Molecular, cellular and *in vivo* comparison of PREOB® and BM-MSC reveals a superior osteogenic potential for PREOB®

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BACKGROUND

Bone Therapeutics is an advanced biotechnology company addressing high unmet medical needs in the field of bone fracture repair and bone fracture prevention. The company develops a range of innovative, differentiated, cell products administrable via a minimally invasive percutaneous technique directly into the bone lesion site; a unique proposition in the market.

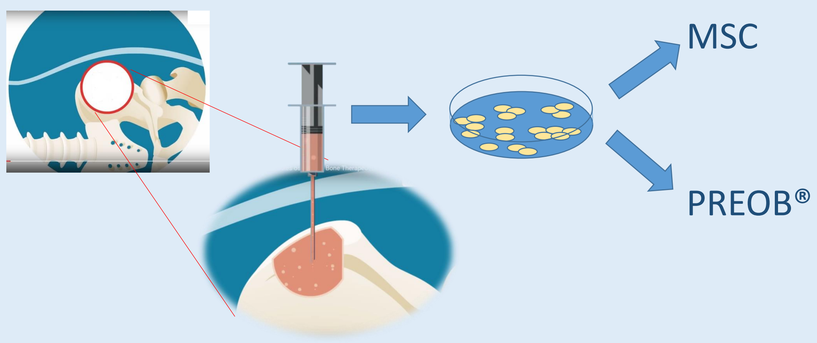
The company has developed a regenerative autogenic osteoblastic cell product, named PREOB®, derived from bone marrow which is currently in two pivotal Phase III trials for osteonecrosis and non-union fractures and in a Phase II trial for severe osteoporosis. The purpose of our study was to directly compare PREOB® *vs.* non-differentiated mesenchymal stromal cells (MSC) for their *in vitro* osteogenic characteristics and their *in vivo* osteogenic potential in order to determine which cellular type would be the most adapted for bone fracture repair.

METHODS

1. Culture and expansion of MSC and PREOB® cells

Bone marrow aspirate from iliac crest

In vitro culture process



2. *In vitro* characterization

Cells were characterized *in vitro* by morphology, immunophenotype (FACS), gene expression (qRT-PCR) and differentiation potential.

3. *In vivo* assessment of efficacy

Subcutaneous injection over the calvaria of nude mice of

- MSC cells
- PREOB® cells
- excipient (control -)

Bone formation evaluated by

- Radiology (Faxitron®)
- Histomorphometry

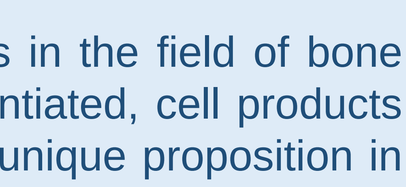
RESULTS

1. Gross characterization of the cells

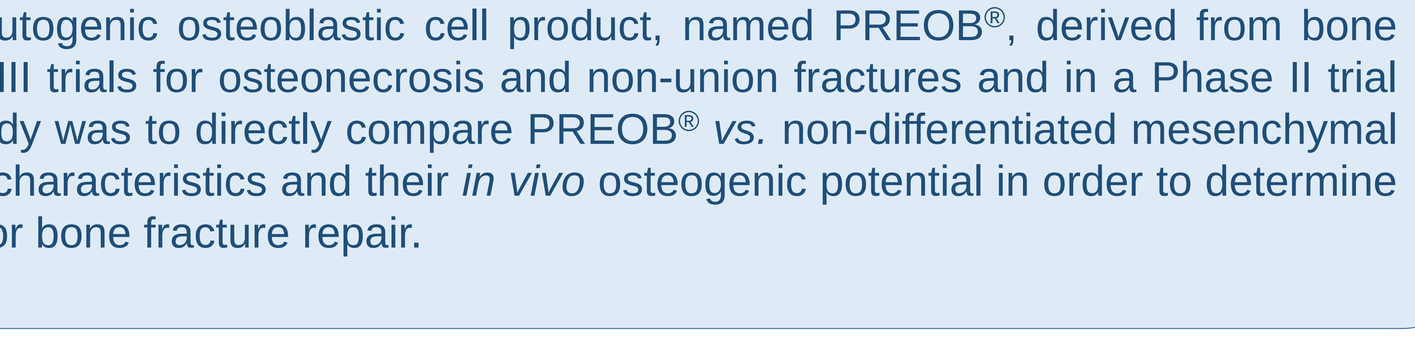
Cell population	Mesenchymal marker	cell-cell adhesion marker	Hematopoietic marker	Osteoblastic markers	
	FACS analysis (% of positive cells)				Enz. act. analysis
	CD73	CD44	CD45	ALP	ALP enz. (mUi/mg tot. prot.)
MSC	99 ± 1 (n=9)	98 ± 2 (n=16)	2 ± 0 (n=9)	21 ± 8 (n=16)	108 ± 86 (n=2)
PREOB®	99 ± 1 (n=19)	100 ± 0 (n=20)	2 ± 0 (n=19)	**72 ±19 (n=20)**	**411 ± 115 (n=7)**

Cell morphology

BM-MSC PREOB®



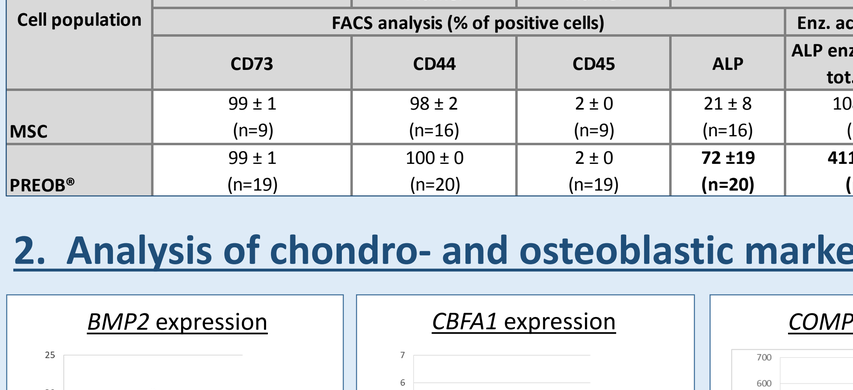
2. Analysis of chondro- and osteoblastic markers gene expression



PREOB® also expressed significantly higher levels of *ALPL* (fold change (FC) >12), *MMP13* (FC > 96), *COL1A1* (FC > 5) and *BGLAP* (FC > 4) compared to MSC

3. Bone Formation in nude mice

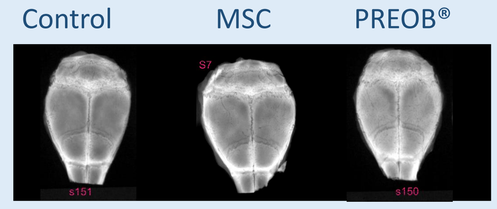
Histological evaluation of the bone formation



Control MSC PREOB®

Radiological evaluation of the bone formation

Control MSC PREOB®



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CONCLUSIONS

PREOB® displays superior osteogenic capacity to BM-MSCs and is therefore a better candidate for the treatment and the prevention of bone fractures.