Maternal embryonic leucine zipper kinase inhibitor OTSSP167 has preclinical activity in multiple myeloma bone disease

Joséphine Muller,[1,a] Arnold Bolomsky,[2,a] Sophie Dubois,[1] Elodie Duray,[1] Kathrin Stangelberger,[2] Erwan Plougonven,[3] Margaux Lejeune,[1] Angélique Léonard,[3] Caroline Marty,[4] Ute Hempel,[5] Frédéric Baron,[1,6] Yves Beguin,[1,6] Martine Cohen-Solal,[4] Heinz Ludwig,[2] Roy Heusschen[1,b] and Jo Caers[1,6,b]

[1]Laboratory of Hematology, GIGA-I3, University of Liège, Belgium; [2]Wilhelminen Cancer Research Institute, Department of Medicine I, Wilhelminenspital, Vienna, Austria; [3]PEPs (Products, Environments, Processes), Chemical Engineering, Liège, Belgium; [4]INSERM-UMR-1132, Université Paris Diderot, France; [5]Institute of Physiological Chemistry, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Germany and [6]Department of Hematology, CHU de Liège, Belgium

[a]Joséphine Muller and Arnold Bolomsky are co-first authors. [b]Roy Heusschen and Jo Caers are co-senior authors.

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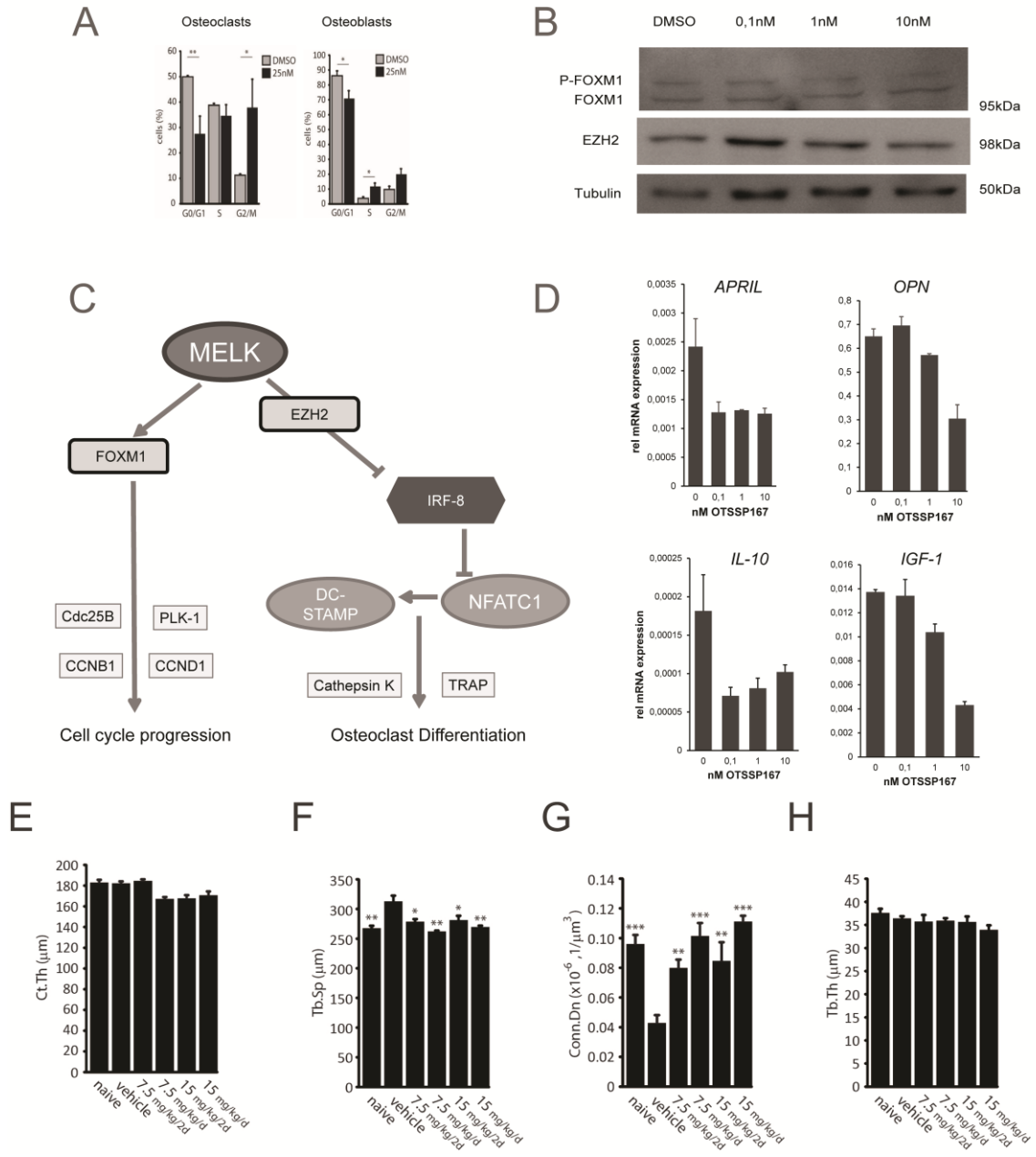
Correspondence: jo.caers@chu.ulg.ac.be

Suplemental figure 1



Supplemental figure 1: (A) Cell cycle analysis demonstrating G2/M cell cycle arrest following treatment of RAW264.7 cells and BMSC-TERT with OTSSP167. (B) Western blots for MELK, FOXM1, EZH2 and tubulin realized on protein samples obtained from RANKL-stimulated RAW264.7 cells, treated with different concentrations of OTSSP167 (C) Diagram illustrating the pathways regulated by MELK in osteoclast differentiation. (D) mRNA expression of IGF-1, OPN, IL-10 and APRIL by RANKL-stimulated RAW264.7 cells, treated with different concentrations of OTSSP167. (E) Bone parameters obtained by CTAn analysis on µCT results illustrating cortical thickness (Ct.Th), (F) trabecular separation (Tb.Sp), (G) trabecular connectivity density (Conn.Dn) and (H) trabecular thickness (Tb.Th).

Supplemental Table 1

Gene	Forward	Reverse
hMELK	AAACCCAAGGGTAACAAGGA	ACAGTATGCCCATGCTCCAA
mMELK	CTTGGATCAGAGGCAGATGTTTG	GCTGTAATCTTGCATGACCCAGG
mFOXM1	CACTTGGATTGAGGACCACTT	GGAGACCCTGGTTCCAGTG
mEZH2	CAGATAAGGGCACCGCAGAA	ACATTCAGGAGGCAGAGCAC
osteoclast function		
mIRF8	AAGGGCAGGCCCAACAAG	ACAGCTGCTCTACCTGCACCAG
NFATc1	TGAGGCTGGTCTTCCGAGTT	CGCTGGGAACACTCGATAGG
CTSK	CAGCAGAGGTGTGTACTATG	GCGTTGTTCTTATTCCGAGC
TRAP	TCCTGGCTCAAAAAGCAGTT	TCCTGGCTCAAAAAGCAGTT
CALCR	CTTCCATGCTGATCTTCTGG	CAGATCTCCATTGGGCACAA
mCDC25B	GCCACCTCTCGGTCTTTGAG	TGGTCTGTGTAAGAGTGGTAACC
mPLK1	CTTCGCCAAATGCTTCGAGAT	TAGGCTGCGGTGAATTGAGAT
mCCNB1	GCGTGTGCCTGTGACAGTTA	CCTAGCGTTTTTGCTTCCCTT
CCND1	TGACTGCCGAGAAGTTGTGC	CTCATCCGCCTCTGGCATT
cytokines		
mIGF-1	AGACAGGCATTGTGGATGAG	CTTGTGTTCTTCAAATGTACTTCC
mOPN	CTCCAATCGTCCCTACAGTCG	CTGTGGCATCAGGATACTGTTCATC
mIL-10	GACTGCCTTCAGCCAGGTG	CGATAAGGCTTGGCAACCC
mAPRIL	ACAGAGCTGCAAAGCCTAAGG	GAGATTTCGCCCCATCCTTC
osteoblast function		
All genes assessed with pre-designed Taqman assays		
reference genes		
b-act	TCTGGCTCCTAGCACCATG	AAAACGCAGCTCAGTAACAG
B2MG	GCTACGTAACACAGTTCCAC	TGATGCTTGATCACATGTCTCG
RPLP0	Taqman assay	

Supplemental table 1: Sequences of primers used for qRT-PCR.