

# The Pezcoller Foundation *Journal*

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## Editorial: November 2004

“Gateways to apoptosis” is the fascinating theme of Stanley J. Korsmeyer’s lecture presented to our readers in this issue.

This was actually the lecture which the winner of the 2004 Pezcoller

Foundation – AACR International Award for Cancer Research gave on March 28 in Orlando at the AACR Meeting.

We are grateful to Stan Korsmeyer for allowing us to offer this pivotal work for careful reading and reflection, hoping that it could

inspire new pro-gress along this interesting path.

We are awaiting the choice of the winner of the 2005 Pezcoller Foundation – AACR International Award for Cancer Research, which will be made in the next

few weeks by the Scientific Selection Committee at the Foundation’s Headquarters in Trento.

The deadline for the 2005 Pezcoller Foundation – FECS

Recognition for Contribution to Oncology is on January 31 and nominations are to be directed to the FECS Office in Brussels Belgium.

In this issue we are publishing the first but not the final draft of the 17<sup>th</sup> Pezcoller Symposium to be held in Trento, Italy from June 16-18, 2005.

The topic of this

Symposium will be “Molecular Understanding of Solid Tumors”.

Gios Bernardi M. D.

The Pezcoller Foundation President and Editor



Award Ceremony - Trento, Italy

## GATEWAYS TO APOPTOSIS

Stanley J. Korsmeyer, M.D. and Eric Dr. Smith

Howard Hughes Medical Institute, Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Department of Pathology and Department of Medicine, Harvard Medical School, Boston, MA 02115, USA.

*Programmed cell death and its morphologic manifestation of apoptosis is a conserved pathway that in its basic tenets appears to operate in all metazoans<sup>1,2</sup>. The evolutionary advent of differentiated cell types may have necessitated controlling death as well as division in order to keep neighboring cells inter-dependent and ensure the proper balance of each cell lineage. This fundamental pathway, clearly required for successful embryonic development, for crafting organs and other complex multi-cellular structures, is also essential in the adult to maintain cellular homeostasis in normal tissues. This is especially critical in long-lived mammals that must integrate multiple physio-logical as well as pathological death signals, which for example includes regulating the response to infectious agents. Gain- and loss-of-function models of genes in the core apoptotic pathway indicate that the violation of cellular homeostasis can be a primary pathogenic event that results in disease. Evidence indicates that insufficient apoptosis can manifest as cancer or autoimmunity, while accelerated cell death is evident in acute and chronic degenerative diseases, immunodeficiency and infertility.*

*The BCL-2 family of proteins constitutes a critical intracellular checkpoint in the intrinsic pathway of apoptosis. We noted that the cardinal member, the BCL-2 protooncogene, inhibits apoptosis in the genesis of lymphoma. We and others cloned the t(14;18) chromosomal breakpoint of human follicular lymphoma and demonstrated that this fusion increases expression*

*of BCL-2<sup>3-5</sup>. Transgenic mice bearing a Bcl-2-Ig minigene recapitulating the chromosomal translocation provided a definitive link to cancer, as they developed follicular hyperplasia due to extended cell survival, which progressed to high-grade lymphomas<sup>6</sup>. We localized BCL-2 to mitochondria and established that the survival effect resulted from the blocking of apoptosis by BCL-2. Thus, BCL-2 plays a primary role in oncogenesis by inhibiting apoptosis and is the first member of a new category of oncogenes—regulators of cell death.*

*The BCL-2 family was extended by identifying conserved homologs - including BAX, the first partner protein that promotes apoptosis - that in concert function as a major control point in the core apoptotic pathway<sup>7</sup>. The susceptibility to cell death is determined by the competing interests of pro- and antiapoptotic BCL-2 members. The BCL-2 family can be divided into three main subclasses, defined in part by the homology shared within four conserved regions termed BCL-2 homology (BH) 1-4 domains, roughly corresponding to a helices which dictate structure and function (Figure 1). The antiapoptotic members - BCL-2, BCL-X<sub>L</sub>, MCL-1, A1, and BCL-W - display high conservation in all four BH1-4 domains. The structure of a BCL-X<sub>L</sub> monomer revealed that its BH1, BH2 and BH3 domains are in close proximity and create a hydrophobic pocket that can accommodate a BH3 domain of a pro-apoptotic member. The “multidomain” pro-apoptotic members (BAX, BAK) possess BH1-3 domains,*

although they appear to require an activation event, perhaps to expose the hydrophobic face of their BH3 domain before they can interact with BCL-X<sub>L</sub> or BCL-2. In contrast, the pro-apoptotic molecule BID, isolated based on its ability to bind both BAX and BCL-2, has homology only within the minimal death domain, the BH3 amphipathic a helix, prompting the title “BH3-only”<sup>8</sup>. Cells doubly deficient for the pair of “multidomain” pro-apoptotic molecules BAX and BAK proved resistant to all tested intrinsic death pathway stimuli.

BAX and BAK together constitute a requisite gateway to the intrinsic pathway operating at both the mitochondrion<sup>9</sup> and the endoplasmic reticulum (ER)<sup>10</sup> (Figure 2). In viable cells multidomain BAX and BAK exist as monomers. Inactive BAX resides in the cytosol or is loosely attached to membranes and its pocket is occupied by its C-terminal helix. Upon receipt of a death signal BAX inserts into the mitochondrial outer membrane (MOM) as homooligomerized multimers. Inactive BAK that resides at the mitochondria also undergoes an allosteric conformational activation in response to death signals, which includes its oligomerization and the permeabilization of the MOM with release of intermembrane space (IMS) proteins including cytochrome c. The precise mechanism whereby IMS proteins are released is still under active investigation.

Recently, we explored a second gateway for BAX/BAK where 10-15% localizes to and functions at the endoplasmic reticulum, the currency there being the regulation of steady state intraluminal ER Ca<sup>2+</sup> levels and consequently the amount of Ca<sup>2+</sup> released upon a death stimulus<sup>10</sup>. Over-expression of BCL-2 was noted to prevent cell death by the passive release of ER Ca<sup>2+</sup> when thapsigargin was used to block the sarcoplasmic/endoplasmic reticulum Ca<sup>2+</sup> ATPase (SERCA) reuptake pump. Either over-expression of BCL-2 or loss of BAX, BAK leads to reduced resting ER Ca<sup>2+</sup> concentrations and a secondary decrease in Ca<sup>2+</sup> uptake by mitochondria. Selective reconstitution of individual

organelles enabled the classification of death signals based on their dependence on an ER Ca<sup>2+</sup> gateway and/or a mitochondrial gateway to apoptosis. Signals reliant upon the ER gateway include the Ca<sup>2+</sup> - dependent lipid second messengers such as C<sub>2</sub>-ceramide and arachidonic acid as well as pathologic oxidative stress. In contrast, activated BH3-only proteins kill cells as long as BAX or BAK is present at the mitochondria irrespective of Ca<sup>2+</sup> stores. Finally, a number of classic death signals utilize both gateways.

Functioning upstream of the multidomain members are the “BH3-only” proapoptotic molecules BID and BAD, which through their post-translational modification interconnect extracellular death and survival cues with the core apoptotic pathway (Figure 2). A proapoptotic cascade exists in which BH3-only members act as death ligands that induce allosteric activation of multidomain BAX and BAK. Specific BH3-only molecules appear to serve as “sentinels,” selectively responding to distinct proximal death/survival signals. For example, the extrinsic pathway is triggered by the engagement of cell surface death receptors, which then activate caspase-8 that cleaves p22 BID to connect with the intrinsic death pathway. A newly exposed glycine following cleavage in an unstructured loop is N-myristoylated enhancing the translocation and targeting of a p7/myr-p15 BID complex to mitochondria<sup>11</sup>. A reconstituted mitochondrial assay reveals that tBID serves as a membrane-targeted ligand, which requires its intact BH3 domain to trigger oligomerization of BAK or BAX to release cytochrome c. The pro-apoptotic activity of BH3-only molecules is apparently kept in check by either transcriptional control or post-translational modification. For example, NOXA and PUMA are under p53 mediated transcriptional control in response to DNA damage. BAD is switched on and off by its phosphorylation in response to growth/survival factors, providing a connection to the established importance of extracellular factors in promoting cell survival. BIM responds to multiple stimuli.

*The impact of these members has been evidenced in vivo, as our mouse models deficient for BID or BAD develop malignancies, demonstrating that even the upstream BH3-only molecules are required to maintain cellular homeostasis and to suppress tumorigenesis in select cell types.*

*In summary, activation of BH3-only molecules either directly or indirectly results in the activation of BAX, BAK and actually requires BAX, BAK for executing apoptosis. In contrast, anti-apoptotics such as BCL-2 or BCL-X<sub>L</sub> serve a principal, although perhaps not an exclusive, role of binding and sequestering BH3-only molecules preventing BAX, BAK activation<sup>12</sup>. Unresolved issues include whether all BH3-only molecules function identically or whether subsets exist that might reflect their marked variation in binding preferences. Recently, short peptides of the  $\alpha$  helical BH3 domains provided evidence for a two-class model in which BAD-like BH3 regions occupy anti-apoptotic pockets serving as “sensitizing” domains capable of displacing BID-like “activating” domains which induce the oligomerization, activation of BAX, BAK<sup>13</sup>.*

*Looking further, we asked whether a greater rationale exists for the localization of BCL-2 members to intracellular organelles, especially the mitochondria. In viable cells, we found BAK complexed with VDAC2, a low abundance isoform restricted to mammals that interacts specifically with the inactive conformer of BAK on the MOM, thus connecting mitochondrial physiology and the core apoptotic pathway (Figure 3A). Knockout cells reveal that VDAC2, but not the highly abundant VDAC1, inhibits apoptosis and is required to keep the potentially lethal BAK molecule inactive. Activated BH3-only molecules displace VDAC2 from BAK enabling BAK homo-oligomerization and apoptosis<sup>14</sup>. Additionally, in a recent study using liver mitochondria, an approach combining proteomics, genetics, and physiology found BAD resides in a functional holoenzyme complex together with its kinase PKA, its phosphatase PPI,*

*WAVE-1 as an “A Kinase Anchor Protein” and glucokinase(GK)<sup>15</sup> (Figure 3B). The association of these candidates was confirmed by co-localization, co-IP, and microcystin-PP1 pull downs. A Bad<sup>-/-</sup> loss-of-function model proved BAD is necessary to assemble the complex. Bad-deficient hepatocytes lack the complex and display diminished mitochondrial GK activity and blunted mitochondrial respiration in response to glucose. Glucose deprivation results in dephosphorylation of BAD and BAD-dependent death. Non-phosphorylatable BAD<sup>3SA</sup> cells indicate the phosphorylation of BAD regulates GK activity. Bad-deficient and Bad<sup>3SA</sup> mice both display defects in glucose homeostasis. Ultimately, BAD serves an unanticipated role in integrating glycolysis and apoptosis, two major pathways critical for cell survival.*

*Finally, the identification of critical control points in the apoptotic pathway has provided rational targets for the development of a new generation of potential therapeutics. Recently, we successfully generated modified BH3  $\alpha$ -helices that bound with increased affinity to multidomain BCL-2 member pockets<sup>16</sup> (Figure 3C). Using a novel hydrocarbon crosslinking strategy termed “hydrocarbon stapling,” we generated BH3 peptides called “stabilized alpha-helix of BCL-2 domains” (SAHBs), which proved to be helical, protease-resistant, and cell-permeable. A SAHB of the BH3 domain from BID specifically activated the apoptotic pathway to kill leukemia cells and effectively inhibited the growth of human leukemia xenografts in vivo.*

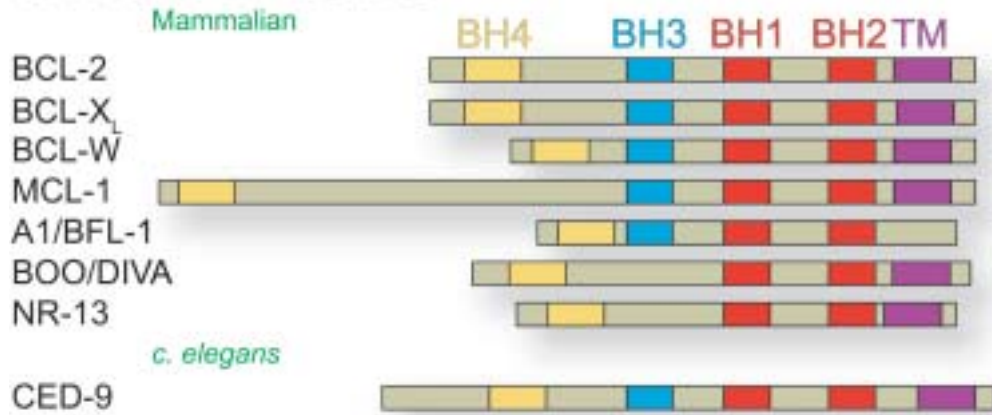
*Much remains to be done to further extend and integrate death pathways. We anticipate that apoptosis will become more fully interwoven with the fabric of other physiological pathways it is charged with monitoring. Overall we are pursuing a combination of genetics, biochemistry, and structural biology to define the mammalian apoptotic pathway, integrating it from death signals to final effector mechanisms.*



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## ANTI-APOPTOTIC



## PRO-APOPTOTIC

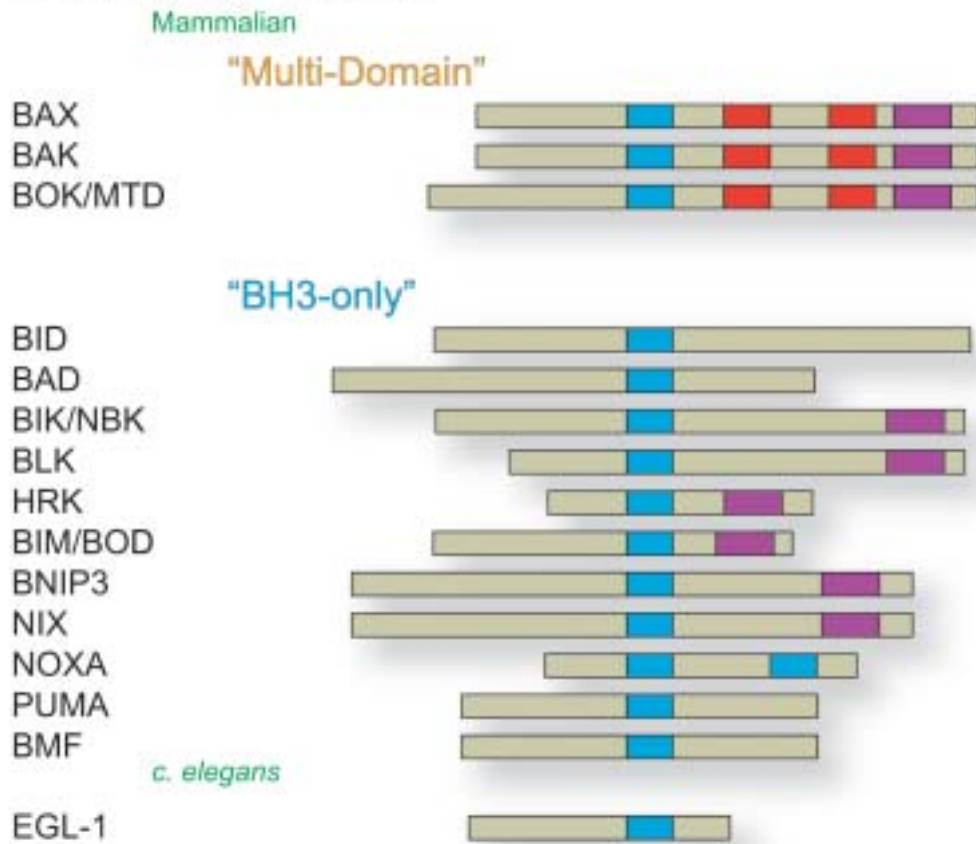


Figure 1. Summary of anti-apoptotic and pro-apoptotic BCL-2 family members.

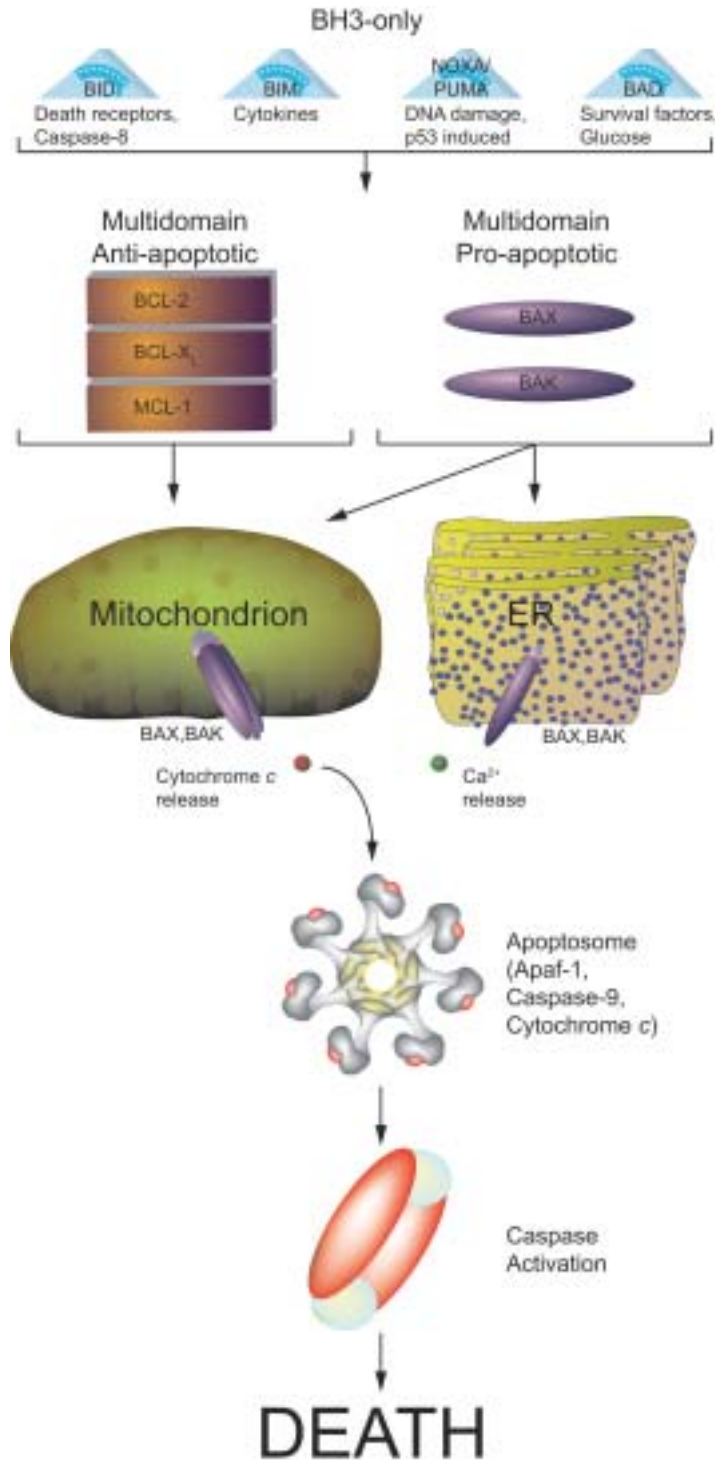


Figure 2. The apoptotic pathway, incorporating the mitochondrial and ER BAX/BAK gateways.

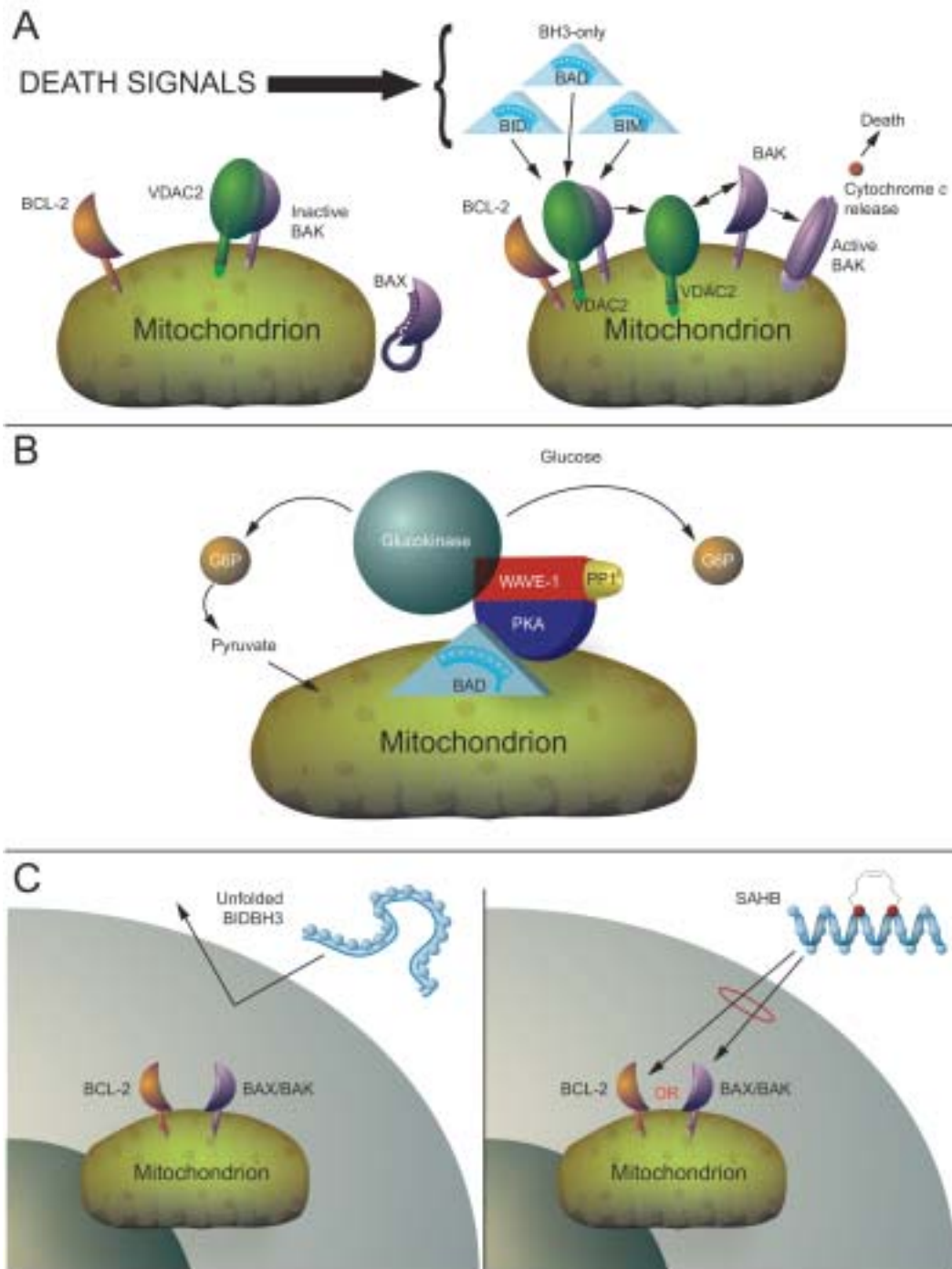


Figure 3. BCL-2 family members play additional roles, including A) BAK interacting with mitochondrial metabolic channel VDAC2, B) BAD complexing with glucokinase and other molecules to partake in glycolysis, and C) modified BID serving as a potential therapeutic agent.



## 2005 - Pezcoller Foundation-FECS Recognition for Contribution to Oncology

*The Federation of European Cancer Societies and the Pezcoller Foundation are pleased to announce the “2005 Pezcoller Foundation-FECS Recognition for Contribution to Oncology”.*

*The Pezcoller Foundation was established in 1982 through a most generous donation from Professor Alessio Pezcoller, a dedicated Italian surgeon, who devoted his life to his profession. Professor Pezcoller not only made important contributions to medicine, but through his generosity and foresight has provided his lifetime’s savings for others to do likewise.*

*In the past, until 1997, the Pezcoller Foundation gave an award in collaboration with the European School of Oncology. The Pezcoller Foundation-FECS Recognition for Contribution to Oncology builds up upon this tradition.*

*In 2005 in collaboration with the Federation of European Cancer Societies, the Pezcoller Foundation-FECS Recognition for Contribution to Oncology will be awarded to a single individual for his/her professional life dedication to the improvement of cancer treatment, care and research.*

*Nominations for the 2005 Pezcoller Foundation-FECS Recognition for Contribution to Oncology will be accepted for candidates regardless of race, sex or nationality. Institutions, groups or associations are*

*not eligible. Self nominations will not be considered. Candidates must be nominated on the official form by one who is, or has been, affiliated with a university or medical institution.*

*A curriculum vitae and description of the professional contribution to Oncology of the candidate should be included with the application form.*

*Nominators are requested to keep their nomination confidential and to refrain from informing the nominee. The awardee will be selected by an International Committee appointed by the FECS President with the agreement of the Council of the Pezcoller Foundation. The decision by the Pezcoller Foundation concerning the 2005 winner will be taken in April 2005.*

*The award consists of a prize of € 30.000 and a commemorative plaque.*

*The award ceremonies will be held in Paris, during ECCO 13 (The European Cancer Conference) and in Rovereto (Italy).*

**Questions about the nomination process** should be directed to the FECS – Federation of European Cancer Societies – Avenue E. Mounier, 83 – B-1200 Brussels – Tel. 32 2 7752931 – Fax 2 7750200 – e-mail: [carine@fecs.be](mailto:carine@fecs.be)

*Completed nomination form must be received by **31 January 2005** in order to be considered.*

*Nomination forms and supporting documents should be sent to:*

**2005 Pezcoller Foundation FECS Recognition for Contribution to Oncology.**

**Federation of European Cancer Societies  
Avenue E. Mounier, 83 – B-1200 Brussels.**

17th Pezcoller Symposium, Trento, Italy  
June 16-18, 2005

## Molecular Understanding of Solid Tumors

*Co-Chairmen: William R. Sellers and Enrico Mihich*

*Program Committee: Yusuke Nakamura, Alex Matter, Pier Paolo Pandolfi,  
Marco Pierotti*

*The Symposium will have five sessions in which each half-hour talk will be followed by half-hour discussions, thus providing ample opportunities for stimulating interactions and scientific cross-fertilization. A session of posters in the areas discussed will be included; the three best posters will be recognized by an award.*

*The first session will be focused on the identification of molecular characteristics of different types of cancer as well as of individual neoplasias within a given type. This session will be followed by two sessions focused on the identification of molecular targets for sensitivity including consideration of novel targets that may not have been validated as yet but which may provide*

*examples of molecular heterogeneity and specificities of potential therapeutic relevance. The fourth session will be devoted to a discussion of signaling pathways and their identification by modern technologies. These four sessions will emphasize the opportunity to characterize individual tumors and project their response to rationally designed individualized intervention. The fifth session will be devoted to the clinical relevance of the various topics discussed at the meeting.*

*Speakers who have accepted to participate in the Symposium include Mike Stratton, Ron DePinho, Anton Berns, William Kaelin, Paul Workman, Bruce Ponder.*

*For scientific aspects of the program, contact: [enrico.mihich@roswellpark.org](mailto:enrico.mihich@roswellpark.org)*

*For local arrangements and administrative matters, contact: [pezcoller@pezcoller.it](mailto:pezcoller@pezcoller.it)*

The Pezcoller Foundation  
**Journal**

news from the Pezcoller Foundation world

**General Information**

**Travel and local Arrangements**

Fondazione Alessio Pezcoller  
Via Dordi, 8 - 38100 Trento (Italy)  
Phone 0039 - 0461 - 980250 - Fax 980350  
[www.pezcoller.it](http://www.pezcoller.it) - E-mail: [pezcoller@pezcoller.it](mailto:pezcoller@pezcoller.it)

**Scientific arrangements**

Enrico Mihich -. Roswell Park Cancer Institute  
Elm & Carlton Streets, Buffalo, NY 14263 - USA  
Phone 716 - 845 - 3314 - Fax 3351  
E-mail: [enrico.mihich@roswellpark.org](mailto:enrico.mihich@roswellpark.org)

**Location**

The Conference will be held at Sala Palazzo Calepini  
Via Calepina, 1 - 38100 Trento (Italy)  
Phone 0039 - 0461 - 980250 - Fax 980350  
Attendance at the meeting will be limited to a maximum  
of 100 participants.

**Registration Fee**

€ 200 payable at the time of acceptance, directly to  
the symposium venue. The registration fee includes:  
participation to the symposium, congress kit, working  
lunches, coffee breaks, symposium dinner.

**Posters**

A limited number of poster presentations will be  
accepted on a competitive basis.

Poezcoller-Begnudelli Awards will be given to the 3  
best posters.

To submit a poster, send a one-page abstract to Dr. Mihich  
(by 30 April, 2005. E-mail [ann.toscani@roswellpark.org](mailto:ann.toscani@roswellpark.org))

For this event attendance credits have been requested.

**Hotel accommodations (bed and breakfast)**

Hotel Trento	€ 87,00	Tel. 0039 0461 271000
Hotel Accademia	€ 90,00	Tel. 0039 0461 233600
Hotel America	€ 60,00	Tel. 0039 0461 983010
Hotel Monaco	€ 57,00	Tel. 0039 0461 983060

Messages will be taken during the Symposium at the  
Foundation Headquarters

Tel. 0039 0461 980250 – fax 980350

**Registration Form**

**Molecular Understanding of Solid Tumors**

Trento - Italy  
June 16 -18, 2005

Last Name: .....

First Name: .....

Affiliation: .....

.....

Address: .....

.....

.....

Phone: ..... Fax: .....

E-mail: .....

**Registration fee: € 200**

**Hotel reservations:**

Hotel: .....

Date of arrival .....

Date of departure .....

Signature ..... Date .....

**Important:**

Please pre-register by fax (0039)-0461-980350  
or e-mail: [pezcoller@pezcoller.it](mailto:pezcoller@pezcoller.it)

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*Editor:* Giuseppe Bernardi MD