Anti-Myeloma Drug Melflufen Inhibits RANKL-stimulated Osteoclastogenesis by Suppressing Proliferation of CD14+ Precursor Cells

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Disclosures

- Konstantin Byrgazov (Oncopeptides AB, employment)
- Ana Slipicevic (Oncopeptides AB, employment)
- Fredrik Lehmann (Oncopeptides AB, employment, equity)
- Joachim Gullbo (Oncopeptides AB, founder, equity, consultancy; Theradex Oncology, employment)
- Rolf Larsson (Oncopeptides AB, equity)
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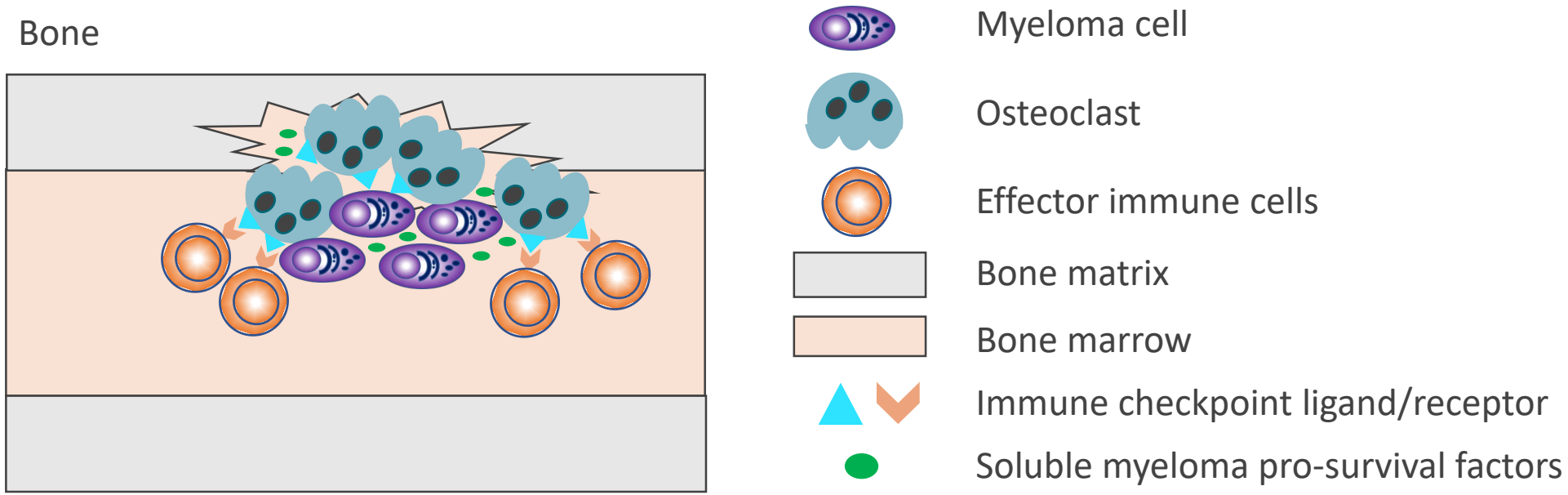
Myeloma bone disease and osteoclasts

- Myeloma bone disease (MBD): pathological fractures, bone destruction, bone pain (Terpos et al., Blood Cancer 2018)
- Local excessive osteoclastogenesis promoted by myeloma cells (Marino and Roodman, 2018)
- Osteoclastogenesis: differentiation of CD14+ monocytes into osteoclasts (OC) stimulated by RANKL and M-CSF
- Patients receiving anti-myeloma drug melflufen (MFL) are reported to have increase physical activity and reduce pain in HORIZON trial (Oriol et al., Poster 3477)



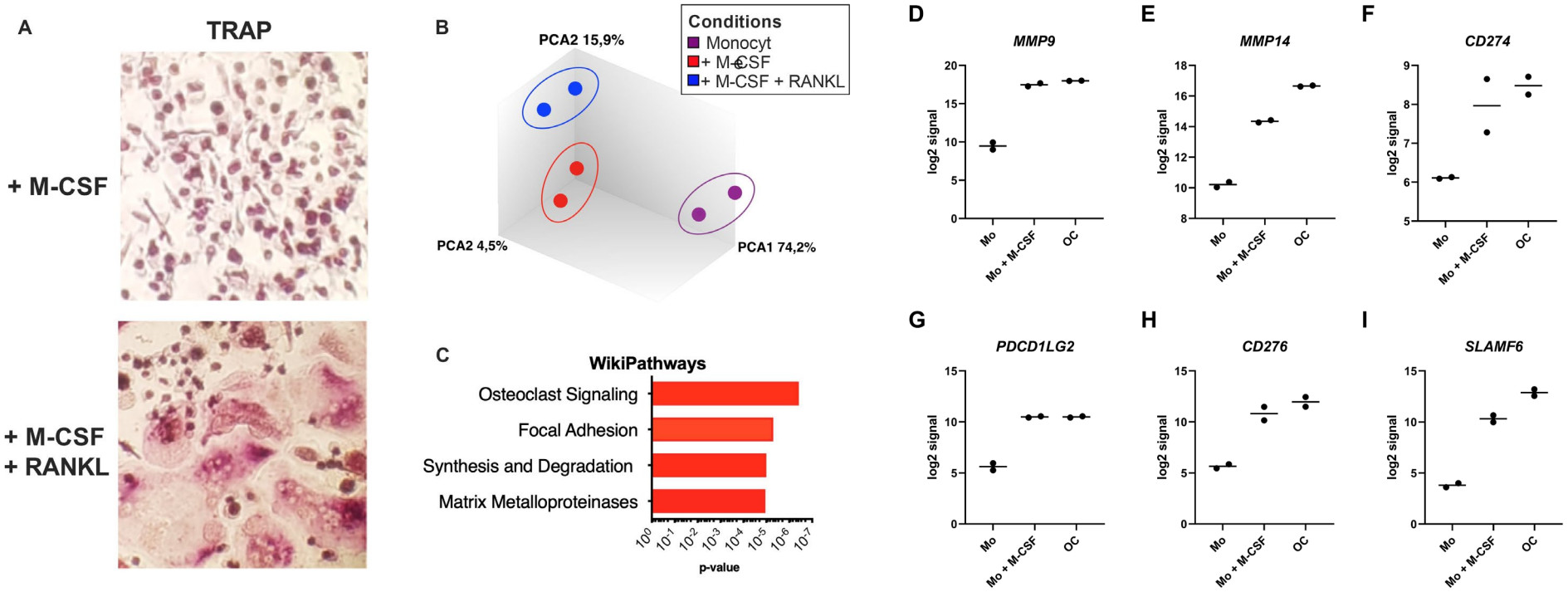
In vitro Osteoclastogenesis

(A) CD14+ monocytes show OC morphology and tartrate-resistant acid phosphotase (TRAP) staining upon incubation with M-CSF and RANKL.

(B) Principal component analysis of gene expression of monocytes alone or incubated with M-CSF and M-CSF + RANKL

(C) Pathways enriched in gnee expression of monocytes incubated with M-CSF and RANKL

(D-I) Expression of selected genes encoding immune suppressive molecules MMP9, MMP14, CD274/PD-L1, PCD1LG2/PD-L2, CD276/B7-H3

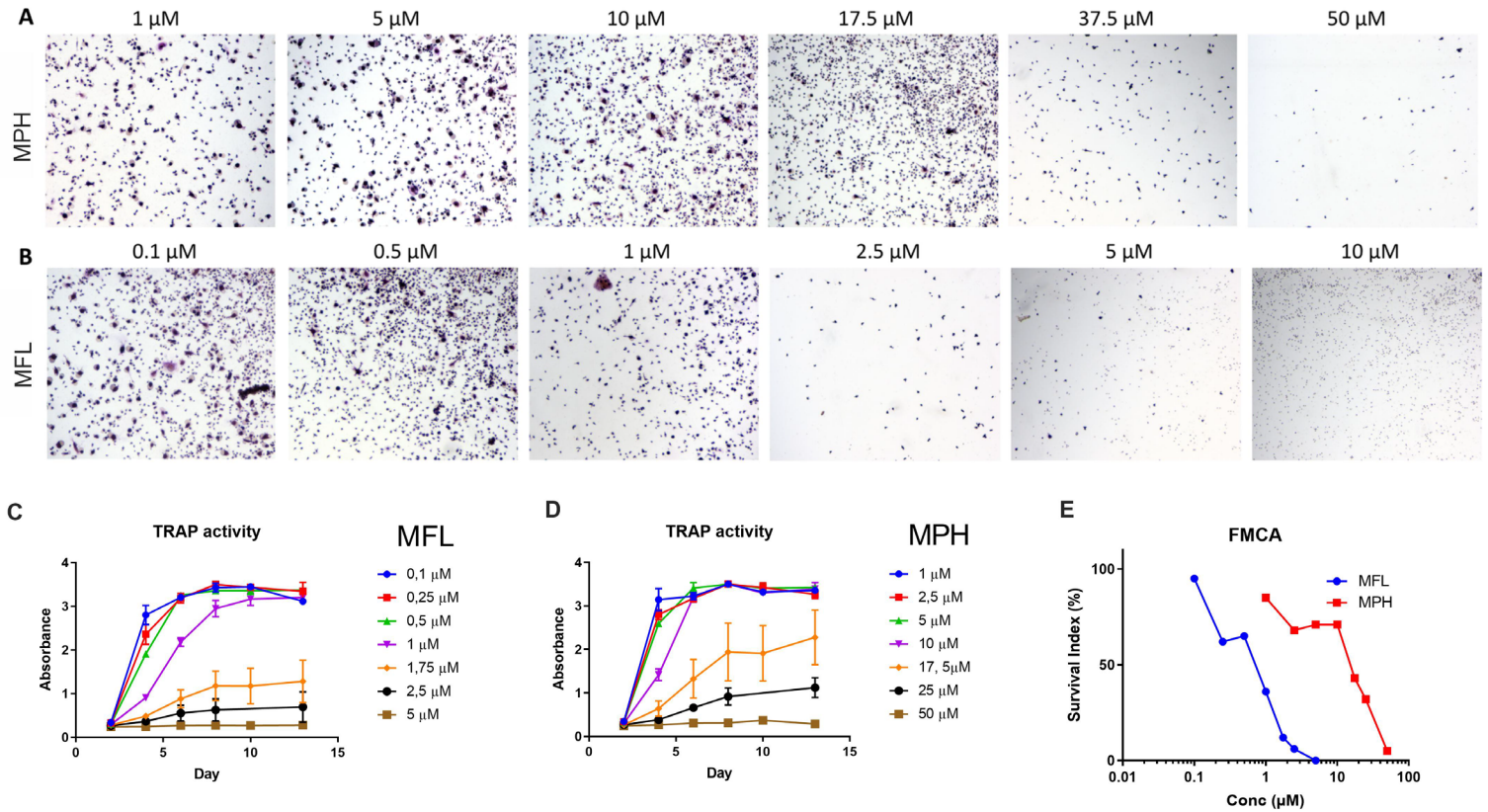


Effect of melflufen and melphalan on *in vitro* osteoclastogenesis

(A-B) Effects of melphalan (MPH) and melflufen (MFL) on in vitro osteoclastogenesis shown by TRAP activity staining

(C-D) Kinetics of osteoclastogenesis in the presence of increasing concentrations of MFL and MPH showing 10-fold higher activity of MFL

(E) Cytotoxicity of MFL and MPH in CD14+ monocytic precursor cells



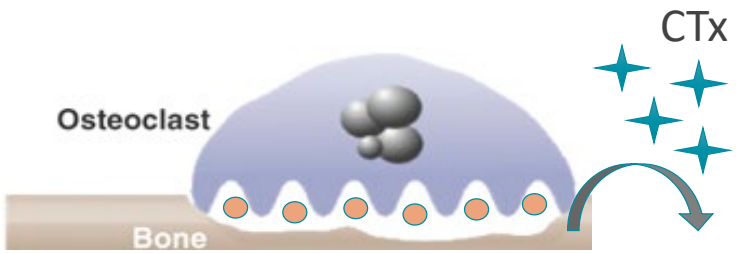
Effect of melflufen and melphalan on *in vitro* osteoclastogenesis

(A) Schematic representation of an osteoclasts bone resorbing activity: collagen fragment (CTx) release and inorganic matrix (brown) degradation

(B) Melflufen and melphalan effects on osteoclast-mediated inorganic matrix degrdation: white – 100% degradation, brown – no degradation

(C) Drug concentration-dependent effects of melflufen (MFL) and melphalan (MPH) on collagen fragment release

A



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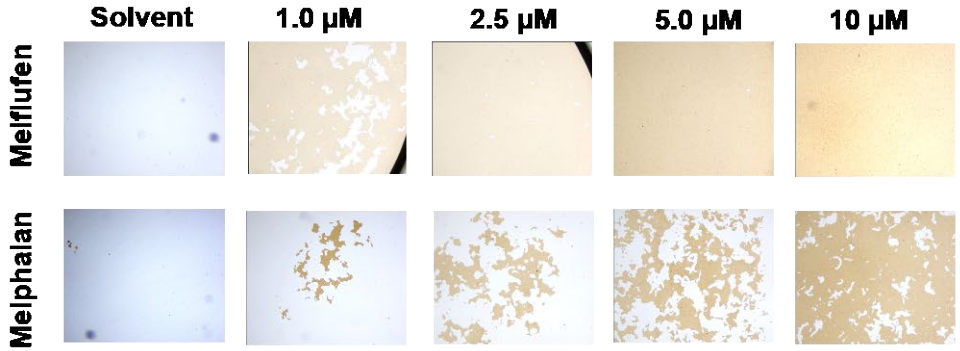

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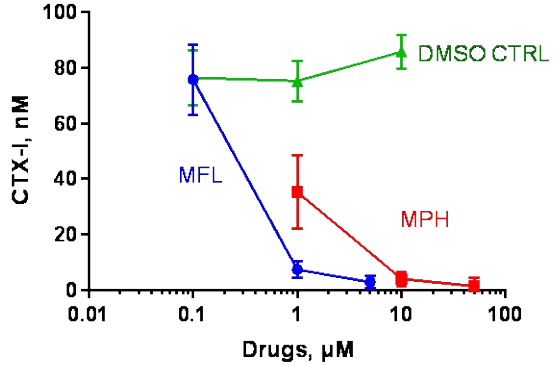

Conclusions

- Osteoclastogenesis is associated with increased bone resorption and immunosuppressive microenvironment.
- Osteoclastogenesis up-regulates expression of genes encoding immunosuppressive molecules PD-L1, PD-L2, B7-H3, and MMP14 which negatively regulate T and NK cell reponses: PD-L1 and PD-L2 are responsible for T cell exhaustion (Boussiotis, NEJM 2017), B7-H3 negatively regulates CD8+ T and NK cells (Lee et al., Cell Res 2017), MMP14 promotes shedding of MICA thus attenuating NK cell response (Liu et al., J Immunol 2010).
- Melflufen **inhibits** osteoclastogenesis and bone resorption (both inorganic matrix and collagen release) at physiologcially achievable concentrations and **10-fold** more efficient than melphalan.