Confrontation cultures of embryonic stem cell-derived embryoid bodies and multicellular tumour spheroids:

a novel *in vitro* model for the study of tumour-induced angiogenesis

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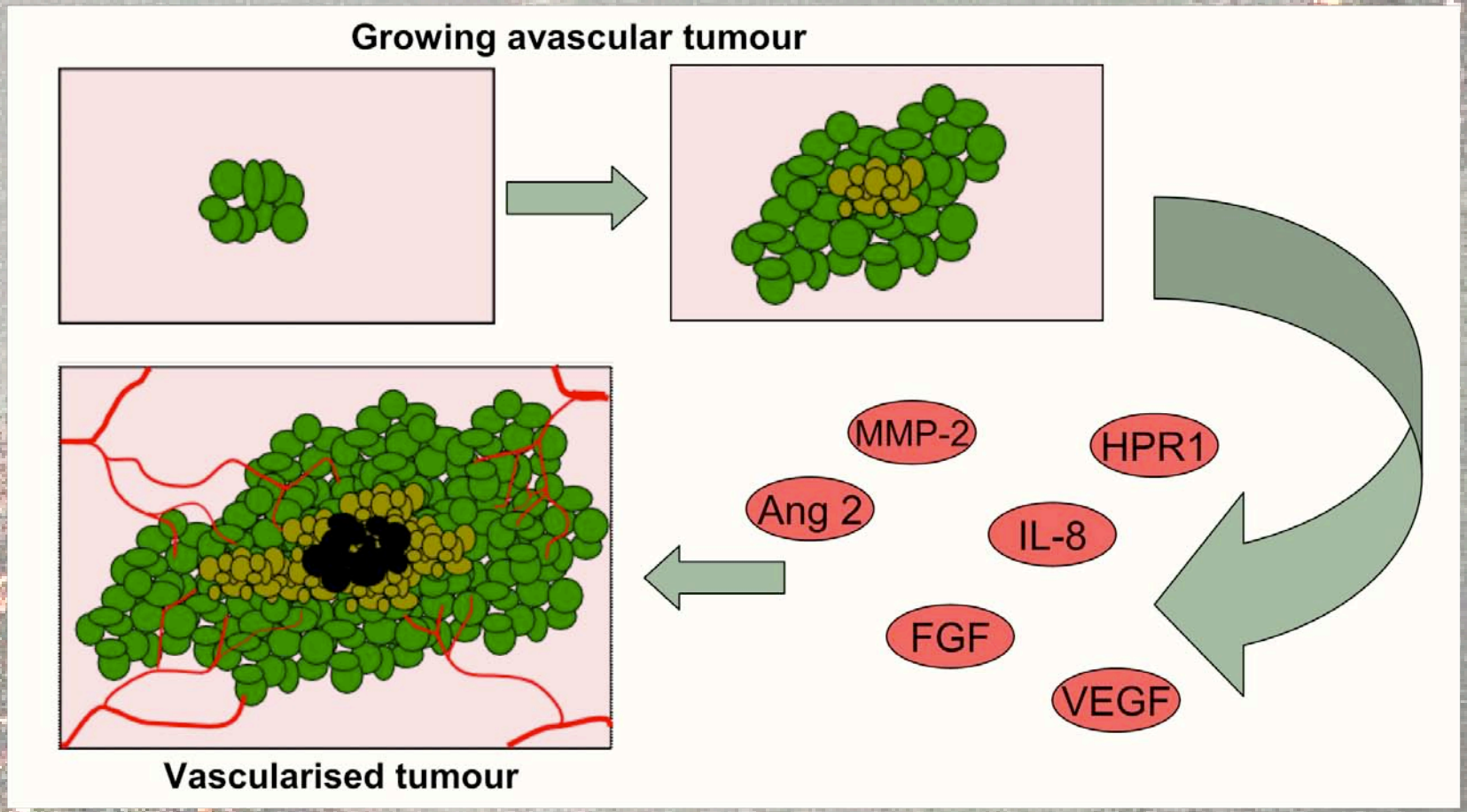
Introduction

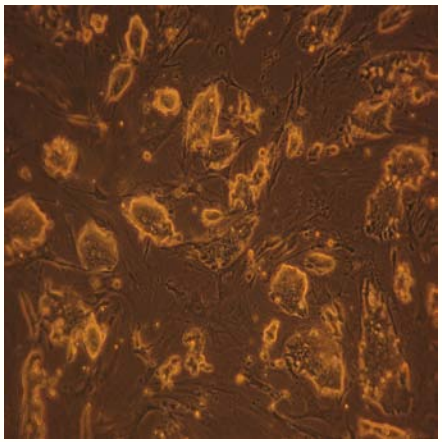
- Cancer is currently one of the leading causes of death in the modern world (22,8%)
- Vascularisation is a prerequisite for tumour growth and metastasis
- Anti-angiogenic therapy is one of the most promising strategies against cancer
- Large number of animals needed for pharmaceutical screenings

Tumour-induced angiogenesis *in vivo*

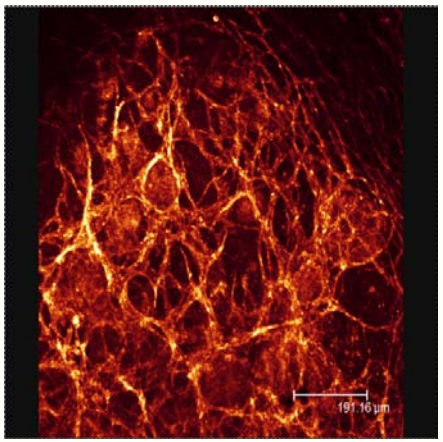


Stem cells are

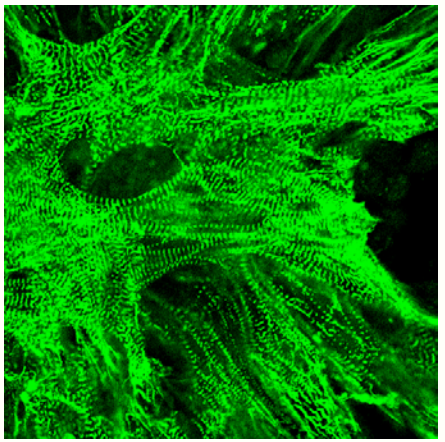
- self renewing *in vitro*
- Pluripotent (embryonic) or multipotent (adult)
- Exhibit and maintain a stable diploid karyotype



In vitro ES Cell culture



Vascular differentiation



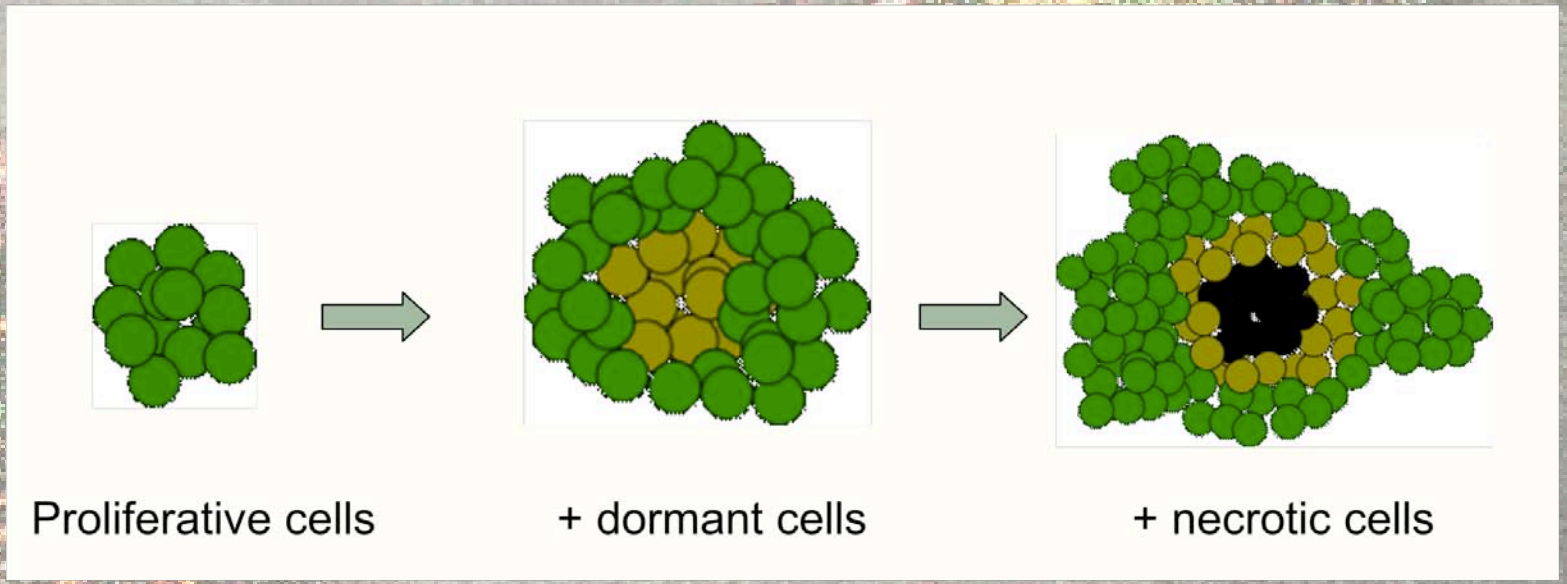
Cardiac differentiation

Differentiation of stem cells

- Stem cells are growing on inactivated embryonic fibroblasts till confluency
- Trypsinised stem cells are transferred into spinner flasks to form embryoid bodies (EBs)
- Four days old EBs were collected for experiments and treated for additional time needed for the experimental setup

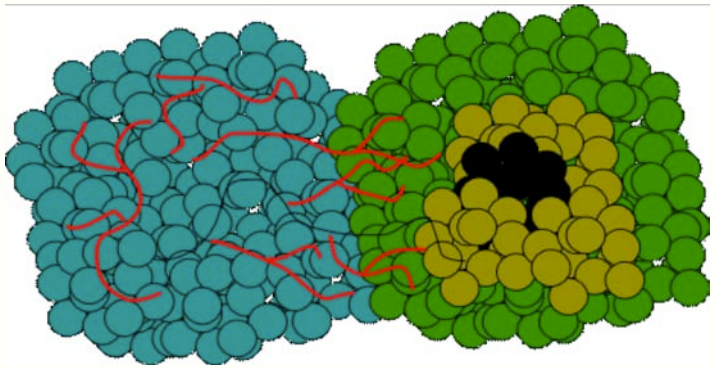
Tumour cells

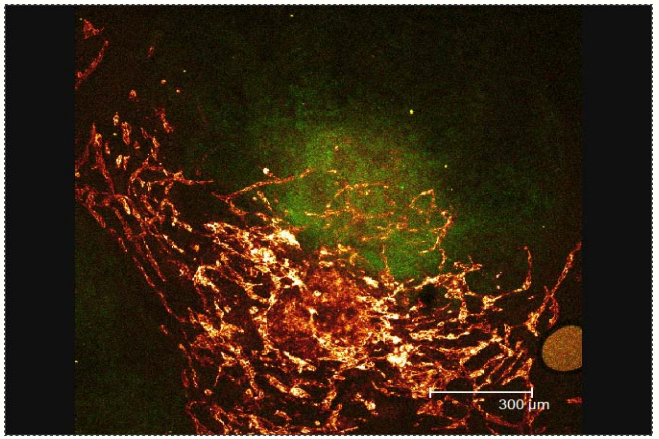
- Are self renewing
- Can be cultured as multicellular spheroids
- Behave similar to tumours *in vivo*



Our hypothesis:

- Co-cultures of embryonic stem cell-derived embryoid bodies and multicellular tumour spheroids

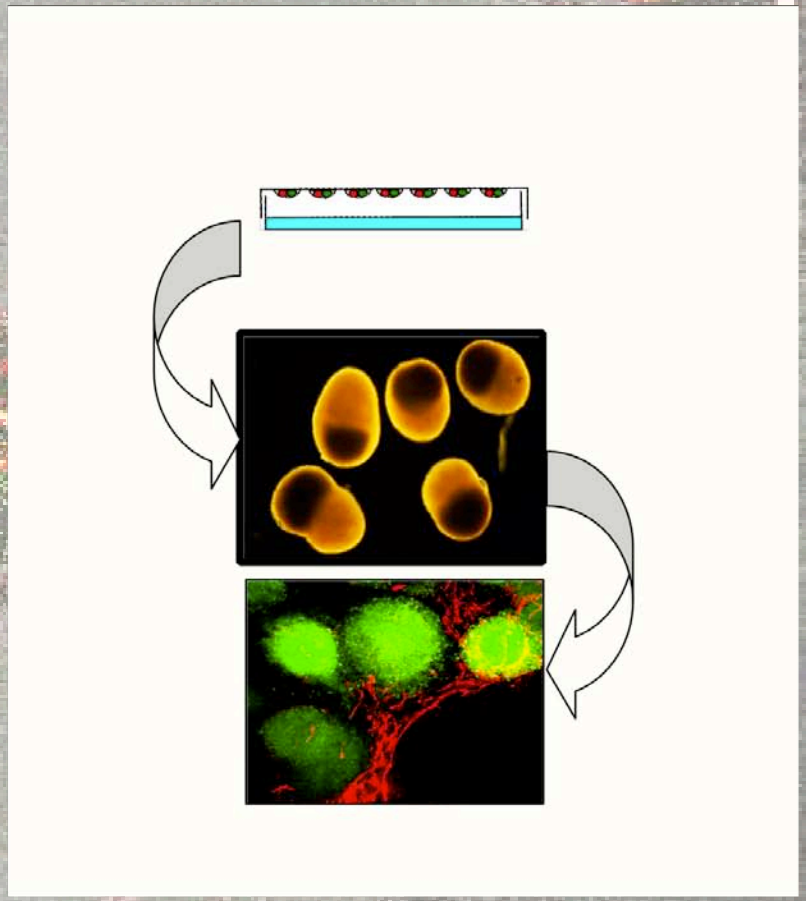




tumour-induced angiogenesis *in vitro*

The co-culture model

- EBs and tumour spheroids were brought in close contact by the hanging drop technique
- After 24h the two parts grew together and were plated on petriperm dishes for additional time needed
- Tumour-induced angiogenesis was analysed by CLSM, FACS and PCR

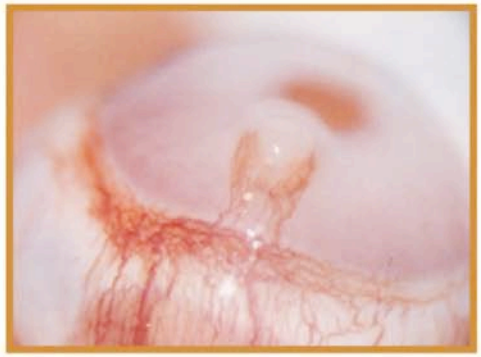


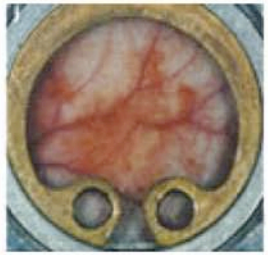
Possibilities of co-cultures

- Human tissue testing (hESC)
- Exclusion of additional inflammatory processes from the animal
- Exclusion of metabolic activity effects from the animal

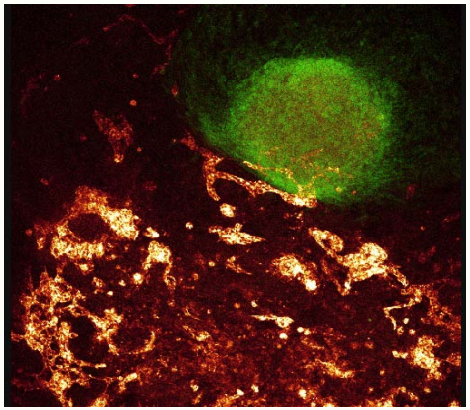
In vivo models that can be replaced by the co-culture model

1. Rabbit corneal assay
2. Hamster cheak pouch assay
3. Cranial window assay
4. Skin chamber assay

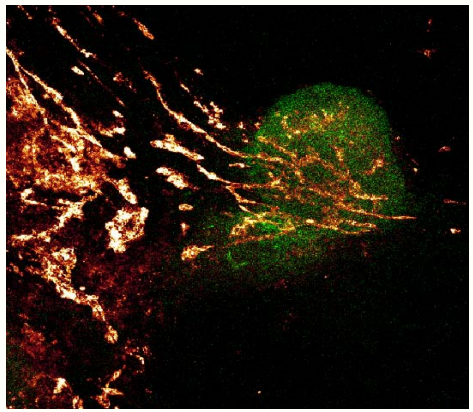




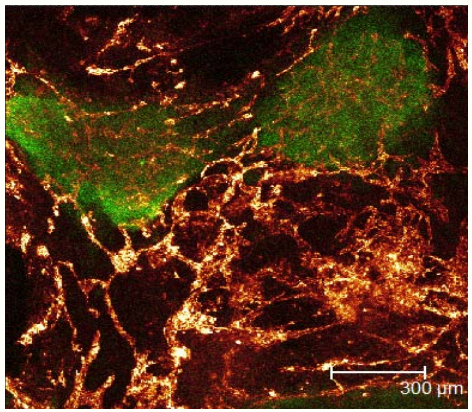
Newly differentiated endothelial cells invade the tumour tissue forming vessel-like structures



2 day old

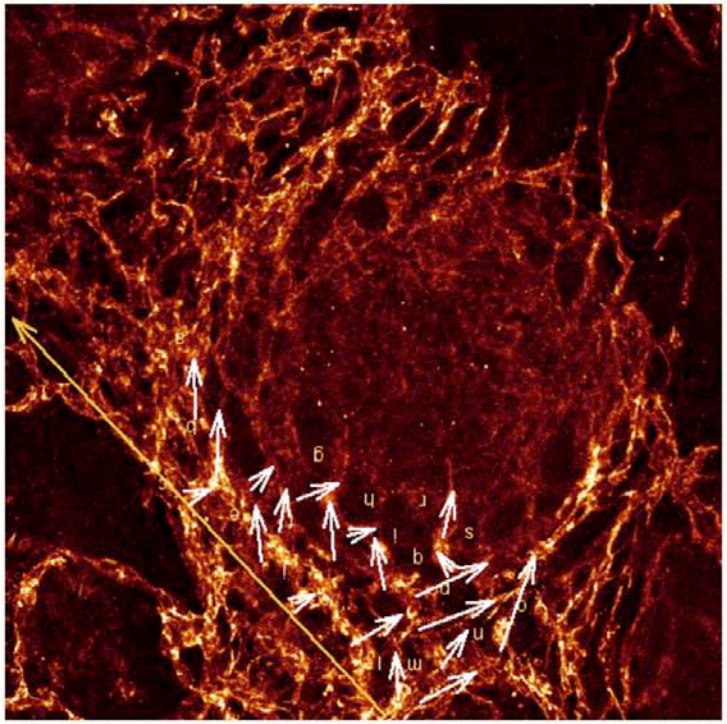


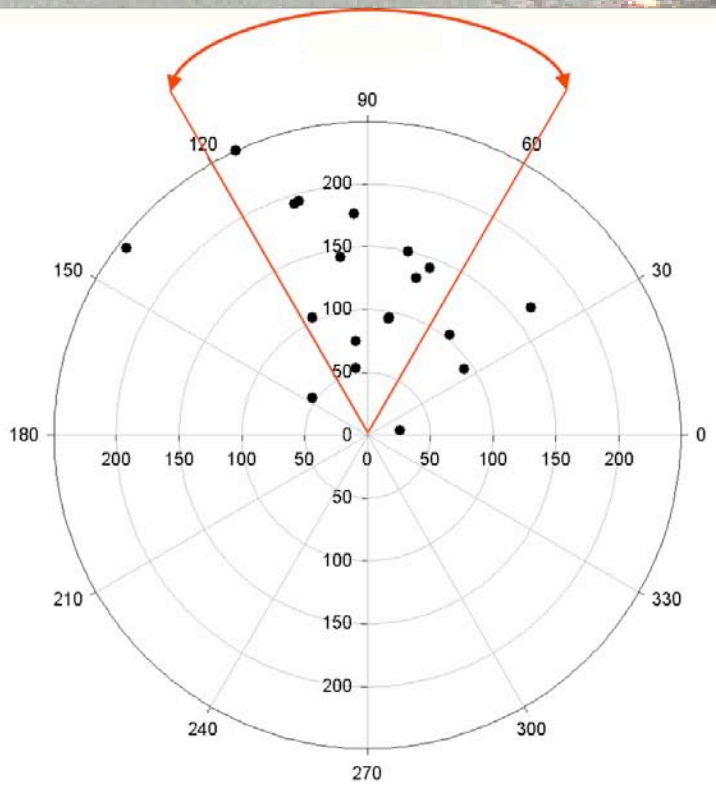
4 day old



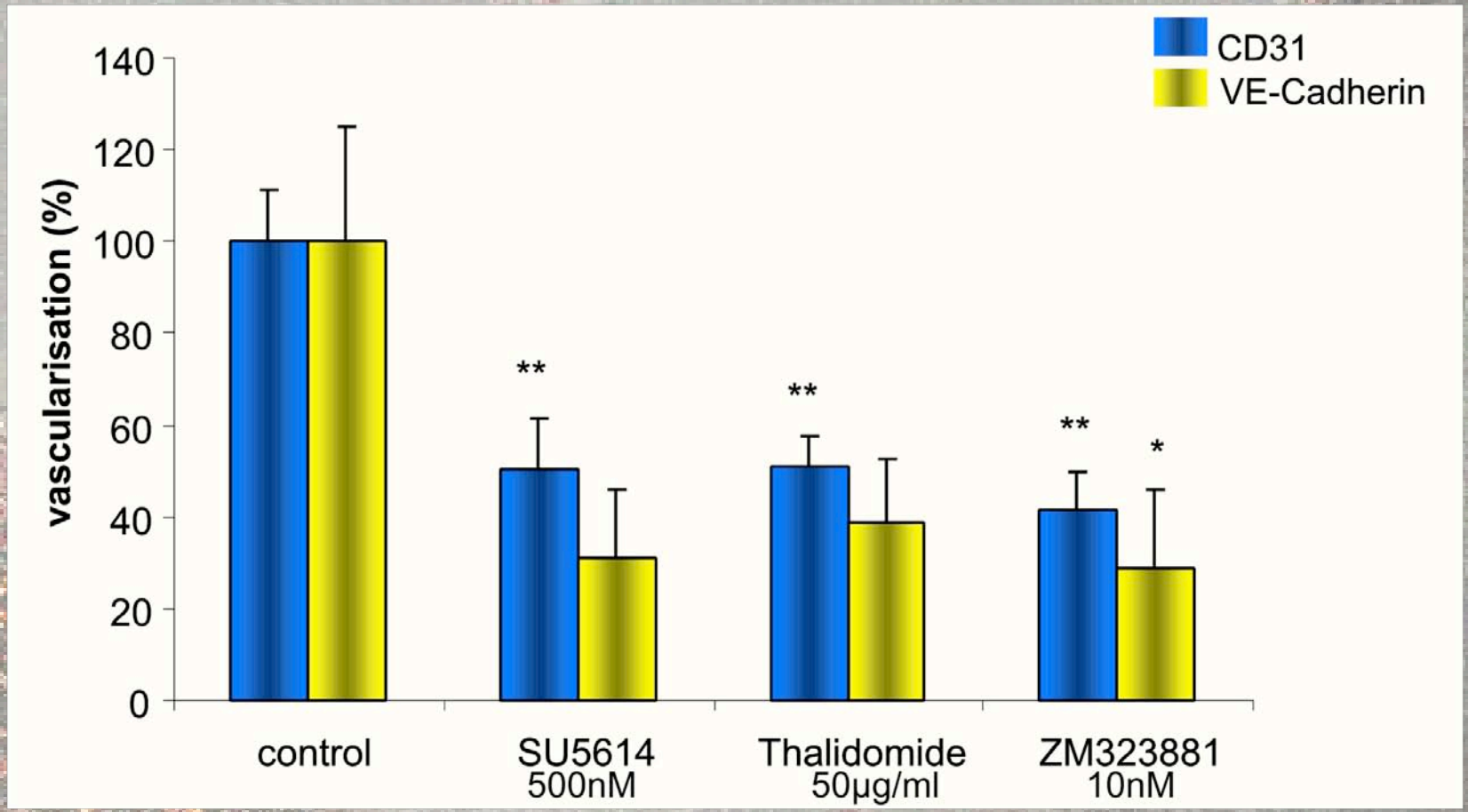
6 day old

Vessel-like structures show tumour-directed growth

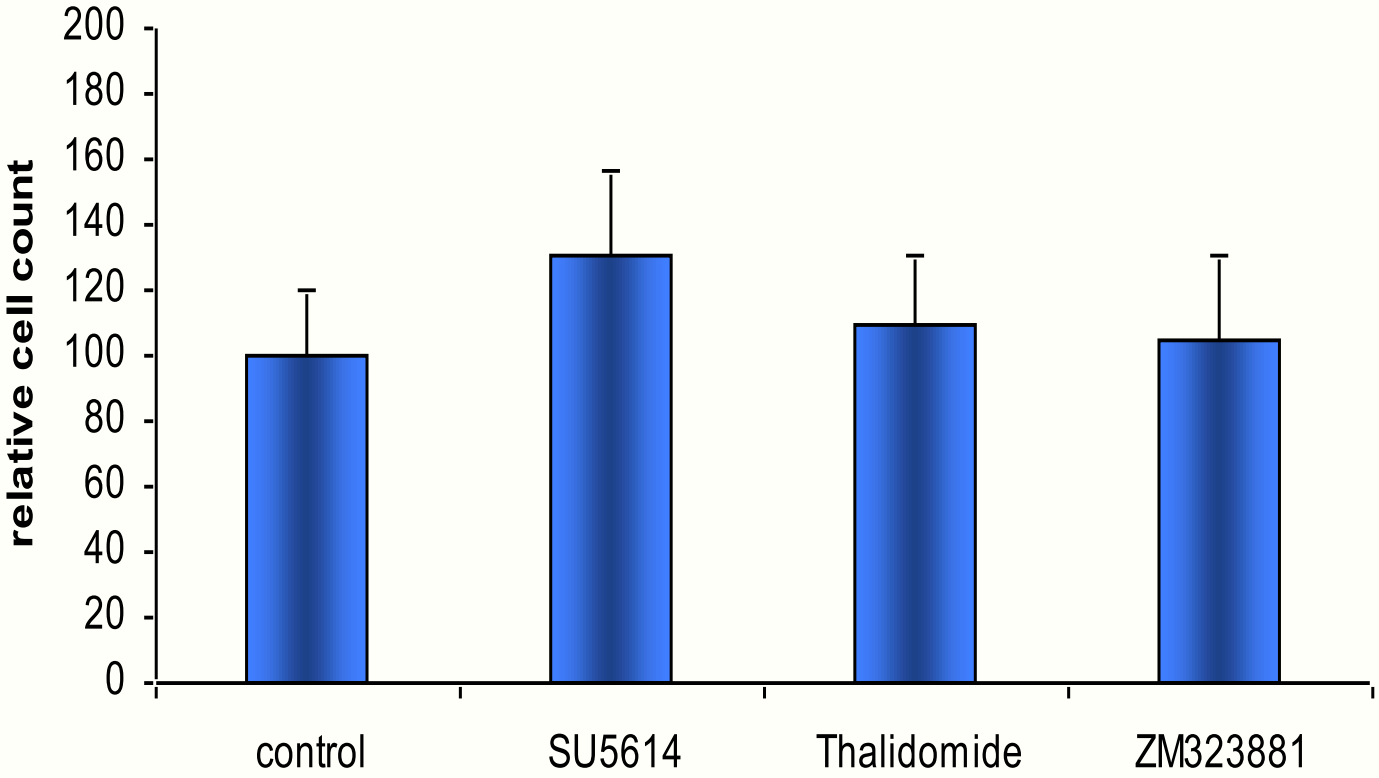




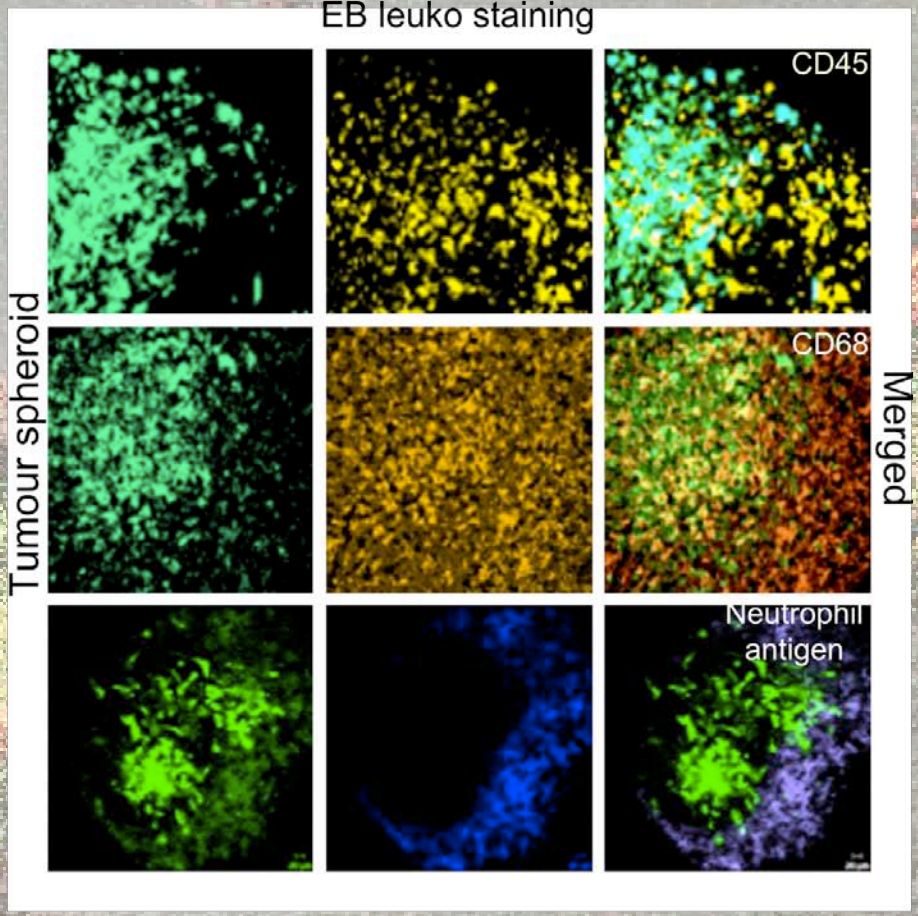
The anti-angiogenic agents inhibit the branching and sprouting of PECAM and VE-Cadherin positive structures



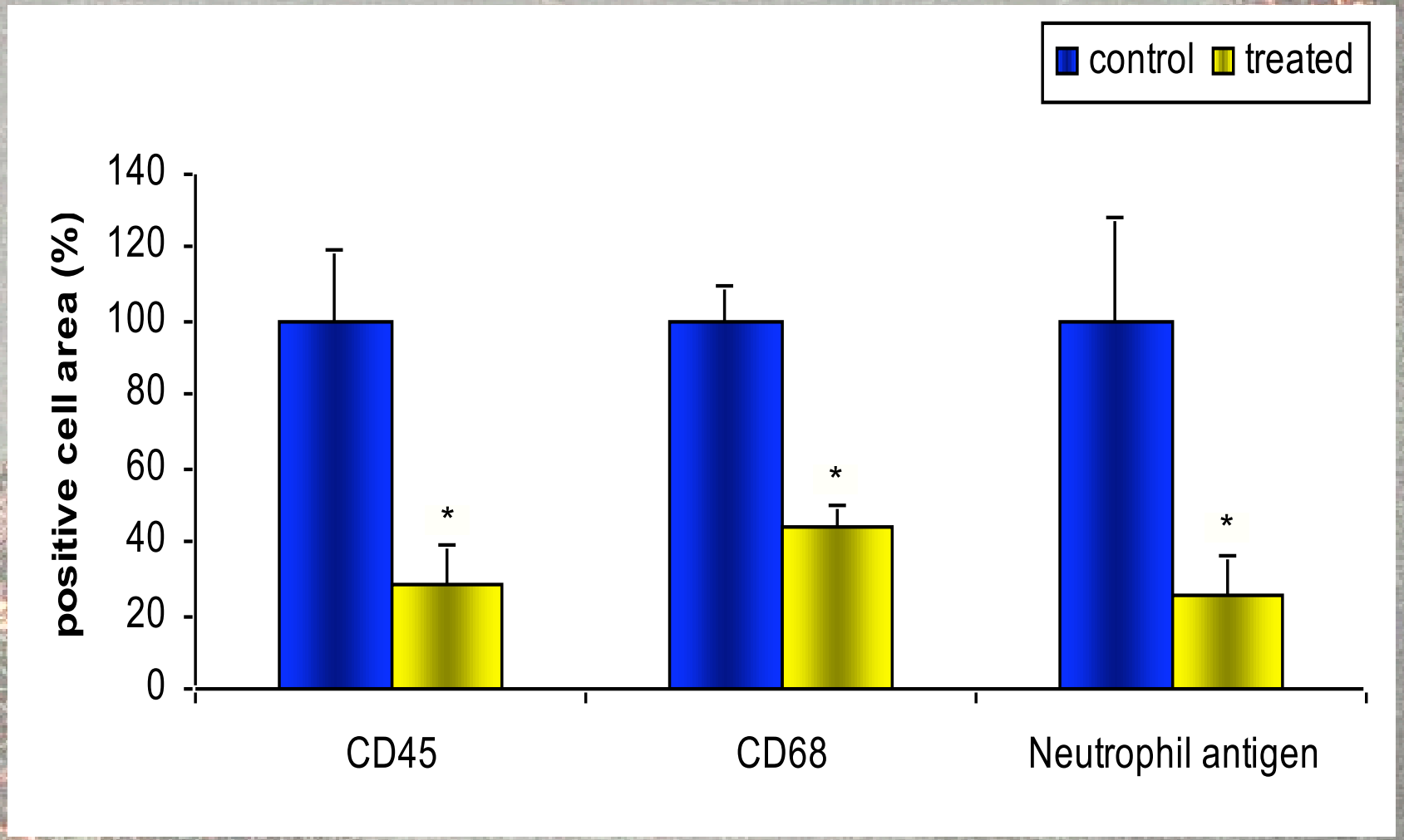
SU5614, Thalidomide and ZM323881 do not significally change the number of PECAM positive cells



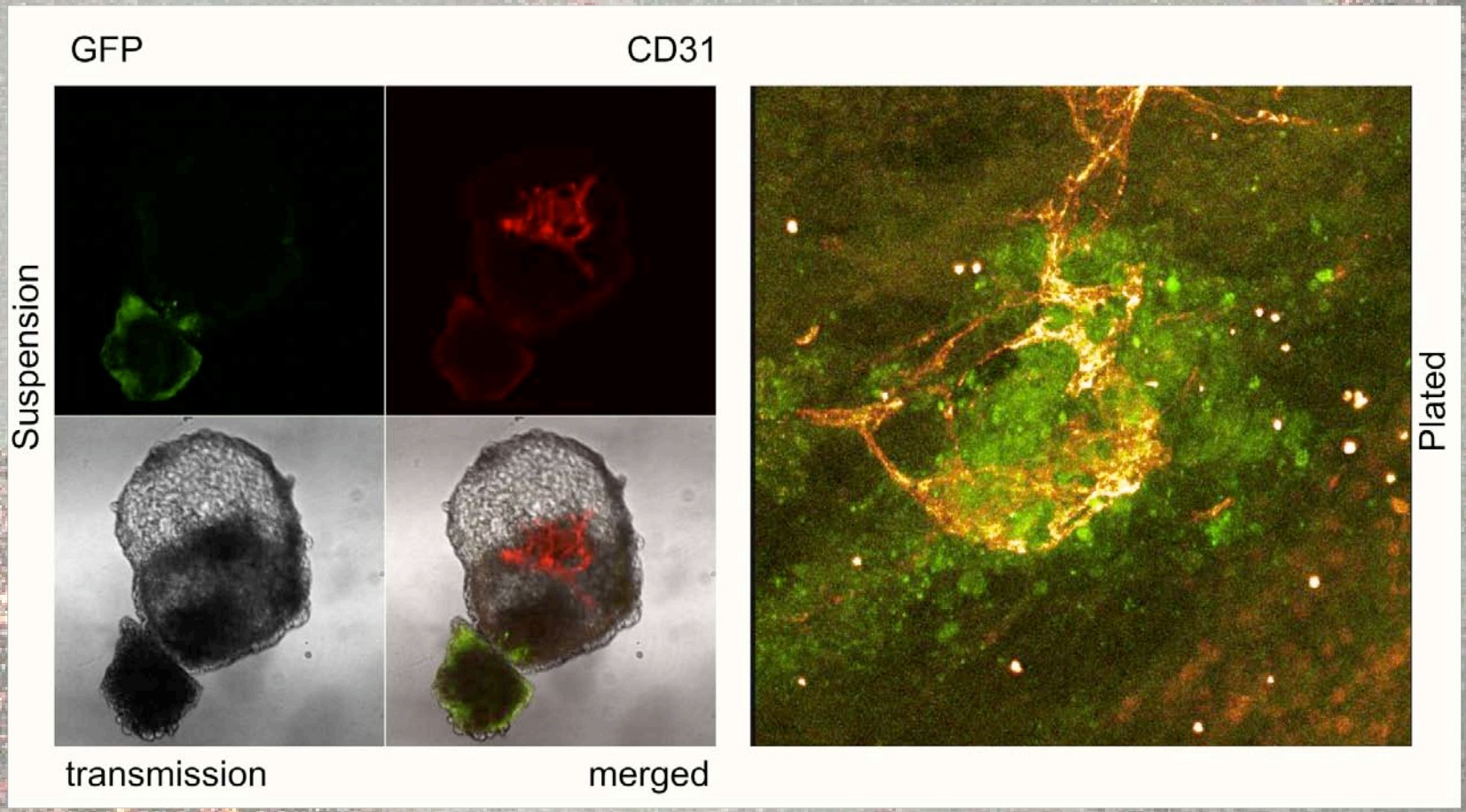
During co-culturing inflammatory cells invade the tumour spheroid creating a further pro-angiogenic micro-enviroment



The anti-angiogenic agent SU5416 inhibits leukocyte differentiation



Co-culturing of human ES cells derived embryoid bodies and tumour spheroids also result in the vascularisation of tumour tissue



Summary

- ✓ Interactions between EBs and tumour spheroids result in vascularisation and infiltration of the tumour tissue with inflammatory cells
- ✓ Tyrosine kinase inhibitors and thalidomide inhibit the formation and branching of developing vessels
- ✓ However they do not decrease the number of $CD31^+$ cells
- ✓ Tyrosine kinase inhibitors are reducing leukocyte invasion of the tumour tissue

Conclusion

Since they mimic host- tumour interactions, co-cultures have a great potential to reduce the number of animals needed for drug screening.

Outlook

- Further analysis of anti-angiogenic agents on human embryonic stem cell derived co-cultures
- Investigation of the role of axon guidance proteins in tumour induced angiogenesis

Thank You!

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