Human Endothelial Progenitor Cells (EPCs) role in Tumor Angiogenesis.

J. Sharma[1], R. Punzalan[1], C. DiMaggio[1], N. Taw[1], M. Majdoub[1], J. Passarini, N. Liles[1], G. Velazquez[1], S. Sharma[1], N. Amezcua[1], C. Sharma[1], M. Jani[1], M. Sharma[1], M.E. Harris-White[2], S. Sharma[2]

1. Stem Cell Biology, Celprogen, San Pedro, CA, US., 2. UCLA/VA-GLAHS, Los Angeles, CA, USA.

Request for off-prints should be addressed to Jay P. Sharma[1]; Email: cancerstemcells@celprogen.com

CELPROGEN®

Stem Cell Research and Therapeutics

Celprogen Inc.***
1871 N. Gaffey St, Ste. A & B, San Pedro, CA 90731
www.celprogen.com

Human Endothelial progenitor cells (EPCs) isolated from peripheral blood have the following CD34, VEGFR-2, or AC 133 (CD133) and Aldehyde dehydrogenase antigen-positive cells, which may home to site of neo-vascularization and differentiate into endothelial cells. Endothelial Cells contribute to tumor angiogenesis, and can originate from sprouting from neighboring pre-existing vessels. The bone marrow-derived circulating EPCs can contribute to tumor angiogenesis and growth of certain tumors. In this study we observed EPCs labeled with GFP contribute to Breast, Brain and Prostate Cancer tumor angiogenesis in mouse tumor explants. This study confirms the EPCs play a major role in tumor angiogenesis in Breast, Brain and Prostate cancers as indicated in the tumor explants.

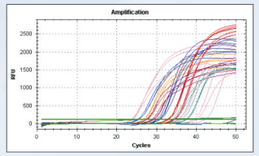
Method: Primary Normal Aortic Arch tissue was obtained from consented patients during surgical procedure in Celprogen's Endothelial Cell Complete Growth Medium. The Aortic Arch Tissue sections were processed as primary endothelial cell culture and the other section was processed and cultured as Endothelial Progenitor cells in Endothelial Complete Growth Media and matrix. After 14 days in culture the cells were characterized for Endothelial Progenitor markers: CD34, VEGFR-2, CD133 and Aldehyde dehydrogenerase antigen positive cells.The cell based assay system enabled one to perform a high throughput characterization of these endothelial progenitor cells.

Animal studies: In this study five female SCID nude mice were injected subcutaneously with Breast Cancer Stem Cells (3-) in their mammary fat pat 1000 cells per mouse. On the third day 83,000 labeled Endothelial Progenitor cells with GFP were at the site of subcutaneous tumor injection site. At day 20 the mice were euthanized and the tumor tissues were sectioned and analyzed under Fluoresence inverted microscope. The following major organs were harvested Brain, Lungs, Liver, Kidney and Bone Marrow and analyzed for neo-vascularization of metastasis at these secondary tumor sites.

Results: The results are indicated in the following Figures 1-9, and Graph 1.

Conclusions: This study has demonstrated that Endothelial Progenitor cells play a major role in Tumor angiogenesis in Breast Cancer implants. The results indicate that endothelial progenitor cells participate in the neo-vascularization of tumor growth in in-vivo model systems.

Fig1. SCID nude mice injected simultaneously with 1000 Breast Cancer Stem Cells per mouse and on the third day 83,000 labeled Endothelial Progenitor cells with GFP were injected subcutaneously at tumor site. The present image is at day 20.



Graph1. Human Endothelial Progenitor cells Real Time - PCR for the following genes: CD34, VEGFR-2, CD133, Aldehyde Dehydrogenase, GAPDH.
(Real Time - PCR: CFX96™ Real-Time System C1000™ Thermal Cycler)

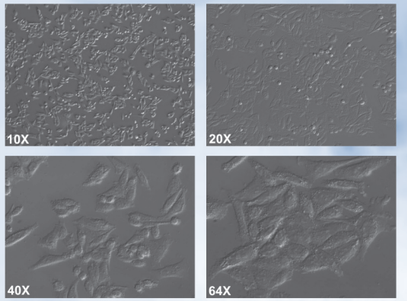


Fig2. Human Endothelial Progenitor Cells at the following magnifications grown in Celprogens Media and Matrix.

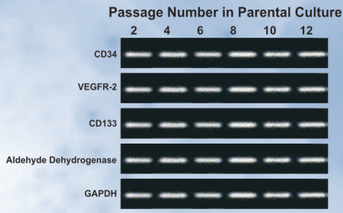


Figure4. Human Endothelial Progenitor Cells gene expression profile of up to twelve passages when maintained in Celprogen's Media and ECM.

•RT-PCR analysis of CD34, VEGFR-2, CD133, Aldehyde Dehydrogenase, and GAPDH was used as an internal control. *n* = 10.

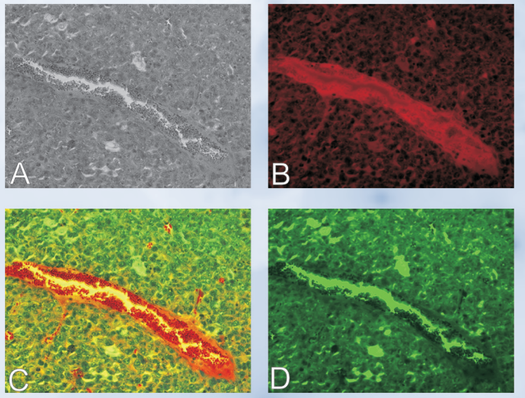


Fig3. Human Breast Cancer (3-) tumor tissue, labeled with GFP Endothelial Progenitor Cells, localized near the blood vessels. A. Brightfield, B. Texas Red, C. Texas Red & GFP (Tumor Angiogenesis), D. GFP labeled only.

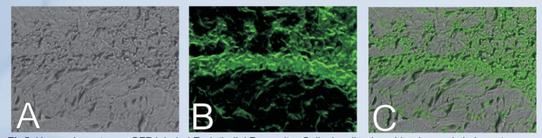


Fig5. Human Lung tumor GFP labeled Endothelial Progenitor Cells, localized neoblood vessels in Lung tumor.
A. Brightfield image, B. Fluorescent image of GFP labeled Endothelial Progenitor Cells, C. Overlay of Brightfield and Fluorescent image.

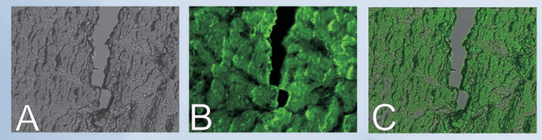


Fig6. Human Liver tumor GFP labeled Endothelial Progenitor Cells, localized neoblood vessels in Liver tumor.
A. Brightfield image, B. Fluorescent image of GFP labeled Endothelial Progenitor Cells, C. Overlay of Brightfield and Fluorescent image.

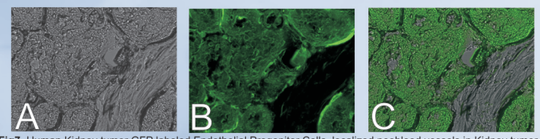


Fig7. Human Kidney tumor GFP labeled Endothelial Progenitor Cells, localized neoblood vessels in Kidney tumor.
A. Brightfield image, B. Fluorescent image of GFP labeled Endothelial Progenitor Cells, C. Overlay of Brightfield and Fluorescent image.

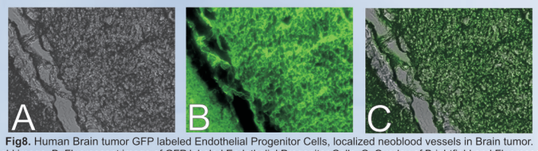


Fig8. Human Brain tumor GFP labeled Endothelial Progenitor Cells, localized neoblood vessels in Brain tumor.
A. Brightfield image, B. Fluorescent image of GFP labeled Endothelial Progenitor Cells, C. Overlay of Brightfield and Fluorescent image.

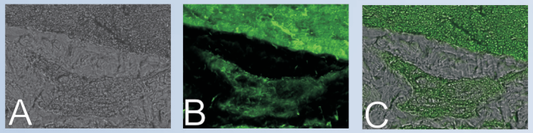


Fig9. Human Heart tumor GFP labeled Endothelial Progenitor Cells, localized neoblood vessels in Heart tumor.
A. Brightfield image, B. Fluorescent image of GFP labeled Endothelial Progenitor Cells, C. Overlay of Brightfield and Fluorescent image.

Acknowledgement:
The authors would like to thank the following individuals
for their technical support and critical review: O. Sobhy and M. Warden.

Visit Celprogen Inc. in Booth No.1440