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The effect of denosumab treatment on osteoclast precursor cells in postmenopausal osteoporosis

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Concerns have been raised about a rebound phenomenon in bone turnover upon discontinuation of denosumab in osteoporotic patients. Findings from the FREEDOM and FREEDOM Extension post hoc analysis encompassing 1475 patients, unveiled an increase in the fracture risk following denosumab cessation to similar levels observed in untreated patients. Our aim was to investigate the effect of denosumab on the osteoclast precursor cell population in order to elucidate the cellular mechanisms of this phenomenon.

Blood samples were obtained from 10 osteoporotic postmenopausal women, 6 months following the last dose of denosumab (60mg SC). Peripheral mononuclear cells were initially isolated and stained for CD14, M-CSFR, CD11b and TNFRII receptors. Osteoclast precursors were identified utilising fluorescent-activated cell sorting (FACS) analysis, as cells expressing CD14+/MCSFR+, CD14+/CD11b+ or CD14+/TNFR-II+. Our control group comprised a historical cohort of 69 postmenopausal women previously recruited for the TRIO study, investigating the effect of bisphosphonate treatment on osteoclast precursor cells (pre-treatment baseline measurements were utilised for comparisons).

Results are presented in Table 1. Data are presented as Mean ± Standard Deviation (SD) or median (IQR, interquartile range) based on whether a normal distribution was observed.

Median duration of denosumab administration was 3 years (range 0.5 to 8 years).

Denosumab administration resulted in an increase in the number of CD14+/CD11b+ cells indicating a block in the differentiation of osteoclast precursors to osteoclasts during denosumab therapy. This could explain the offset of treatment upon discontinuation and the reason a rebound increase in bone turnover is observed.

Table 1
Comparison of the CD14+ cells which were positive for M-CSFR, CD11b, TNFR-II between the two groups.

	Denosumab (N=10)	TRIO cohort (N=69)	p-value
%CD14+/MCSFR+	2.57 ± 1.87	1.7 (1.7)	0.361*
%CD14+/CD11b+	5.65 (23.08)	3.07 ± 1.73	0.001*
%CD14+/TNFRII+	1.93 ± 1.6	2.56 ± 1.47	0.224$
*Independent samples Mann Whitney U test	$Independent Samples t-test		

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Mir-342-3p regulates ostoclastogenesis in arthritis-associated osteoclast precursors

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Background: Rheumatoid arthritis is associated with bone destruction mediated by osteoclasts (OC), which originate from blood-derived myeloid precursors. MicroRNAs (miRNA) are key regulators of cellular processes, including osteoclastogenesis. Aiming at identifying key regulators of pathogenic OC in the context of arthritis, we have investigated the role of miR-342-3p in osteoclactogenesis.

Methods: OC were derived from $CD11b^+$ and $CD11c^+$ cells isolated from mouse bone marrow. In vitro studies were performed using the murine RAW264.7 cell line transfected with either miR-342-3p mimics or inhibitors. Cell survival and motility were assessed. We used Illumina RNA-Seq and in silico approach to identify putative targets of miR-342-3p, and dual luciferase assay to validate the binding. Anti-miR-342-3p was delivered in vivo to circulating $Ly6C^{high}$ monocytes using the DMAPAP/DOPE cationic liposome upon intravenous injection of mice with K/BxN serum-transfer arthritis (STA). Arthritis severity and bone histomorphometric parameters were analyzed.

Results: The expression level of miR-342-3p was increased in OC from STA mice, and transiently up-regulated in the early phase of osteoclastogenesis. Using the RAW264.7 cell line, we showed that miR-342-3p neutralization significantly inhibits the motility, proliferation and survival of OC precursors, resulting in decreased OC numbers and resorption activity. Upon miR-342-3p neutralization, 1185 genes were significantly deregulated, which were associated with locomotion and apoptosis pathways. In vitro assays validated ADAM17 as a target for miR-342-3p. Neutralization of miR-342-3p in $Ly6C^{high}$ precursors of STA mice increased ADAM17 expression level in OC progenitors.

Conclusions: Our data suggest that the miR-342-3p/*ADAM17* axis promotes the early phase of osteoclastogenesis by enhancing the cell survival and motility of OC precursors. The up-regulation of miR-342-3p in OC may also represents a biomarker of increased osteoclastogenic potential of inflammatory precursors in arthritis.

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The non-erythropoietic analogue cibinetide inhibits osteoclastogenesis *in vitro* and increases bone density in mice

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Erythropoietin (Epo), the master regulator of erythropoiesis, has also been implicated in a wide range of non-erythroid activities, including tissue protection. These functions are mediated *via* two receptors: hematopoiesis *via* the Epo receptor (EpoR) homodimer and tissue protection *via* a heteromer composed of EpoR and CD131. Cibinetide (ARA290) is a non-erythropoietic analogue of Epo, acting selectively on the heteromer complex. Our published findings that Epo stimulates osteoclast precursors and entrains a decrease in bone density, raise questions regarding the underlying molecular mechanisms.

Unexpectedly, we found that cibinetide injections in 12-week-old female mice (120 μg/kg thrice weekly for one month) resulted in a significant increase in tissue mineral density in cortical bone by 5.8% (1416.4 ± 39.27 vs 1338.74 ± 16.56 mgHA/cm^3) and in trabecular bone by 5.2% (1056.52 ± 30.94 vs 1004.13 ± 16.91 mg HA/cm^3) ($p< 0.05$), as measured using microCT.