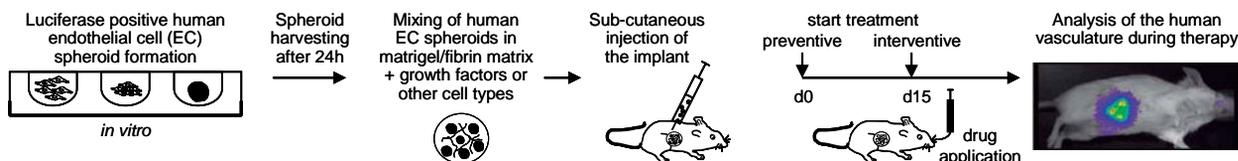


## Angiogenesis

Angiogenesis, the formation of new blood vessels from pre-existing ones, is a physiological process during growth and development. Beyond that angiogenesis is mandatory for tumor growth and is involved in other pathological disorders (e.g. psoriasis, macular degeneration). The complexity of the angiogenic cascade limits cellular approaches towards the study of angiogenic endothelial cells (EC). In turn, manipulatory *in vivo* angiogenesis assays are complex, multicellular and do not include human endothelial cells. The spheroid-Based *in vivo* Angiogenesis Assay takes advantage of human EC spheroids which form the basis for the development of a functional human vasculature in mice.

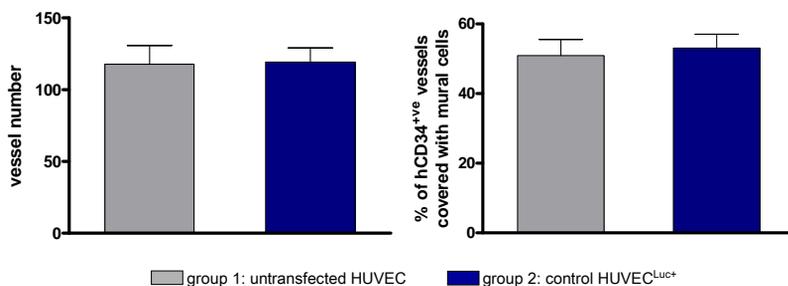
## Assay procedure



**Figure 1:** Assay procedure. Primary human endothelial cells are transduced by a lentivirus encoding a luciferase-neomycin fusion protein (HUVEC-LLN). Spheroids formed by HUVEC-LLN are mixed in a matrigel/fibrin matrix which is subcutaneously injected in SCID mice. The matrix mixture contains VEGF-A/FGF-2 or fibroblasts/smooth muscle cells (SMC) for stimulation of angiogenesis. The treatment may be started directly after implant injection (preventive study) or after a mature human vasculature has been established (interventive study). The growing human vasculature is monitored by bioluminescence imaging during therapy. Finally, the matrix plug is removed and the luciferase signal is analysed *ex vivo*. Optional, a part of the plug is analyzed for human microvessel density, pericyte-coverage and/or perfusion.

## Assay features

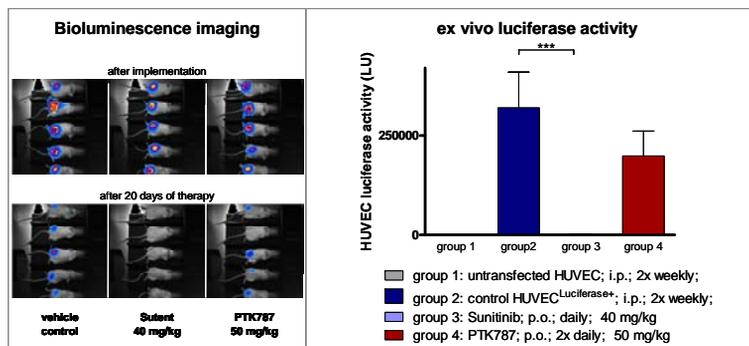
**Figure 2:** Assay features. Human EC form a human vasculature that anastomoses with the mouse vasculature upon stimulation with VEGF-A/FGF-2 or addition of fibroblasts/SMCs. The first perfused vessels are detectable at day 4 to day 6. After 20 days of *in vivo* growth a well established vasculature with around 40 - 60 % pericyte-covered and perfused vessels is established [1]. HUVEC-LLN form a human vasculature with no detectable difference to untransfected cells when analysing vessel number and coverage



[1] Alajati, A et al.(2008) Nat Methods. **5**(5): 439-45

## Study example

**Figure 3:** Study example. Sunitinib and PTK787 were tested in the *in vivo* Angiogenesis Assay using HUVEC-LLN. Bioluminescence imaging of the mice was performed during therapy. After implementation the mice showed similar luciferase signal which was strongly reduced in the Sunitinib treated group after 20 days of treatment (left). After removing the plug the analysis of the *ex vivo* luciferase activity demonstrates a comparable result (right). The new technique allows monitoring the effect of the compound during therapy and a rapid and most objective analysis after plug removal.



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