A					C					
	Case	Gender	Age years	Location	Histology	Case	Gender	Age Years	Location	Histology
	1	F	55	Sacrum	Secondary OS, telangiectatic	OSR07	М	19	Tibia	Osteoblastic
						OSR13	F	9	Femur	Telangiectatic
	2	М	62	Pelvis	Osteoblastic	OSR17	F	13	Tibia	Osteoblastic
	3	Μ	18	Femur	Osteoblastic with	OSR18	M	14	Tibia	Osteoblastic
						OSR20	M	12	Femur	Osteoblastic
					telangiectatic	OSR24	F	14	Femur -	Osteoblastic
		••		-	component	OSR25	M	13	Femur	Osteoblastic
	4	М	22	Femur	Osteoblastic	OSR26	M	19	Femur	Osteoblastic
	5	М	26	Femur	Osteoblastic	OSR27	F _	15	Femur	Osteoblastic
	6	М	27	Femur	Osteoblastic	OSR28	+	9	l ibia	Osteoblastic
	7	М	28	Femur	Osteoblastic	OSR32	M _	19	Femur	Osteoblastic
	8	E	80	Femur	OS on Paget Osteoblastic	OSR35	F	19	l ibia	Fibroblastic
		Г	00			OSR39	M	18	l ibia	Osteoblastic
	9	F	27	Femur	Low grade OS, Parosteal	OSR45	M	21	Femur	Osteoblastic
		·	21			05R40	IVI	18	Femur	Chondroblastic
	10	М	18	Humerus	Osteoblastic with	05R47	IVI	14	Femur	
			10			05R48	IVI N4	8	Humerus	Osteoplastic
					telangiectatic	05R49		24	Tibia	
					component	05K00	Г	0	Tibla	
	11	М	16	Femur	Osteoblastic	OSE01		29	Femur	Osteoplastic
в	12	М	16	Humerus	Chondroblastic	03E02	M	12	Femur	Chondroblastic
34 mRNA	0.2					OSE08	F	20	Femur	Osteoblastic
	-					OSE09	M	16	Tibia	Osteoblastic
	<b>6</b> 0.1					00E00	M	25	Humerus	Osteoblastic
						OSE15	F	15	Femur	Telangiectatic
e II	e 0.02					OSE16	M	13	Tibia	Osteoblastic
lti∨	<b>ö</b> 001-				90 ¬	00210		10	TIDIQ	Coloopidolio
rela	0.01									
R	0									
		<b>ှိ</b> <u>1 2 3</u>	8456	7 8 9 10 1						
	0.00	E -1	Р	atients	-34 ssic					.11
NA						I IIn	• L			11.1 1
ШШ	⊆ <sup>0.004</sup>	<b>4</b>   T		$\top$	exp		1.11			
34	.0.003	3			- 09 <u>a</u>	111111	L.III	1111		
Ę	8 0.002	2 -			R		ш			
ive	X				50 -	<b>ਸ਼੶ਸ਼੶ਸ਼੶ਸ਼੶ਸ਼</b>	╷┛╷┚╷┚╷┚	<b>┛╷┛╷┛╷┚</b> ╷	╷┛╷┛╷┛╷┚	└ <b>╷┻╷┻╷┻╷┻╷┻╷</b> ┻╷
elat	- 0.001					R07 R13 R17 R18 R18 R20 R24 R24	R25 R26 R27 R27 R27	R32 R35 R39 R39 R39	R47 R47 R48 R49 R50 R50	
_										

Supplementary Data 1: Human osteosarcoma expressed IL-34. (A) Clinical features of 12 osteosarcoma treated at the University Hospital of Nantes (Department of Orthopaedic Surgery, France). (B) IL-34 expression by quantitative PCR performed in human osteosarcoma biopsies (patients 1-12) and in human HOS, U2OS, Saos2 and MG63 osteosarcoma cell lines. IL-34 mRNA expression was normalized to the HPRT expression. (C) Clinical characteristics of 27 osteosarcoma patients and (D) relative IL-34 expression in the corresponding biospie specimens from GEO bank. OS: osteosarcoma.



**Supplementary Data 2: Validation data of the IL-34 and M-CSF expression by transduced HOS osteosarcoma cells.** HOS osteosarcoma cells transduced by M-CSF (**A**) and IL-34 (**B**) overexpressed *in vitro* the corresponding cytokines compared to the control cells (empty vector, CT) at the transcriptional level [as demonstrated by quantitative PCR (**A**, **B**)] and at the protein level [as demonstrated by ELISA assay (**A**) and flow cytometry (**B**)]. Similarly, M-CSF and IL-34 were also overexpressed compared to control group (pLv105 vector) in explanted tumour tissues established from inoculation of M-CSF- or IL-34-transduced HOS osteosarcoma cells (**C**). For quantitative PCR, HPRT gene expression was used as the invariant control.



Supplementary Data 3: TNF- $\alpha$  and IL-1 $\beta$  increase IL-34 mRNA expression in various osteosarcoma cells. (A) U2OS osteosarcoma cells were stimulated with increased dose (1–25 ng/ml) of TNF- $\alpha$  or IL-1 $\beta$  for 8 h. After incubation, IL-34 mRNA levels were determined by RTqPCR, normalised to GAPDH. Error bars represent the standard deviation between 3 independent experiments (B) MG63 osteosarcoma cells cultured on plastic chamber on microscope glass slides were treated or not with 10 ng/mL of TNF- $\alpha$  or IL-1 $\beta$  for 24 h. IL-34 expression detected by Alexa Fluor 488-conjugated antibody (green), actin filaments detected by alexa fluor 546-conjugated phalloidin (red), and nuclei stained by DAPI (blue) were observed by confocal microscopy. A representative experiment is shown.



**Supplementary Data 4: IL-34 is expressed by osteosarcoma cells.** IL-34 expression was analyzed by imunoshistochemistry in human osteosarcoma biopsies. In all samples studied, most of osteosarcoma cells showed a positive immunostaining for IL-34 (A-D). (A) Multinucleated cells (arrow) closely located to tumour cells (asterik) also expressed IL-34 staining. (B) In contrast to osteosarcoma cells (asterik), no IL-34 expression was observed in osteocytes. Osteosarcoma cells exhibited a nuclear (C) and a cytoplasmic immunostaining (D).



**CD31** 



**Supplementary Data 5: IL-34 and M-CSF increase the neo-formation of CD31<sup>+</sup> vessels in a mouse osteosarcoma model.** Human HOS osteosarcoma cells over-expressing IL-34 (HOS-IL34), M-CSF (HOS-MCSF) or transduced by the empty vector (HOS) were inoculated in close proximity to the tibia of female Rj:NMRI-nude mice. All mice were sacrificed by cervical dislocation at an equivalent volume of 1500 mm<sup>3</sup> and immunohistochemical assessment of CD31 was carried out, as previously done for the CD146 staining.



Supplementary Data 6: IL-34 and M-CSF modulate angiogenesis *in vivo*, in a mouse model of Matrigel<sup>®</sup> plug Assay. (A) Representative photographs of plugs excised on day 14 containing PBS (negative control); FGF-2 (350 ng/mL) used as a positive control, alone or with IL-34 (500 ng/mL), M-CSF (500 ng/mL). (B) Haemoglobin quantification: values represent mean  $\pm$  SEM of the haemoglobin content in Matrigel<sup>®</sup> plugs excised from C57Bl/6 mice (n=6 to 7 per experimental group). \* p<0.05, \*\* p<0.01 and \*\*\* p<0.001 compared to the FGF-2 control group.

Α

В



**Supplementary Data 7: Western blot quantification.** Bar graphs represent the relative densitometric values of phosphoproteins detected in Figure 3. The luminescence detected with a Charge Couple Device camera was quantified using the GeneTools programme (Syngene, Cambridge, United Kingdom).