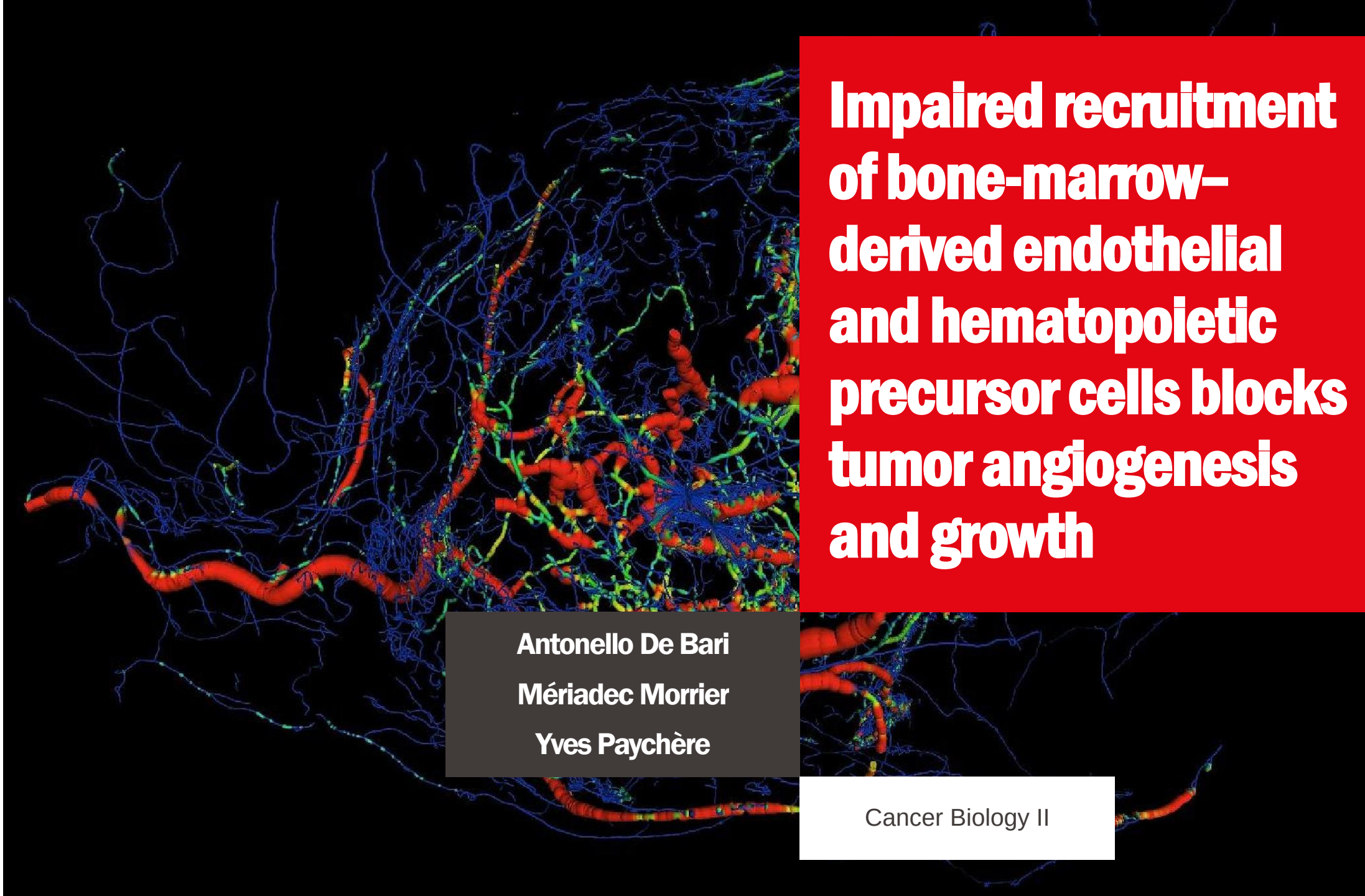

# Impaired recruitment of bone-marrow–derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth

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# Introduction and Methods

# Mouse models: ID mutant, rosa-26

- Id1+/– Id3–/– double-mutant mouse embryos display vascular malformations
- Adult mice with reduced Id gene dosages cannot support neo-angiogenesis when challenged with tumor

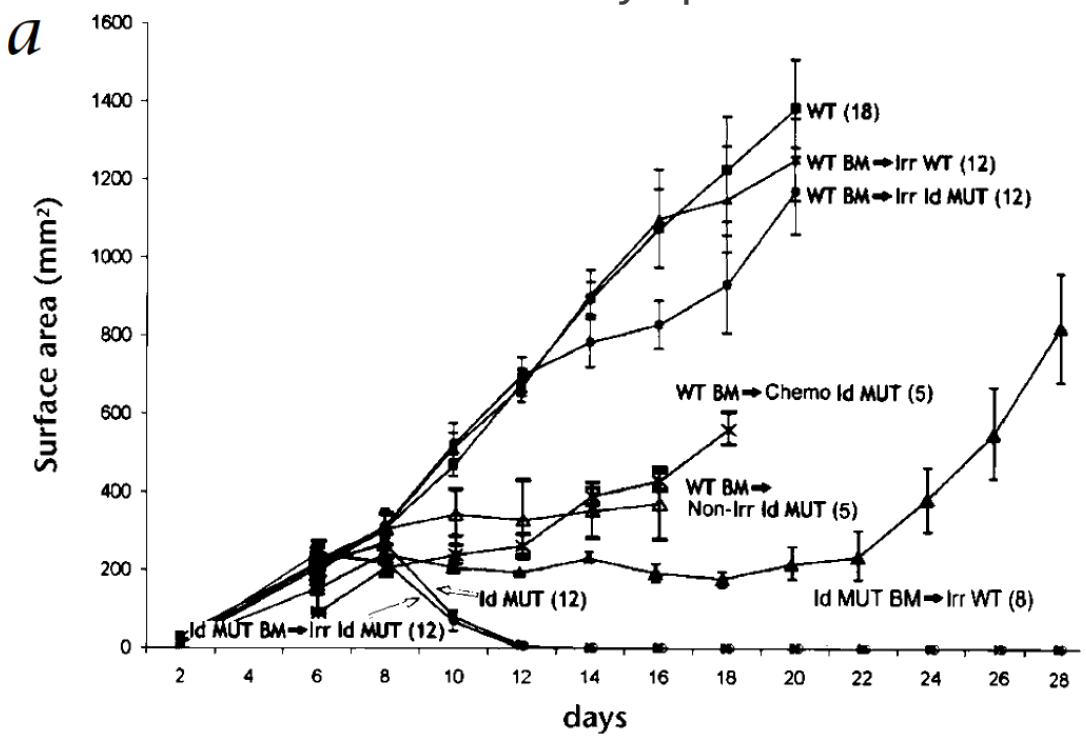
- Rosa-26 mice express the β-gal transgene in all tissues
  - Used as WT bone marrow (BM) donor

- Von Willebrand factor (vWF) is an endothelial cell marker up-regulated by angiogenesis factors

# Results

# Transplantation of BM restores Id-mutant angiogenic defects

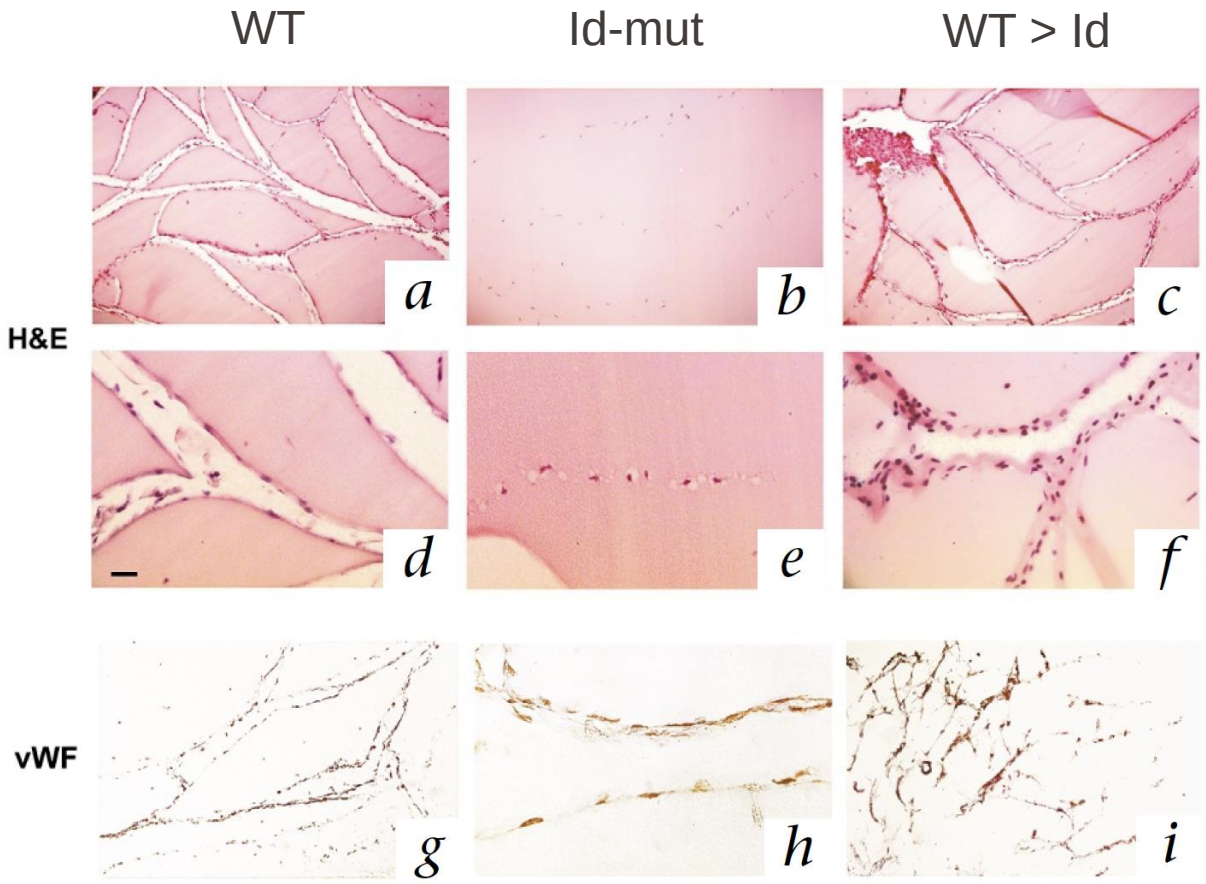
BVRV2 lymphoma




Irradiation and engraftment. 4 weeks later, tumor challenge:

- WT BM transplantation allows tumor growth
- BM-derived precursor cells are required for tumor growth (early stages at least)
- The tumor growth is not likely to be due to vascular alterations caused by the irradiation

# Transplantation of BM restores Id-mutant angiogenic defects



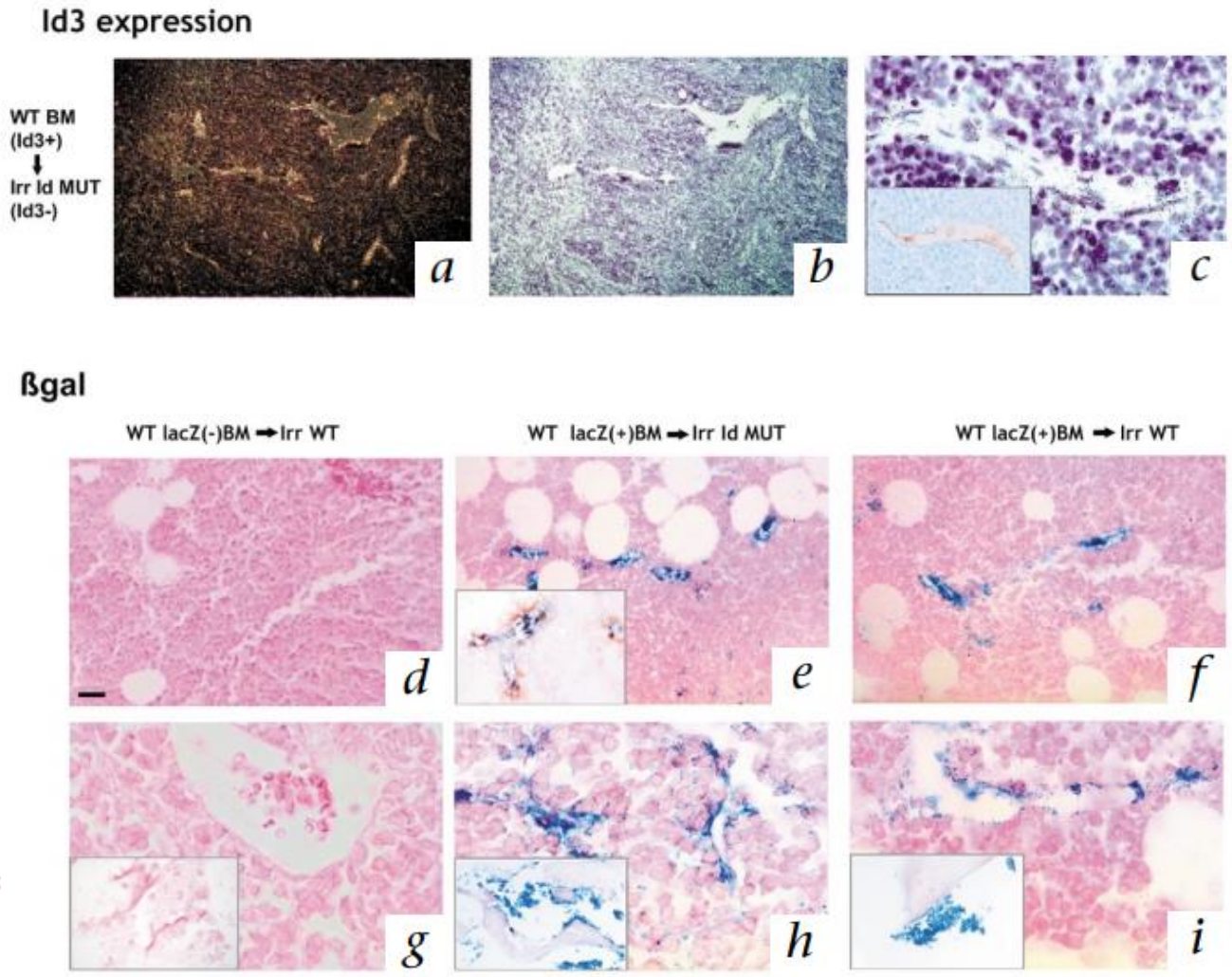

- VEGF-loaded Matrigel plug assay

| | WT | Id-mut | WT > Id |
|---|---|---|---|
| Vascular channel formation | Yes | No | Yes |

- Numerous vWF+ sprouting vessels (g, i)

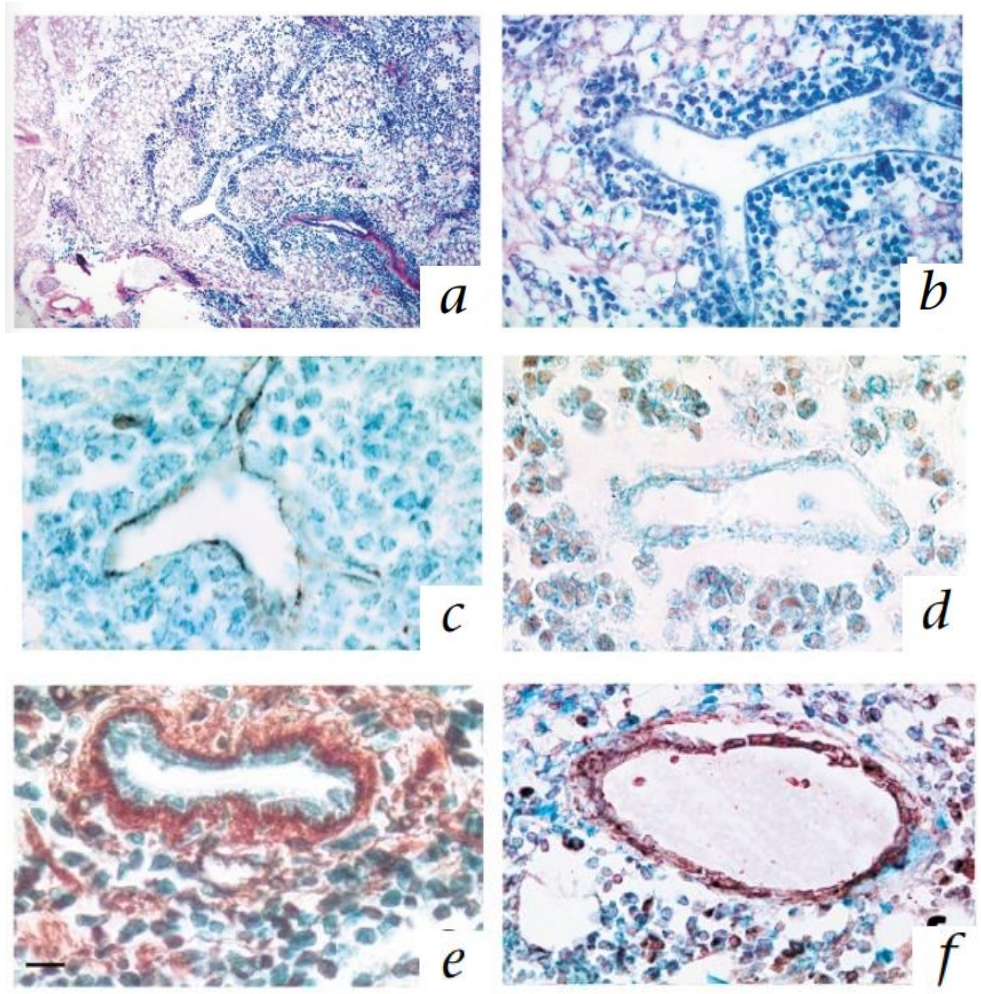
"These data demonstrate that wild-type BM contributes to the restoration of vascular channels in Id-mutant host mice in a non-tumor setting"

# Incorporation of BM-derived CEPs into neovessels



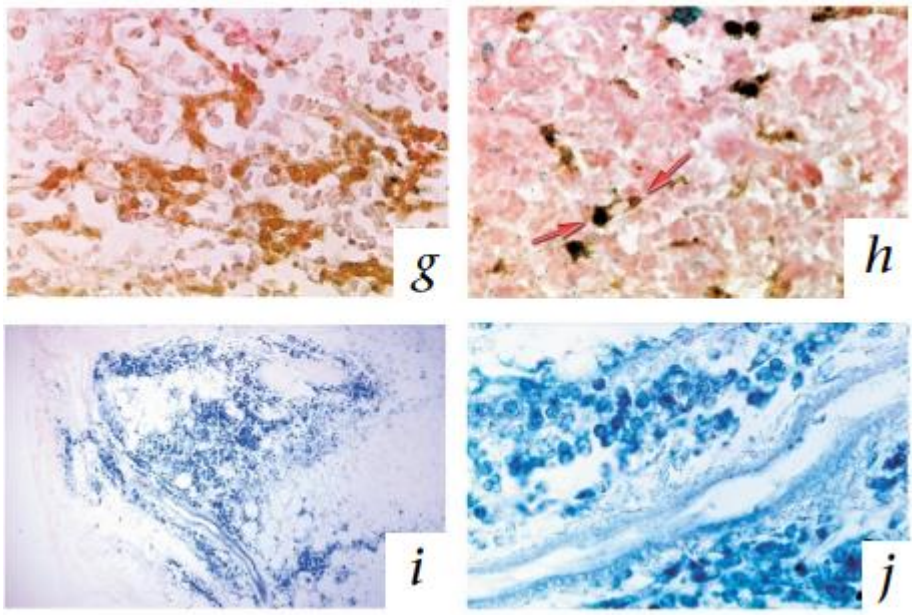

- ID3 detected by RNA in situ (a) and colocalize with vWF (c)
- LacZ+ vessels with vWF (e)
- Even in WT mice, BM-derived cells are incorporated into tumor vasculature

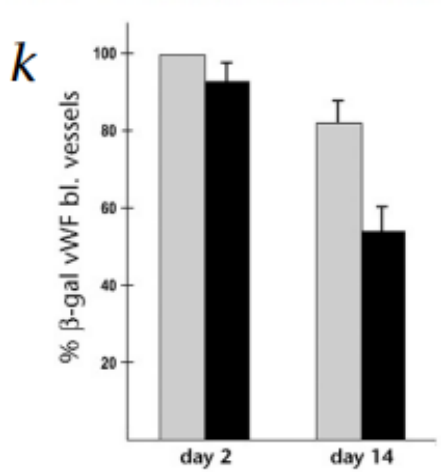
# Transplantation of VEGF-mobilized cells into lethally irradiated ld-mutant mice rescue hematopoiesis




- transplantation of VEGF-mobilized LacZ+ cells and tumor implantation
- LacZ+ cells detected in blood vessels
- vWF+ LacZ+ vessels (C) surrounded by VEGFR1+ LacZ+ mononuclear cells (d)
- The VEGFR1+ cells are of myeloid origin (MOMA+) (e)
- the cells in the lumen are of endothelial origin (VE-cadherin+) (f)

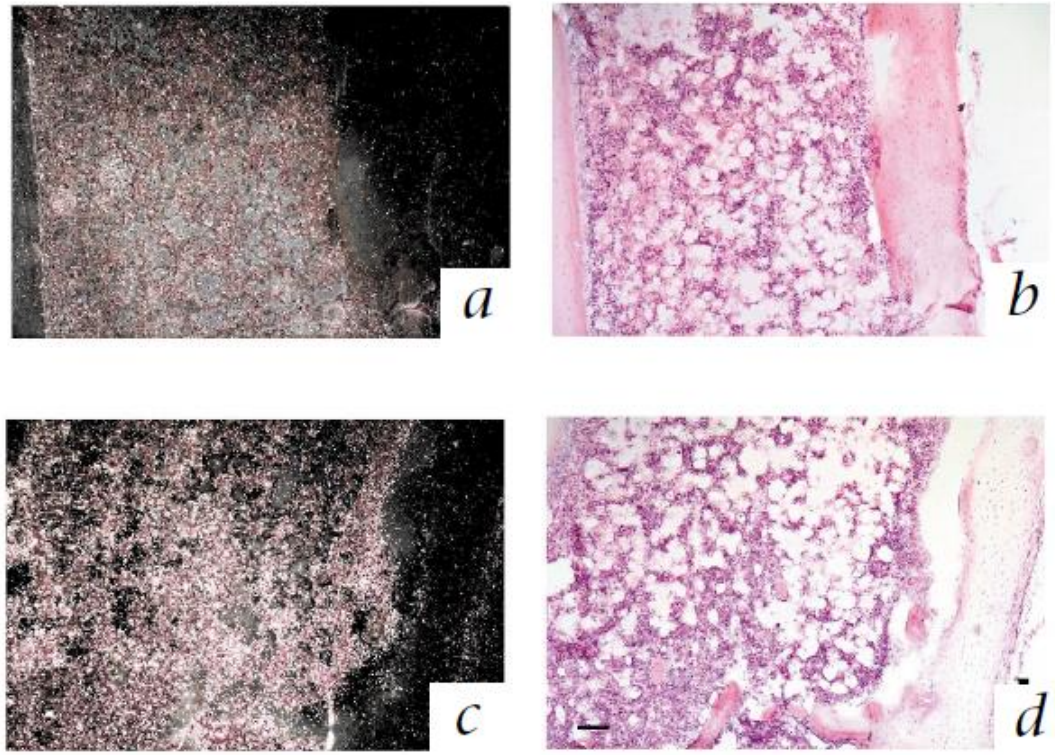
# Tumor type influence



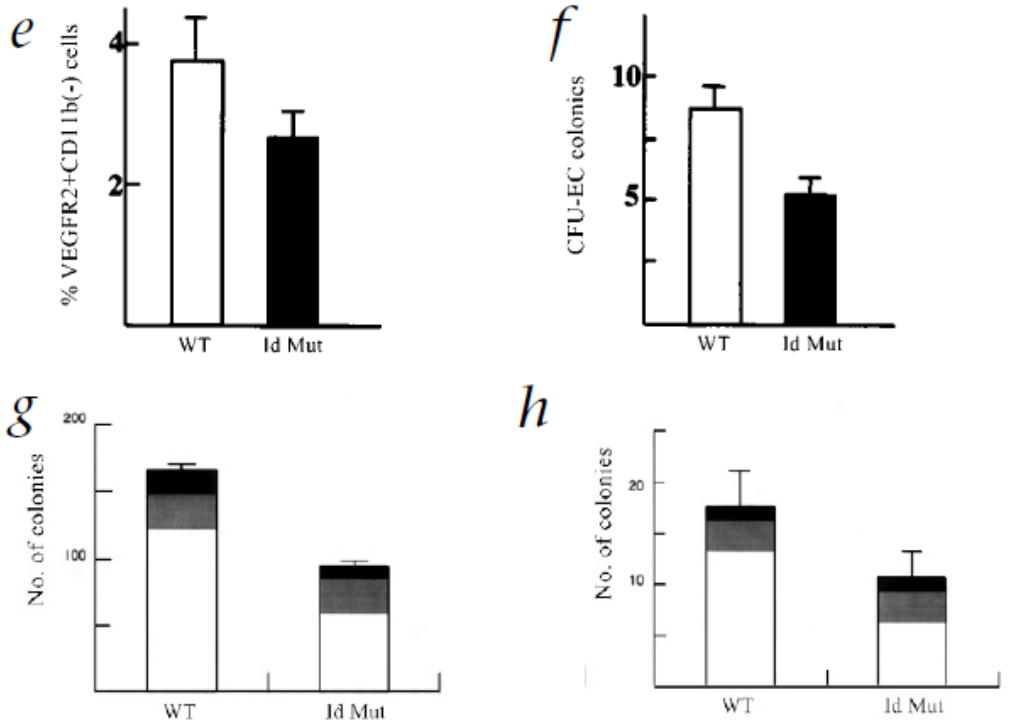

- ID mutant micro-transplanted with wt BM → wt cells incorporated into tumor vessels (β-gal stained in brown) (g and h)
- Same results if we change the tumor type (B6RV2 to LLC) (i,j)
- The tumor type influence the persistence of BM-derived precursors in tumor angiogenesis

# VEGF induces ID3 expression in the BM




- WT mice were infected with AdVEGF or control virus
- VEGF induced ID3 expression (shown in c and d)

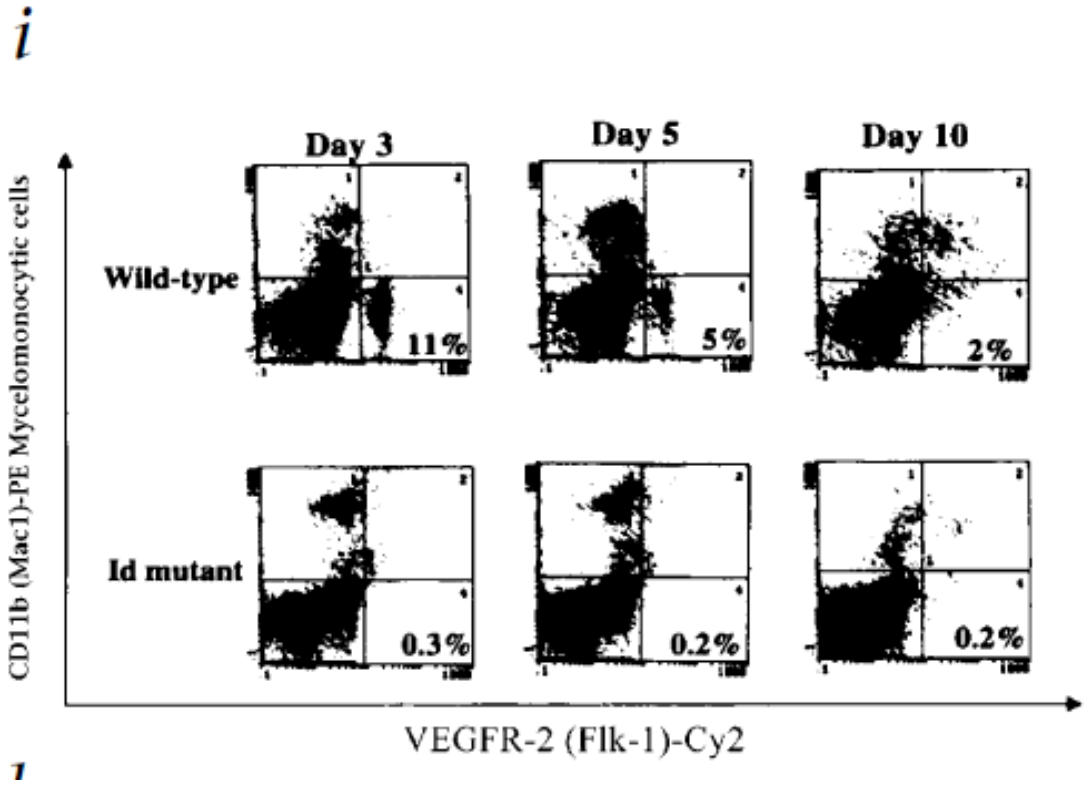
# Different cell populations between wild type and ID mutants




- Less VEGFR2(+) CD11(-) in ID mutant BM
- Less BM (g) and splenic (h) hematopoietic progenitors
- But hypothesis :

*Their impaired recruitment is more likely to be responsible for tumor resistance in Id-mutant mice than the absolute decrease in the number of the BM precursors.*

# VEGF fails to recruit VEGFR2+ CD11- CEPs in ID mutants




- WT and mutant mice were infected with AdVEGF
- Induced apparition of VEGFR2+ Cd11- cells among PBMC in WT but not in ID mutant
- Colony forming assay shows that these were CEPs rather than mature ECs

# Same results when inoculating B6RV2 cells and precisions on the role of VEGFR2

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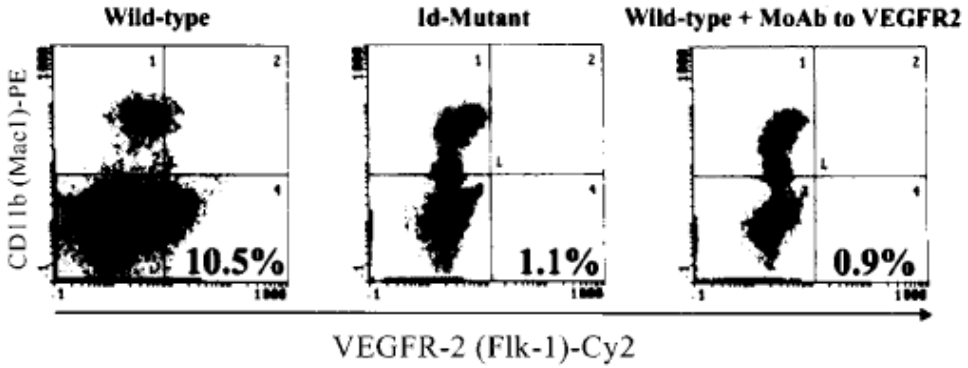
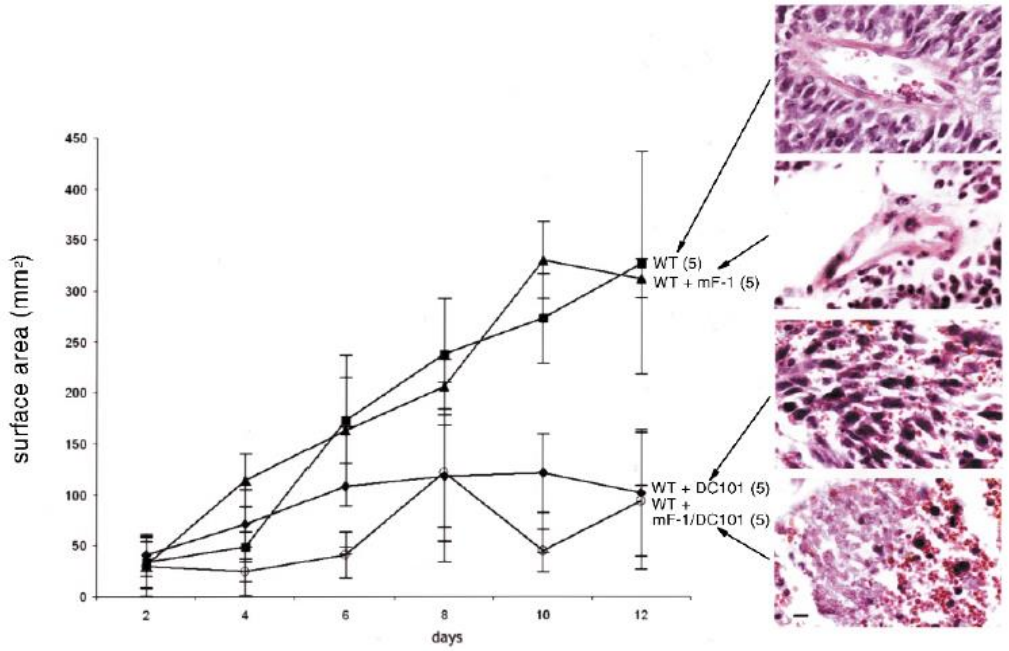



- WT and mutant mice were inoculated with B6RV2 cells
- VEGFR2 helps but is not necessary in this process (cf. third condition)

- VEGFR1+CD11b+ are only modestly fewer in ID mutants but failed to expand to VEGF in long-term BM cultures

# Inhibiting VEGFR signaling blocks tumor growth

→ Final hypothesis: Both VEGFR2+ CEPs and VEGFR1+ myeloid precursors would be needed in early angiogenesis ?




- Tumoric (LLC/B6RV2) mice treated with anti-VEGFR1 (mF-1) or anti VEGFR2 (DC101) or both antibodies

**Conclusion: "Collectively, these data suggest that inhibition of both VEGFR1 and VEGFR2 signaling blocks early phases of tumor growth by blocking the recruitment of VEGF-responsive BM precursors."**

# Thank You for your attention