Paper 2: Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells

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Short introduction to the paper

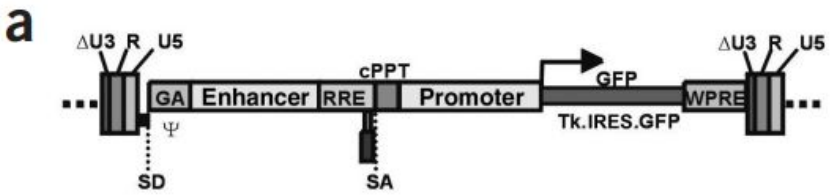
The paper is about the bone marrow transplantation of cells transfected with lentiviral vectors expressing genes from transcription-regulatory elements of Tie2 gene into mice, and aims to show that Tie2 expressing cells are involved in the formation of endothelial cells at angiogenic tumor sites. Tie2 is a receptor for Ang1 and Ang2; VEGF and Ang2 are driver growth factors in tumor angiogenesis. Transfection of the Tie2 vector associating with GFP allows to visualize the presence of Tie2 expressing mononuclear (TEM) cells at angiogenic sites.

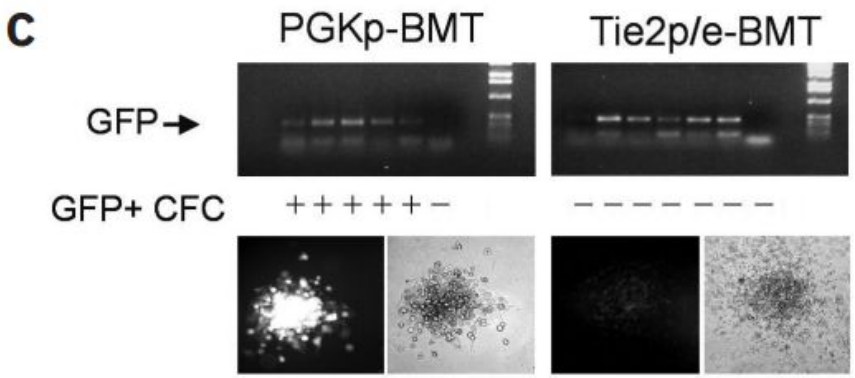
Tie2: marker known to be expressed by endothelial cells. Found in the paper to be expressed by a distinct hematopoietic population that “homed” to the tumor and interacted with endothelial cells at the tumor periphery, subtype of BM derived progenitor. We want to know if vascular ECs originate from BM progenitors.

PGK-p: gene ubiquitously expressed in all cells. Serves as a control for lentiviral transfection.

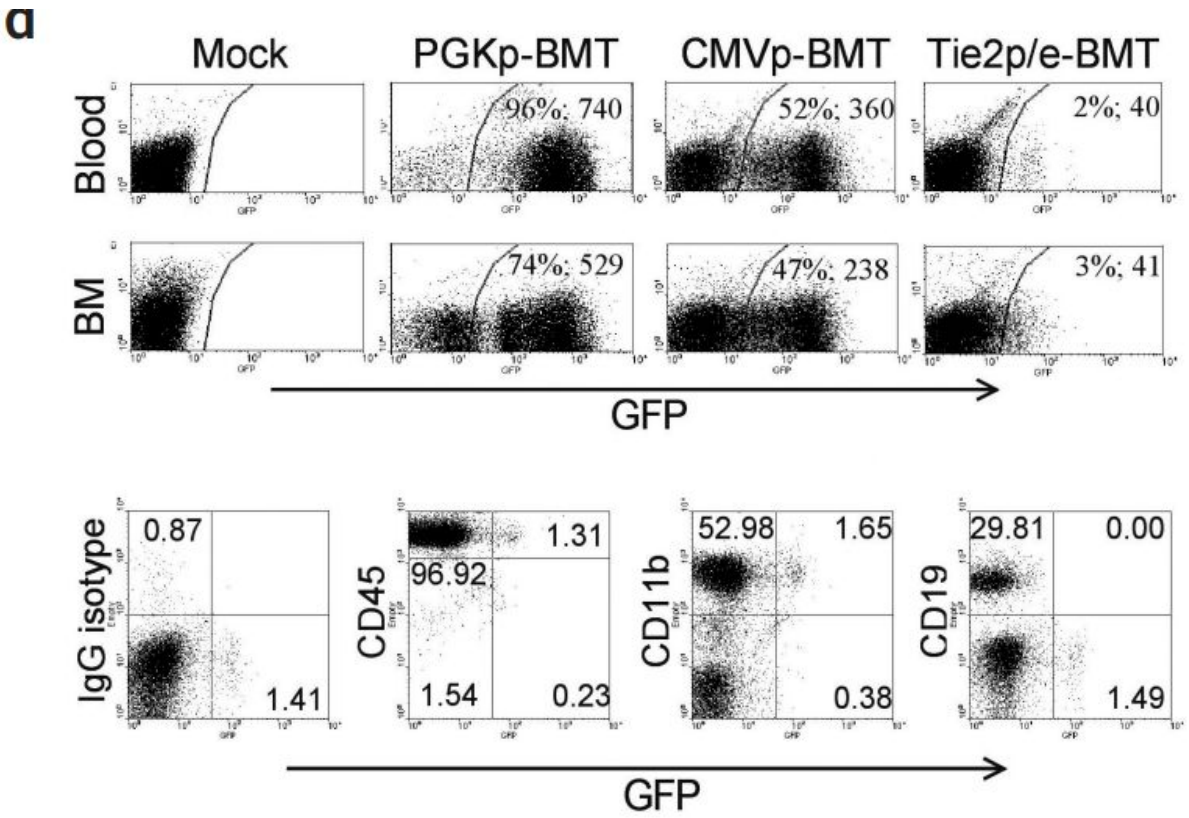
Unexpected result in the paper: no BM derived endothelial cells were found in tumors when BM progenitors expressing Tie2 were transplanted. According to the experiments described in this paper, the BM does not contribute vascular endothelial cells to the tumor stroma.

Figure 1



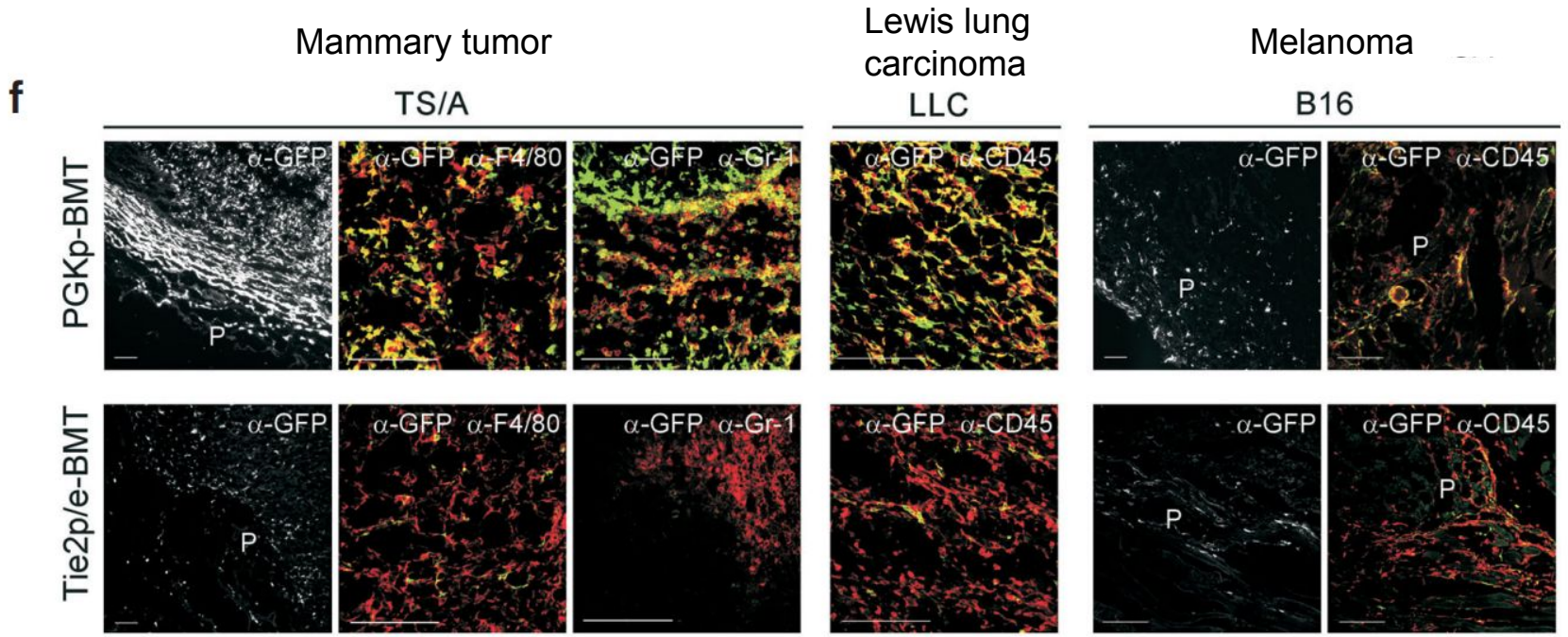


- Control for transfection efficiency of the Tie2p/e lentiviral vector
- Subfig c: PCR analysis of GFP sequence and fluorescence microscopy of hematopoietic colonies from the bone marrow of transplanted mice. GFP is not expressed in Tie2 positive hematopoietic cells.



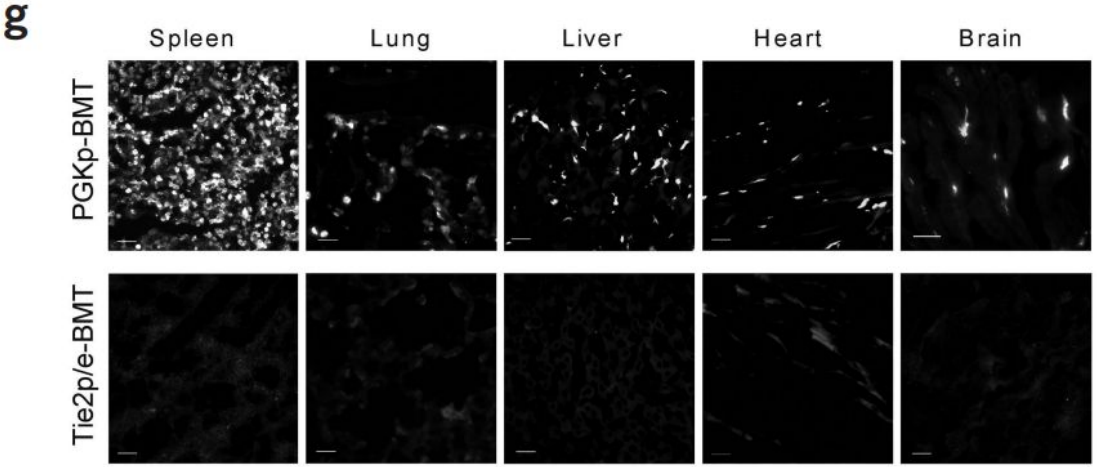
- Subfig. d: FACS analysis of GFP expression. Tie2 expression in blood and bone marrow is very weak (2-3%) compared to PGK and CMV (controls)
- Subfig e: circulating GFP+ cells after BMT were hematopoietic (CD45 +) and of myeloid lineage (CD11b+ CD19–)

Figure 1 continued



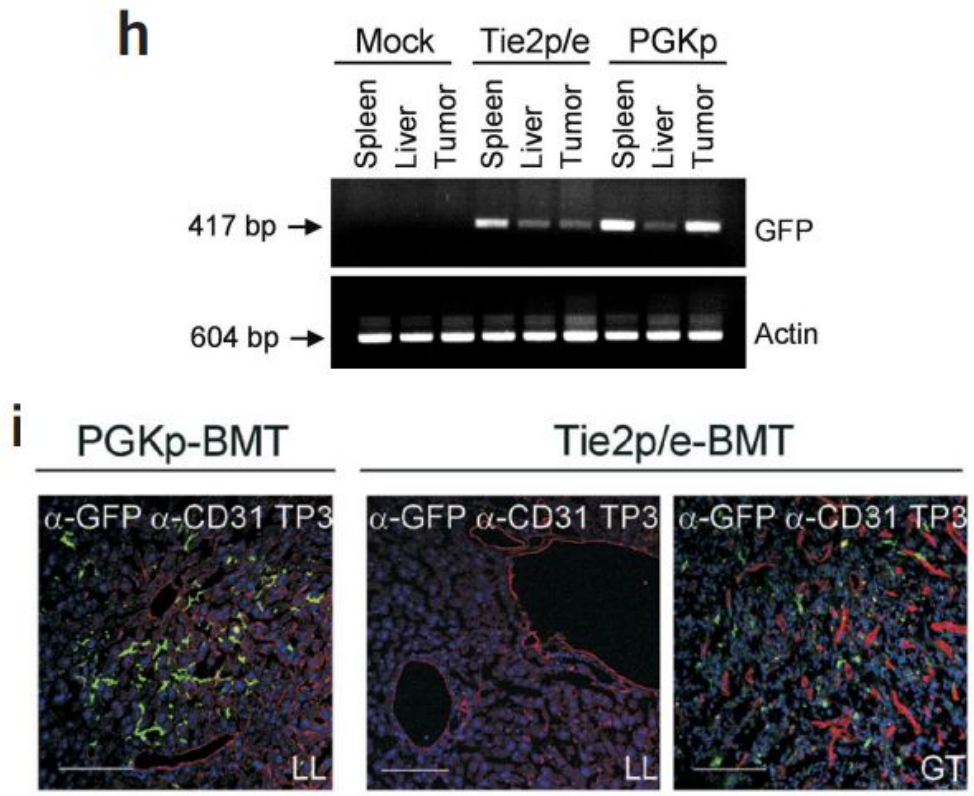
- Gr-1 marker for granulocytes and F4/80 for macrophages
- Some colocalization of GFP and F4/80 in Tie2+ cells

Figure 1 continued



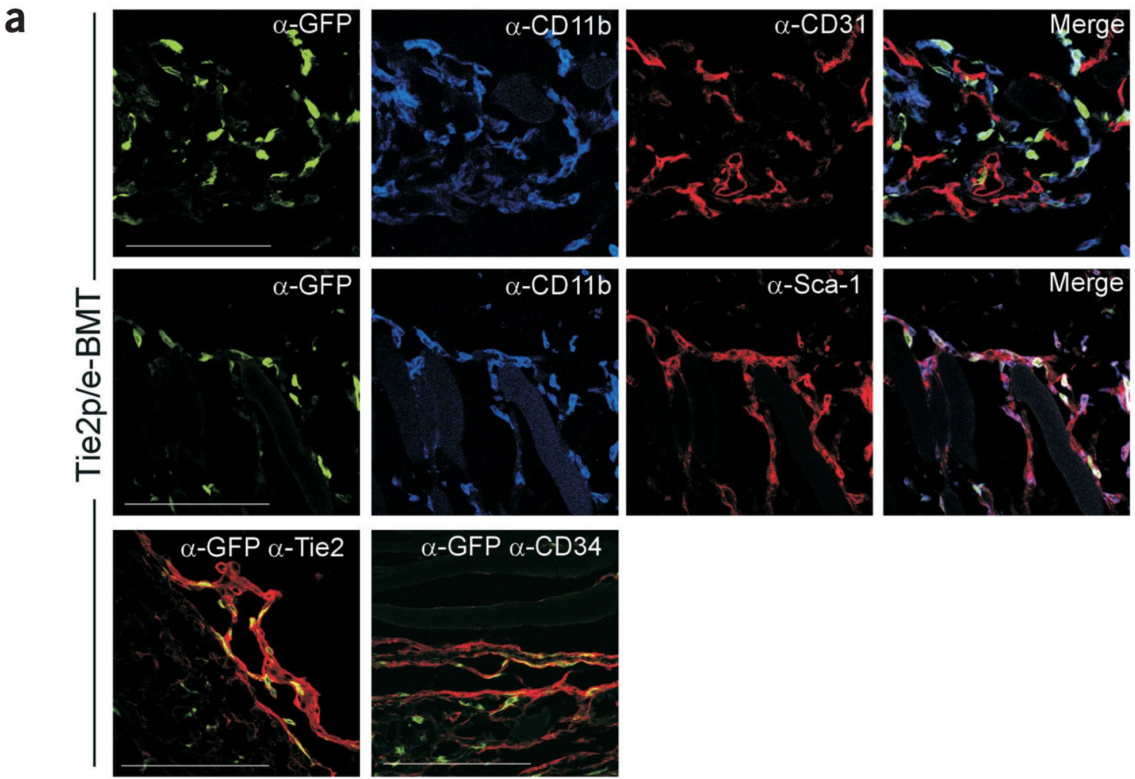
- GFP immunostaininng of non-cancerous organs from BMT mice
- Tie2 is not expressed in these stable non-angiogenic tissues, despite the presence of BM derived cells (GFP+ PGK vector expression in these normal tissues)
- The absence of Tie2 GFP signal shows that the endothelial cells forming the vessels do not originate from BMDCs

Figure 1 continued



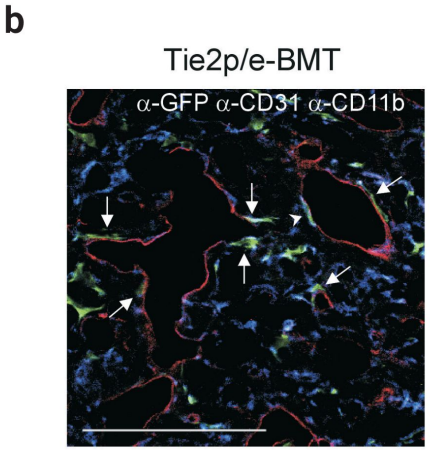
- Subfig h: PCR for the GFP sequence on spleen, liver and tumor DNA
- Subfig i: immunostaining for GFP (green), CD31 (red) and nuclear DNA (blue).
- CD31 : marker for vascular cell adhesion and signaling molecule
- We see that Tie2 is present in the granular tissue that surrounds regenerating liver lobules (LL) in BMT mice after partial hepatectomy.
- The presence of Tie2 in liver recovering from hepatectomy shows that Tie2 BMDCs are recruited for normal angiogenesis.

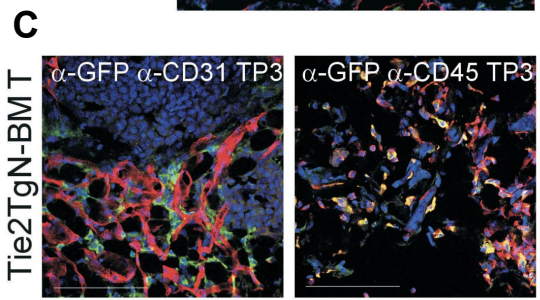
Figure 2: immunofluorescence of tumors

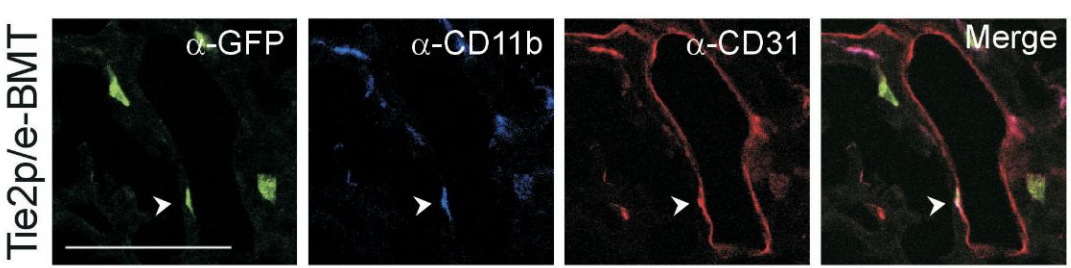


→ Tie2 expressing cells are not endothelial cells (don't overlap with CD31+ cells) but are myeloid cells (overlap with CD11b+ cells)

Figure 2 continued







→ Tie2 expressing cells are localized closely to the vessels. They surround the vessels like pericytes, but they are myeloid cells.

Figure 2

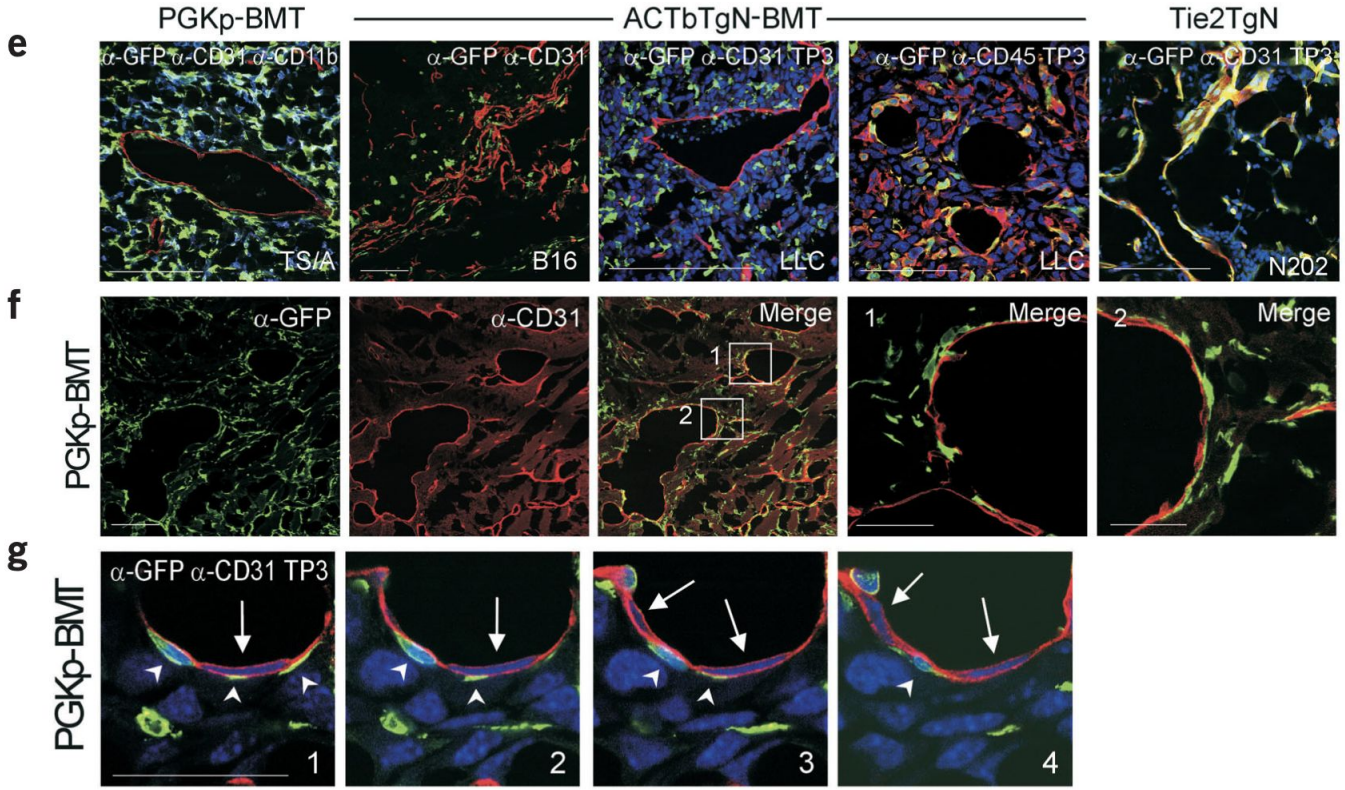
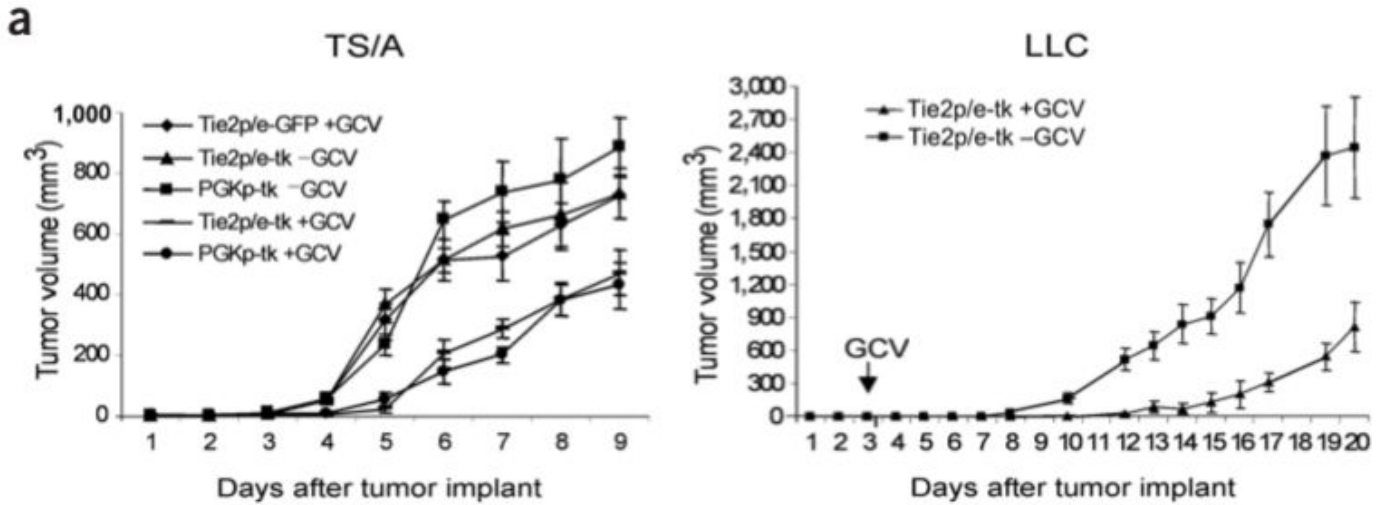
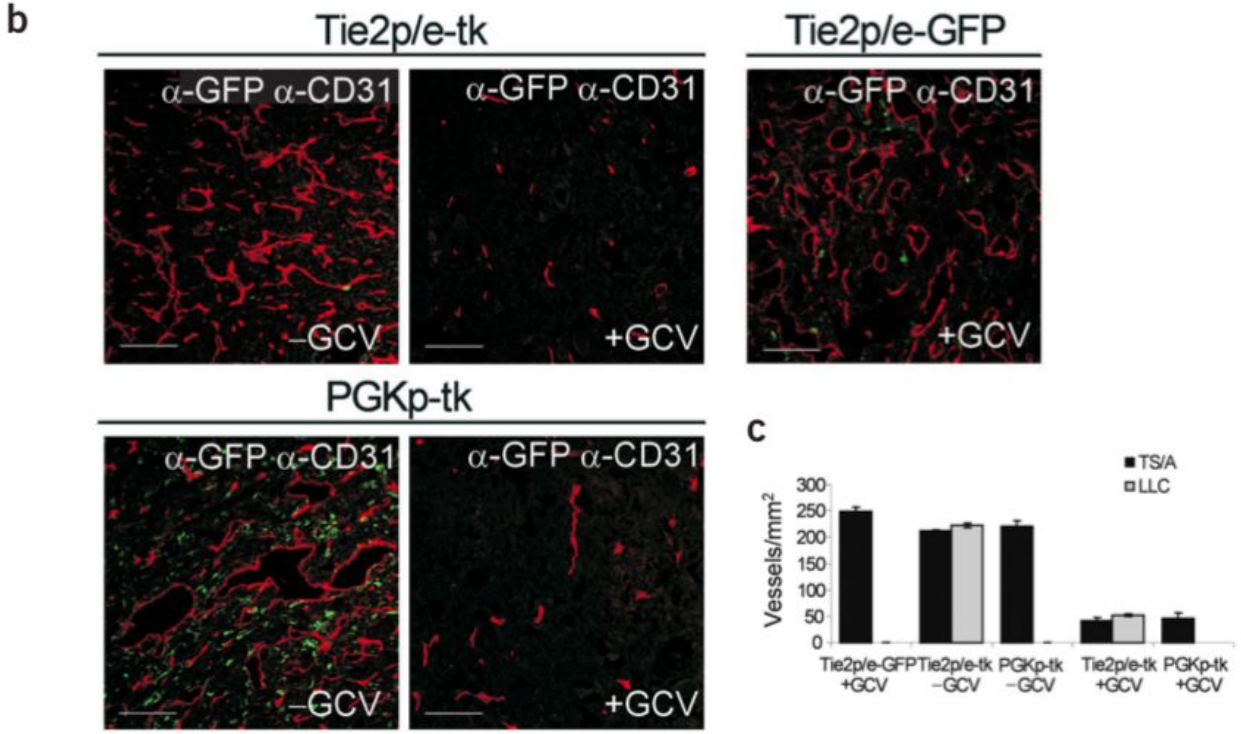

PGKp-BMT
ACTbTgN-BMT
Tie2TgN
e
α-GFP α-CD31 α-CD11b
TS/A
α-GFP α-CD31
B16
α-GFP α-CD31 TP3
LLC
α-GFP α-CD45 TP3
LLC
α-GFP α-CD31 TP3
N202
f
PGKp-BMT
α-GFP
α-CD31
Merge
1
2
Merge
1
Merge
2
g
PGKp-BMT
α-GFP α-CD31 TP3
1
2
3
4

Figure 3: Selective in vivo elimination of the Tie2p/e-vector-targeted cells inhibited tumor angiogenesis and slowed tumor growth.



→ delay in the appearance of tumor and slower tumor growth in mice where Tie1p/e cells were selectively killed, compared to the control mice.

Figure 3 continued



→ lower vascular density in GCV-treated Tie2p/e-tk-BMT and PGKp-tk-BMT mice than in the controls and complete absence of infiltrating GFP-positive cells.

→ Same results in PGKp-tk-BMT and Tie2p/e-tk-BMT mice so the later are responsible for most bone marrow–dependent proangiogenic activity in the tumors studied.

Conclusions

- Tie2 expressing cells derived from BM do not seem to evolve into endothelial cells in blood vessels
- Hematopoietic stem cells can be genetically modified to target tumor angiogenesis and reduce tumor growth.
- Genetically modified hematopoietic stem cell transplantation represents a promising approach for cancer therapy → potential of exogenous gene delivery through hematopoietic stem cells to target specific cells and tissues in a therapeutic context.