, Pan J (2020) Zoledronic acid regulates the synthesis and secretion of IL-1beta through Histone methylation in macrophages. *Cell Death Discov* 6: 47.
- Yu G, Wang LG, Han Y, He QY (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 16: 284–287.
- Zhang Q, Atsuta I, Liu S, Chen C, Shi S, Shi S, Le AD (2013) IL-17-mediated M1/M2 macrophage alteration contributes to pathogenesis of bisphosphonate-related osteonecrosis of the jaws. *Clin Cancer Res* 19: 3176–3188.
- Zhu W, Xu R, Du J, Fu Y, Li S, Zhang P, Liu L, Jiang H (2019) Zoledronic acid promotes TLR-4-mediated M1 macrophage polarization in bisphosphonate-related osteonecrosis of the jaw. *FASEB J* 33: 5208–5219.