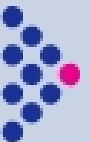


The Effect of Myeloma Cells on Bone Metabolism is Heterogenous and Correlates with Underlying Genetic Lesions and Bone Disease *In Vivo*

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Multiple Myeloma

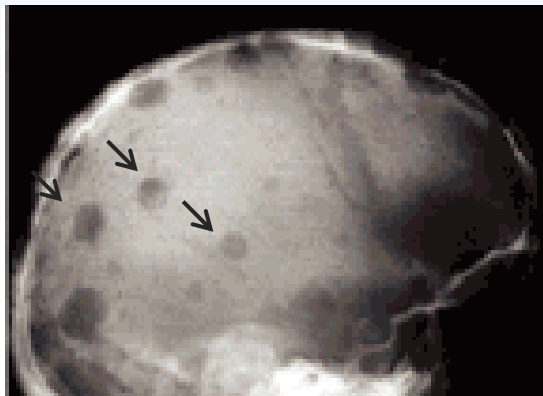
- **Malignant plasma cell tumour located at multiple sites in the bone marrow compartment**
- **MM cells are dependent on the bone marrow microenvironment for survival, growth and differentiation**
- **MM cells actively modulate their environment inducing angiogenesis and osteolytic bone destruction**
- **Patients differ with regard to clinical characteristics**

Molecular Classification of Multiple Myeloma

	<i>Frequency</i>	<i>Predominant CGN finding</i>	<i>Clinical outcome</i>
Hyperdiploid	45%	Increased number of chromosomes	favourable
Non-hyperdiploid	40%		
Cyclin D1 translocation	18%	t(11;14)	neutral
MMSET translocation	15%	t(4;14)	unfavourable (FGFR3 upreg in 75%)
MAF translocation	8%	t(14;16)	unfavourable

Multiple Myeloma Bone Disease

- important clinical feature of MM is osteolytic bone disease
- osteolytic lesions cause bone pain, cord compression, pathological fractures and hypercalcemia

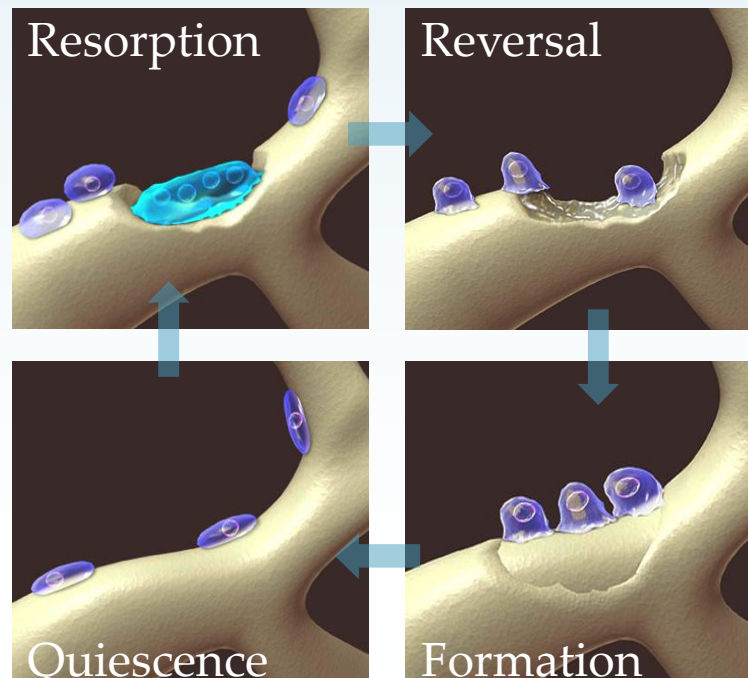


Kuehl and Bergsagel., 2002

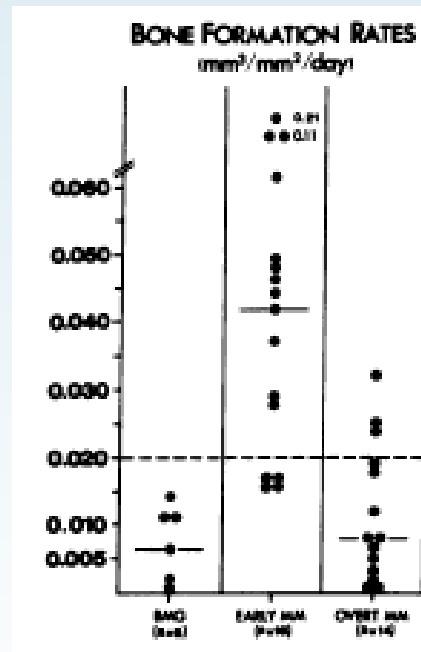
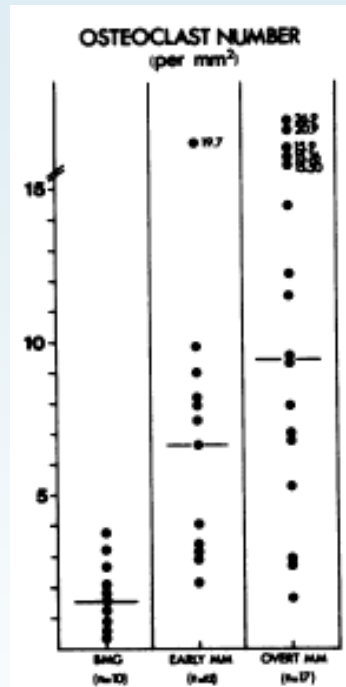


Multiple Myeloma Bone Disease

- Results from the uncoupling of normal homeostatic bone remodeling
- Coupled bone turnover is a process that is active throughout most adult life and maintained by the opposing actions of osteoclasts and osteoblasts.



Abnormal bone remodelling in multiple myeloma patients



Bataille et al., 1991

- resorption and formation become uncoupled resulting in an increase in bone resorption and a decrease in bone formation

Patients differ with regard to Bone Disease

IgH translocation	D-type Cyclin	% of all patients	Bone Lesions
t (11;14)	D1	15-20%	94%
-	D1	30-35%	86%
t (4;14) or t (14;16)	D2	15-20%	55%

Gene expression profiles distinguishing subgroups of patients may account for heterogeneity between patients

- correlation with recurrent IgH translocation partners
- t (11;14) associated with high incidence of bone disease

The effect of MM cells on osteoblast differentiation and function is heterogenous and correlates with underlying genetic lesions and bone disease in vivo

Project Design

Do *in vivo* models based on TC classification of Human Myeloma Cells lines (HMCL) recapitulate heterogenous bone disease observed in clinical subgroups?

Establish additional *in vivo* models

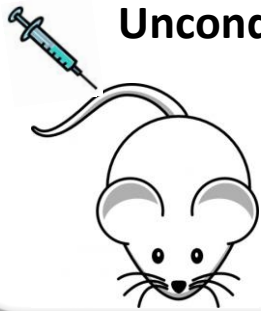


Compare to existing KMS12BM t(11;14) model



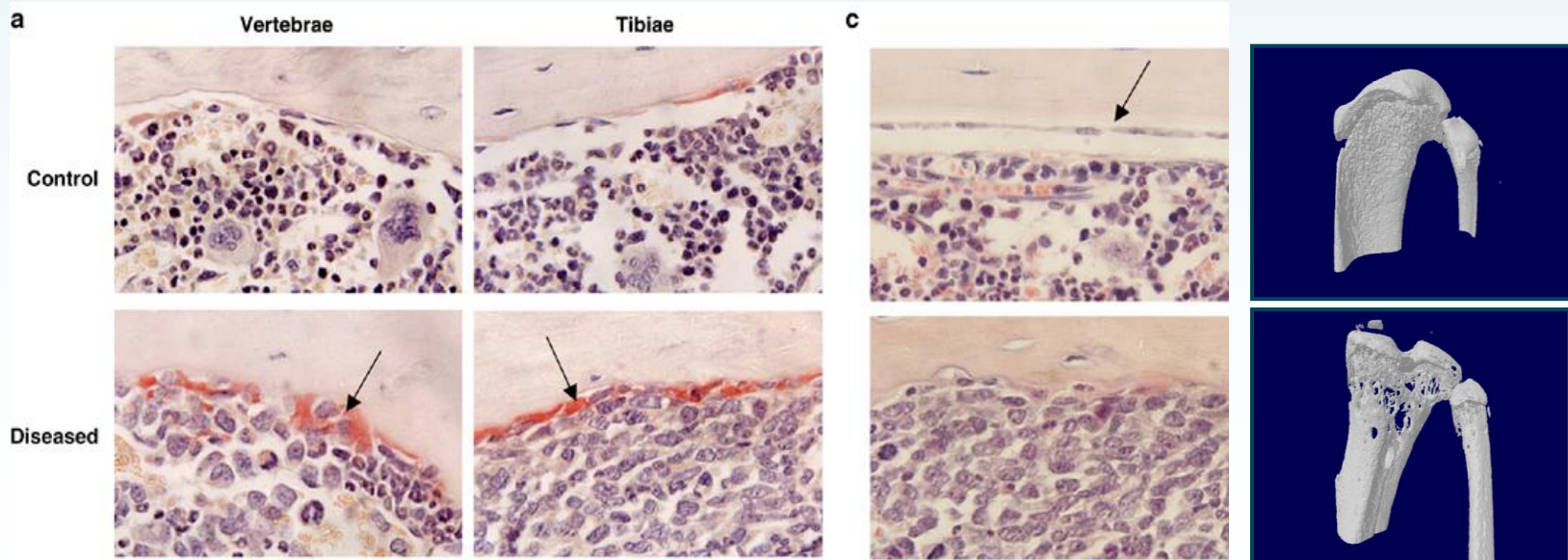
elucidate molecular mechanisms involved in the development of bone disease

KMS12BM model of multiple myeloma

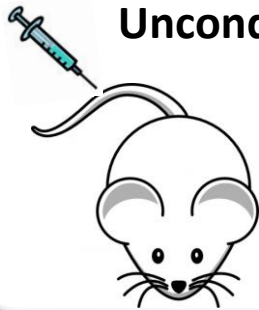


Unconditioned β 2M Nod/SCID mice injected IV with KMS12BM cells

- t(11;14) translocation
- cells engraft within the bone marrow



Establishing Xenogeneic Models of Alternative Genetic Subgroups of Multiple Myeloma



Unconditioned β 2M Nod/SCID mice injected IV with labelled HMCL

- HMCL from different TC groups
- assessed 8 weeks post IV injection

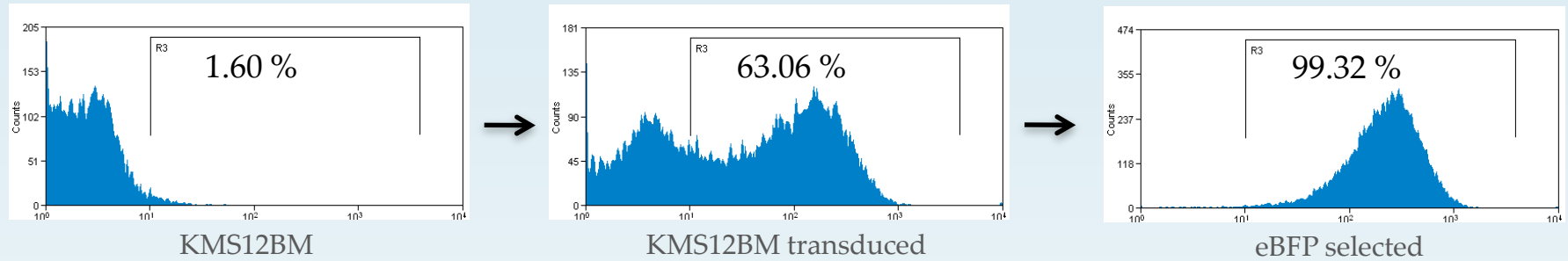
Criteria

- medullary engraftment
- little extramedullary disease

Analysis

- *in vivo* live animal imaging
- FACS
- IHC

a) HMCL transduced with luciferase.eBFP constructs

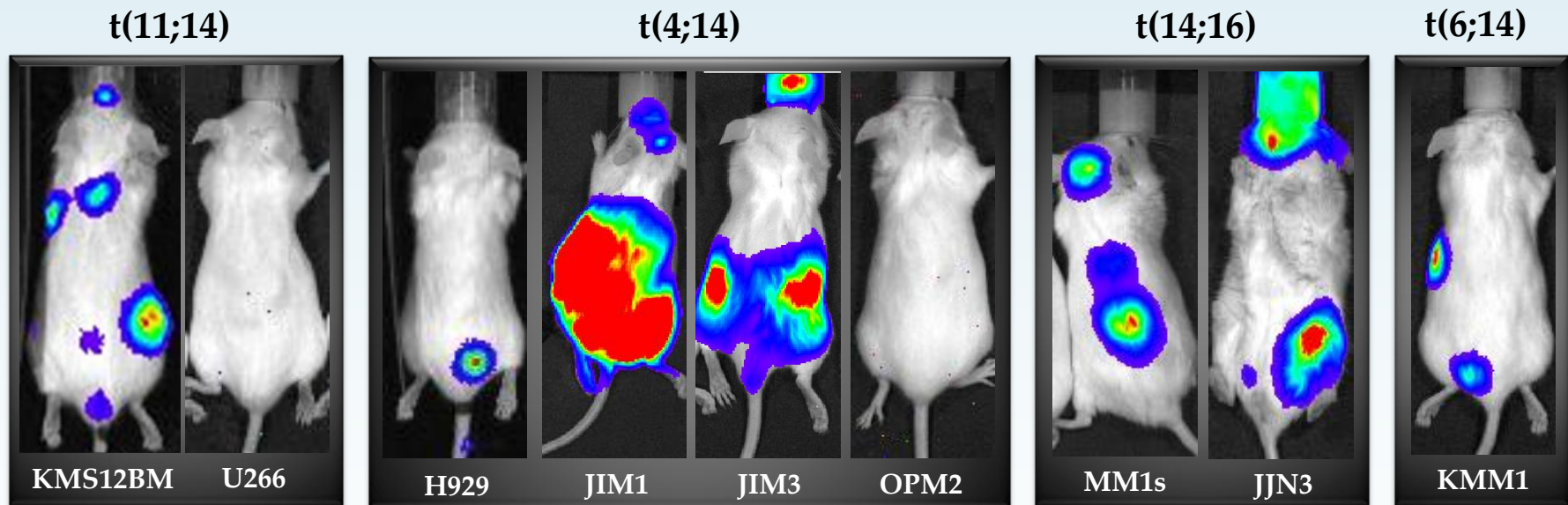


b) HMCLs then injected into 7 week old β 2M Nod/SCID mice

t(4;14)	t(6;14)	t(11;14)	t(14;16)
NCI H929	KMM1*	KMS12 BM	JJN3
OPM2*		U266	MM1S
JIM-1			
JIM-3			

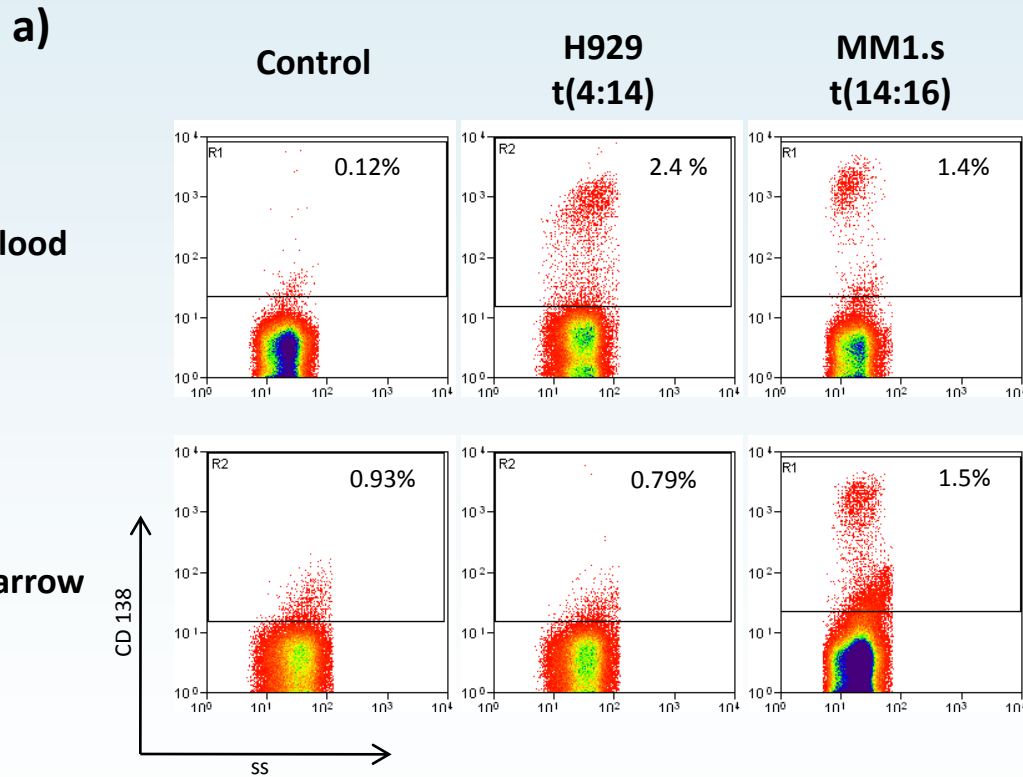
c) Mice were sacrificed after 8 weeks and analysed

In vivo imaging of IV injected HMCL

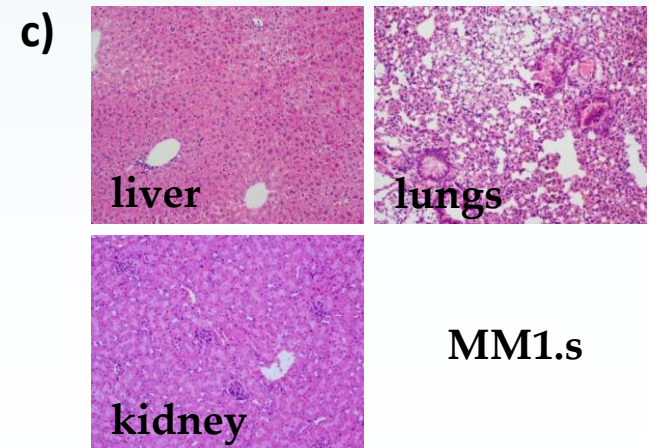
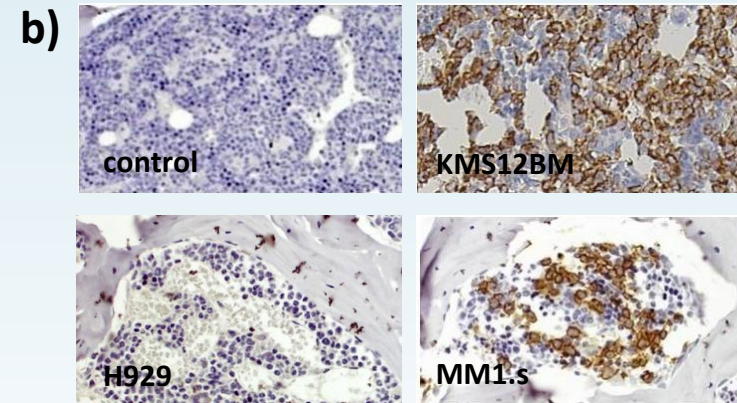


➤ U266, OPM2 do not engraft following tail vein injection

MM1.s cells engraft in the bone marrow of non-irradiated recipients



CD138 (syndecan I)
- marker of mature plasma and myeloma cells



Comparison of t(11;14) vs t(14;16) in vivo models

MM1.s

- Different genetic lesion – t(14;16)
- Lower incidence of bone disease
- Medullary engraftment
- Minimal extramedullary tumours, ie resemble human disease

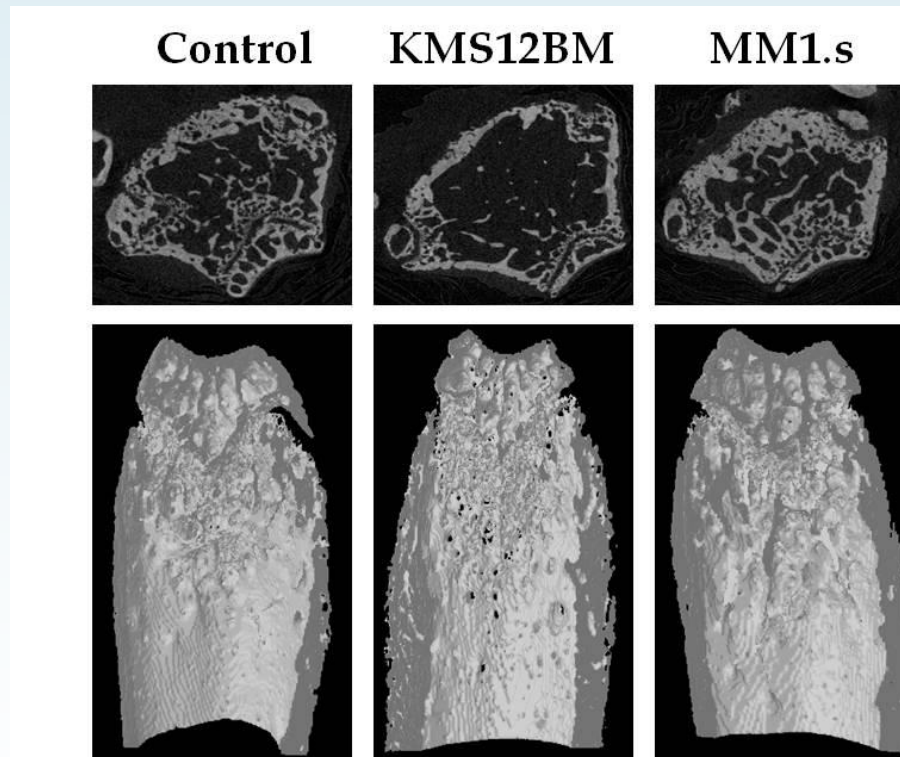


Unconditioned β 2M Nod/SCID mice injected IV with
KMS12BM and MM1.s cells

- sacrificed after 8 weeks

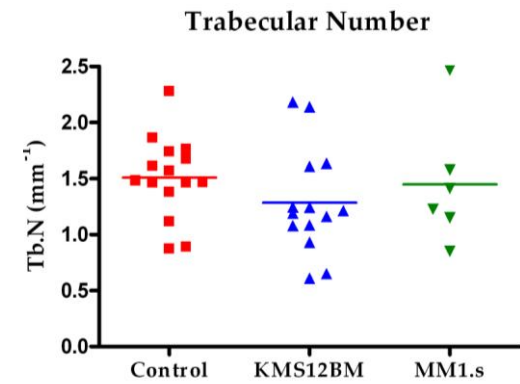
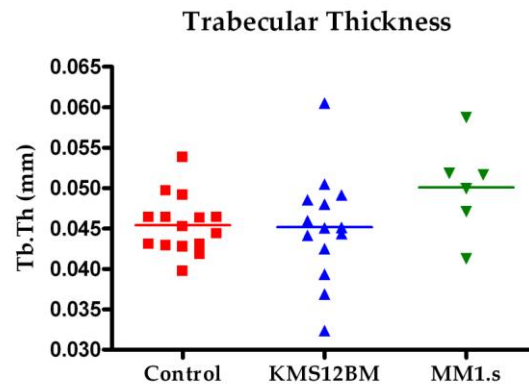
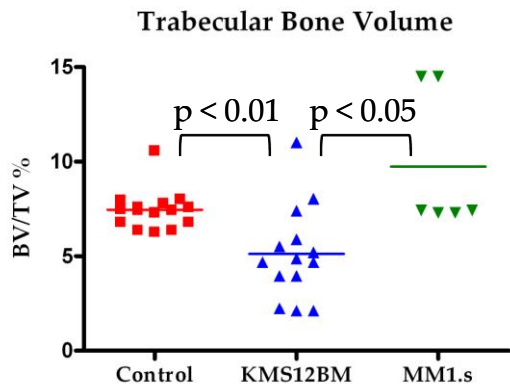
- analysed by uCT, static histomorphometry, FACS and IHC

Assessing Bone Disease in MM1.s model - micro-computed tomography (uCT) analysis



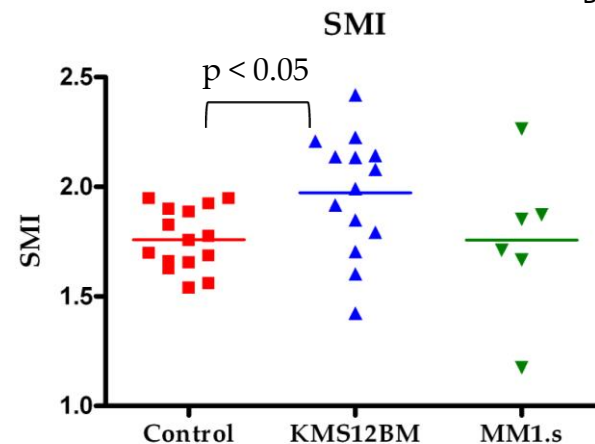
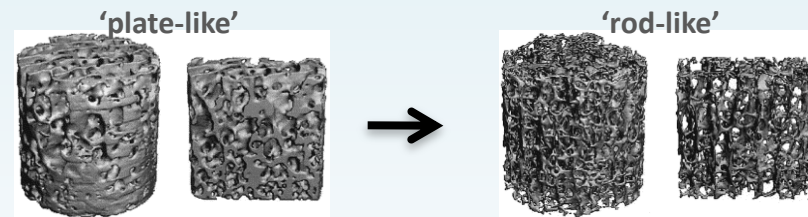
- MM1.s diseased animals do not develop osteolytic lesions

uCT analysis reveals differences in femoral bone morphology between KMS12BM and MM1.s models



Structure Model Index (SMI)

- Quantified based on a differential analysis of the triangulated bone surface of a structure
- Scored between 0 (plate-like) and 3 (rod-like)



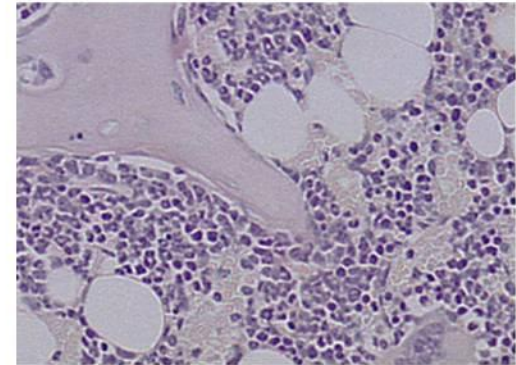
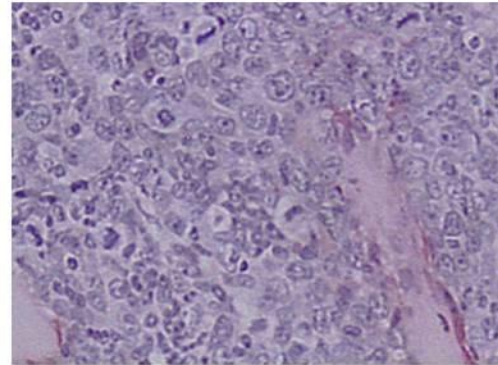
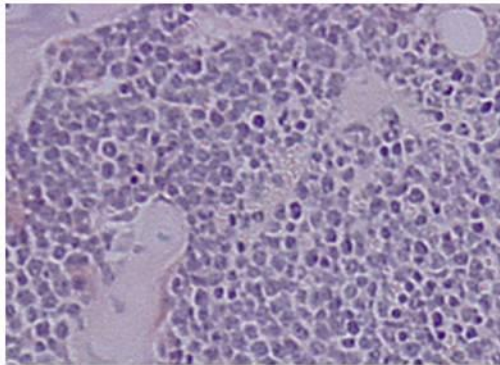
Osteoclast Analysis (TRAP stained)

Control

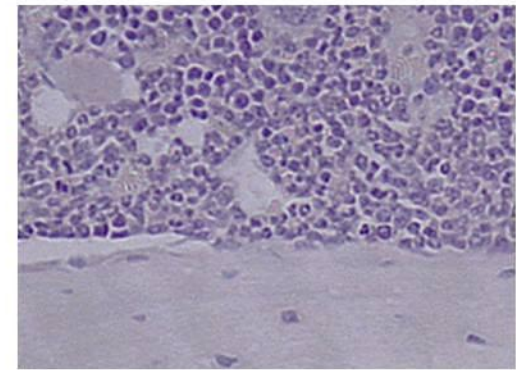
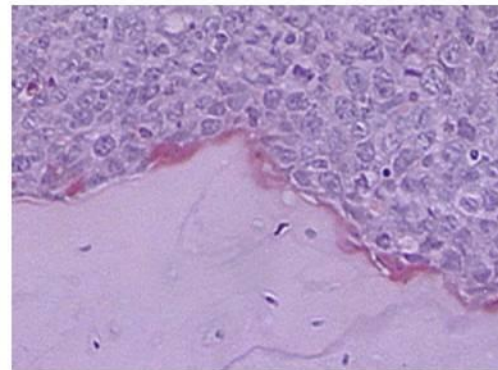
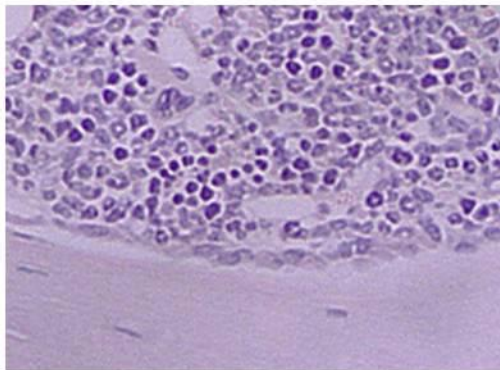
KMS12BM

MM1.s

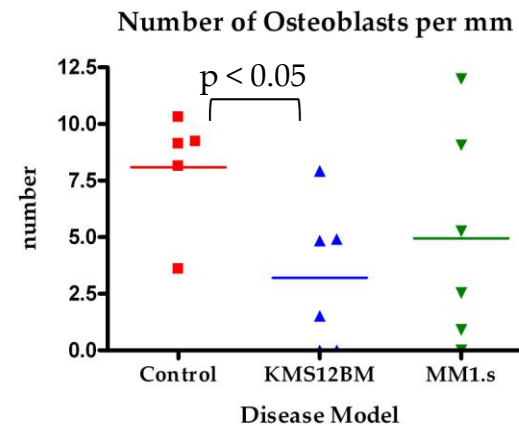
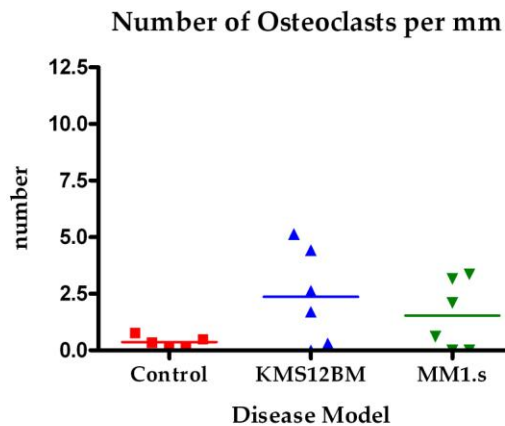
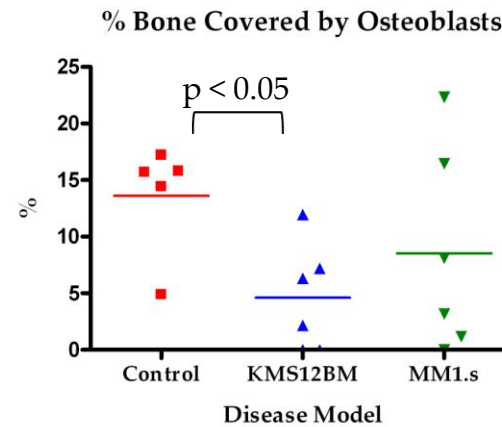
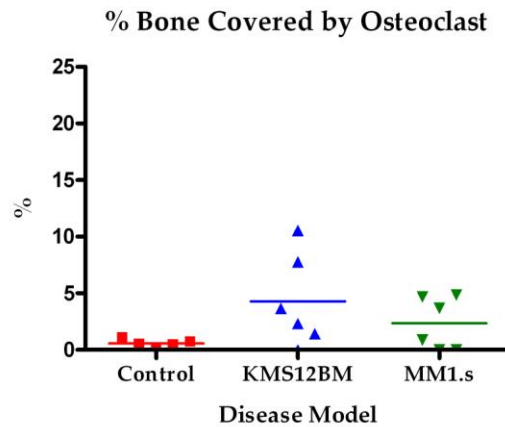
Trabeculae



Cortical



Quantification of osteoclasts and osteoblasts (static histomorphometry)



In Summary...

- In vivo models established with different genetic human myeloma cell lines display differences in patterns of bone loss and disturbances in bone metabolism which reflect the clinical disease

	t(11;14)	t(14;16)
Clinical bone disease	++	+/-
Xenogeneic model :	KMS12BM	MM1.s
Medullary engraftment	yes	yes
Extra medullary engraftment	no	minimal
Osteolytic lesions	yes	no
Perturbed bone biology	yes	yes

Future Work...

- Increase sample size
- Functional validation of osteoblast numbers
- Comparison of gene expression profiles of stromal cells and pre-osteoblasts at different stages of the disease

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