



The Pezcoller
Foundation

Journal



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October 2009

We are glad to publish the fourth “Korsmeyer Lecture”. We are grateful to Dr Napoleone Ferrara for his kindness to let us publish the lecture entitled “Tumor angiogenesis: VEGF-dependent and independent pathways” given in Padua at VIMM the day before the Award ceremony in Trento on May 8. Ferrara is the prestigious recipient of the 2009 Pezcoller Foundation-AACR International Award for Cancer Research.

I like to remind that the annual Stanley J. Korsmeyer Lectureship has been started by the Pezcoller Foundation in 2006 in accordance with the AACR American Association for Cancer Research and the VIMM Venetian Institute of Molecular Medicine in Padua (Italy). The goal of this event is to honour the fundamental contribution of the late S. Korsmeyer, an international leader in the field of cancer biology, whose pioneering observations opened the molecular era of programmed cell death. S. Korsmeyer was the recipient of the Pezcoller Foundation-AACR International Award for Cancer Research in 2004. He presented his last European lecture by the VIMM immediately before receiving the Pezcoller Award. Unluckily he passed away from cancer a few months later. We wish to remember Stanley Korsmeyer every year with this lecture given by the recipient of the Award.

The 2009 Symposium entitled “Unconventional therapeutic targets in cancer” took place in Trento last June and was very successful for the high level of the five sessions and for the large participation. As usual we gave also the “Pezcoller Begnudelli Awards” for the best posters to three young deserving researchers: Ulrich Bogdan,

University of Regensburg, Germany; Francesca Bersani e Riccardo Taulli, University of Torino, Italy; Gianfranco Distefano, HSR (San Raffaele Foundation) Milano, Italy.

We are also glad to introduce the “focus and goals” of the next 22nd Pezcoller Symposium which will be held in Trento on June 10-12, 2010 and will be entitled “RNA Biology and Cancer”. The program of the Symposium has been developed by Dr Rene Bernards (National Cancer Institute, Amsterdam), Dr Witold Filipowicz (Friedrich Miescher Institute for Biomedical Research, Basel) and Dr David Livingston (Dana Farber Cancer Institute, Boston); they will co-chair the meeting in cooperation with Dr Enrico Mihich (Dana Farber Cancer Institute). The focus of the Symposium will be on the functional importance of various non-coding RNA molecules in cancer.

It's a pleasure to mention that on September 11th in Rovereto we gave the 2009 Pezcoller Foundation-ECCO Recognition for Contribution to Oncology to Dr Françoise Meunier, General Director of EORTC, Bruxelles. We also presented her during the joint 15th ECCO - 34th ESMO Multidisciplinary Congress with a Plenary Lecture.

We are also presenting on the last pages the call for the 2011 Pezcoller Foundation-AACR Award for Cancer research.

*Gios Bernardi MD
The Pezcoller Foundation President*

Picture on front page: during the 2009 Pezcoller Symposium from the left D. Livingston, G. Bernardi, E. Mihich.

The Stanley J. Korsmeyer memorial lecture

Tumor angiogenesis: VEGF-dependent and independent pathways

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Introduction:

Angiogenesis is a complex process that results in the establishment of microvascular networks, required for pre/postnatal development and for tissue repair in the adult. Indeed, the cardiovascular system is the first organ system to develop and reach a functional state in an embryo. Importantly, without the onset of angiogenesis, most tumors cannot grow beyond 1 to 2 mm due to diffusion limitations and thus may remain dormant.

The observation that tumor growth can be accompanied by increased vascularity was reported more than one century ago. In 1939, Ide et al. postulated the existence of a tumor-derived blood vessel growth stimulating factor. In 1968, Greenblatt and Shubik demonstrated that transplantation of tumor cells promotes blood vessel proliferation even when a Milipore filter is interposed between the tumor and the host, suggesting that the neovascu-

larization is mediated by diffusible factors produced by tumor cells. In 1971, Folkman proposed that anti-angiogenesis might be an effective approach to treat human cancer. Subsequently, several putative angiogenic factors were described, including aFGF, bFGF, EGF, TGF α , etc.

History of VEGF

In 1983, Senger et al. described the identification in the conditioned medium of a guinea-pig tumor cell line of a protein able to induce vascular leakage in the skin, which was named "tumor vascular permeability factor" (VPF). The authors proposed that VPF could be a mediator of the high permeability of tumor blood vessels. However, these efforts did not yield the full purification of the VPF protein. The lack of amino acid sequence data precluded cDNA cloning and establishing the identity of VPF. Therefore, very limited progress in

elucidating the role of VPF was possible during the following several years.

In 1989, we reported the isolation of an endothelial cell mitogen from the supernatant of bovine pituitary cells, which we named “vascular endothelial growth factor” (VEGF). The NH₂-terminal amino acid sequence of VEGF did not match any known protein in available databases. Subsequently, Connolly’s group at Monsanto Co. reported the isolation and sequencing of VPF. By the end of 1989, we isolated cDNA clones encoding bovine VEGF₁₆₄ and three human VEGF isoforms: VEGF₁₂₁, VEGF₁₆₅ and VEGF₁₈₉. The Monsanto group described a human VPF clone, which encoded a protein identical to VEGF₁₈₉. These studies indicated that, unexpectedly, a single molecule was responsible for both mitogenic and permeability-enhancing activities. The finding that VEGF is potent, diffusible and specific for vascular endothelial cells led to the hypothesis that this molecule might play a role in the regulation of physiological and pathological growth of blood vessels.

Molecular and biological properties of VEGF-A

VEGF belongs to a gene family which also includes VEGF-B, C, D, E, and placenta growth factor. Multiple isoforms of VEGF, ranging from 121 to 206 amino acids, can be generated by alternative exon splicing. These isoforms differ in their ability to bind heparin, which determines their bioavailability, and may play distinct roles in angiogenesis during development. In addition, extracellular proteolysis regulates VEGF activity. Early studies showed

that plasmin is able to cleave heparin-binding VEGF isoforms at the COOH-terminus to generate bioactive and diffusible fragments. More recently, other investigators reported that MMP3 is able to generate VEGF proteolytic fragments, which are biologically and biochemically very similar to those resulting from plasmin cleavage.

VEGF promotes growth of vascular endothelial cells derived from arteries, veins and lymphatics. VEGF also induces a strong angiogenic response *in vivo*. VEGF-A is also an important survival factor for endothelial cells. While in most circumstances VEGF functions as a paracrine mediator, autocrine roles for VEGF in survival of hematopoietic stem cells and endothelial cells have been described. Three tyrosine kinase receptors bind members of the VEGF gene family: VEGFR-1 (Flt-1), VEGFR-2 (KDR) and VEGFR-3. Moreover, co-receptors, such as heparan sulphate proteoglycans and neuropilins, may facilitate activation of VEGFRs. VEGF-B and PlGF bind selectively to VEGFR-1. VEGF-A is the main ligand for VEGFR-2. VEGFR-1 and VEGFR-2 are expressed in vascular endothelial cells, monocytes, macrophages and hematopoietic stem cells. All VEGF-A isoforms can bind VEGFR-1 and VEGFR-2. Despite the fact that VEGF binds to VEGFR1 with ~10 fold higher affinity than VEGFR2, it is mainly VEGFR2 that mediates VEGF signaling in endothelial.

Role of VEGF-A in tumor angiogenesis in mouse models

Experiments with neutralizing antibodies and other inhibitors demonstrated that blockade of

the VEGF pathway is sufficient to significantly suppress angiogenesis associated with solid tumor growth in several models. Both subcutaneous and orthotopic models have been used to test the effects of inhibitors of the VEGF/VEGFR pathway on the growth of a variety of tumor cell lines. Mab A4.6.1, (the murine precursor of bevacizumab), was initially shown to suppress the growth of human rhabdomyosarcoma, glioblastoma, and leiomyosarcoma cells implanted in immunodeficient mice. Since then, Mab A4.6.1/bevacizumab has been tested on a wide range of human tumor cells implanted subcutaneously or orthotopically. Together, these studies demonstrate that Mab A4.6.1/bevacizumab is effective in reducing tumor vessel density and suppressing tumor growth even as a single agent, regardless of tumor location and route of administrations. Shojaei *et al.* examined the differences among various syngeneic murine tumor cell lines in terms of responsiveness to VEGF blockade. They found that tumor cells that are relatively insensitive to VEGF blockade exhibit a greater ability to recruit CD11b⁺Gr⁺ myeloid cells compared to the sensitive ones. Subsequent studies identified the secreted protein Bv8 as a myeloid cell-derived mediator of tumor angiogenesis. Recent studies indicate that not only frankly malignant tumors, but also benign or premalignant tumors may be sensitive to anti-VEGF therapies. Inhibition of VEGF-A has been shown to suppress the angiogenic switch, resulting in a substantial increase in survival, in the Apc^{+/min} mouse model of intestinal polyposis. Furthermore, Korsisaari *et al.* tested the efficacy of anti-VEGF treatment in a mouse

model of multiple endocrine neoplasia type 1 (Men1) and found that tumors in animals that received anti-VEGF treatment were growth-arrested, resulting in reduced serum prolactin level and increased lifespan of mice.

Clinical trials in cancer patients with VEGF inhibitors

Several VEGF inhibitors have been developed as anti-cancer agents including a humanized anti-VEGF-A monoclonal antibody (bevacizumab), various small molecules inhibiting VEGFR-2 signal transduction and a VEGF receptor chimeric protein.

The clinical trial that resulted in FDA approval of bevacizumab (February 2004) was a randomized, double blind, phase III study in which bevacizumab was administered in combination with irinotecan, 5FU, leucovorin (IFL) chemotherapy as first line therapy for previously untreated metastatic colorectal cancer. Median survival and progression-free survival were significantly increased by the addition of bevacizumab survival. Although bevacizumab was generally well tolerated, some toxicities were observed including gastrointestinal perforation and arterial thromboembolic complications. Hypertension requiring medical intervention with standard anti-hypertensive therapy developed in 11% of bevacizumab treated patients and is now recognized as a class effect of VEGF blockers. Also, bevacizumab combined with paclitaxel in women with previously untreated metastatic breast cancer provided a significant improvement in progression free survival. Combining

bevacizumab with paclitaxel and carboplatin in patients with previously untreated, nonsquamous, non-small-cell lung carcinoma (NSCLC) provided a significant improvement in the primary endpoint of overall survival. Also, combining bevacizumab with 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) in patients with previously treated metastatic colorectal cancers provided a significant improvement in survival. Most recently, bevacizumab has been approved by the FDA also for the treatment of renal cell carcinoma, in combination with interferon- α , and glioblastoma multiforme. Besides bevacizumab, several other types of VEGF inhibitors are being developed. Among these, a variety of small molecule RTK inhibitors targeting the VEGF receptors are at different stages of clinical development. The most advanced are Sunitinib (Sutent[®]) and sorafenib (Nexavar[®]). Sunitinib inhibits tyrosine phosphorylation of several RTKs including VEGFRs, PDGFR, c-kit and Flt-3. Sunitinib is FDA-approved for the treatment of Gleevec-resistant gastro-intestinal stromal tumor (GIST) and for metastatic renal cell carcinoma. Sorafenib is a raf kinase inhibitor that also inhibits VEGFR-2 and -3, PDGFR- β , Flt-3 and c-kit. Sorafenib has been approved by FDA for advanced renal cell carcinoma (RCC) and inoperable hepatocellular carcinoma.

Role of VEGF-A in intraocular neovascular syndromes

VEGF-A mRNA expression is correlated with neovascularization in several animal models

of retinal ischemia. This is consistent with the fact that VEGF-A gene expression is up-regulated by hypoxia. In 1994 it was reported that the levels of VEGF-A are elevated in the aqueous and vitreous humor of human eyes with proliferative retinopathy secondary to diabetes and other conditions. Subsequently, animal studies using various VEGF inhibitors have directly demonstrated the role of VEGF as a mediator of ischemia-induced intraocular neovascularization. Age-related macular degeneration (AMD) is the most common cause of severe, irreversible vision loss in the elderly. AMD is classified as nonexudative (dry) or exudative (wet or neovascular) disease. Although the exudative form accounts for ~10-20% of cases, it is responsible for 80-90% of the visual loss associated with AMD. Verteporfin (Visudyne[®]) photodynamic therapy (PDT) has been approved by the FDA for only predominantly classic lesions, in which 50% or more of the lesion consists of classic choroidal neovascularization (CNV). Pegaptanib sodium (Macugen[®]) an aptamer that binds to the VEGF₁₆₅, but not to VEGF₁₂₁ or the proteolytic fragments of VEGF-A was approved in December 2004 for all angiographic subtypes of neovascular AMD. Although both treatments can slow the progression of vision loss, only a small percentage of treated patients experience any improvement in visual acuity.

Ranibizumab (Lucentis[®]) is a recombinant, humanized Fab that binds to and potentially neutralizes the biological activities of all known human VEGF-A isoforms, as well as

the proteolytic cleavage products VEGF₁₁₀ or VEGF₁₁₃. Ranibizumab has been evaluated in two large, phase III, multicenter, randomized, double-masked, controlled pivotal trials in different neovascular AMD patient populations.

The MARINA trial randomized subjects with minimally classic (less than 50% of the lesion consisting of classic CNV) or occult without classic CNV to monthly sham injections or monthly intravitreal injections of one of two doses of ranibizumab. A significantly greater proportion of ranibizumab subjects avoiding moderate vision loss than sham-injected subjects. Moreover, on average, ranibizumab-treated subjects gained vision at one year or two years compared with baseline, while sham-injection subjects lost vision. A significantly larger percentage of subjects treated with ranibizumab gained ≥ 15 letters than did the sham-injection group.

The ANCHOR trial randomized subjects with predominantly classic CNV to verteporfin PDT with monthly sham ocular injections or to monthly intravitreal injections of one of two doses of ranibizumab with a sham PDT procedure. In the primary analysis at one year, the study met its primary endpoint, with a significantly greater proportion of ranibizumab subjects avoiding moderate vision loss compared with subjects treated with verteporfin PDT. In addition, on average, ranibizumab-treated subjects gained vision at one year compared with baseline, while verteporfin PDT subjects lost vision, and a significantly larger percentage of subjects treated with ranibizumab gained ≥ 15 let-

ters at one year than did the verteporfin PDT group. In June 2006, ranibizumab was approved by the FDA for the treatment of all subtypes of neovascular AMD.

Conclusions and perspectives

Research conducted over the last two decades has established that VEGF plays an essential role in the regulation of embryonic, postnatal physiologic angiogenesis processes, including normal development and cyclical ovarian function. A variety of animal models have generated much information on the biology of VEGF and the therapeutic potential of VEGF/VEGFR inhibitors in cancer. There is also clear evidence that targeting VEGF-A is a meaningful approach for the therapy of cancer and age-related macular degeneration. However, further studies are required to establish optimal dosages and therapeutic regimens. It appears likely that cancer therapy will be combinatorial in most cases.

A particularly active area of research concerns the elucidation of the mechanisms of refractoriness or acquired resistance to anti-VEGF therapy. Tumor cell-intrinsic or treatment-induced expression of angiogenic factors have been implicated. Recent studies have provided evidence that, at least in some murine models, refractoriness to anti-VEGF therapy is related to the ability of the tumor to recruit CD11b⁺Gr1⁺ myeloid cells, which in turn promote VEGF-dependent angiogenesis. Further work is needed to determine whether these findings also apply to human tumors.

Pezcoller Foundation-ECCO

2009 Recognition for Contribution to Oncology



Françoise Meunier

In 2009, the Pezcoller-ECCO Recognition for Contribution to Oncology has been presented to Doctor Françoise Meunier for her unique contribution to clinical cancer research in Europe as a scientific leader and mentor. Françoise Meunier is the Director General of the European Organisation for Research and Treatment of Cancer. She has been selected among qualified candidates by the following Award Committee: Alexander M.M. Eggermont (NL) President of ECCO and Chair, Gios Bernardi (IT) President of the Pezcoller Foundation, Franco Cavalli (CH) Foundation for Research and Cure of Lymphomas, Tessin, Raffaella Giavazzi (IT) "Mario Negri"

Institute for Pharmacological Research, Bergamo, Jacques Pouyssegur (F) Institute of Signalling, Developmental Biology and Cancer Research, Nice Ulrik Ringborg (SE) Karolinska Institute, Stockholm.

The award ceremonies have been held in Rovereto (Italy) on September 11 and in Berlin during the joint 15th ECCO - 34th ESMO Multidisciplinary Congress as a Plenary Lecture.

The EORTC is a unique pan-European academic clinical research organization operating as a non-profit association under Belgian law with the mission to develop, conduct, coordinate and stimulate high-quality translational and clinical research



to improve the standards of patient cancer care by developing innovative drugs and more effective therapeutic strategies. Françoise Meunier has led the coordination and administration of all EORTC activities since 1991 with the mandate to promote the EORTC as a major European organisation in the field of oncology with a network of 2,500 oncologists in over 300 universities and a headquarters staff of 160 representing 17 different nationalities. As General Director, she is responsible for the organisation of scientific activities, public relations and medium-term EORTC strategy as defined by the EORTC Board.

From the left: Guglielmo Valduga, Ugo Rossi, Giovanni Kessler, Margherita Cogo, Gios Bernardi, Françoise Meunier, Alexander Eggermont

2010 Pezcoller Symposium

RNA Biology and Cancer

June 10-12, 2010 - Trento, Italy

The program of the Symposium has been developed by Dr Rene Bernards (National Cancer Institute, Amsterdam), Dr Witold Filipowicz (Friedrich Miescher Institute for Biomedical Research, Basel) and Dr David Livingston (Dana Farber Cancer Institute, Boston); they will co-chair the meeting in cooperation with Dr Enrico Mihich (Dana Farber Cancer Institute). The focus of the Symposium will be on the functional importance of various non-coding RNA molecules in cancer.

The topics to be discussed will include the opportunities offered by small RNA as tools for cancer drug development, the role of non-coding RNAs, the biochemistry of small RNAs,

the function of micro-RNA in cancer, and RNAs as diagnostics and therapeutics in cancer. Each 30 minute talk by the 18 invited speakers will be followed by 25 minutes of discussion, thus providing ample opportunity for interaction and cross-fertilization among participants. A session of posters in the areas discussed at the Symposium will be included.

Poster abstracts should be submitted to Dr Mihich [enrico_mihich@dfci.harvard.edu] before April 15, 2010, and will be reviewed for acceptance by Drs Livingston and Mihich. For scientific matters contact Dr Livingston [david_livingston@dfci.harvard.edu], for local arrangements and travel matters contact Pezcoller Foundation secretariat office [pezcoller@pezcoller.it].

Pezcoller Foundation-AACR

Call for 2011 International Award for Cancer Research

The prestigious Pezcoller Foundation-AACR International Award for Cancer Research was established in 1997 to annually recognize a scientist:

- who has made a major scientific discovery in basic cancer research or who has made significant contributions to translational cancer research;
- who continues to be active in cancer

research and has a record of recent, note-worthy publications;

- whose ongoing work holds promise for continued substantive contributions to progress in the field of cancer.

The Award is intended to honor an individual scientist. However, more than one scientist may be co-nominated and selected to share

the Award when their investigations are closely related in subject matter and have resulted in work that is worthy of the Award. In the rare event that there are dual winners of the Award, the cash award will be shared equally between them, and the AACR Executive Committee will determine which of the two co-recipients will present the Pezcoller-AACR Award Lecture at the AACR Annual Meeting.

Candidates for the Award will be considered by a prestigious international Selection Committee of renowned cancer leaders appointed by the President of the AACR and the Council of the Pezcoller Foundation. The Committee will consider all nominations as they have been submitted; the Committee may not combine submitted nominations, add a new candidate to a submitted nomination, or otherwise make alterations to the submitted nominations. After careful deliberations by the Committee, its recommendations will be forwarded to the Executive Committee of the AACR and the Council of the Pezcoller Foundation for final consideration and determination.

Selection of the Award winner will be made on the basis of the candidate's scientific accomplishments. No regard will be given to race, gender, nationality, or religious or political view.

The Pezcoller Foundation was established in 1980 by Professor Alessio Pezcoller, a dedicated Italian surgeon who made important contributions to medicine during his career and who, through his foresight, vision and generous gift in support of the formation of the Foundation, stimulated others to make significant advances in cancer research. Previously the Pezcoller Foundation, gave a major biennial award for outstanding contributions to cancer and cancer-related biomedical science, in collaboration

with the ESO-European School of Oncology.

The American Association for Cancer Research (AACR) was founded in 1907 by eleven physicians and scientists dedicated to the conquest of cancer and now has over 25,000 laboratory, translational, clinical and epidemiological scientists engaged in all areas of cancer research in the United States and in more than 60 other countries around the world.

The AACR is dedicated to its mission of preventing and curing cancer through the communication of important scientific results in a variety of forums including publications, meetings and training and educational programs. Because of the commitment of the Pezcoller Foundation and the AACR to scientific excellence in cancer research, these organizations are now collaborating annually on the presentation of the Award. This will strengthen international collaborations and will be a catalyst for advancements in cancer research internationally.

The winner of the Pezcoller Foundation-AACR International Award for Cancer Research will give an award lecture during the AACR Annual Meeting (April 2011), and the memorial Korsmeyer lecture at the VIMM in Padua and will receive the award in a ceremony at the Foundation's headquarters in Trento, Italy (May, 2011). The award consists of a prize of € 75.000 and a commemorative plaque.

Nomination Deadline: September, 2010

Questions about the nomination process:

Monique P. Eversley, Staff Associate - American Association for Cancer Research, 17th Floor, 615 Chestnut Street, Philadelphia, PA 19106-4404 - Tel. +1 (267) 646-0576;

E.mail: eversley@aacr.org - www.aacr.org

Save the date!

22nd Pezcoller Symposium

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RNA Biology and Cancer



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