

# ***Therapy in osteosarcoma: Biological Rationale for Future Studies***

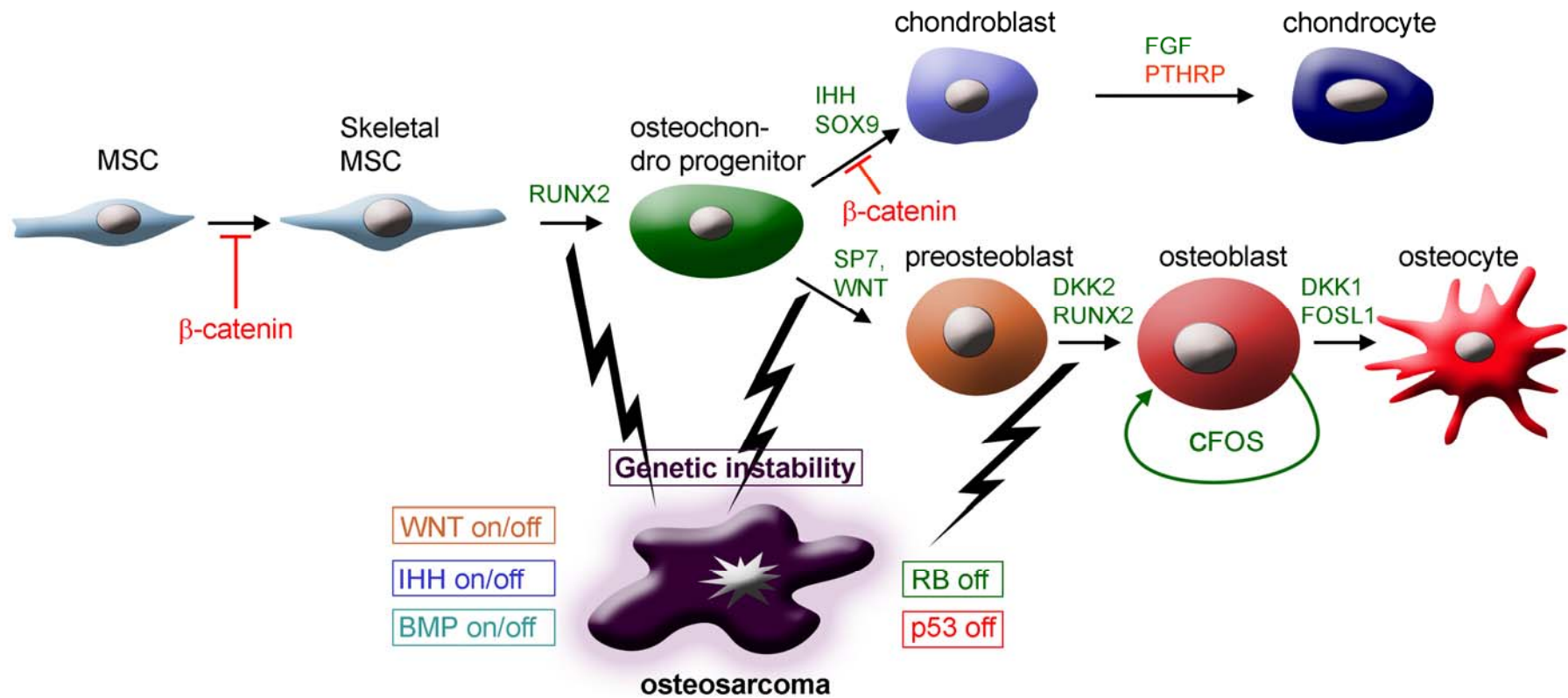
Results from the EuroBoNet consortium

Edited version

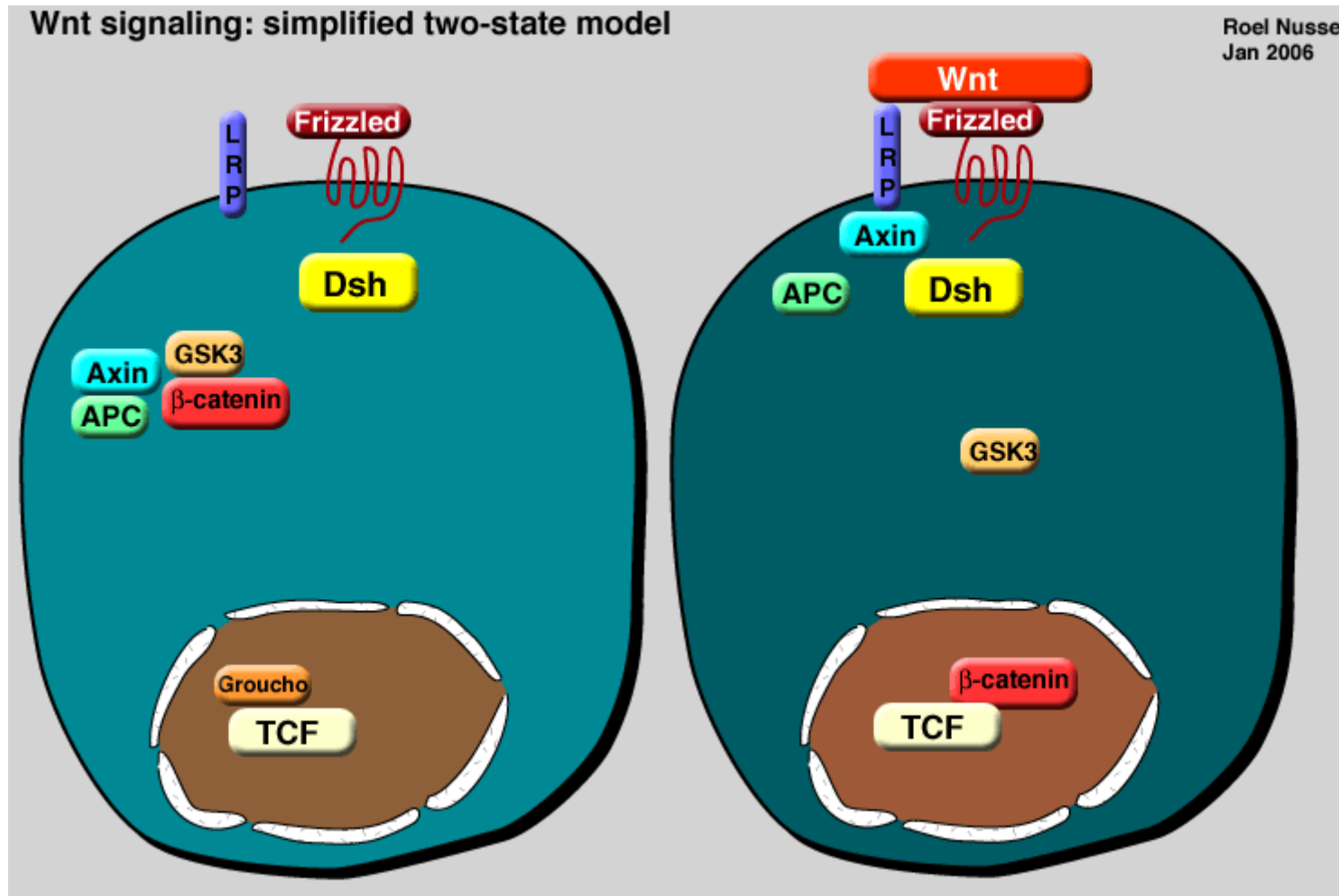


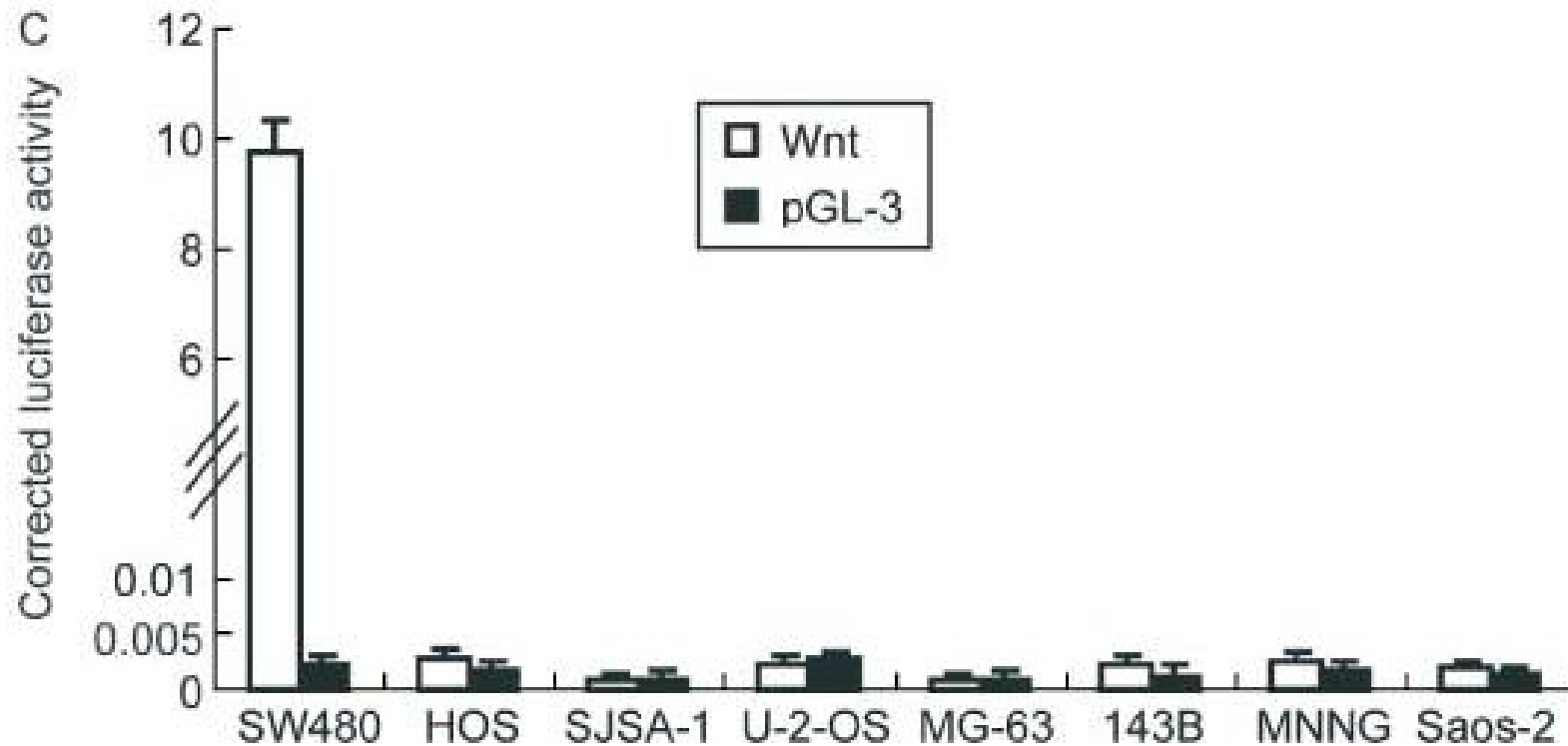


- Developmental pathway components: **Wnt/ $\beta$ -catenin** signaling
- Tyrosine kinases: **Ezrin**
- Immunsystem: **Macrophages**



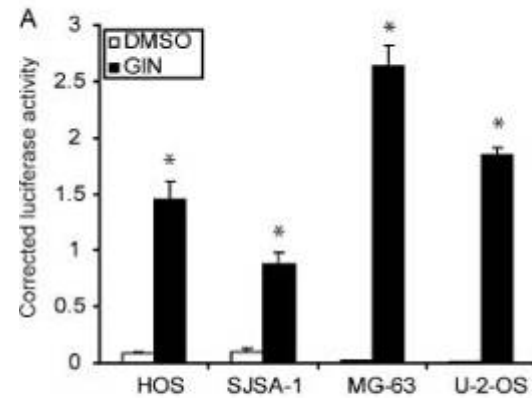
- **Colorectal e.o. tumors: aberrant upregulation**



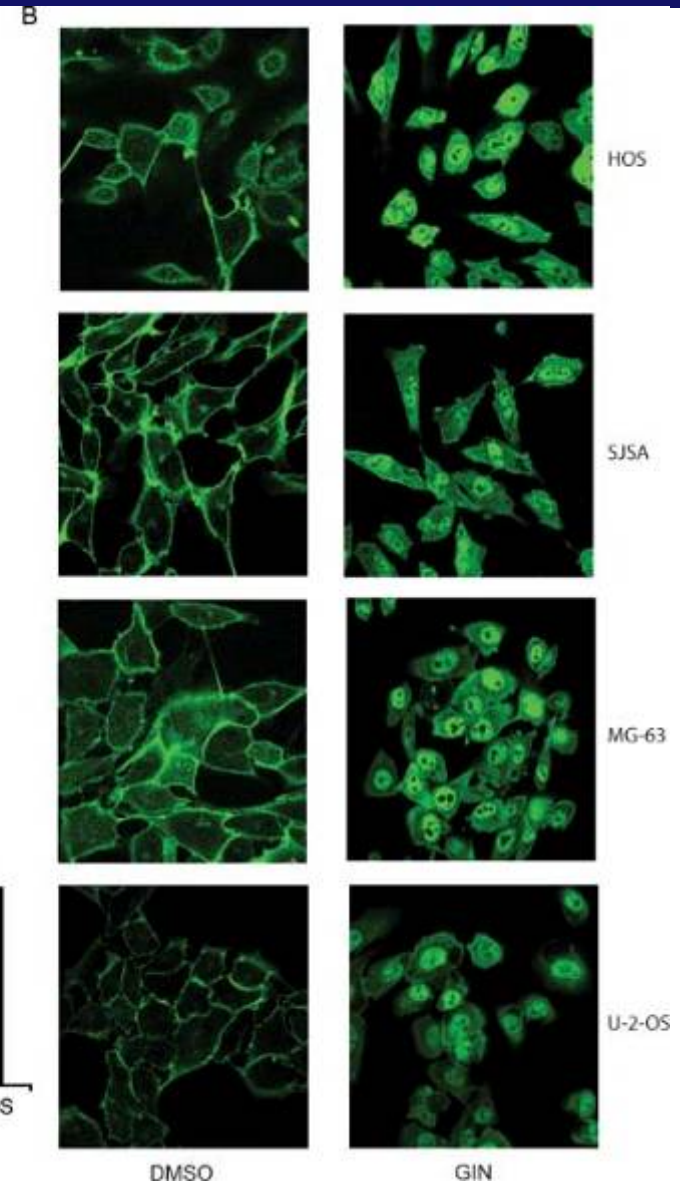
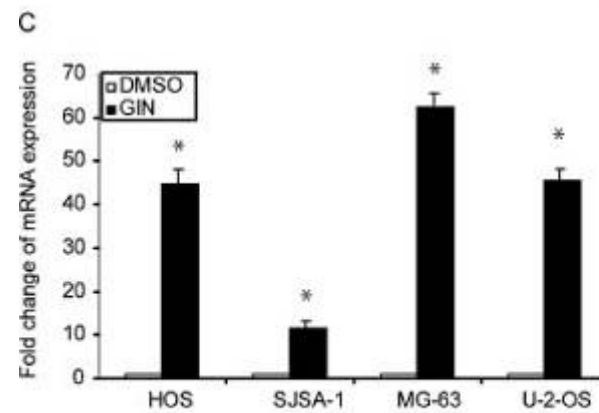


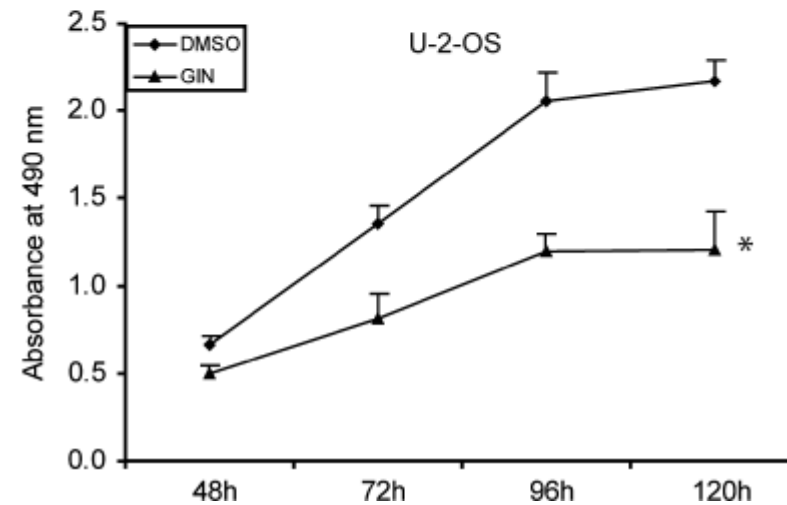
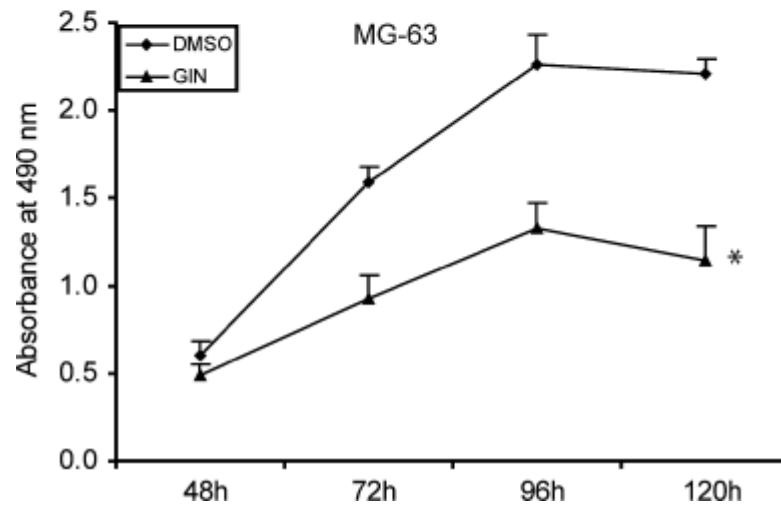
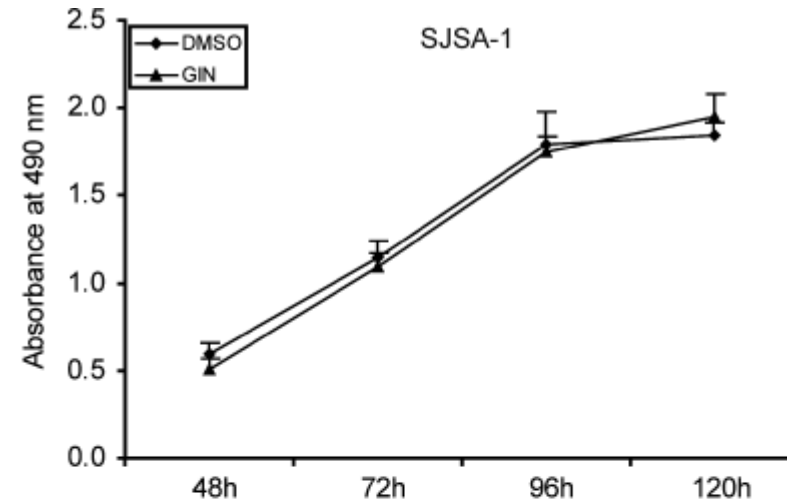
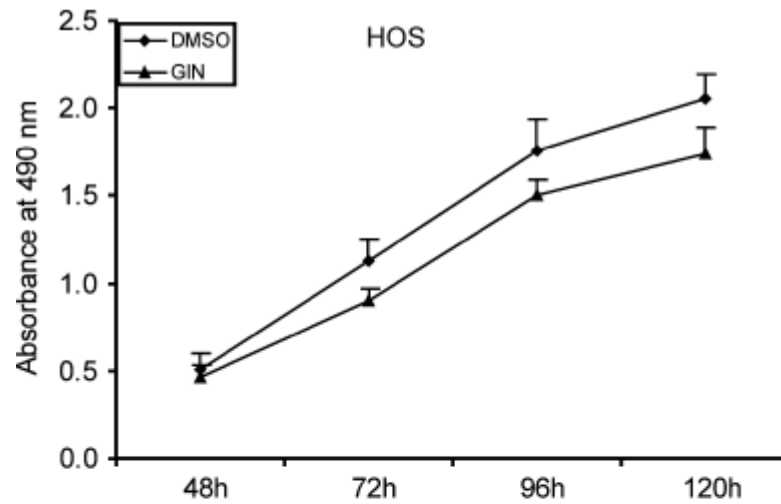
Cai et al., *J Pathol* 2010; **220**: 24–33

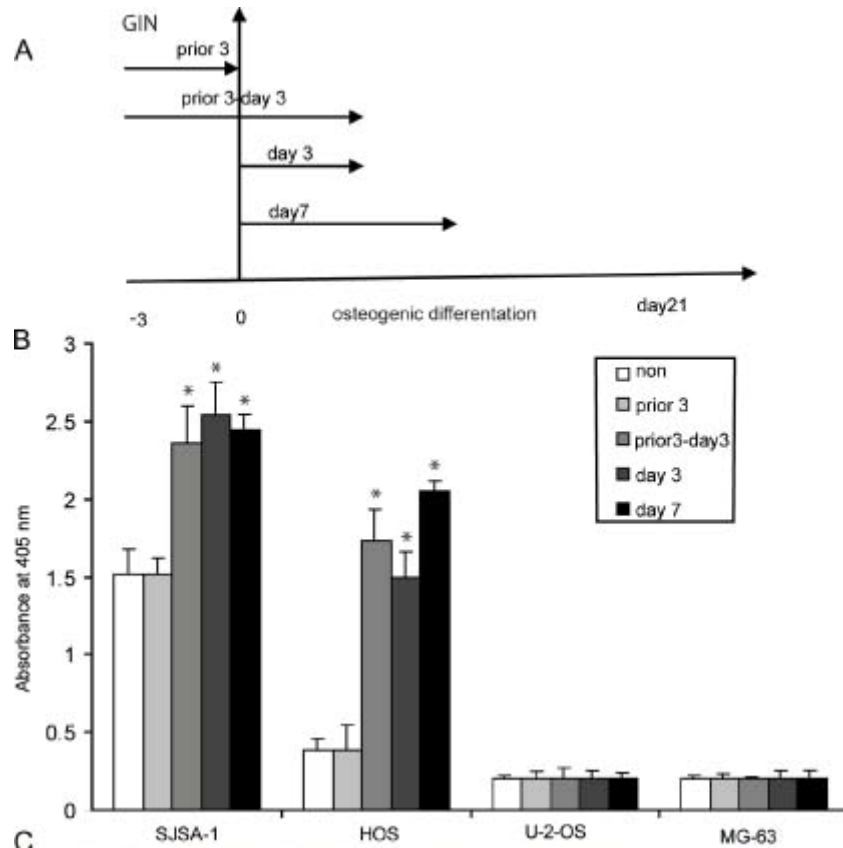
Luciferase activity



Axin2 mRNA expression

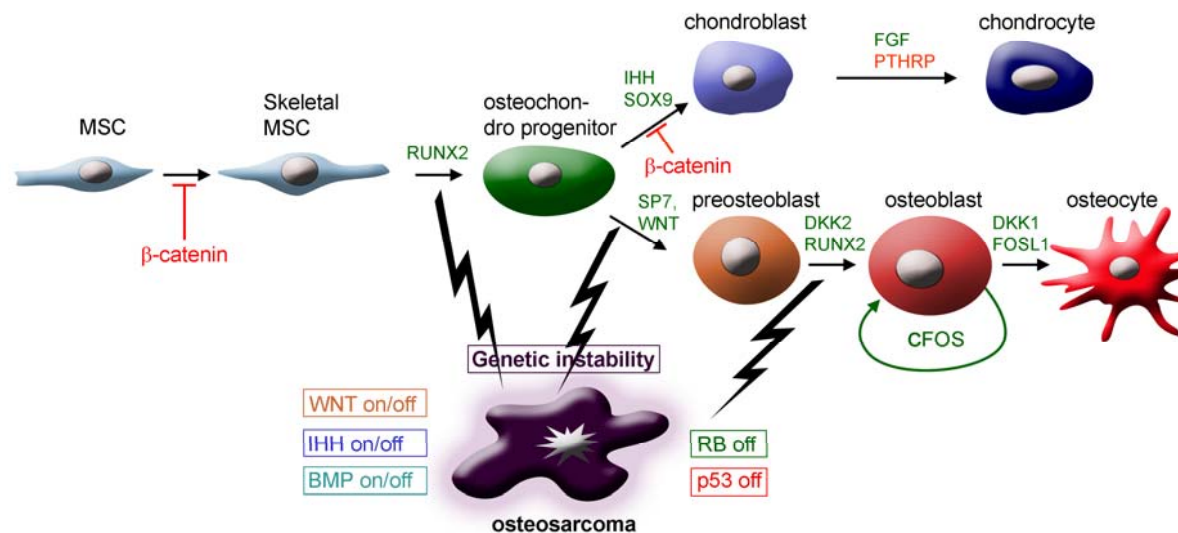


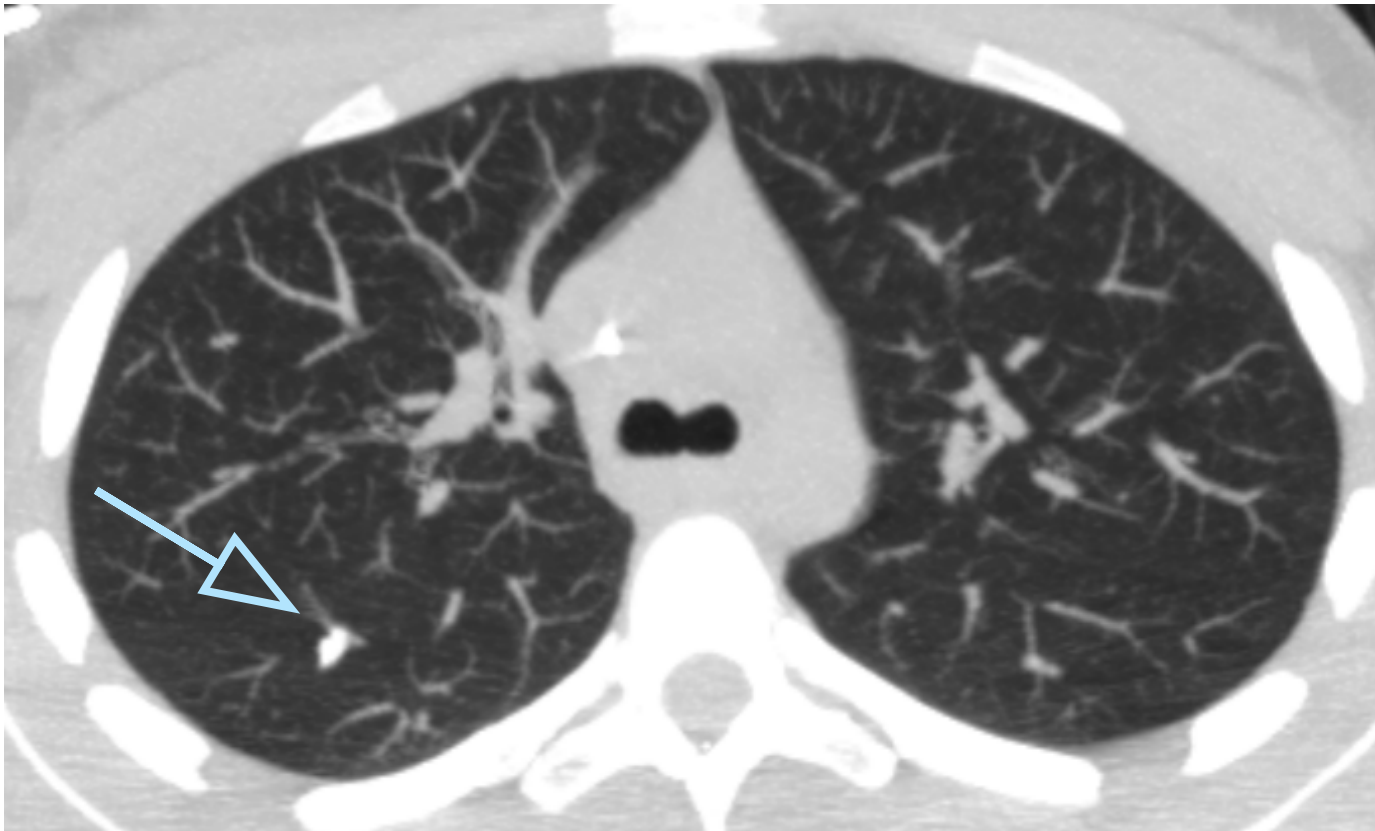




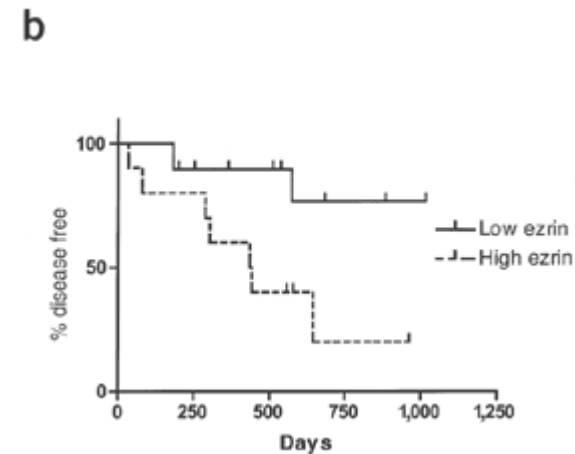
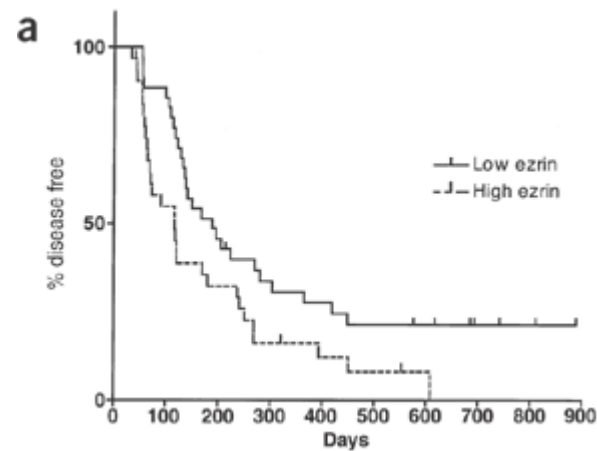
$\beta$ -glycerophosphate, dexamethasone, ascorbic acid

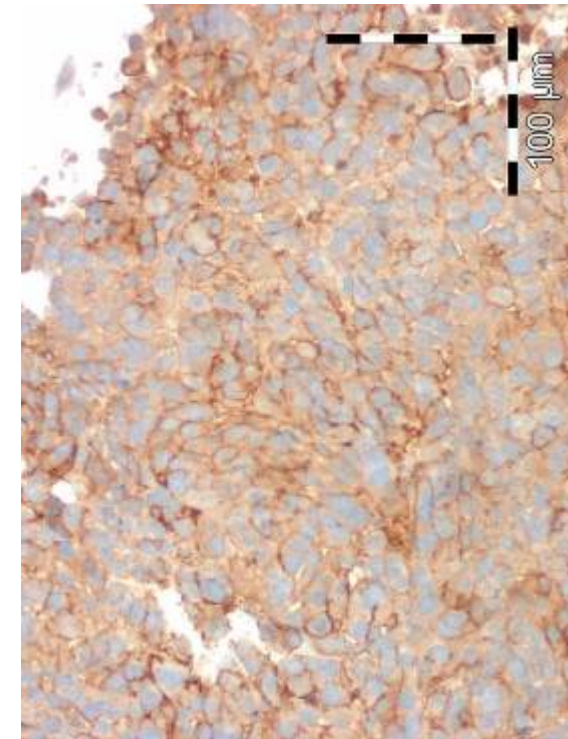
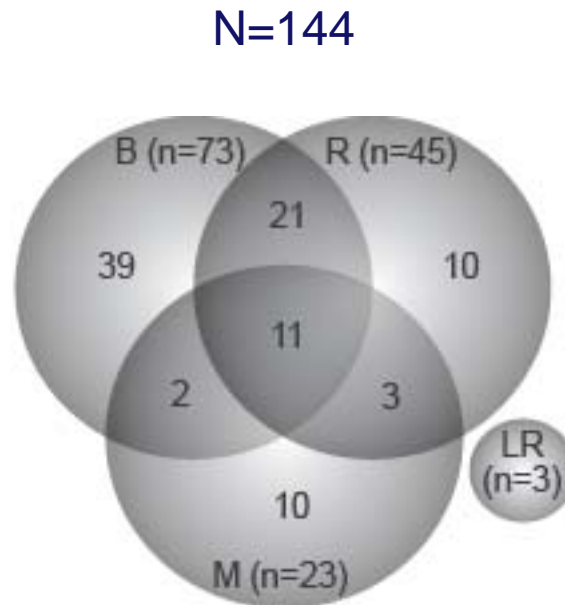
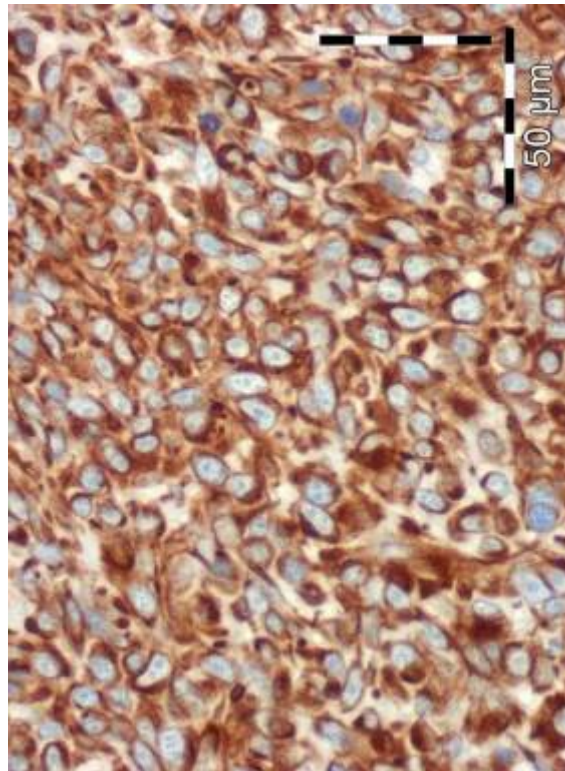
- **Canonical WNT is not constitutively active in osteosarcoma**
- **Activation of WNT can cause**
  - Inhibition of proliferation
  - Stimulation of differentiation
- **Stimulation of WNT may suppress osteosarcoma**





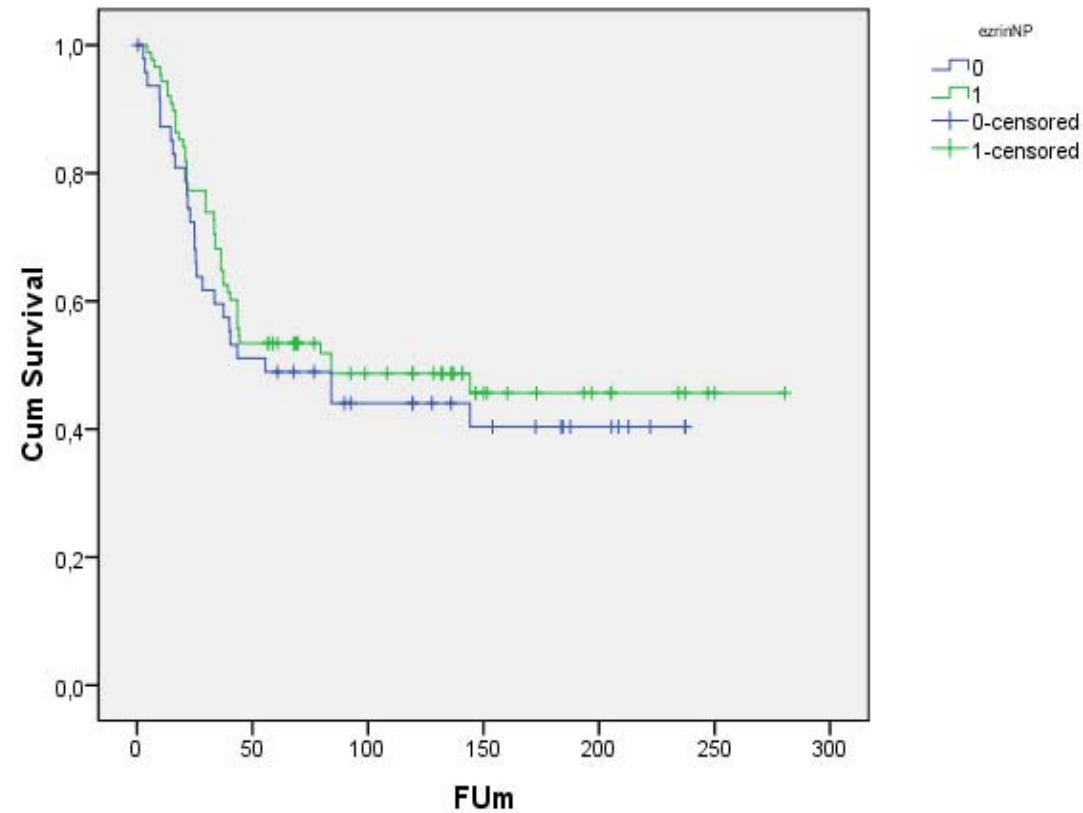
- Ezrin is a protein-tyrosine kinase substrate involved in adhesion, migration and cytoskeleton organisation
- Mouse model microarray study identified ezrin as mediator for metastasis in osteosarcoma (Khanna, 2001)
- Ezrin expression associated with poor survival in osteosarcoma clinical samples (n = 19) (Khanna, 2004)
- Small molecule inhibitors for ezrin (AACR 2009)





*Alex Mohseny, in preparation*

Survival Functions



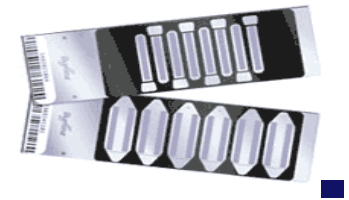
P = 0.7

- **Collection of osteosarcoma pré-CT biopsies from the EuroBoNet network of excellence**

- [www.EuroBoNet.eu](http://www.EuroBoNet.eu)

- **Selection of groups**

- Patients developing metastases within 5yrs (n=34)
  - Patients without metastases within 5yrs (n=19)



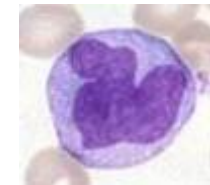
- **Genome wide expression profiling with Illumina Human-6 v2 BeadChips with 48,701 different probes**

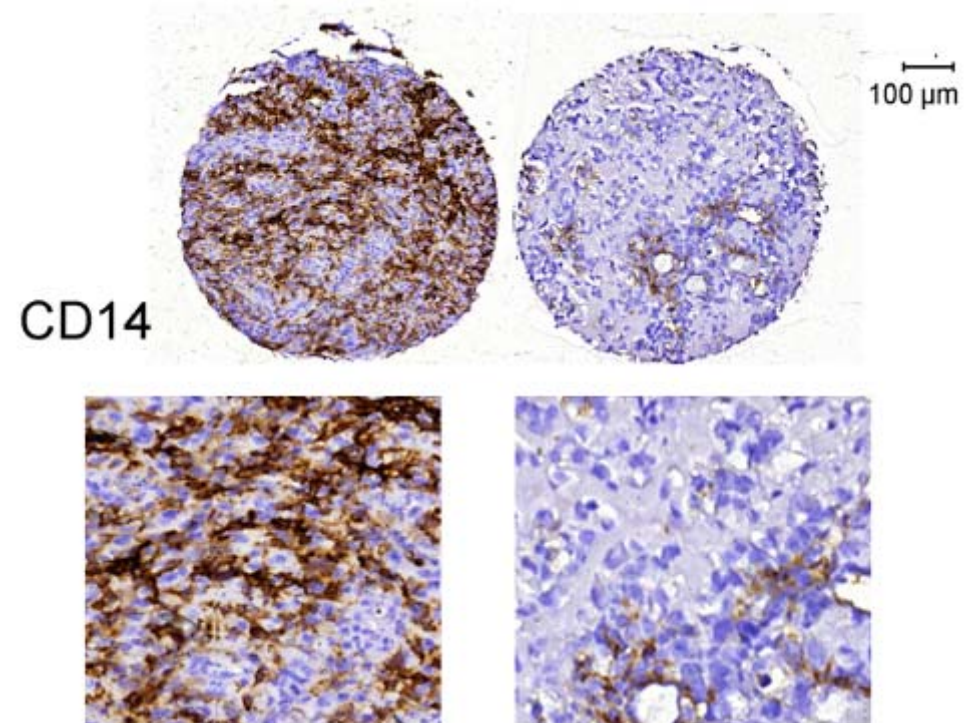
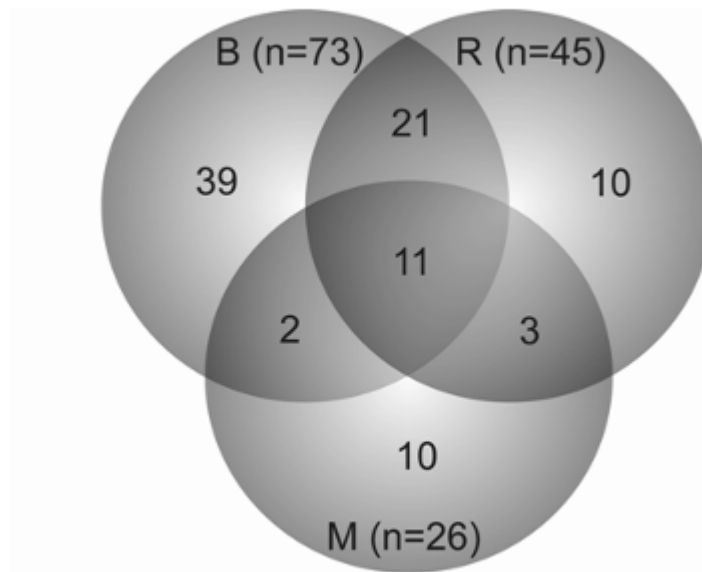
- **Detection of differentially expressed genes (DE)**

- Limma Smyth (2004) *Stat Appl Genet Mol Biol*
  - BH FDR-adjusted p-values  $<0.05$

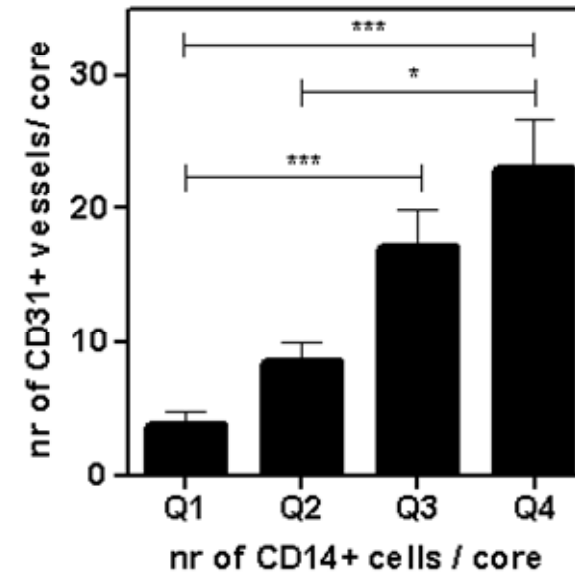
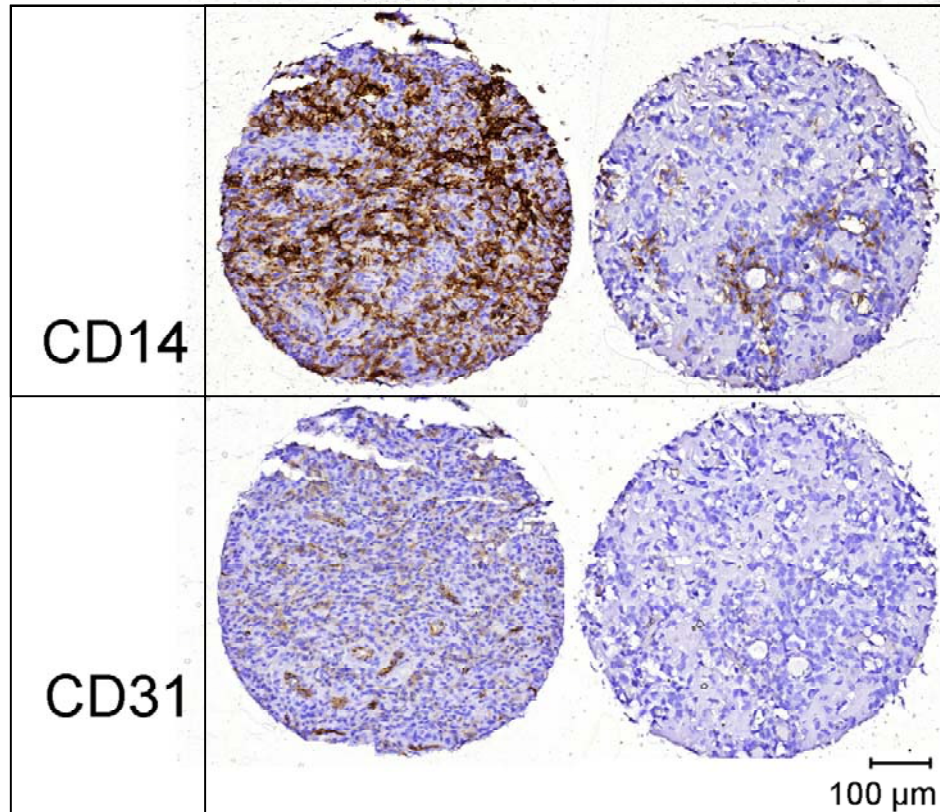


- **139 probes significantly DE**
- **High expression of macrophage-associated probes in patients who did not develop metastases within 5yrs**
  - ~50% of all significantly DE probes (possible) macrophage-association
  - E.g.:
    - *CD14 LPS receptor*
    - *CSF2RA colony stimulating factor 2 receptor*

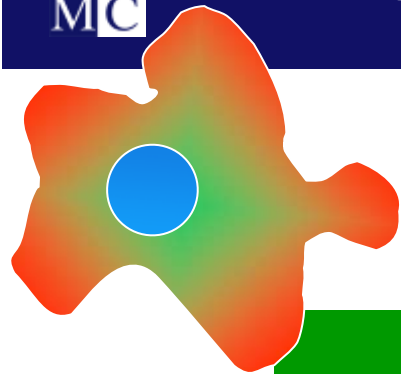




OS TMA with 145 samples from 88 patients



MoAbs Neomarkers



'anti-tumor'

"Classical activation"



'pro-tumor'

"Alternative activation"

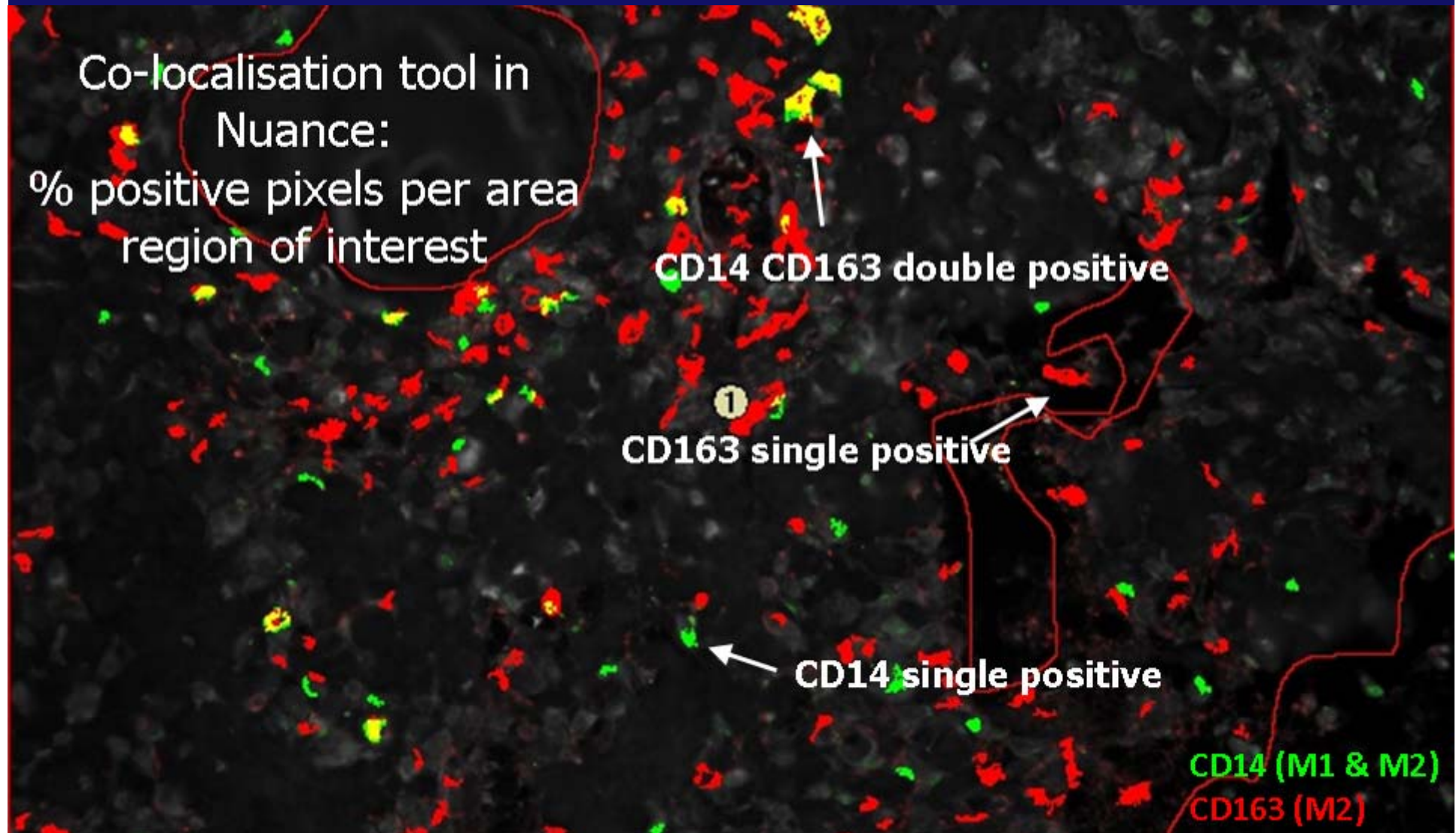
M1



M2

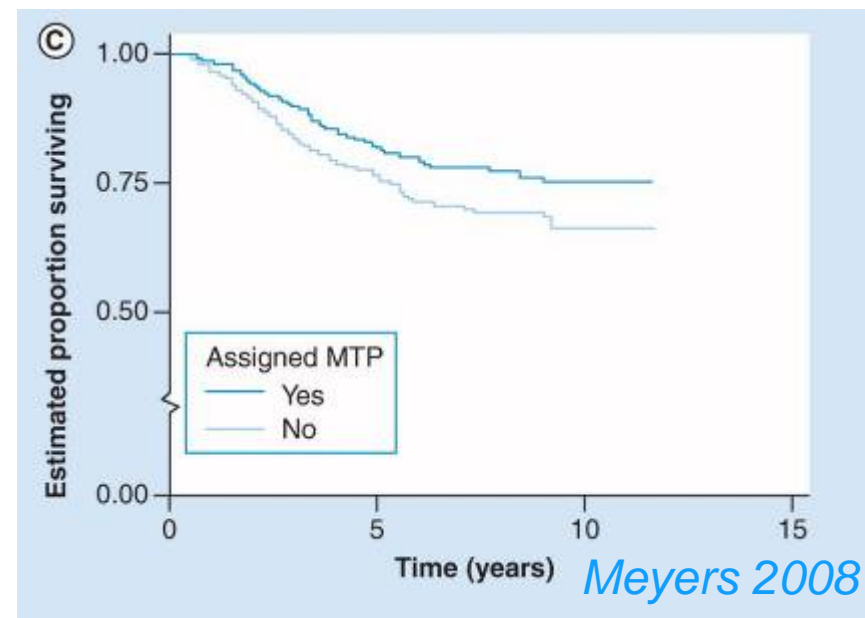
Higher expression of MHC molecules  
(incl HLA II)  
Pro-inflammatory cytokines: IL-10<sup>low</sup>/IL-12<sup>high</sup>, TNF- $\alpha$ , IL-6  
Reactive oxygen species  
Activation of adaptive immune system  
Expression of CCR7

Anti-inflammatory cytokines: IL-10<sup>high</sup>/IL-12<sup>low</sup>  
Pro-angiogenic VEGF, CXCL8, PDGF  
Expression of matrix metallo-proteinases  
Suppression of adaptive immune system  
Expression of scavenger receptors eg CD163  
MSR1

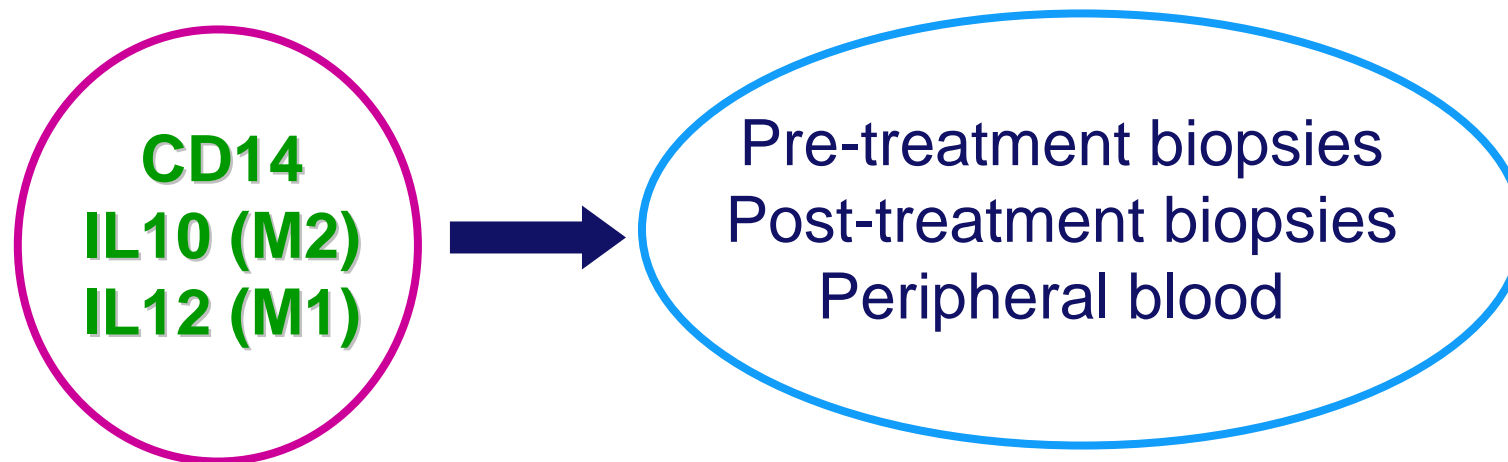


M1, antitumor, HLAII – M2, protumor, CD163

- **Liposomal Muramyl Tripeptide, L-MTP-PE, Mepact, Mifurmatide**
- **Activation of monocytes and macrophages**
- **Successful trial in xenografts and dogs**
- **Phase III trial improved survival from 70 to 78% in primary localized disease**



- Our findings that macrophages are associated with less metastases and better survival provide a rationale for applying MTP-PE as adjuvant treatment for osteosarcoma
- Monitoring efficacy of treatment:



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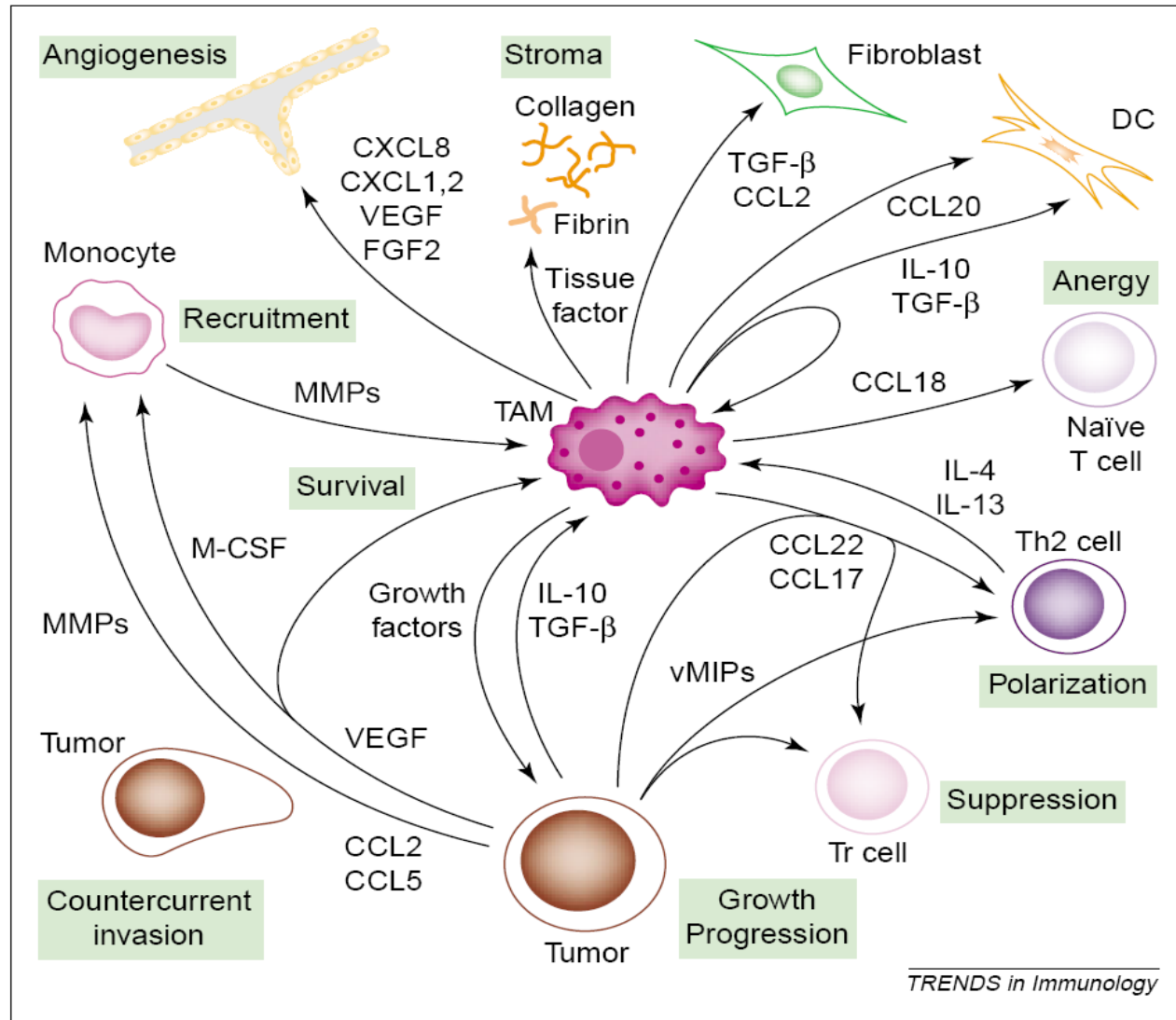
## EuroBoNet

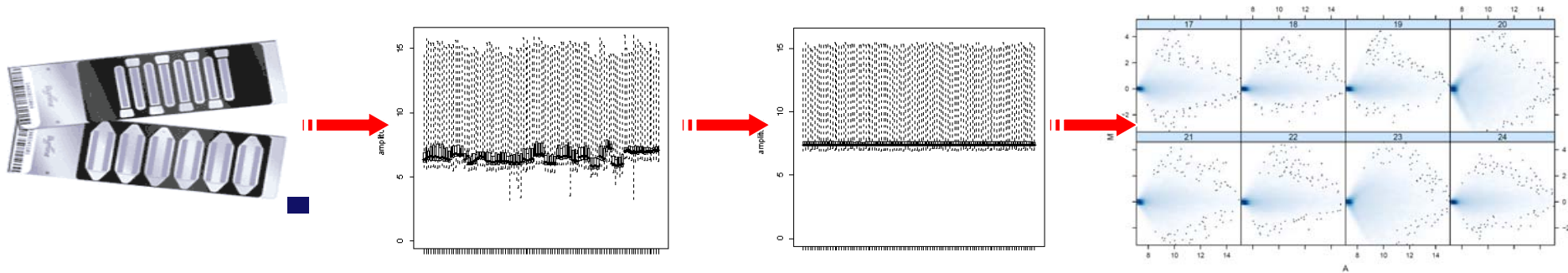
Esther Hauben, Leuven, Belgium  
 Eberhard Korsching, Münster, Germany  
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 Konstantin Agelopoulos, Münster, Germany  
 Ola Myklebost, Oslo, Norway  
 Fredrik Mertens, Lund, Sweden  
 Horst Bürger, Münster, Germany



"We came up with a pill that cures everything...but I'm warning you, it's gonna be expensive."

# Tumor associated macrophages

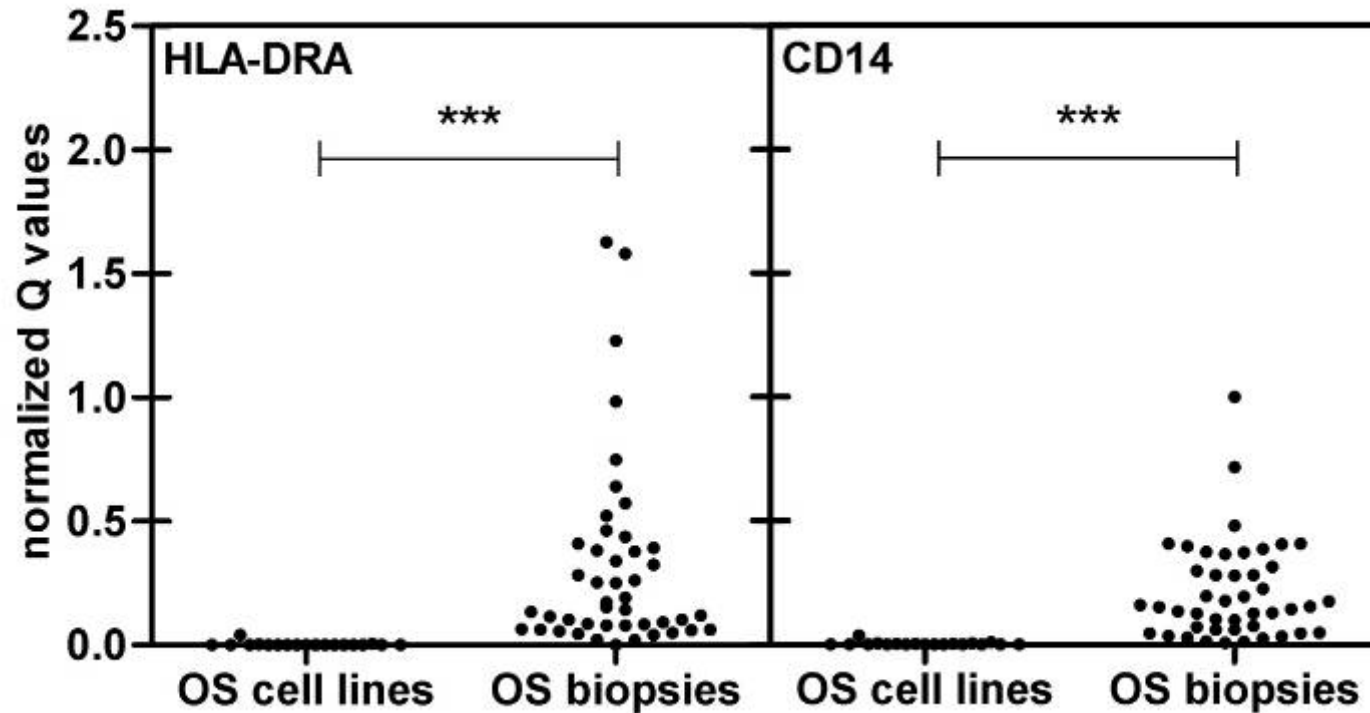




Illumina Human-6 v2  
BeadChips

Variance stabilizing  
transformation and robust  
spline normalisation

Removal of outlier arrays:  
arrayQualityMetrics



No expression of macrophage-associated genes  
*HLA-DRA* and *CD14* by 19 osteosarcoma cell lines