Project no.: 518198

Project acronym: TUMOR-HOST GENOMICS

Project title:

Genome-wide analysis of signaling pathways in regulation of the interactions between tumor and host cells: Applications for cancer therapy

Instrument: STREP

Thematic Priority:

Thematic Priority 1, LSHC-CT-2005-518198

Life sciences, genomics and biotechnology for health

Title of report:

Publishable Final Activity report

Period covered: from 1.11.2008 to 31.10.2009 **Date of preparation:**

Start date of project: 1.11.2005 **Duration:** 48 months

Project coordinator name: Petri Salvén

Project coordinator organisation name: University of Helsinki

PUBLISHABLE FINAL ACTIVITY REPORT

1. PROJECT EXECUTION

2. DISSEMINATION AND USE

ANNEX – FINAL PLAN FOR USING AND DISSEMINATING THE KNOWLEDGE

1. Project execution

The summary description of project objectives

The workplan entails development of novel advanced functional genomics instruments, technologies and methods to study tumor-host interactions in cancer, and to apply these techniques to the identification of molecules and processes in normal cells which could be targeted by novel anti-cancer therapeutic agents. In addition, we will develop targeted lentiviruses which would allow in vivo delivery of therapeutic agents into tumors. Functional validation of the discovered targets and developed delivery systems will be performed in vivo models of murine tumor growth and dissemination. For purely technical reasons, melanoma and prostate cancer models are planned to be utilized first. However, tumor-host interactions are universally essential for the growth and dissemination of any malignant disease, and the results of the experiments will be applicable for any kind of human cancer. The work has significant exploitation potential and relevance for health in the understanding of the molecular mechanisms of tumor-host interactions, and in the treatment of cancer.

The specific aims of the project are:

1. To develop targeted lentiviral libraries for inhibition of selected major cell signaling pathways

2. To identify tumor-derived factors that lead to increased angiogenesis and recruitment of stromal cells contributing to a microenvironment permissible for tumor growth

3. To identify host-derived factors that induce tumor cell growth and tumor stem cell self-renewal

4. To identify endothelial/BM cell-specific cis-regulatory elements for use in lentiviral in vivo targeting vectors

5. To test in vivo the effect of targeted lentiviruses in inhibition of tumor growth and metastasis

Contractors involved

Partic. Role*	Partic. no.	Participant name	Participant short name	Country	Date enter project	Date exit project
CO	1a)	University of Helsinki	UH	Finland	Mont 1	Month 48
CR	1b)	University of Helsinki	UH	Finland	Month 1	Month 48
CR	1c)	University of Helsinki	UH	Finland	Month 1	Month 48
CR	2	Leids Universitair Medisch Centrum	LUMC	Netherland	Month 1	Month 48
CR	4	Universitá Vita-Salute San Raffaele	USR	Italy	Month 1	Month 48

Co-ordinator contact details

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Background

The tumor microenvironment consists of cells of hematopoietic and mesenchymal origin, including inflammatory cells, stem and progenitor cells, fibroblasts, endothelial cells and vascular mural cells. Tumor cell growth is known to depend on the interaction of tumor cells with such stromal cells. For example, growing tumor needs to recruit normal endothelial and vascular mural cells to form its blood vessels. In addition, tumor cells induce stromal cells to secrete factors that contribute to tumor cell growth and invasion. Stromal cell -dependent interactions represent an attractive target for cancer therapy, because normal cells are genetically stable, and would not be expected to develop resistance to therapeutic agents. The development of such therapies is hampered by the fact that the molecular mechanisms behind tumor-stroma interactions are often poorly understood.

Summary of main results and impact

The main achievements of the TUMOR-HOST GENOMICS project have been:

1) Designed and cloned dominant negative constructs targeting 8 signaling pathways (**partners 1a-c**).

2) Lentiviral packaging of the dominant negative constructs (**partner 1a-c**).

3) Completed in silico prediction of enhancer elements for BM cell-specific gene expression (**partner 1c**).

4) Completed in silico prediction of enhancer elements for stromal cell-specific gene expression (**partner 1c**).

5) Completed in silico prediction of enhancer elements for endothelial cell-specific gene expression (**partner 1c**).

7) Completed cell-cycle specific screening format (**partners 1c & 2**).
The screen was performed in U2OS and control RPE cells. 273 and 453 genes orthologous to Drosophila cell cycle regulators from were identified as cell cycle regulators in the human U2OS and RPE cell screens, respectively. The genes, which potentially affect tumor cell growth, are discussed in report to deliverable 3.3.

8) Completed signaling pathway specific screening format (**partners 1c & 2**).
In summary, we have screened three different signaling pathways using kinome cDNA expression, and one using kinome siRNAs. The most important finding from these studies has been the identification of two novel kinases regulating the Shh pathway, DYRK2 and MAP3K10 (Varjosalo et al., **Cell 2008**). Both of these kinases were also required for Shh signaling, as revealed by a kinome-wide siRNA screen. Using cell-lines deficient in different components of the Shh pathway we further mapped the activities of these kinases to the GLI transcription factors, and showed

that they are required for pathway function in cultured cells and also affected Shh signaling *in vivo*.

9) Potential genes affecting tumor cell growth (**partner 1c**).
Primary screening to identify regulator of the cell cycle in human cells (U2OS cells) was performed using laser scanning cytometry (Acumen) in triplicates (total 96-well 24 plates=8x3). Subsequent treatment of U2OS cells with dsRNAs corresponding to 105 different genes resulted in decreased number of cells, and 15 dsRNAs resulted in G1 arrest. Thus, we have identified a large number of genes affecting tumor cell growth (Table I). Of particular interest in the area of tumor-host interactions are the following genes that affect the cell cycle phenotypes: FGFR3 and PDGFRA, receptors for fibroblast growth factors (FGFs) and platelet-derived growth factor (PDGF) that could respond to the respective ligands secreted by host cells.

10) Potential genes affecting signaling pathways involved in tumor-host interactions (**partners 1c & 2**).
We identified two kinases, DYRK2 and MAP3K10, which are required for Shh signaling in NIH-3T3 cells (Varjosalo et al., 2008). Of these, DYRK2 directly phosphorylates GLI2 and GLI3 and induces their degradation. MAP3K10, in turn, appears to act in a more indirect fashion, binding to and phosphorylating multiple other proteins that regulate the Hh pathway, including GSK3β, DYRK2 and Kif3a (Varjosalo et al., 2008). Because of the many connections of MAP3K10 to different pathway components, its mechanism of action is likely to be complex, and requires further study. Of the identified kinases, the positively acting kinase, MAP3K10 is a potential target for siRNA mediated and/or chemical inhibition in cancer.

11) Generation of Tie2-GFP; PGK-GFP; Tie2-tk; PGK-tk transgenic mice (**partner 3**).

12) Analysis of TEM phenotype and functions, analysis of the phenotypes and functions of other BM-derived stromal cell populations in tumors (**partners 3 & 1a**). We investigated the phenotype of TEMs by analyzing GFP^+ cells from Tie2-GFP transgenic mice, as well as by using expressional profiling. We also studied the phenotype of human TEMs. Part of these results have been published (De Palma et

al., **Cancer Cell 2005**; Venneri et al., **Blood 2007**). Our own results also indicate that BM-derived endothelial cells do not significantly contribute to tumor- or cytokine- induced neoangiogenesis. Instead, BM-derived periendothelial cells are mobilized from the BM during andiogenesis and tumor growth, and these cells associate closely with the vascular structures (Purhonen et al., **PNAS 2008**).

13) Identification of kinases that modulate BMP/Smad signalling and elucidation of the mechanism by which BMP7 blocks TGF-b induced invasion of breast cancer cells and the importance BMP signaling in tumor cells on metastatic properties of breast cancer towards bone (**partners 1c & 2**).

14) Elucidation of the effect dominant negative targeted lentiviruses for Gli3, Smad7, Socs1, Socs3 and Stat3 (from WP 2) on cancer cell proliferation/survival and invasion in vitro **(partners 1a,c & 2).**

Lentiviruses encoding dominant negative regulators of the pathways Gli3, Smad7, Socs1, Socs3 and Stat3 (from WP 2) were used for transduction of MCF10 cell lines. Preliminary data suggest that ectopic expression of Socs3 and Spry 1 does not affect basal and TGF-β induced invasion. Moreover Socs1 expression has only minor effect on TGF-β induced invasion.

15) Completed analyses of the growth kinetics and metastasis of tumors in mice with transgenic TEM cells, and the feasibility of using TEMs as gene delivery vehicles for therapeutic molecules (**partner 3**).

In Tie2-IFN mice, we observed increased tumor cell apoptosis, inhibition of angiogenesis, and recruitment and activation of immune cells, as compared to transplanted control mice. Prototypical IFN-inducible genes were strongly upregulated in the tumors, but not in the organs of Tie2-IFN mice. Importantly, our strategy did not impair myelopoiesis or wound healing detectably in the mice, and was much more effective than systemic IFN-alpha administrations, which were significantly toxic. These promising results may justify the development of preclinical models that better assess the therapeutic potential of this new IFN delivery strategy. (De Palma et al., **Cancer Cell 2008**).

Expected end results of the project

Stromal cell -dependent interactions represent an attractive target for cancer therapy, because normal cells are genetically stable, and would not be expected to develop resistance to therapeutic agents. The development of such therapies is hampered by the fact that the molecular mechanisms behind tumor-stroma interactions are often poorly understood. The project aims to develop novel tools and methods to study tumor-host interactions in cancer, and to apply these techniques to the identification of molecules and processes in normal cells, which could be targeted by novel anti-cancer therapeutic agents.

The present project is designed to increase understanding concerning central mechanisms on which tumor growth is depended on. Therefore, the findings may potentially combat all types of human cancer, since tumor-host interactions including but not limited to tumor angiogenesis are universally essential for the growth and dissemination of any malignant disease. Unraveling the molecular basis of tumor-host interactions not only increases our knowledge about these pathophysiological processes, but also allows us to affect cancer by targeting normal cells that are genetically stable, and would not be expected to develop resistance to the novel therapeutic agents. Therefore, the work has significant exploitation potential and relevance for health in the understanding of the molecular mechanisms of tumor-host interactions, and in the treatment of cancer.

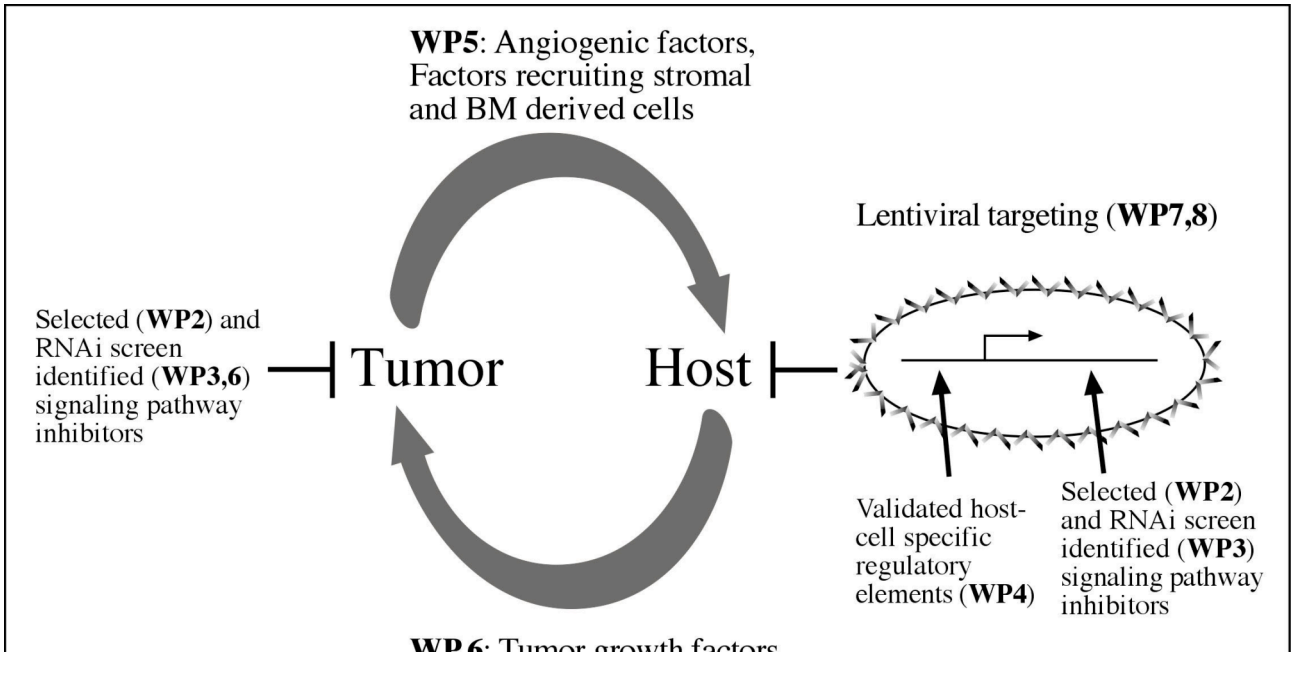

Figure 1. Summary of the original science and technology objectives of the project. *In vivo* testing of pathways affecting tumor-host interactions takes place in mouse tumor and stem cell transplantation models (please see WPs 5&8). Abbreviations: BM, bone marrow; RNAi, RNA interference; WP, work package.

Intentions for use and impact

The results of the project will be used as follows:

1. One aim of the present project is to develop and test technology and methods of advanced functional genomics, and apply these technologies to the identification of novel therapeutic targets in cancer.

2. Potential target genes for the treatment of cancer that will allow the search and preclinical and clinical validation of respective lead compounds. Tumor-host interactions are universally essential for the growth and dissemination of any malignant disease, and the results of the experiments could in principle ultimately be applicable for any kind of human cancer.

3. Cancer has and will continue to have a major medical and economical impact in all of the EU member states. In the present project, we perform functional genomic analyses in vitro and in vivo in murine tumor and angiogenesis models in order to find gene targets crucial for the tumor-host interactions. These genes and regulatory pathways would offer potential targets for further research and future therapeutic interventions. We expect the present project to potentially have wide social, economic, and public health implications if the obtained knowledge and discovered therapeutic targets eventually lead to improved strategies to treat cancer.

2. Dissemination and use

The main exploitable result of the project so far has been the human Tie2-expressing monocytes (TEMs) (US patent application published as US 2008/0057043), which may provide a novel, biologically relevant marker of angiogenesis and represent a previously unrecognized target of cancer therapy.

The project has resulted in several publications in high-impact international, peer-reviewed journals. Research has also been highlighted in scientific meetings, and in journals aimed at medical professionals in different European languages. Participants in the consortium have also presented the data in scientific meetings.

Verified data will be made available for public in digital form, for example via the websites of the project (http://research.med.helsinki.fi/tumorhostgenomics/), those of respective research groups (see for example http://www.ltdk.helsinki.fi/cancerbio/Salven/default.htm), and that of the Finnish Centre of Excellence in Cancer Biology (http://research.med.helsinki.fi/coecancerbio/). In addition, where relevant, information on new findings has been disseminated to the general public, in the form of press releases, and articles in journals and newspapers. After patent protection, verified data will be made available for public in digital, machine readable form, for example via a distributed annotation server (DAS), which can be linked to DAS clients, such as the Ensembl site server.

Annex 1 – Final plan for using and disseminating the knowledge

1. Exploitable knowledge and its Use

Overview table

Exploitable Knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
1. Human Tie2-expressing monocytes (TEMs)	Possible target for cancer therapy	Therapeutics, diagnostics and/or prognosis	Searching for a suitable commercial partner	US patent application published 2008	Partner 4

1. Recent studies have suggested that human CD14+ monocytes can be divided into two main subsets according to the expression of CD16, a Fc gamma receptor III. CD14highCD16– cells are the most abundant monocytes in peripheral blood and are thought to represent classical monocytes that mediate inflammatory responses ('inflammatory' monocytes), whereas CD14lowCD16+ cells are a less characterised subset, which are thought to represent the precursors of tissue-resident macrophages and are referred to as 'resident' monocytes. It was found that a subset of CD14lowCD16+ monocytes expressed the TIE2 angiopoietin receptor. These Tie2-expressing monocytes (TEMs), but not the other monocytes, markedly promoted angiogenesis in xenotransplanted human tumours. In human cancer patients, TEMs were observed in the blood and were specifically recruited to the tumours, where they represented the main monocyte population distinct from tumour-associated macrophages (TAMs). In vitro, TEMs migrated towards Angiopoietin-2, a TIE2 ligand released by activated endothelial cells and angiogenic vessels, suggesting a homing mechanism for TEMs to tumours (Venneri et al., **Blood 2007** Feb 27). Human TEMs may provide a novel, biologically relevant marker of angiogenesis and represent a previously unrecognized target of cancer therapy.

Patent application:
Inventors: Luigi Naldini; Michele De Palma; Mary Anna Venneri
Applicant: Fondazione Centro San Raffaele del Monte Tabor (50%), Fondazione Telethon (50%).
US patent application was published as US 2008/0057043

2. Dissemination of knowledge

Results have been and will be published without delay in international, peer-reviewed journals. Research has been and will be highlighted in scientific meetings, and in journals aimed at medical professionals in different European languages. Participants in the consortium have also presented the data in scientific meetings. Verified data will be made available for public in digital form, for example via the websites of the project (http://research.med.helsinki.fi/tumorhostgenomics/), those of respective research groups (see for example http://www.ltdk.helsinki.fi/cancerbio/Salven/default.htm), and that of the Finnish Centre of Excellence in Cancer Biology (http://research.med.helsinki.fi/coecancerbio/). In addition, where relevant, information on new findings has been and will be disseminated to politicians, and where applicable, to the general public, in the form of press releases, and articles in journals and newspapers. After patent protection, verified data will be made available for public in digital, machine readable form, for example via a distributed annotation server (DAS), which can be linked to DAS clients, such as the Ensembl site server.

Overview table

Planned /actual Dates	Type	Type of Audience	Countries addressed	Size of audience	Parner responsible / involved
2006	*Project website*	*Research and general public*	worldwide		*Parner 1 / Partners 2,4*
2006	*Publication 1: Noda et al. in Oncogene*	*Research*	worldwide		*Partner 2*
2006	*Publication 2: Inamitsu et al. in FEBS*	*Research*	worldwide		*Partner 2*
2007	*Publication 3 (review): Varjosalo et al. in J Cell Sci*	*Research*	worldwide		*Partner 1c*
2007	*Publication 4: Itoh et al. Curr Opin, Cell Biol.*	*Research*	worldwide		*Partner 2*
2007	*Publication 5: Heldin et al. in Curr Opin Cell Biol.*	*Research*	worldwide		*Partner 2*
2007	*Publication 6: Venneri et al. In Blood*	*Research*	Worldwide		*Partner 4*
2007	*Publication 7: Wirzenius et al. in J Exp Med.*	*Research*	Worldwide		*Partner 1b*
2007	*Publication 8: Dennler et al. in Cancer Res.*	*Research*	Worldwide		*Partner 2*
2007	*Publications 9:Lewis et al. in Cancer Res.*	*Research*	Worldwide		*Partner 4*
2007	*Publications 10 (review): De Palma et al. in Trends Immunol*	*Research*	Worldwide		*Partner 4*

2008	*Publications 11: ten Dijke et al. in Angiogenesis.*	*Research*	Worldwide		*Partner 2*
2008	*Publications 12: Kodach et al. in Gastroenterology*	*Research*	Worldwide		*Partner 2*
2008	*Publication 13: Purhonen et al. in Proc Natl Acad Sci.*	*Research*	Worldwide		*Partner 1a / 1b*
2008	*Press release (22.4.2008)*	*General public*	Finland / worldwide		*Partner 1a*
2008	*News article* in **ScienceDaily** 24.04.2008	*General public*	Worldwide		*Partner 1a*
2008	*Newspaper article* in **Helsingin Sanomat** 29.04.2008	*General public*	Finland	*5 million*	*Partner 1a*
2008	*Newspaper article* in **Turun Sanomat** 08.05.2008	*General public*	Finland	*5 million*	*Parner 1a*
2008	*Publication 14: Varjosalo et al. in Cell*	*Research*	Worldwide		*Partner 1c*
2008	*Publication 15: De Palma et al. in Cancer Cell*	*Research*	Worldwide		*Partner 4*
2008	*Publication 16: Lohela et al. in Am J Pathol.*	*Research*	Worldwide		*Partner 1b*
2008	*Journal article in* **New Biotechnology** Vol. 25, 2008	*General public*	Worldwide		*Partner 1*

2009	*Publications 17 (review): Pardali E and Ten Dijke P. in Frontierd in Bioscience*	*Research*	Worldwide		*Partner 2*
2009	*Publication 18: Itoh et al. Lab Invest.*	*Research*	Worldwide		*Partner 2*
2009	*Publication 19: Pucci et al. Blood*	*Research*	Worldwide		*Partner 4*
2009	*Publication 20 (review article): M. De Palma and L. Naldini in Biochim Biophys Acta – Reviews on Cancer*	*Research*	Worldwide		*Partner 4*
2009	*Publication 21: Liu et al. J Cell Sci.*	*Research*	Worldwide		*Partner 2*
2010/2011	*Publication of project data in digital machine readable form.*	*Research*	Worldwide		*Partner 1 / Partners 2,4*

Journal articles and news articles:

- News article: Stem Cell Type Supposed To Be Crucial For Angiogenesis And Cancer Growth Does Not Exist? **ScienceDaily** 24.04.2008 http://www.sciencedaily.com/releases/2008/04/080422113003.htm

- Newspaper article: Kantasolut eivät ehkä kasvatakaan syöpäsuonistoa. **Helsingin Sanomat** 29.04.2008

- Newspaper article: Suomalaisjohtoinen tutkimus paljastaa väärinkäsityksiä: Uusia syöpähoitoja kehitelty tutkimalla kuviteltuja kantasoluja. **Turun Sanomat** 08.05.2008

- Journal article: Recent highlights of EU-funded medical research projects: Provided by CORDIS, the official information service for participation in the EU research programmes **New Biotechnology** Vol. 25, 2008

Publications:

1. Noda D, Itoh S, Watanabe Y, Inamitsu M, Dennler S, Itoh F, Koike S, Danielpour D, ten Dijke P, Kato M. (2006) ELAC2, a putative prostate cancer susceptibility gene product, potentiates TGF-b/Smad-induced growth arrest of prostate cells. **Oncogene**, 25:5591-600.

2. Inamitsu M, Itoh S, Hellman U, ten Dijke P, Kato M. (2006) Methylation of Smad6 by protein arginine N-methyltransferase 1. **FEBS** Lett. 580:6603-11.

3. Varjosalo, M., Taipale J. (2007) Hedgehog signalling. **J Cell Sci**. Jan 1;120(Pt 1):3-6. Review.

4. Itoh S and ten Dijke P. (2007) Negative regulation of TGF-b receptor/Smad signal transduction. **Curr Opin. Cell Biol**. 2007 19:176-84.

5. Heldin C-H and ten Dijke P. (2007) Cellular signaling. **Curr Opin Cell Biol**. Apr;19(2):109-11.

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9. Lewis CE, De Palma M, Naldini L. (2007) Tie2-expressing monocytes and tumor angiogenesis: regulation by hypoxia and angiopoietin-2. **Cancer Res**. 15;67(18):8429-32. Review.

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12. Kodach LL, Bleuming SA, de Miranda NFCC, Wiercinska E, Musler AR, Peppelenbosch MP, Dekker E, van den Brink GR, van Noesel CJM, Morreau H, ten Dijke P, Offerhaus GJA and Hardwick JCH. (2008) The Bone Morphogenetic Protein Pathway is inactivated in the majority of Sporadic Colorectal Cancers **Gastroenterology**.134(5):1332-41. Epub 2008 Mar 4

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S. Coffelt, C.E. Lewis, L. Naldini, J.M. Brown, N. Ferrara, M. De Palma
Elusive identities and overlapping phenotypes of pro-angiogenic myeloid cells in tumors. **Am J Pathol**, in press.

Petersen M, Pardali E, van der Horst G, Cheung H, van der Pluijm G, ten Dijke P. Smad2 and Smad3 have opposing roles in breast cancer bone metastasis by differentially affecting tumor angiogenesis. **Oncogene** in press.

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P. Andreu, M. Johansson, N. I. Affara, F. Pucci, T. Tan, S. Junankar, L. Korets, J. Lam, David T., D.DeNardo, L. Naldini, K. E. de Visser, **M. De Palma,** L.M. Coussens FcRγ activation regulates inflammation-associated squamous carcinogenesis. **Cancer Cell**, in press.

Hawinkels LJAC, Kuiper P, Wiercinska E, Verspaget HW, Pardali E, Sier CFM, ten Dijke P. Soluble endoglin is generated via MMP-14 mediated cleavage and inhibits tumor angiogenesis. Submitted for publication.

Petersen M, Buijs JT, Pardali E, van der Horst G, Cheung H, ten Dijke P, van der Pluijm G. Constitutive activation of activin receptor-like kinase 2 in human breast cancer cells inhibits metastatic progression and osteolytic bone lesions. Submitted for publication.

Manuscripts in preparation:

R. Mazzieri, K. Alitalo, M. De Palma, L. Naldini et al. Angiopoietin-2 mediates pulmonary metastasis in a breast cancer model.

Hawinkels LJAC, Verspaget HW, Wiercinska E, van der Zon JM, Koelink PJ, Lindeman JHN, ten Dijke P, Hommes DW, Lamers CBHW, Sier CFM. Interaction between colon cancer cells and fibroblasts generates myofibroblasts via activation of transforming growth factor-β1.

Conferences and meetings where partners have presented results of the project:

Partner 1:

Petri Salvén:

- 3rd Systems Radiation Biology Workshop, Rovaniemi, Finland (12.1.2009**)**
- Biocenter Oulu Day Symposium, Oulu, Finland (17.3.2009**)**

- British Microcirculation Society's meeting, Birmingham, UK (29.-31.3.2009**)**
- Tumor-Vessel Interface, Kloster Seeon, Germany (19-22.9.2009**)**
- Swiss and German societies for Microcirculation, Bern, Switzerland (8-10.10.2009)
- Finnish Red Cross Blood Service 60th Anniversary Symposium. Espoo, Finland (September 2008)

Kari Alitalo:

- AACR Annual meeting 2006, Washington DC, USA (3.-6.4.2006)
- Fondation Louis-Jeantet, Geneve, Switzerland (26.-29.4.2006)
- Karolinska Institutet, Huddinge, Sweden (11.5.2006)
- International Vascular Biology Meeting, Leiden, The Netherlands (6.-7.6.2006)
- 24th Gonference on Microcirculation, Amsterdam, The Netherlands (1.-8.9.2006)
- Genomics and Cancer, DKFZ, Heidelberg, Germany (13.-15.9.2006)
- Symposium on Angiogenesis, Maastricht, The Netherlands (16.-17.9.2006)
- Josephine Nefkens Symposium, Rotterdam, The Netherlands (1.12.2006)
- Targeting the Kinome Symposium, Basel, Switzerland (3.-4.12.2006)
- AACR annual meeting, Los Angeles, USA (13.-18.4.2007)
- Fondation Ipsen, Spineto, Italy (19.-25.5.2007)
- 4th IRCC International Cancer Conference, Candiolo, Italy (7.-9.6.2007)
- Gordon Research Confrences, USA (22.-25.8.2007)
- Danish Cancer Society XIII Symposium, Copenhagen, Denmark (26.-27.8.2007)
- 4th European Meeting on Vascular Biology…, Bristol, UK (17.-18.9.2007)
- The Tumor-Vessel Interface, Kloster Seeon, Germany (22.-24.9.2007)
- Capri Science Conferences, Capri, Italy (6.-14.10.2007)
- 6th International Symposium on Translational Research in Oncology (11.-14.10.2007
- Dublin, Ireland)
- Tumor-host Interactions and Angiogenesis, Ascona, Switzerland (26.-29.10.2007)
- Pacific Angiogenesis Meeting, Jeju, South Korea (10.-16.11.2007)
- Fundación Centro Nacional de Investigaciones, Madrid, Spain (14.-18.12.2007)
- AACR Annual Meeting, San Diego, USA (11.-17.4.2008)
- ESH Angiogenesis Meeting, Paris, France (8.-11.5.2008)
- Barcelona BioMed Conference, Barcelona, Spain (18.-21.5.2008)
- 11th Annual Meeting of the American Society of G., Boston, USA (30.5.-1.6.2008)
- IVBM Conference, Sydney, Australia (1,-6.6.2008)
- Gordon Research Conferences, Oxford, UK (3.-5.8.2008)
- Angiogenesis: Molecular Mechanisms, Kloster Seeon, Germany (20.-22.9.2008)
- Conference on Angiogenesis, Beijing, China (26.-28.9.2008)
- EMBO workshop, Banz Monastery, Bad Staffelstein, Germany (6.-8.10.2008)
- 3rd Mayo Clinic Symposium, Rochester, USA (22.-27.10.2008)
- 15th Scientific Symposium of the Lilly Foundation, Madrid, Spain (26.-27.3.2009)
- Paterson Institute for Cancer Research, University of Manchester, UK (7.4.2009)
- AACR 100th Annual Meeting, Denver, Colorado, USA (17.-22.4.2009)
- World Congress of Nephrology. Milan, Italy (22.-26.5.2009)
- EMBO Workshop on Lymphatic and Blood Vaculature:
- From Models to Human Disease, Helsinki, Finland (4.-5.6.2009)
- ESH International Conference on Angiogenesis, Helsinki, Finland (6.-7.6.2009)
- Angiogenesis, GRC, Salve Regina University, Newport, MA, USA (2.-7.8.2009)
- 19th Annual Biocity Symposium, Turku, Finland (20.-21.8.2009)
- Sino-Finn Symposium, Biocenter, Helsinki, Finland (24.8.2009)
- 5th European Meeting on Vascular Biology and Medicine, Marseille, France (14-15.9.2009)
- The Tumor-Vessel Interface: Cellular Mechanisms of Tumor Progression and Metastasis, Kloster-Seeon, Germany (19-22.9.2009)
- Joint Meeting 2009 of the GfMVB and the Swiss Society

for Microcirculation (SSM), Bern, Switzerland (8-9.10.2009)

Jussi Taipale:

- Joint Meeting of the Spanish and British Societies for Developmental Biology, Spain (24.-27.9.2008)
- European Life Scientists Organisation, Germany, Dresden (1-4.9.2008)
- EMBO Young Investigators Meeting, Germany (15.6.2008)
- CIFAR meeting, Canada (15-16.2.2008)
- Sigrid Jusélius Symposium on Genetics of Development, Helsinki, Finland (2007)
- Mitocheck meeting, EMBL, Heidelberg, Germany (2007)
- Taipale EMBO Young Investigator Meeting EMBL, Heidelberg, Germany (2007)
- ELSYS Meeting, Enschede, the Netherlands (2007)
- Hedgehog Signaling in Cancer and Stem Cells, Rome, Italy (2006)
- EMBL Biennial Meeting, From Functional Genomics to Systems Biology, Heidelberg, Germany (2006)

Partner 2

Peter ten Dijke:

- TGF-b signal transduction in human diseases. TGF-b signal transduction meeting, Uppsala, Sweden (12-14 May, 2006).
- Misregulation of TGF-b signal transduction in human diseases. Erasmus University, Rotterdam (11 July, 2006).
- TGF-b signal transduction in cancer and angiogenesis. Federation of European Connective Tissue Societies, Oulu, Finland (July 1-5, 2006).
- Subversion of TGF-b signaling in human disease. Tsukuba Symposium on Signal transduction and disease, Tsukuba, Japan (30-31 August, 2006)
- TGF-b signaling and vascular diseases. Tokyo University, Japan (1 September, 2006).
- TGF-b signaling in vascular development and diseases. The Beatson workshop. TGF-b and cancer, Glasgow, UK (15-17 September, 2006).
- Subversion of TGF-b signaling in human disease. Karolinska Institute, Stockholm, Sweden (16 December, 2006)
- Misregulation of TGF-b signaling in human diseases. Novartis, Boston, USA (14 February, 2007)
- TGF-b siganling in cancer. University of Trondheim, Norway (16 April, 2007)
- Misregulation of TGF-b signaling in human disease. Department of Experimental Oncology. Campus IFOM-IEO, Milan, Italy (April 7, 2008)
- TGF-b receptor signaling in angiogenesis. 28th Sapporo Cancer Seminar meeting. Hokkaido, Japan (26-27 June, 2008)
- Misregularion of TGF-b signaling in human disease. Novartis Shanghai, China (1 July, 2008)
- TGF-β receptor signal transduction in tumor angiogenesis. EndoTrack FP6 meeting, On the tracks of signaling, Tuscany, Italy, November 9-11, 2009
- TGF-β receptor signaling and vacular dysfunction and disease. Signal Transduction and Disease, Biochemical societies of Belgium, the Netherlands and Germany. Aachen, Germany, September 27-30, 2009.
- TGF-β signaling and vascular morphogenesis. Signal Transduction meeting, Ixtapan de la Sal, Mexico, in Sept, 6-9, 2009.
- TGF-β signaling and disease. Novartis, London, UK, 30 July, 2009.
- TGF-β signaling and disease. Merck, Darmstadt, Germanym, June, 2009.
- TGF-β signaling and disease Bayer, Wuppertal, Germany, June 2009.
- TGF-β receptor signaling and angiogenesis Faseb meeting TGF-β signaling in development and disease, Tuscon, USA, 14-19 July, 2009.

- TGF-β receptor signaling in vascular development and disease Angiogenesis meeting Helsinki, Finland, 5-8 June, 2009.

Partner 4

Luigi Naldini:

- The NCRI Cancer Conference 2009, Birminghan,UK (10/09).
- Annual Meeting of the Myelin Project, Toronto,Canada (10/09). Cancer Center Seminar Series, University of Minnesota, Minneapolis, USA (02/08)
- The EMBO Workshop 2009, Turin, IT (09/09).
- The EMBO Meeting 2009, Amsterdam, NL (08/09).
- XII Annual Meeting of the American Society of Gene Therapy (ASGT), San Diego, USA (05/09)
- 6th BSGT Annual Conference, University of London, UK (04/09)
- Wells Seminar Series, Indiana University School of Medicine, Cancer Research Institute, Indianapolis, USA (02/08)
- Seminar at Institute of Bioengineering and Nanotechnology (IBN), Singapore (02/09)
- XVI Annual Meeting of the European Society of Gene and Cell Therapy (ESGCT), Brugge, Belgium (11/08)
- 20th Meeting of the European Association for Cancer Research (EACR), Lyon, France (07/08)
- iSBTc 22nd Annual Meeting & Associated Programs, Boston,USA, (11/07)
- ESGT 15th Annual Congress,2007, Rotterdam,Netherlands (10/07)
- ECCO 14, 2007, Barcelona, Spain (09/07)
- 13th Annual Meeting of the International Society for Cell Therapy (ISCT), Sidney Australia (06/07)
- RNA MEETING Istituto Pasteur-Fondazione Cenci Bolognetti,Roma(06/07)
- CANCER SCIENCE 3, Spineto,Italy, (05/07)
- KEYSTONE SYMPOSIA Meeting, Banff, Canada (03/07)
- KEYSTONE SYMPOSIA Inflammation and Cancer Meeting, Santa Fe, New Mexico (02/07)
- The LSD JAPAN meeting, Tokyo, Japan (11/06)
- XIV Annual Congress of the European Society of Gene Therapy, Athens,Greece (09/06)
- 31st FEBS CONGRESS Molecules in Health & diseases, Istanbul, Turkey (06/06)
- 9th ASGT Annual Meeting, Baltimore, Maryland (06/06)
- V°Convegno Nazionale "Cellule Staminali e Progenitori Emopoietici Circolanti", Rome (05/06)
- AACR 97th Annual Meeting, Washington, DC (04/06)
- Gordon Research Conference on Viral Vectors for Gene Therapy, Ventura, California (03/06)
- AACR Special Conference in Cancer Research "Anti-Angiogenesis and Drug Delivery to Tumors: Bench to Bedside and Back, Boston

Michele de Palma:

- Joint Meeting 2009 of the GfMVB and the Swiss Society for Microcirculation (SSM). Bern, Switzerland, Oct 2009
- European Association for Cancer Research (EACR) Special Conference on Inflammation and Cancer. Berlin, Germany, Sept 2009
- 7th International Symposium on the Biology of Endothelial Cells. Vienna, Austria, Sept 2009
- Gordon Research Conference on Angiogenesis and Microcirculation. Salve Regina University, Newport, RI, Aug 2009
- Educational Session, 100th American Association for Cancer Research Annual Meeting, Denver, Colorado, Apr 2009
- Speaker and Chair, 3rd European Conference on Tumor Angiogenesis and Antiangiogenic Therapy. Padova, Italy, Nov 2008
- Plenary lecture, Cytokine 2008. Montreal, Canada, Oct 2008
- Kloster-Seeon Meeting on Angiogenesis: Molecular Mechanisms and Functional Interactions. Seeon, Germany, Sept 2008

- Chair, 22nd Annual Meeting - European Macrophage and Dendritic Cell Society. Brescia, Italy, Sept 2008
- Vascular differentiation and remodeling meeting, Frankfurt, Germany, July 2008
- French Society of Cell and Gene Therapy (SFTCG). Giens, France, June 2008
- Plenary lecture, Cytokine 2008. Montreal, Canada, Oct 2008
- NCRI Cancer Conference, Cancer Research UK, Birmingham, UK, Oct 2007
- Plenary lecture and Young Investigator Award, European Society of Cell and Gene Therapy (ESCGT), Rotterdam, Netherlands
- Tumor-Vessel Interface, Kloster-Seeon Meeting, Seeon, Germany, Sept 07
- Gordon Research Conference on Angiogenesis and Microcirculation, Salve Regina University, Newport, RI, Aug 2007

3. Publishable results

The published research papers are listed below.

1. Noda D, Itoh S, Watanabe Y, Inamitsu M, Dennler S, Itoh F, Koike S, Danielpour D, ten Dijke P, Kato M. (2006) ELAC2, a putative prostate cancer susceptibility gene product, potentiates TGF-b/Smad-induced growth arrest of prostate cells. **Oncogene**, 25:5591-600.

2. Inamitsu M, Itoh S, Hellman U, ten Dijke P, Kato M. (2006) Methylation of Smad6 by protein arginine N-methyltransferase 1. **FEBS** Lett. 580:6603-11.

3. Varjosalo, M., Taipale J. (2007) Hedgehog signalling. **J Cell Sci**. Jan 1;120(Pt 1):3-6. Review.

4. Itoh S and ten Dijke P. (2007) Negative regulation of TGF-b receptor/Smad signal transduction. **Curr Opion. Cell Biol**. 2007 19:176-84.

5. Heldin C-H and ten Dijke P. (2007) Cellular signaling. **Curr Opin Cell Biol**. Apr;19(2):109-11.

6. Venneri MA, De Palma M, Ponzoni M, Pucci F, Scielzo C, Zonari E, Mazzieri R, Doglioni C, Naldini L. (2007) Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer. **Blood**. Jun 15;109(12):5276-85. Epub 2007 Feb 27

7. Wirzenius M, Tammela T, Uutela M, He Y, Odorisio T, Zambruno G, Nagy JA, Dvorak HF, Ylä-Herttuala S, Shibuya M, Alitalo K. (2007) Distinct vascular endothelial growth factor signals for lymphatic vessel enlargement and sprouting. **J Exp Med.** Jun 11;204(6):1431-40. Epub 2007 May 29.

8. Dennler S, Andre J, Alexaki I, Li A, Magnaldo T, ten Dijke P, Wang X-J, Verechia F, Mauviel A. (2007) Induction of Sonic hedgehog mediators by TGF-ß: Smad3-dependent activation of Gli2 and Gli1 expression in vitro and in vivo. **Cancer Res.** Jul 15;67(14):6981-6.

9. Lewis CE, De Palma M, Naldini L. (2007) Tie2-expressing monocytes and tumor angiogenesis: regulation by hypoxia and angiopoietin-2. **Cancer Res**. 15;67(18):8429-32. Review.

10. De Palma M, Murdoch C, Venneri MA, Naldini L, Lewis CE. (2007) Tie2-expressing monocytes: regulation of tumor angiogenesis and therapeutic implications. **Trends Immunol**. 28(12):519-24. Epub 2007 Nov 5. Review.

11. ten Dijke P, Goumans M-J, Pardali E. (2008) Endoglin in Angiogenesis and Vascular Diseases. **Angiogenesis.** 11(1):79-89.

12. Kodach LL, Bleuming SA, de Miranda NFCC, Wiercinska E, Musler AR, Peppelenbosch MP, Dekker E, van den Brink GR, van Noesel CJM, Morreau H, ten Dijke P, Offerhaus GJA and Hardwick JCH. (2008) The Bone Morphogenetic Protein Pathway is inactivated in the majority of Sporadic Colorectal Cancers **Gastroenterology**.134(5):1332-41. Epub 2008 Mar 4

13. Purhonen S., Palm J., Rossi D., Kaskenpää, N., Rajantie I., Ylä-Herttuala S., Alitalo K., Weissman I.L., and Salven P. (2008). Bone marrow-derived circulating endothelial precursors do not contribute to vascular endothelium and are not needed for tumor growth. **Proc Natl Acad Sci** U S A. 6;105(18):6620-5. Epub 2008 Apr 28.

14. Varjosalo, M., Bjorklund, M., Cheng, F., Syvanen, H., Kivioja, T., Kilpinen, S., Sun, Z., Kallioniemi, O., Stunnenberg, H. G., He, W. W., et al. (2008). Application of active and kinase-deficient kinome collection for identification of kinases regulating hedgehog signaling**. Cell** 133, 537-548.

15. De Palma M, Mazzieri R, Politi LS, Pucci F, Zonari E, Sitia G, Mazzoleni S, Moi D, Venneri MA, Indraccolo S, Falini A, Guidotti LG, Galli R, Naldini L. (2008) Tumor-targeted interferon-alpha delivery by Tie2-expressing monocytes inhibits tumor growth and metastasis. **Cancer Cell**. 7;14(4):299-311.

16. Lohela M, Heloterä H, Haiko P, Dumont DJ, Alitalo K. (2008) Transgenic Induction of Vascular Endothelial Growth Factor-C Is Strongly Angiogenic in Mouse Embryos but Leads to Persistent Lymphatic Hyperplasia in Adult Tissues. **Am J Pathol**. 173(6):1891-901. Epub 2008 Nov 6.

17. Pardali E and ten Dijke P. (2009) Transforming Growth Factor-b Signaling and Tumor Angiogenesis**. Frontiers in Bioscience**. 1;14:4848-61. Review.

18. Itoh F, Itoh S, Carvalho RLS, Adachi T, Ema M, Goumans MJ, Larsson J, Karlsson S, Takahashi S, Mummery C, ten Dijke P, Kato M. (2009) Poor vessel formation in embryos from knock-in mice expressing ALK5 with L45 loop mutation defective in Smad activation. **Lab Invest**. 89(7):800-10.

19. F. Pucci, M.A. Venneri, D. Biziato, A. Nonis, D. Moi, A. Sica, C. Di Serio, L. Naldini, M. De Palma (2009) A distinguishing gene signature shared by tumor-infiltrating Tie2-expressing monocytes (TEMs), blood "resident" monocytes and embryonic

macrophages suggests common functions and developmental relationships. **Blood**. 23;114(4):901-14.

20. M. De Palma[§] and L. Naldini. (2009) Tie2-expressing Monocytes (TEMs): Novel Targets and Vehicles of Anticancer Therapy? **Biochim Biophys Acta – Reviews on Cancer**. 1796(1):5-10

21. Liu, Z Kobayashi, K van Dinther M, van Heiningen SH, Valdimarsdottir G, van Laar T, Scharpfenecker M, Lowik CWGM , Goumans MJ, ten Dijke P, and Pardali E. (2009) VEGF and TGF-beta type I receptor kinase inhibitor synergistically promote blood vessel formation by inducing integrin alpha5 expression. **J. Cell Sci**., 122:3294-302.