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Antiangiogenic Therapy of Breast Cancer

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13. ABSTRACT (Maximum 200 Words) Cancer growth and spread depends on the orchestrated proliferation of tumor-associated blood supply. Cancer cells release signals that instruct the body to build new blood vessels (angiogenesis) required to feed the tumor as it increases in size. Pharmacological agents, i.e. proteins and derivatives, that interfere with angiogenesis, in cancer bearing mice, stop cancer growth and lead to its regression. Animal modeling has revealed that repeated administration of large amounts of such antiangiogenic proteins is required for anti-cancer effect. This may be logistically difficult to achieve in larger beings such as humans. A remedy to this problem would involve a combined cell and gene therapy approach. We propose that normal tissue such as marrow stromal cells (MSCs) can be harvested from patients and engineered to secrete therapeutic proteins. The tissue would be genetically engineered in the laboratory and subsequently returned to the patient as an implant releasing on a continuous basis therapeutic proteins that interfere with cancer growth and spread. We have already developed and published many of the key components required to develop this novel therapeutic modality and have shown promising initial results with Interleukin-12-secreting MSCs in a mouse model of breast cancer.				
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Annual Summary Report for Postdoctoral Fellowship BC011316 "Genetically Engineered Autologous Cells for Antiangiogenic Therapy of Breast Cancer" DAMD 17-02-1-0477

1. TRAINING ACCOMPLISHMENTS AND AWARDS

Firstly, excellent mentoring has been provided to me by my supervisor, Dr. Jacques Galipeau, not only with regards to the experimental aspect of medical research but also in terms of training for the eventual transition from a senior postdoctoral fellow to a junior scientist. This has included my increasing implication in grant proposal writing and in helping graduate students and summer students with their research projects, as well as my greater involvement with colleagues and collaborating investigators which has led to co-authorships on four recently published/submitted manuscripts in the past year:

- Annabi, B., Thibeault, S., Lee, Y.T., Bousquet-Gagnon, N., **Eliopoulos, N.**, Barrette, S., Galipeau, J., Richard Béliveau. Matrix Metalloproteinase Regulation of Sphingosine-1-Phosphate-Induced Angiogenic Properties of Bone Marrow Stromal Cells. *Experimental Hematology* 31: 640-649, 2003.
- Annabi, B., Lee, Y.T., Turcotte, S., Naud, E., Desrosiers, R.R. Champagne, M., **Eliopoulos, N.**, Galipeau, J., Béliveau, R. Hypoxia Promotes Murine Bone Marrow-Derived Stromal Cell Migration and Tube Formation. *Stem Cells* 21: 337-347, 2003
- Annabi, B., Naud, E., Lee, Y.T., Bousquet-Gagnon, N., **Eliopoulos, N.**, Galipeau, J., Richard Béliveau. Paracrine Regulation of Bone Marrow Stromal Cells Migration and Tube Formation: Implications in Tumor Angiogenesis. *Submitted* 2003.
- Al-Khalidi, A., **Eliopoulos, N.**, Lejeune, L., Martineau, D., Lachapelle, K., Galipeau, J. Postnatal Bone Marrow Stromal Cells Elicit a Potent VEGF-Dependent Neo-Angiogenic Response. *Gene Therapy*, 10(8): 621-629, 2003.

Further training to aid in the effective execution of the granted proposal was achieved by being one of 99 selected of more than 175 applicants to attend the American Association of Cancer Research (AACR) Edward A. Smuckler Memorial Pathobiology of Cancer Educational Workshop in July 2002. There, I was also one of about 70 participants for poster presentations and was very honoured to have been chosen to receive the 2002 American Association of Cancer Research, Edward A. Smuckler Award for Basic Science (please see appended AACR newsletter). The study presented was entitled "Gene-Modified Autologous Bone Marrow Stroma Embedded in Matrigel™ Leads to a Neovascularized Implant for Prolonged *In Vivo* Systemic Delivery of Functional Erythropoietin" by **Eliopoulos, N.**, Al-Khalidi, A., and Galipeau, J. This work was included in a manuscript that was very recently published "A Neovascularized Organoid Derived from Retrovirally-Engineered Bone Marrow Stroma Leads to Prolonged *In Vivo* Systemic Delivery of Erythropoietin in Non-Myeloablated, Immunocompetent Mice" by **Eliopoulos, N.**, Al-Khalidi, A., Crosato, M., Lachapelle, K., and Galipeau, J. *Gene Therapy*, 10(6): 478-489, 2003.

In addition, due to the fact that an understanding of how the immune system works is beneficial and often essential in cancer research, I followed the Introductory Course in Immunology given by the American Association of Immunologists (AAI) in June 2003.

Moreover, I had the opportunity to attend and participate at the 9th Annual Bionorth Ottawa Life Sciences International Conference November 2002 where I presented a poster entitled "Matrix-Embedded Marrow Stromal Cells as Efficient Vehicle of Erythropoietin Long-Term in Mice" by **Eliopoulos, N.**, Al-Khalidi, A., Crosato, M., Lachapelle, K., and Galipeau, J., as well as at the 6th Annual International Conference of the American Society of Gene Therapy (ASGT) June 2003 where I presented a poster of work completed this past year entitled "Genetically Engineered Autologous Marrow Stromal Cells Sequestered Within a Human-Compatible Bovine Collagen

Matrix for Prolonged and Reversible *In Vivo* Systemic Delivery of Functional Erythropoietin in Mice” by **Eliopoulos, N.**, Lejeune, L., Martineau, D., and Galipeau, J. (please see abstract attached).

The results in this latter abstract were included in the article (please see the appended manuscript) “Human-Compatible Collagen Matrix for Prolonged and Reversible Systemic Delivery of Erythropoietin in Mice from Gene-Modified Marrow Stromal Cells” by **Eliopoulos, N.**, Lejeune, L., Martineau, D., and Galipeau, J. *Submitted to Gene Therapy May 2003*. As discussed below, the findings in this manuscript provided the proof-of-concept and better tools for testing our transgenic cell therapy approach for the treatment of breast cancer.

Furthermore, training has also been provided by regular lab meetings and discussions with supervisor, colleagues and collaborators as well as by attending lectures by invited speakers.

2. RESEARCH ACCOMPLISHMENTS

There have been some minor modifications in the specific objectives of the proposal that was funded July 2002. Studies initiated with the antiangiogenic gene *sflt1*, a soluble vascular endothelial growth factor (VEGF) receptor were technically challenging and unsuccessful and since the contingency plan was that other antiangiogenic molecules may consequently be assessed, other transgenes were investigated and extensive studies in the past year initiated with one, Interleukin-12 (IL-12). As with the originally proposed *sflt1*, IL-12 is an antiangiogenic protein with therapeutic actions involving VEGF reduction. I have included here below the rationale for selecting and utilizing IL-12, and a summary of what we have accomplished thus far encompassing initial *in vivo* results utilizing IL-12-secreting marrow stroma cells (MSCs) for cancer treatment. Also described below is a description of proof-of-concept work which led to abstracts and publications in the past year, all supporting our cell and gene therapy strategy for the antiangiogenic therapy of breast cancer. Lastly, we have included a modified statement of work.

2.1 Rationale for Utilization of IL-12 Gene-Modified MSCs

There are many plasma-soluble proteins with putative antiangiogenic activity, and a rational choice must be made for coupling with our proposed MSC-based delivery system. The difficulties that we encountered with *sflt1* were a strong incentive to explore alternative transgenes. We expect a favourable bias if transgenes are encoding for a native secreted antiangiogenic protein. Hence, the idiosyncrasies [abnormal protein folding, poor solubility, short half-life] of truncated gene products, such as endostatin, may be avoided. For these reasons, we selected IL-12, a potent antiangiogenic cytokine.

IL-12 is produced mostly by antigen presenting cells, such as macrophages, monocytes and dendritic cells, is a heterodimeric Th1 cytokine with strong abilities to provoke cancer cell apoptosis, to stimulate a cellular immune response, and to generate an antiangiogenic effect¹⁻³. The angiostatic activity of IL-12 is due to the induction of the chemokine interferon- γ inducible protein and monokine induced by IFN- γ ⁴⁻⁶. Investigators utilizing a murine breast cancer model reported that the antiangiogenic properties of IL-12 are due to a reduction of VEGF, decrease in matrix metalloproteinase-9 and increase in tissue inhibitor of matrix metalloproteinase-1⁷. However, it was noted in phase I/II studies in advanced cancers that significant toxicity arose from recombinant human IL-12 administration⁸⁻⁹. Therefore, long-term delivery of IL-12, avoiding toxic peaks seen with intermittent IV infusions, would be desirable. This problem was addressed experimentally with gene therapy-based approaches. In a systemic cell and gene therapy approach, IL-12 mediated antiangiogenesis was observed in SCID mice bearing human or murine tumors that were co-inoculated with IL-12-secreting gene-modified 3T3 fibroblasts¹⁰. In a Phase I study, autologous fibroblasts genetically engineered to secrete IL-12 and injected in the peritumoral surrounding showed promise for the treatment of disseminated cancer¹¹. Though

encouraging, these IL-12 delivery strategies are imperfect. As an aggregate, these reports support the notion that IL-12 is an effective anti-cancer cytokine with robust antiangiogenic activity in cancer and is amenable to delivery via a cell therapy delivery platform. However, an optimal means of delivery, sustained and sufficient IL-12 (yet not too much) in breast cancer patients remains to be defined in pre-clinical models such as we propose below.

2.2 Hypothesis and Specific Objectives

We propose that autologous MSCs can be genetically engineered as synthetic endocrine neo-organoids to secrete antiangiogenic gene products. This novel biopharmaceutical will permit sustained, pharmacologically-relevant production of therapeutic exogenous proteins targeting cancer-associated angiogenesis. We will test our transgenic cell therapy strategy with the use of IL-12 as paradigm antiangiogenic transgene.

Our specific objectives are: (1) Develop retrovectors expressing IL-12 and other antiangiogenic genes. (2) Perform gene transfer into primary murine MSCs and assess if gene modified MSCs can secrete biologically significant levels of functional antiangiogenic gene products with *in vitro* assays. (3) Test in mouse models of breast cancer (4T1-Balb/c metastatic model, DA3-Balb/c non-metastatic model, and MDA235-nude mice xenograft model) that implantation of MSCs secreting antiangiogenic proteins leads to a measurable anti-tumor and antiangiogenic effect. IL-12 secreting MSCs will be evaluated in absence and presence of other antiangiogenic gene product-secreting MSCs.

2.3 Experimental Aims Accomplished

- The cDNA of IL-12 was incorporated in a retroviral vector and retroviruses generated.
- Normal cultured murine MSCs from C57Bl/6 and Balb/c mice were transduced, as per our published protocol.
- Polyclonal populations, as well as transduced clones were selected and independently expanded for characterization.
- Enzyme-linked immunosorbent assay (ELISA) was performed on supernatants from polyclonal and monoclonal populations of transduced MSCs. All these IL-12 transduced cells were thus determined to secrete *in vitro* over 20 ng/10⁶ cells/24hrs. Control empty vector gene-modified MSCs were similarly generated and analyzed.
- Initial *in vivo* experiments were performed to characterize our genetically engineered MSCs in the 4T1 breast cancer model and to similarly test our hypothesis in another cancer model, B16 melanoma. Mice were first inoculated with cancer cells and 24hrs later polyclonal populations of gene-modified MSCs from normal female Balb/c or C57Bl/6 mice were implanted in the 4T1 breast or B16 melanoma cancer-bearing isogenic mice, respectively, subcutaneously within a matrix scaffolding. For the therapeutic strategy here explored, we embedded MSCs in the human-compatible viscous collagen matrix compound that we have shown, as detailed in our appended manuscript, allows formation of a retrievable synthetic endocrine organoid.
- Tumor growth was monitored and compared to appropriate controls (tumor but no MSCs, control vector transduced MSCs). As illustrated below, these initial experiments show the anti-tumor activity of polyclonal preparations of IL-12 secreting murine MSCs in the B16-C57Bl/6 melanoma mouse model (Figure 1) and in the 4T1-Balb/c breast cancer model (Figure 2), chosen because it is an animal model of stage IV human breast cancer.

2.4 Preliminary Results in Support of Hypothesis

We have generated compelling data which was included in articles published in the last year as well as in a recently submitted manuscript that strongly predict that a "transgenic cell therapy"

approach with antiangiogenic gene product-secreting MSCs would be feasible for the treatment of breast cancer. We have conducted extensive "proof-of-principle" studies (summarized here below) to analyze the utility of MSCs as a delivery vehicle for functional plasma soluble proteins in mice. Moreover, we initiated *in vivo* studies evaluating the use of IL-12-secreting MSCs in a mouse model of breast cancer as well as in a melanoma model.

2.4.1 High-Level Erythropoietin Production from Genetically Engineered Marrow Stromal Cells Implanted in Non-Myeloablated, Immunocompetent Mice (*work published in 2003*)

We determined that Epo gene-modified murine MSCs can engraft in normal immunocompetent C57Bl/6 mice, without the requirement of conditioning immunosuppressive therapy such as chemotherapy or radiotherapy, and subsequently express sufficient pharmacologically-relevant amounts of Epo in the systemic circulation. We showed that intraperitoneal implantation of engineered MSCs leads to sustained production of a synthetic plasma protein. This data was included in our recently published article entitled "A Neovascularized Organoid Derived from Retrovirally-Engineered Bone Marrow Stroma Leads to Prolonged *In Vivo* Systemic Delivery of Erythropoietin in Non-Myeloablated, Immunocompetent Mice" by Eliopoulos, N., Al-Khaldi, A., Crosato, M., Lachapelle, K., and Galipeau, J. *Gene Therapy*, 10(6): 478-489, 2003. We showed a dose response relationship between the number of implanted gene-modified MSCs and the extent of the therapeutic effect, also taking into account the level of protein secretion *in vitro* prior to implantation. However, there are some legitimate concerns about this route of delivery. Firstly, the biodistribution of the cells in the abdominal cavity is unknown and there is the theoretical concern that MSCs may relocate in undesirable organ compartments. Furthermore, if undesirable side effects from the implant were to occur, it would be impossible to remove the "implant" from the abdominal cavity. For these reasons, we then explored an alternative delivery route.

2.4.2 MatrigelTM-Embedded Marrow Stroma and Direct Protein Delivery in Circulation (*work published in 2003*)

Specifically, we investigated the use of "matrix" embedded MSCs which are subsequently implanted in the subcutaneous space, the concept being that an artificial subcutaneous "organoid" would be formed from which the plasma protein would be produced. We observed that murine MSCs embedded in MatrigelTM, not only survive but actually elicit and participate in a major neoangiogenic response. In sum, we found that MSCs have the ability to effectively recruit and participate in angiogenesis and arteriogenesis *de novo*, and that MSC-released VEGF plays an integral role in the observed angiogenic response. This data was included in article entitled "Postnatal Bone Marrow Stromal Cells Elicit a Potent VEGF-Dependent Neo-Angiogenic Response" by Al-Khaldi, A., Eliopoulos, N., Lejeune, L., Martineau, D., Lachapelle, K., Galipeau, J. *Gene Therapy*, 10(8): 621-629, 2003).

These findings lead us to speculate that Epo-secreting MSCs would elicit a similar angiogenic response, and since these cells directly participate in new blood vessel formation, that their plasma protein would be efficiently released directly into the bloodstream. In addition, since all the cells would be sequestered within a subcutaneous "implant", safety would be enhanced since removal of the organoid could be done with very little morbidity. Therefore, we embedded Epo-secreting MSCs in MatrigelTM and tested the "neovascularized organoid" concept. Indeed we found that this "delivery platform" led to sustained, significant Epo production in test mice. This data was also included in the article mentioned in section 2.4.1.

2.4.3 Effectiveness of Viscous Collagen Matrix for Supporting Marrow Stromal Cells *In Vivo* (*work submitted for publication in 2003*)

We then desired to optimize the subcutaneous delivery route for eventual use in large mammals and humans. Matrigel is a semi-solid matrix derived from a murine sarcoma cell line and contains a wide array of mouse proteins. It will be impossible to use this material as a matrix in humans or large mammals because of immunological incompatibility. Therefore, a "mammal" compatible matrix needed to be defined. We have tested a variety of matrixes and have found that an FDA-

approved, bovine collagen-based viscous matrix will indeed support mouse MSCs as efficiently as Matrigel. Our data was just recently submitted for publication consideration in *Gene Therapy* (please see manuscript attached entitled “Human-Compatible Collagen Matrix for Prolonged and Reversible Systemic Delivery of Erythropoietin in Mice from Gene-Modified Marrow Stromal Cells” by Eliopoulos, N., Lejeune, L., Martineau, D., and Galipeau, J.). In this article, we also demonstrate the retrievability safety feature of our neo-organoid approach. Therefore, our results strongly indicate that matrix implants containing genetically engineered MSCs can be effectively used as platforms for the systemic delivery of Epo or any other therapeutic plasma soluble protein, such as IL-12. The ease of implantation and removal makes this approach clinically desirable.

2.4.4 Effect of IL-12 Secreting Marrow Stromal Cells on Tumor Growth

As illustrated in Figures 1 and 2 below, we demonstrated in a pilot study with 10^5 B16 melanoma or 4T1 breast cancer cells a decrease in tumor progression when a polyclonal population of 5×10^5 IL-12-secreting MSCs were implanted subcutaneously embedded in a human compatible viscous collagen matrix in C57Bl/6 or Balb/c mice, respectively. Control mice received tumor cells alone or with control vector modified MSCs. The next experiments will examine monoclonal populations of IL-12 MSCs (secreting more IL-12), a greater ratio of MSCs to breast cancer cells, as well as combinations of IL-12 MSCs with MSCs secreting other antiangiogenic gene products.

Figure 1. Effect of IL-12-Secreting Marrow Stroma on B16 Melanoma Tumor Volume Over Time

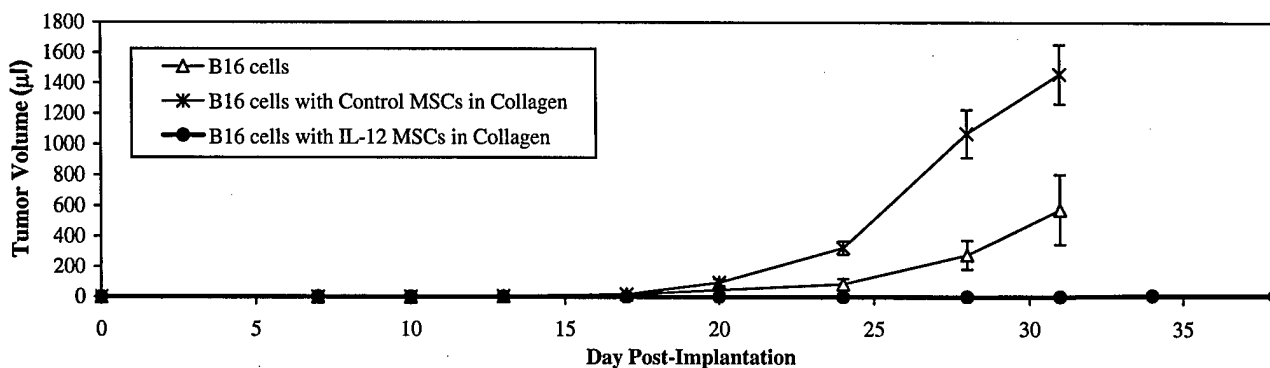
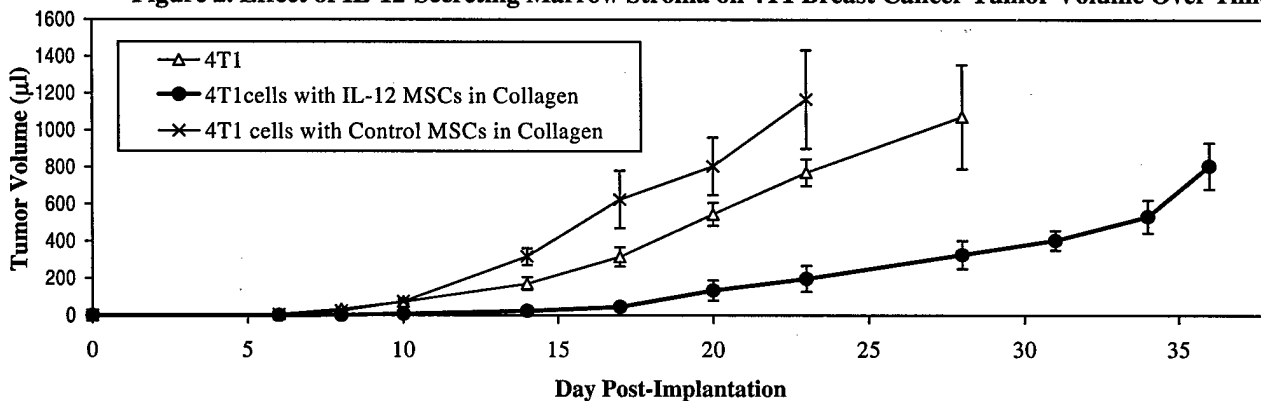


Figure 2. Effect of IL-12-Secreting Marrow Stroma on 4T1 Breast Cancer Tumor Volume Over Time



In conclusion, we have compelling preliminary results that strongly buttress our claim that engineered autologous MSCs may serve to generate a synthetic endocrine neo-organoid and that altogether predict that a “transgenic cell therapy” approach with antiangiogenic protein-secreting MSCs would be feasible for treatment of breast cancer.

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Modified Statement of Work

Genetically Engineered Autologous Cells for Antiangiogenic Therapy of Breast Cancer.

Task 1. Production and characterization of novel retroviral vectors comprising antiangiogenic genes, such as Interleukin-12 (IL-12) Months 1-16:

- a. Constructs: We have already obtained several cDNAs for antiangiogenic proteins, such as that of IL-12.*
- b. Clone the cDNAs into our previously published retrovector constructs and generate replication-free retroparticles. We have already generated one IL-12 containing retrovector.*
- c. Transduce primary marrow stromal cells (MSCs) and determine gene transfer efficiency, vector stability, transgene expression, and antiangiogenic protein secretion levels from polyclonal as well as monoclonal populations. We have already generated polyclonal and monoclonal preparations of murine MSCs secreting IL-12 and assessed in vitro protein secretion levels.*

Task 2. Determination of the therapeutic efficacy of transplanted gene-modified autologous MSCs in breast cancer-bearing mice. The tasks below will be accomplished with antiangiogenic gene product IL-12 with and without other antiangiogenic gene products, Months 17-36:

- a. Implant Balb/c mice (over 50 with controls) with non-metastatic DA3 murine mammary adenocarcinoma and later implant subdermally a removeable organoid comprising viscous collagen-embedded MSCs genetically engineered to secrete the antiangiogenic gene product(s).*
- b. Implant Balb/c mice (over 50 with controls) with metastatic 4T1 murine mammary adenocarcinoma (animal model of stage IV human breast cancer) and later implant subdermally a removeable organoid comprising viscous collagen-embedded MSCs genetically engineered to secrete the antiangiogenic gene product(s). We have already conducted initial in vivo experiments as shown above in the research accomplishments section of this report.*
- c. Monitor tumor growth and correlate with transgene expression assessed by biochemical protein assay on plasma.*
- d. Likewise, implant the gene-modified MSCs subdermally in nude mice (over 50 with controls) bearing breast cancer xenografts (such as MDA235) and determine tumor response as well as extent and duration of transgene expression in MSCs by periodic peripheral blood sample analysis.*
- e. Perform immunohistochemical analysis on tumor sections examining vascular and related structures from all groups of mice.*
- f. Conduct mechanistic analysis of the antiangiogenic effect of IL-12. Specifically, (1) determine if host-derived vascular structures express the IL-12 receptor and reveal effect on cell biology and (2) identify host-derived immune competent cells recruited by IL-12 and analyze their role in the antiangiogenic effect.*

List of Appended Documents

Abstract entitled "Genetically Engineered Autologous Marrow Stromal Cells Sequestered Within a Human-Compatible Bovine Collagen Matrix for Prolonged and Reversible *In Vivo* Systemic Delivery of Functional Erythropoietin in Mice" by **Eliopoulos, N.**, Lejeune, L., Martineau, D., and Galipeau, J.

Manuscript entitled "Human-Compatible Collagen Matrix for Prolonged and Reversible Systemic Delivery of Erythropoietin in Mice from Gene-Modified Marrow Stromal Cells" by **Eliopoulos, N.**, Lejeune, L., Martineau, D., and Galipeau, J. *Submitted to Gene Therapy May 2003.*

American Association of Cancer Research Newsletter Discussing the Edward A. Smuckler Memorial Pathobiology of Cancer Workshop and Award

median of 639 days. The frequency of gene-marked cells in peripheral blood and marrow leukocytes, red cells and platelets was monitored at regular intervals by flow cytometry and PCR and complete blood counts were obtained at the time of each blood draw. Although in some of the animals gene-marking increased transiently after infusion of gene-marked cells or after administration of a selective agent (a chemical inducer of dimerization), no obvious clonal outgrowth was observed based on flow cytometric analysis of multiple hematopoietic lineages. None of the animals that died or were euthanized at the end of the study showed any evidence of leukemia or a myeloproliferative syndrome. We are currently performing clonality analyses in animals with high-level marking to determine clonal fluctuation over time and to rule out the development of oligo or monoclonality. In summary, we have seen no evidence of leukemia after transplantation of gene-modified cells in any of our animals. The follow-up presented here (median 1.75 years) is comparable to 3 to 5 years in children given the shorter life span of dogs and baboons. Further analysis of the clonal engraftment pattern after transplantation will be presented at the meeting.



285. Genetically Engineered Autologous Marrow Stromal Cells Sequestered within a Human-Compatible Bovine Collagen Matrix for Prolonged and Reversible *In Vivo* Systemic Delivery of Functional Erythropoietin in Mice

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Bone marrow stromal cells (MSCs) can be exploited therapeutically in transgenic cell therapy approaches. These autologous cells, with their strong proliferative ability, can be genetically engineered into synthetic endocrine cells, expanded in sufficient number for clinical effect, and returned to the donor. However, should unanticipated complications result from use of gene-modified cells, it would be impossible to remove the MSCs if introduced by intravenous or intraperitoneal injection. Consequently, the capacity to deliver these cells subcutaneously (s.c.) as a retrievable implant would augment the safety of this strategy. Therefore, the aim of this study was to determine if gene-modified MSCs sequestered within the FDA-approved, bovine type I collagen-based material Contigen™ (Bard Canada) can serve as a retrievable implant for systemic delivery of functional erythropoietin (Epo) when injected s.c. in normal immunocompetent mice. We generated a monocistronic retroviral construct comprising murine Epo cDNA, stably transfected GP+E86 packaging cells, and employed retroviruses to transduce primary MSCs from C57Bl/6 mice. A clone of Epo gene-modified MSCs, revealed by ELISA to secrete 3 Units of Epo per 10⁶ cells per 24 hrs, was implanted at 10⁷ MSCs per syngeneic mouse, s.c. (1) without a matrix and (2) mixed in Contigen. In 5 mice implanted with these MSCs without a matrix, the hematocrit (Hct) increased from 57 ± 0.7% (mean ± SEM) prior to implantation, to a peak 70 ± 3.2% at ~3 weeks following implantation, and gradually dropped to a basal 57 ± 2.4% at 7 weeks. In contrast, in mice (n=5) implanted with Contigen-embedded MSCs, the Hct rose from 51 ± 0.2% pre-implantation, to 81 ± 0.9% at ~3 weeks post-implantation, and further climbed, maintaining levels of 82-88% until week 15 and >70% up to ~29 weeks ensuing implantation (p<0.0001 Logrank). Plasma Epo concentration in these mice rose from 2.5 ± 0.4mU/ml pre-implantation to 30-50mU/ml commencing 2 weeks post-implantation and descending to ~15mU/ml at week 16. In a separate experiment to ascertain the implant

retrievability safety feature of the approach, 9 mice were implanted s.c. with Contigen-embedded Epo-secreting MSCs and ~3 weeks later, implants removed from 4 recipients. The Hct in these 4 mice decreased from 77 ± 2.7% at 3 weeks post-implantation to baseline levels of 55 ± 1.2% 2 weeks following implant harvesting, whereas in mice with implant left intact, the Hcts at these time points were 76 ± 2.7 and 80 ± 2.9%. Control mice implanted with Contigen only or Contigen with green fluorescent protein (GFP) gene-modified MSCs had Hcts unchanged from baseline. Flow cytometry analysis revealed GFP-engineered MSCs as mainly CD34-, CD31-, CD45- and CD44+, both prior to and ~3 weeks succeeding implantation mixed in Contigen. In conclusion, this investigation demonstrates that s.c. implanted MSCs embedded in human-compatible matrix Contigen offers a safe reversible approach for delivery of plasma soluble therapeutic proteins, in addition to a more persistent pharmacological effect as compared to non-embedded MSCs.

286. Gene Delivery to Primary Peripheral Blood Lymphocytes: Efficient and Effective Transduction of PBL with Single and Multiple rSV40 Vectors

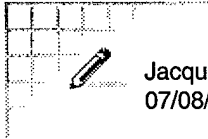
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Background: Gene delivery to primary blood cells, particularly lymphocytes, has been difficult to achieve. Most approaches require or prefer lymphocyte stimulation, are generally inefficient and constrain practical gene delivery to lymphocytes to the small percentages of the total pbl pool that can be handled *ex vivo*. The inefficiencies of single gene delivery to pbl are compounded if one tries to deliver multiple genes. Since recombinant SV40-derived vectors (rSV40s) transduce unstimulated, unselected, pbl permanently and at high efficiency we tested whether these vectors could deliver multiple genes in sequence to pbl. We further asked sequential rSV40 combination gene therapy improved functionality.

Methods: Normal human buffy coats were separated using ficoll, and then depleted of monocytes by plastic adherence. Resulting pbl-enriched populations were transduced without stimulation or selection using one or more rSV40s carrying anti-HIV transgenes. After transduction, cells were cultured with IL-2 and challenged with HIV-1NL4-3 at virus:cell ratios (MOI) of 0.005, 0.01, 0.015 and 0.03. Transgene functionality was measured as inhibition of HIV replication, as assayed by ELISA for supernatant HIV p24. The rSV40s used carried: RT3 and IE8, single chain Fv antibodies (SFv) to HIV reverse transcriptase; Aw, a SFv to HIV integrase; HE, a SFv against CXCR4; RevM10, a dominant negative mutant of HIV Rev; PolyTAR, a polymeric TAR decoy; and (as a control) HBS, hepatitis B surface antigen.

Results: Previous studies had documented simultaneous expression of several transgenes by >95% of unselected cells after sequential rSV40 transduction. Expression of the transgenes used here was demonstrated by Western and Northern blotting, immunostaining and flow cytometry. Effectiveness of SV(HE) gene delivery was tested by FACS analysis, showing decreased cell membrane CXCR4. Singly- and multiply-transduced cells were challenged with progressively higher doses of HIV. It is characteristic of gene delivery to inhibit HIV that protection of transduced cells deteriorates as challenge doses of HIV increase. Thus, most individual transgenes protected pbl completely from HIV challenge at MOI = 0.005, but protected only partially or not at all at higher doses. Only SV(HE), which decreases CXCR4, protected singly at the highest HIV dose, MOI = 0.03. Sequentially delivered combinations of 2 or 3 transgenes generally protected better than any component transgene did alone. Some combinations, e.g., Aw (anti-IN SFv) +



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Human-compatible collagen matrix for prolonged and reversible systemic delivery of erythropoietin in mice from gene-modified marrow stromal cells

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Running Title

Collagen matrix for Epo delivery by MSCs in mice

Summary

Bone marrow stromal cells (MSCs) can be exploited therapeutically in transgenic cell therapy approaches. Our aim was to determine if gene-modified MSCs sequestered within a clinically approved, bovine type I collagen-based viscous bulking material could serve as a retrievable implant for systemic delivery of erythropoietin (Epo). To test this hypothesis, we embedded Epo-secreting MSCs in viscous collagen (Contigen™) and determined the pharmacological effect following implantation in normal mice. Primary MSCs from C57Bl/6 mice were retrovirally-engineered to express murine Epo (mEpo) and 10^7 cells of a clonal population secreting 3U of mEpo/ 10^6 cells/24hrs were implanted subcutaneously in normal C57Bl/6 mice with and without viscous collagen. Without matrix support, Hct rose to >70% for <25 days and returned to baseline by 60 days. However, in mice implanted with viscous collagen-embedded MSCs, the Hct rose to >70% up to 203 days post-implantation ($p < 0.0001$). In parallel, plasma Epo concentration was significantly increased ($p < 0.05$) for >145 days. Moreover, surgical removal of the viscous collagen organoid 24 days ensuing implantation led to reduction of Hct to baseline levels within 14 days. In conclusion, this investigation demonstrates that mEpo⁺MSCs embedded in human-compatible viscous collagen matrix offers a potent, durable and reversible approach for delivery of plasma soluble therapeutic proteins.

Keywords

marrow stroma, collagen, erythropoietin, cell and gene therapy, autologous cells

Introduction

Bone marrow derived stromal cells (MSCs) are an autologous cell type that can be made use of for numerous cell and gene therapy applications. MSCs with their strong expansion ability are amenable to genetic reprogramming with recombinant retroviruses and can consequently be engineered into synthetic endocrine cells for the delivery of plasma soluble therapeutic proteins.^{1,4} In a previous study,⁵ we reported the pharmacological effect on blood hematocrit of a synthetic endocrine organoid derived from erythropoietin (Epo)-transduced MSCs admixed in a basement membrane gel MatrigelTM (Becton Dickinson Biosciences, MA). However, Matrigel is derived from a murine sarcoma cell line, thus non-human compatible, which prohibits its use in clinical studies. Owing to the fact that collagen is an important component of Matrigel, we also evaluated a clinically-applicable bovine type I collagen porous formulation, namely Collagen Matrix (Collagen Matrix Inc., N.J.), as a support vehicle for Epo-releasing MSCs. The observed results were encouraging and buttressed the notion that a bovine collagen-based matrix could serve as a viable platform to support MSCs *in vivo*, though there was much room for formulation improvement. Indeed, there is extensive published experience in testing a wide array of biological and synthetic matrices to support engineered somatic cells *in vivo*.^{6,10} However, we sought a formulation that offered cost-effective, FDA-approved, off-the-shelf convenience, coupled to specific pharmacological properties. Namely, a semi-solid viscosity amenable to syringe injection in the subcutaneous space, long *in vivo* half-life for durability and a chemical composition compatible with MSC survival, such as a neutral pH. Furthermore, the matrix formulation should allow for removal of the organoid when the pharmacological effect is no longer required or if unforeseen side effects were to arise from the embedded gene-modified cells.

ContigenTM (C.R. Bard Inc., GA) is a clinically approved, bovine collagen-based device, formulated as a peri-urethral injectable viscous bulking agent for the treatment of stress urinary incontinence in women.^{11;12} Specifically, it is a nonpyrogenic substance constituted of highly purified dermal collagen, 95% Type I with $\leq 5\%$ Type III, suspended in phosphate buffered physiological saline (35mg/ml). Unlike many clinical-grade collagen formulations, it has a pH of 7.3 and is designed for a long half-life *in vivo*. We therefore tested the utility of this viscous collagen preparation as the matrix component of a human-compatible synthetic endocrine organoid. As a proof-of-concept, we employed murine MSCs retrovirally reprogrammed to secrete a plasma soluble protein – Epo – as the cellular component of a synthetic organoid. We here report that in mice implanted subcutaneously

with viscous collagen-embedded Epo-secreting MSCs, a pharmacological effect arose that was superior to and of significantly longer duration than that seen with MSCs without matrix support. Furthermore, we ascertained the flexibility of the approach by reversing the clinical effect via surgical removal of the organoid implant. These results buttress the use of bovine collagen-based matrices for pre-clinical and clinical studies incorporating autologous, engineered somatic cells – such as MSCs – as a biopharmaceutical platform for long term delivery of plasma soluble therapeutic proteins.

Results

Epo gene-modified marrow stroma

To confirm that mEpo transduced MSCs secrete mEpo *in vitro*, and quantitate the amount, supernatant collected from these MSCs was utilized in an ELISA for human Epo as previously described.⁵ The polyclonal population of Epo⁺MSCs was determined to secrete ~2 U of Epo per 10⁶ cells per 24 hours and the clonal population of Epo⁺MSCs, utilized in the present study, was noted to release ~3 U of Epo per 10⁶ cells per 24 hours. There was no Epo detected in the supernatant collected from control MSCs (data not shown). Flow cytometric analysis of the clonal subset of Epo⁺MSCs utilized experimentally revealed a phenotype of <0.01% CD31+, 98% CD44+, <0.01% CD45+ and 35% CD34+.

Long-term hematocrit of mice implanted subcutaneously with Epo-secreting MSCs

We assessed and compared the long-term effect on hematocrit (Hct) of MSCs delivered subcutaneously, either admixed in viscous collagen or without matrix support. As observed in Figure 2, in C57Bl/6 mice (n=5) implanted with 10⁷ collagen-embedded Epo⁺MSCs, the hematocrit rose from a basal 51 ± 0.2% (Mean ± SEM) pre-implantation to 81 ± 0.9% at 22 days following implantation and remained at values of 82-88% until day 106, and surpassing 70% up to day 203. Control mice implanted with collagen only (Figure 2) or with IRES-GFP engineered MSCs in collagen (data not shown) showed stable baseline Hct levels over time. In contrast, when the identical amount of 10⁷ Epo⁺MSCs were injected subcutaneously without matrix support, the Hct increased from a basal 57 ± 0.7% prior to implantation to a peak value of 70 ± 3.2% at 23 days post-implantation which gradually thereafter decreased, reaching basal value of 57 ± 2.4% at ~63 days (Figure 2). When comparing the long-term impact on Hct, all mice implanted with collagen embedded Epo⁺MSCs sustained a Hct of ≥70% for over 119 days whereas in mice which received unembedded cells, this Hct level persisted for 23 days in 4 of 5 mice (p=0.0001 LogRank). We observed that the decrease in Hct in the matrix group was associated with the physical decrease in size of the implant, likely due to gradual resorption.

Plasma Epo concentration in mice implanted with Epo-secreting MSCs in Contigen

We measured the concentration of mouse Epo in plasma of mice over time with the use of a human Epo ELISA assay. In mice injected with collagen embedded Epo⁺MSCs, plasma Epo

levels rose from a basal 7.5 ± 0.5 mU/ml before implantation to 25-65 mU/ml, as early as 6 days post-implantation (33 ± 2.6 mU/ml), peaking at 14 days (67 ± 17 mU/ml) and slowly falling to levels of 25 ± 7.2 mU/ml at day 93 (Figure 3). Thereafter, the concentration of Epo in plasma further decreased to 15 ± 3.0 mU/ml at day 147. Statistical evaluation of the plasma Epo concentrations in recipient rodents of Epo⁺MSCs embedded in collagen revealed that values detected at days 6 to 147 were significantly different ($P < 0.05$, Student t-test) from pre-experiment baseline measurements (Figure 3). Moreover, although a peak in plasma Epo concentration was observed at day 14, statistical analysis revealed that values measured at days 6 to 93 inclusively were not significantly distinct from one another. It was only from time point day 119 that the concentration of Epo in plasma was significantly different from that measured at day 14 ($P < 0.01$, Student t-test). Further, from day 163 on ensuing implantation, plasma Epo levels detected were not significantly dissimilar from baseline. An ELISA assay specific for detection of human Epo was used to measure mouse Epo as is standard and similarly utilized in other studies.^{5;13;14} Hence, the sensitivity for mEpo being weak,¹⁵ our measured plasma mEpo levels are likely underestimated but remain useful for comparison with other published reports.

Removal of Contigen-embedded MSCs and abolishment of pharmacological effect.

The organoid implant behaves as a synthetic endocrine gland. Its removal should lead to complete abolishment of its pharmacological effects if the bulk of engineered MSCs remain within its framework. To test this hypothesis, nine mice were implanted subcutaneously with Epo⁺MSCs embedded in collagen and implants were removed from 4 randomly chosen mice 24 days later. As illustrated in Figure 4, implants were easily harvested from live mice under anesthesia with no residual matrix remaining post-surgery and no morbidity. The Hct in these 4 mice decreased from $77 \pm 2.7\%$ at 21 days post-implantation to baseline levels of $55 \pm 1.2\%$ within 14 days following implant removal, whereas in mice with implant left intact, the Hcts remained significantly increased at $>75\%$ for the duration of the experiment ($P \leq 0.005$, Student t-test) (Figure 5).

Histological analysis of Contigen implants following removal from mice

Organoids were removed from mice implanted 24 days earlier with (i) collagen matrix only, (ii) EmptyVector engineered MSCs embedded in collagen matrix, in addition to (iii) Epo⁺MSCs admixed in collagen matrix. Macroscopic appearance in all these three groups was

similar. The implant consisted of a soft amorphous core and a rubber-like capsule with small blood vessels on its surface. Hematoxylin and eosin stained histological sections of the MSC-containing organoids were also examined. Figure 6 is a representative example of histological findings. The implants consisted of a large, fragmented avascular center surrounded by a concentric thin band of vascularized matrix material, which was itself covered by a capsule of connective tissue made of mature collagen infiltrated by scant neutrophils and richly vascularized by a loose network of capillaries. The matrix band closely mimicked granulation tissue. The capillary network infiltrated a thin subjacent band of matrix material that contained viable cells. In the implants containing MSCs, the fragments composing the center contained dead cells that showed features of coagulation necrosis (preserved outline of necrotic swollen cells). The inflammatory response was minimal and consisted of neutrophils scattered within the capsule and the neovascularized matrix band. There were no obvious differences between the three groups examined, thus the host-derived response was due to the collagen material and not the MSCs. The collagen matrix material is quite inert as it triggers little inflammation. In particular, the rarity of macrophages or giant cells, hallmarks for the presence of foreign bodies, was notable. There were very few lymphocytes (other than plasma cells) if any at all. There were Russell bodies, i.e. plasma cells with abnormally dilated rough endoplasmic reticulum filled with aggregates of immunoglobulins. The subcutaneous layer contained several dense perivascular groups of plasma cells filled with Russell bodies. There was little neovascularization and angiogenesis did not reach the implant center.

Analysis of MSC phenotype prior to and post implantation in mice within Contigen matrix

To determine the *in vivo* fate of MSCs embedded in Contigen, we GFP reporter labelled MSCs to allow us to distinguish input MSCs from host-derived cellular events recruited within the implant and we used flow cytometry to track the phenotype of GFP⁺MSCs. As demonstrated in Figure 7, flow cytometric analysis revealed these GFP⁺MSCs as <0.04% CD31+, 96% CD44+, <0.1% CD45+ and 15% CD34+, prior to implantation. Flow cytometry analysis performed on GFP⁺ cells retrieved from implants 23 days following Contigen embedding and subcutaneous injection in mice showed the same phenotype as that observed prior to *in vivo* implantation (data not shown).

Discussion

Collagen constitutes a valuable biomaterial for medical purposes. It is utilized for wounds and burns, for eye shields, for protein delivery, for transdermal delivery controlling substance, for tissue engineering, and for cell culture matrices.¹⁶ In this study, we established that a three-dimensional, clinically-approved bovine type I collagen-based viscous bulking preparation can serve as an effective matrix biomaterial for support of MSC-derived synthetic endocrine organoid. The long-term pharmacological effect observed was comparable to what we have previously noted using the mouse-specific Matrigel™ matrix.⁵

Numerous studies have assessed three-dimensional matrices in pre-clinical models of transgenic cell therapy with various cellular vehicles including MSCs, myoblasts, and in many cases, fibroblasts. The type of materials tested fall under two broad categories: biomaterials and synthetic matrices and devices. Both types have been coupled to MSCs engineered to produce plasma soluble proteins, including: hydroxyapatite particles, hyaluronic acid sponge, and collagen-based sponge.^{8;9} Indeed, others and we have previously validated^{5;8;9} the utility of embedding protein-secreting MSCs within a collagen-containing matrix for prolonged pharmaceutical effect. As demonstrated by Daga et al.⁸ with Epo-transduced human MSCs tested *in vivo* in 3 dissimilar matrices, including a collagen sponge, the pharmacological effect was of longer duration than without matrix support. The apex in the Hct upsurge with their collagen sponge device was ~60% at day 28 which remained at levels above 55% to day ~50 ensuing implantation. Another recent investigation led to human plasma factor IX serum levels, above 25, 11.5, and 6ng/ml, for 1 week, 1 month, and 4 months, respectively, in immunocompromised mice by MSCs enclosed in a collagen sponge.⁹ Unlike the viscous collagen material here tested, the collagen materials brought into play in these two studies required a surgical procedure for subcutaneous implantation and led to short lived, modest or subtherapeutic plasma protein levels. Thus, the “injectability” of a viscous collagen matrix coupled to robust long-term support of engineered MSCs as we have here shown stand out as desirable features.

Similarly, mammalian adult somatic cells other than MSCs, such as fibroblasts and myoblasts engineered to produce plasma soluble proteins, have been embedded in an array of collagen-based biomaterials: collagen preparation;¹⁷ rat type I collagen-based lattices;^{18;19} rat tail collagen.^{7;20} Though conceptually related, most of these biomatrices have not been validated for use in humans, and serve mostly as buttressing proof-of-concept experiments validating in a generic sense the use of collagen-based matrix materials.

Synthetic matrices and surgically implanted devices have also been widely tested, including: 5% agarose gel;²¹ growth factor reduced Matrigel;²² alginate-poly-L-lysine-alginate membrane;²³ microcapsules;¹⁰ polyether-sulfone capsules;¹⁴ polytetrafluoroethylene (PTFE) ring;²⁴ hollow fibers constituted of 8% AN69 copolymer (polyacrylonitrile-sodium methallylsulfonate);²⁵ hydroxyethyl methacrylate-methyl methacrylate (HEMA-MMA);²⁶ immunoisulatory devices^{6,27} and hollow microporous polymer membrane.²⁸ An obvious advantage of these strategies is the avoidance of all biological materials in an implant device. However, most have remained at the pre-clinical prototype developmental stage and are still far removed from clinical approval and widespread clinical use.

Others, and we have also shown the use of genetically engineered MSCs for cell and gene therapy applications via intraperitoneal or intravenous administration.^{5,29,30} The main downside to this mode of cell delivery is the inability to remove or retrieve the engineered cells when the pharmacological effect is no longer required or if an unforeseen toxicity were to arise from their use. However, a subcutaneous implantation would be desirable since MSCs confined to a three-dimensional scaffolded organoid would allow for surgical extraction if clinically warranted. Hence, a primary goal in this current study was to demonstrate that a pharmacological effect attained *in vivo* with plasma protein secreting MSCs enclosed in a collagen organoid is obliterated upon implant removal. Indeed, we unambiguously demonstrate that a collagen-based organoid is readily resectable and with its removal leads to the complete reversal of its endocrine effect. This observation also leads us to speculate that the bulk of engineered MSCs sequestered within the implant do not migrate out of the organoid, at least in the first few weeks.

We also noted that although Contigen™ is human-compatible, it was not entirely inert in mice as a modest inflammatory infiltrate was observed following implantation. As illustrated in Figure 6, neutrophils and plasma cells infiltrated the matrix implant capsule. This host-derived inflammatory response was due to the bovine collagen material and not the MSCs as similar observations were made with the collagen matrix in the absence of MSCs. Moreover, a greater cell density was observed in the collagen implant periphery while a sparse and more necrotic cell population was seen in the implant core (Figure 6). There was little neovascularization, as if the collagen material was a barrier for new capillaries. It is possible that the glutaraldehyde cross-linking of this material contributes to prevent its neovascularization. This failure to vascularize most likely contributed to the death

(coagulation necrosis) of implanted MSCs localized in the implant core and to the fragmentation of the organoid material.

Marrow stromal cells have remarkable cellular plasticity and we have previously shown that a significant subset of these will adopt spontaneously an endothelial phenotype *in vivo* when embedded in Matrigel.³¹ Interestingly, we saw no obvious phenotype change in MSCs embedded in Contigen *in vivo*, at least in the first three weeks following implantation, utilizing the GFP reporter labeled MSCs. As reported by others in the field, we noted anchorage-dependent MSCs to be CD31-, CD44+, CD45- *in vitro*³ prior to implantation and remained as such following retrieval from Contigen. We speculate that though collagen-based matrix allows for survival of MSCs, the viscous collagen preparation here used lacks supplementary signals that would otherwise lead to some vasculogenic differentiation as we have previously observed in Matrigel. Though we did not observe any vasculogenic differentiation of MSCs, this did not seem to impede the desirable biopharmaceutical features of the collagen-based organoid in regards to its ability to support long-term, sustained delivery of proteins *in vivo*.

We chose to focus on a market-approved collagen device for a series of reasons. Namely, it is a pH neutral, viscous, collagen-based preparation specifically designed for delivery by injection in subcutaneous soft tissues and for slow resorption rate in humans. Though we presume its original clinical development had absolutely nothing to do with cell therapy, the pharmacological features it possesses are ideal for the purpose of providing a stable extra-cellular matrix environment for engineered autologous adult somatic cells as here described. Furthermore, its widespread clinical use makes it – and comparable devices – conveniently available for off-label use – in appropriate experimental setting – for cell therapy applications in the treatment of disease amenable to delivery of plasma soluble proteins in mammals, including humans. It is conceivable that the approach of plasma soluble protein delivery by viscous collagen encapsulated MSCs can be translated to numerous clinical purposes where a short-term or long-term beneficial effect is needed. Thus, diseases such as cancer, hemophilia, growth or other hormone deficiency, and all diseases amenable to therapeutic plasma protein delivery could be improved via this neo-organoid therapeutic platform.

Materials and methods

Production of retrovector and of retrovirus-producing cells

The retroviral plasmid pIRES-EGFP, containing a multiple cloning site linked by an internal ribosomal entry site (IRES) to the enhanced green fluorescent protein (EGFP) (Clontech Laboratories, Palo Alto, CA), was formerly synthesized in our laboratory.³² The retroviral construct pEmptyVector was generated by removal of IRES-EGFP fragment following NotI digest of pIRES-EGFP and subsequent autoligation of the resulting vector EmptyVector. The retrovector pEpo (Figure1) was constructed by retrieving the mouse Epo cDNA by BamHI digest of our previously reported pEpo-IRES-EGFP⁵ and ligating it with a BglII digest of pEmptyVector.

For the preparation of retrovirus-producing cells GP+E86-Epo, the pEpo construct (10 μ g) was linearized by FspI digest and co-transfected, using lipofectamine reagent (Invitrogen/Life Technologies, Carlsbad, CA), with 1 μ g pEGFPC1 (Clontech), which contains the neomycin resistance gene, into the GP+E86 ecotropic retrovirus-packaging cell line³³ from American Type Culture Collection (ATCC). These cells were grown in Dulbecco's modified essential medium (DMEM) (Wisent Technologies, St. Bruno, QC) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Wisent) and 50 U/ml penicillin, 50 μ g/ml streptomycin (Pen/Step) (Wisent). Stable transfectants were selected in complete media supplemented with 400 μ g/ml geneticin (Invitrogen/Life Technologies). Control GP+E86-EmptyVector virus producers were generated in an identical manner and then sorted based on GFP expression, and GP+E86-IRES-EGFP cells were prepared and sorted as earlier published.⁵

Collection, culture, and transduction of primary murine marrow stroma

One female 15-20g C57Bl/6 mouse (Charles River, Laprairie Co., QC) was sacrificed by CO₂ inhalation, and whole bone marrow obtained by flushing the femurs and tibias with complete media (DMEM supplemented with 10% FBS and 50U/ml Pen/Strep). Cells were plated in tissue culture dishes and ensuing a five day incubation at 37°C with 5% CO₂, the non-adherent hematopoietic cells were discarded and the adherent marrow stromal cells (MSCs) cultured for ~15 passages in complete media. A mixed population of Epo gene-modified MSCs was generated by several transduction rounds of MSCs with retroparticles from subconfluent GP+E86-Epo producers. Specifically, transduction was carried out twice a day for three consecutive days in each of four successive weeks by placing 0.45 μ m filtered retroviral supernatant from virus producers over ~60% confluent MSCs, in the presence of

6µg/ml lipofectamine reagent (Invitrogen/Life Technologies). Supernatant was harvested from these gene-modified MSCs and mouse Epo secretion was evaluated by ELISA specific for human Epo (Roche Diagnostics). This polyclonal population of Epo-secreting MSCs was then plated at very low cell density and resulting single colonies isolated and expanded as clonal populations. A clonal Epo⁺MSC subpopulation secreting 3U Epo/million cells/24 hrs was used for all subsequent experiments. Control EmptyVector engineered MSCs were prepared as described above and IRES-EGFP MSCs were generated as earlier reported (Eliopoulos et al., 2003). Animals were handled under the guidelines promulgated by the Canadian Council on Animal Care and with the Animal Welfare Act Regulations and other Federal statutes relating to animals and experiments involving animals and adheres to the principles set forth in the Guide for Care and Use of Laboratory Animals, U.S. National Research Council, 1996.

Marrow stroma phenotypic analysis

Epo⁺MSCs were analyzed prior to implantation for expression of cell surface antigens. Cells were incubated with the following mAbs after Fc receptor blocking: PE-labeled rat anti-mouse CD45 (clone 30-F11), CD44 (clone IM7), biotin-conjugated rat anti-mouse CD31 (clone 30-F11), CD34 (clone RAM 34), isotypic controls PE labeled rat IgG2a, IgG2b and biotin-conjugated rat IgG2a (All from BD Pharmingen, San Diego, CA). Biotinylated Abs were revealed by TC-streptavidin (Caltag Laboratories, Burlingame, CA, USA). All cells were washed and acquired using a FACS Calibur flow cytometer (BD Immunocytometry systems) and analyzed with Cellquest software.

Marrow stroma implantation for long-term blood sample analysis

For the implantation of “matrix-free” cells, Epo⁺MSCs were trypsinized, concentrated by centrifugation, and 10⁷ cells in 0.5ml of serum-free RPMI media (Wisent) were injected subcutaneously in the right flank of 5 syngeneic C57Bl/6 mice. For the subcutaneous implantations of matrix-embedded MSCs, 10⁷ Epo⁺MSCs were resuspended in 50µl of RPMI media, mixed with ~500µl of a “human-compatible”, FDA approved bovine type I collagen-based material ContigenTM (C.R. Bard Inc, Covington, GA) and implanted by subcutaneous injection in the right flank of 5 syngeneic C57Bl/6 mice. Control mice were implanted with Contigen only or 10⁷ IRES-EGFP MSCs mixed in Contigen (n=3 per group). Blood samples were collected from the saphenous vein of recipient mice with heparinized micro-hematocrit

tubes (Fisher Scientific, Pittsburgh, PA) prior to and every ~1 or more weeks post-implantation and utilized to assess hematocrit (Hct) levels by standard microhematocrit method and plasma mEpo concentrations by ELISA for human Epo (Roche Diagnostics). Mice were followed for over 250 days.

Marrow stroma implantation for implant retrieval

In a separate experiment evaluating the retrievability of MSC-containing collagen implants and reversal of the pharmacologic effect, an additional 9 mice were injected subcutaneously with 10^7 Epo⁺MSCs mixed in ~500ul Contigen. At day 24 post-implantation, the organoids were removed from 4 mice anaesthetized by isoflurane inhalation as illustrated in Figure 4. The organoid was left intact in the remaining 5 “positive control” mice. Blood samples were collected prior to and every ~1week post-implantation from all 9 mice until day 77 and hematocrits assessed by standard microhematocrit method. Supplementary “negative control” mice were generated by subcutaneous administration of EmptyVector engineered MSCs embedded in collagen matrix, IRES-EGFP gene-modified MSCs admixed in collagen matrix, as well as collagen matrix only (n=3 per group). Implants were harvested from these control mice at day 23-24 ensuing implantation.

Contigen implant processing and analysis

All implants recovered from mice were divided in two parts. One part of each sample was fixed with 10% formalin, embedded in paraffin and sections of 5 μ m prepared and stained with hematoxylin and eosin, visualized with a microscope, and digital images saved on a computer. The other part was cut into little pieces and then treated with type IV collagenase (Sigma-Aldrich Canada Ltd, Oakville, Ontario) 1.6 mg/ml, and DNase I (Sigma) 200 μ g/ml in 1X PBS at 37°C for 1 hour. The cells that were recovered were counted and analyzed by flow cytometry for specific cell surface antigen expression as indicated here below.

IRES-GFP gene-modified MSCs were analyzed prior to implantation as well as 23 days following subcutaneous injection of cells mixed in Contigen for expression of cell surface antigens exclusively on GFP positive MSCs. Cells were treated with collagenase as described above and incubated with the following mAbs after Fc receptor blocking: PE-labeled rat anti-mouse CD45 (clone 30-F11), CD44 (clone IM7), biotin-conjugated rat anti-mouse CD31 (clone 30-F11), CD34 (clone RAM 34), isotypic controls PE labelled rat IgG2a, IgG2b and biotin-conjugated rat IgG2a (All from BD Pharmingen, San Diego, CA). Biotinylated Abs

were revealed by TC-streptavidin (Caltag Laboratories, Burlingame, CA, USA). All cells were washed and acquired using a FACS Calibur flow cytometer (BD Immunocytometry systems) and analyzed with Cellquest software.

Acknowledgements

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Titles and legends to figures

Figure 1. Schematic illustration of retroviral construct encoding mEpo cDNA. The retrovector pEpo was generated as described in Materials and methods. The murine erythropoietin (Epo) cDNA sequence was introduced in the multiple cloning site of the control pEmpty Vector constructed by removing IRES-EGFP from our previously reported pIRES-EGFP.

Figure 2. Long-term hematocrit of mice implanted subcutaneously with Epo-secreting marrow stroma with or without Contigen. A clonal population of Epo-gene-modified MSCs secreting 3U of Epo/ million cells/ 24 hours *in vitro* was injected, at 10^7 cells/mouse, either mixed in Contigen (full triangle) or without a matrix support (empty circle), subcutaneously in C57Bl/6 syngeneic mice (n=5 per group). Peripheral whole blood was collected from the saphenous vein for over 250 days and hematocrit measured. Control mice (full square) (n=3) were implanted with Contigen alone. Mean \pm SEM.

Figure 3. Plasma Epo concentration of mice implanted with collagen-embedded Epo-secreting marrow stroma. A clonal population of Epo⁺ MSCs secreting *in vitro* 3U of Epo/ million cells/ 24 hours was injected subcutaneously in syngeneic mice and saphenous vein blood was collected for over 250 days. Plasma was recovered from the peripheral blood and plasma mEpo concentration determined by ELISA specific for hEpo. Mean (n=5) \pm SEM.

Figure 4. Surgical organoid implant retrieval. A group of mice injected with Epo⁺MSCs-containing Contigen implants were anesthetized by isoflurane inhalation and implant removal executed as illustrated. A small skin incision was first performed, exposing the subcutaneous neo-organoid subsequently pulled out by complete and easy detachment from the host.

Figure 5. Removal of organoid implants and effect on hematocrit. Mice were injected subcutaneously with Contigen-embedded Epo⁺MSCs secreting 3U of Epo/ million cells/ 24 hours. At 24 days post-implantation, the neo-organoid was excised out of several recipient mice. Hematocrit was assessed in these mice (full square)(n=4) as well as in animals with implant left intact (empty circle)(n=5).

Figure 6. Histologic analysis of collagen-based organoid implants. Contigen implants without MSCs, with Empty Vector MSCs, or with Epo⁺MSCs were recovered from mice at 23-24 days post-implantation and sections stained with hematoxylin and eosin. (A) Representative section of whole implant illustrating host-derived capsule, Contigen band and Contigen core components (4X magnification). (B) Representative section of implant capsule composed of host-derived tissues including capillary filled with red blood cells (grey arrow), neutrophils (blue arrow), and plasma cell with Russell bodies (yellow arrow) (40X magnification).

Figure 7. Phenotypic analysis of MSCs. IRES-GFP-gene-modified MSCs were analyzed by flow cytometry prior to subcutaneous implantation in mice. Expression of cell surface antigens CD31, CD34, CD44 and CD45 on GFP positive MSCs was evaluated as described in Materials and methods. Analysis performed 23 days following subcutaneous injection of cells *in vivo* mixed in Contigen revealed similar findings (data not shown).

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Figure 1

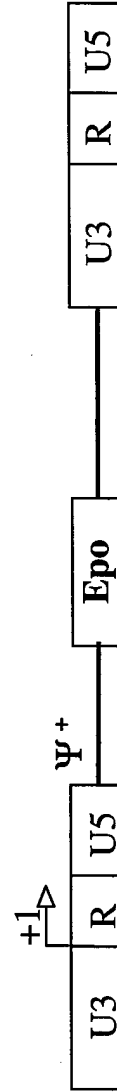
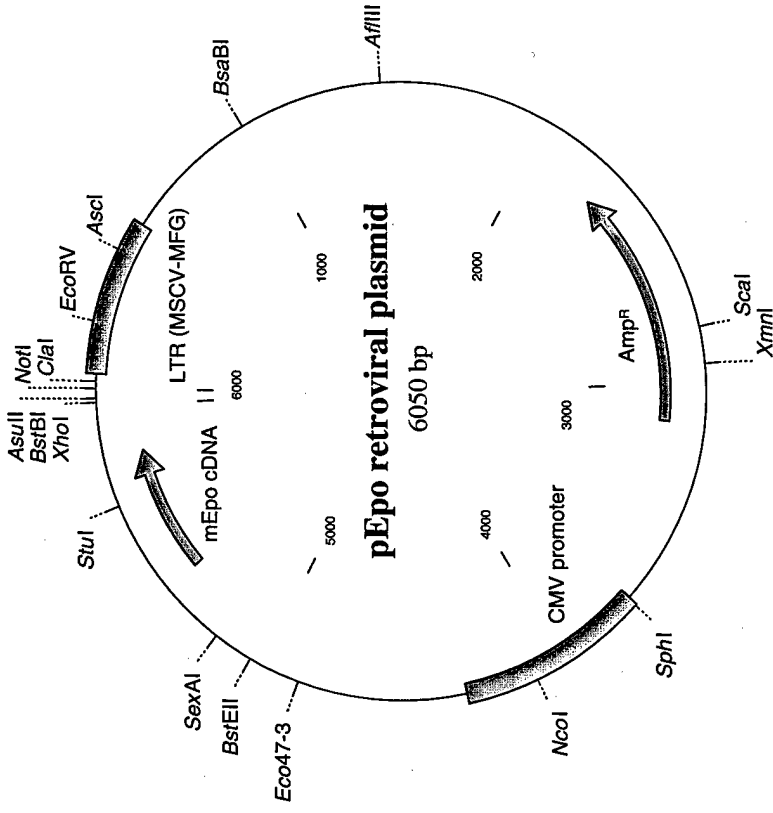


Figure 2

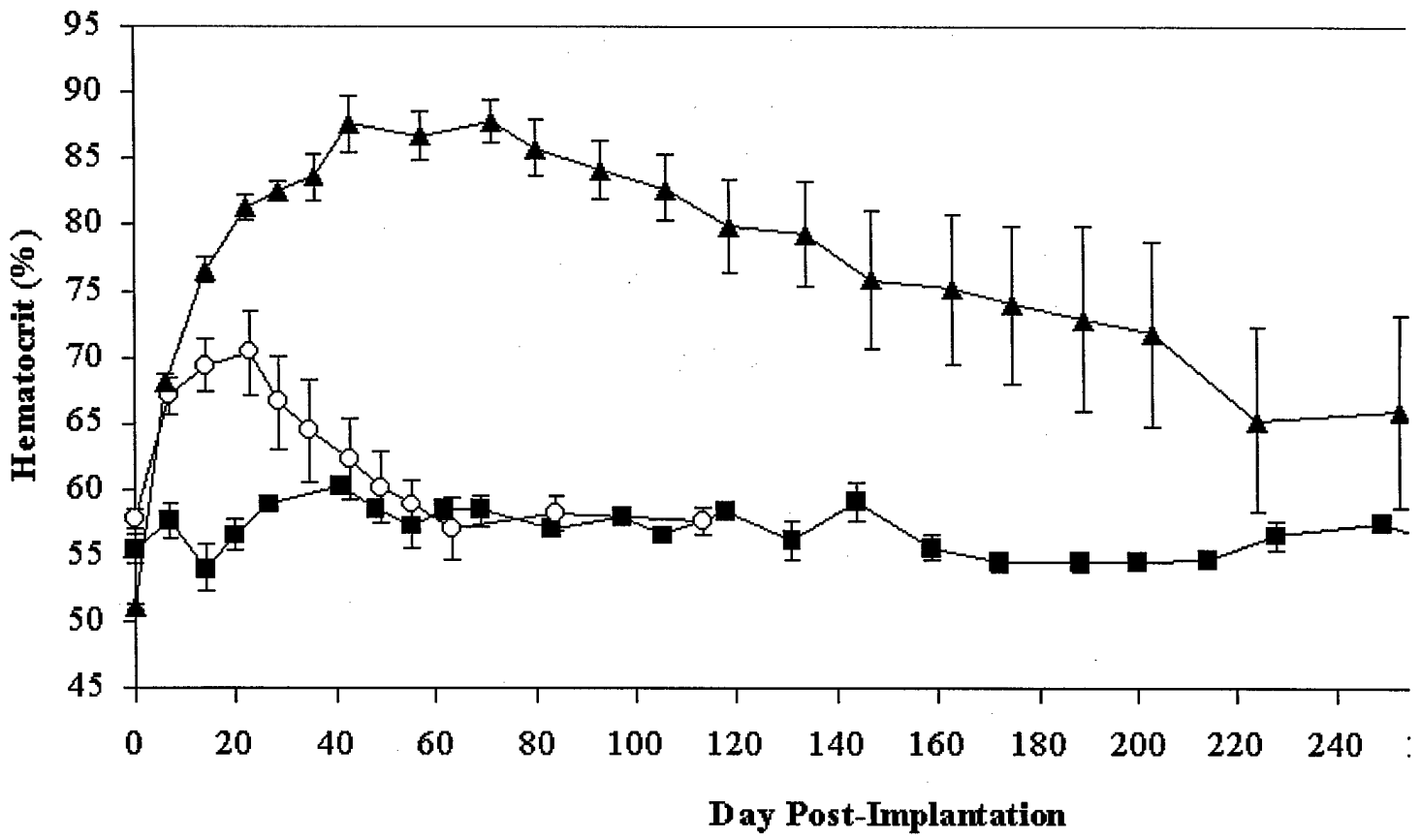


Figure 3

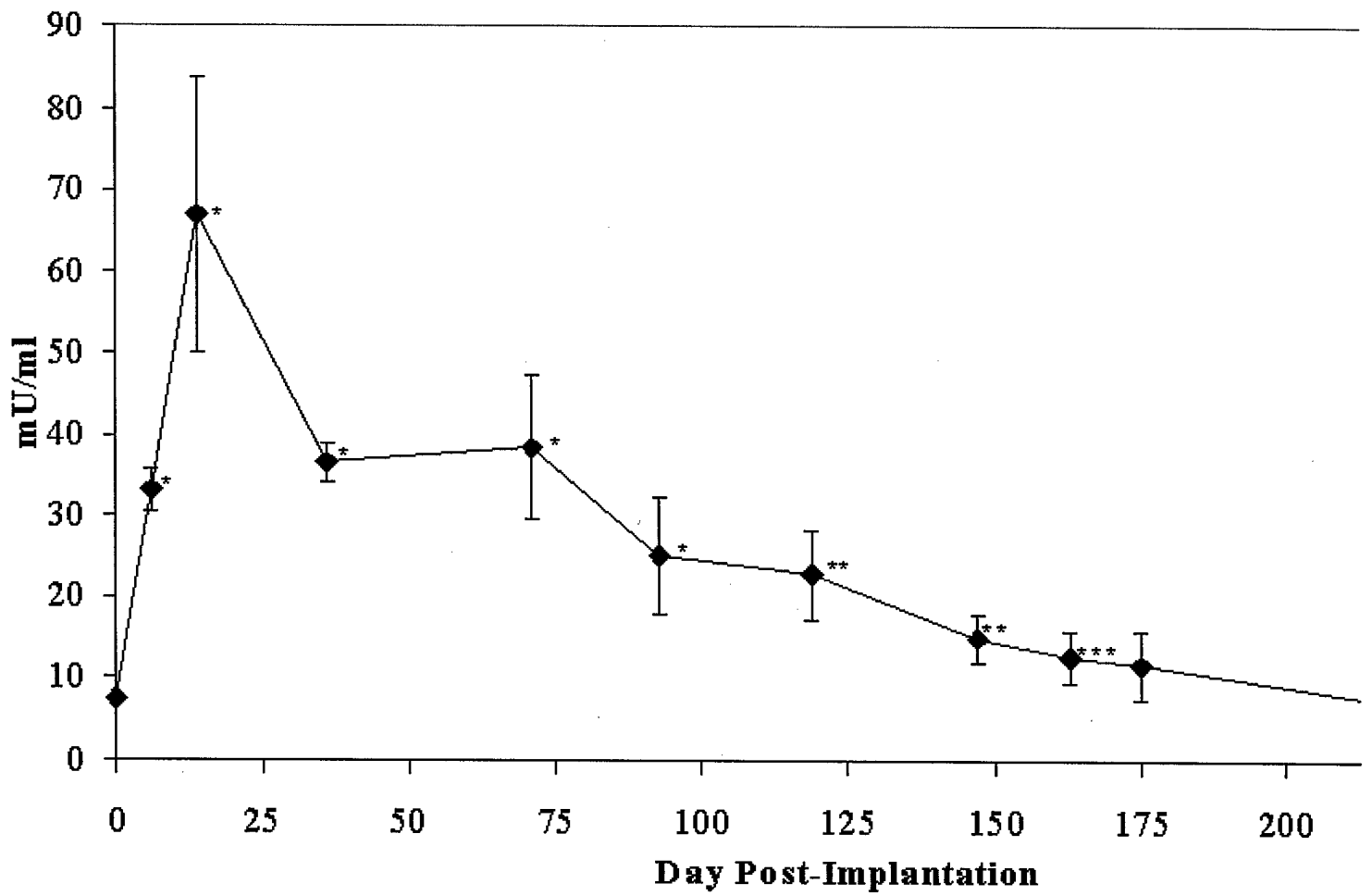


Figure 4

Mouse with Contigen Implant Anesthetized
by Isoflurane Inhalation for Surgery



Skin Incision Exposing Contigen Implant



Beginning of Retrieval of Contigen Implant



Last Step in Retrieval of Contigen Implant



Post-Removal of Contigen Implant



Figure 5

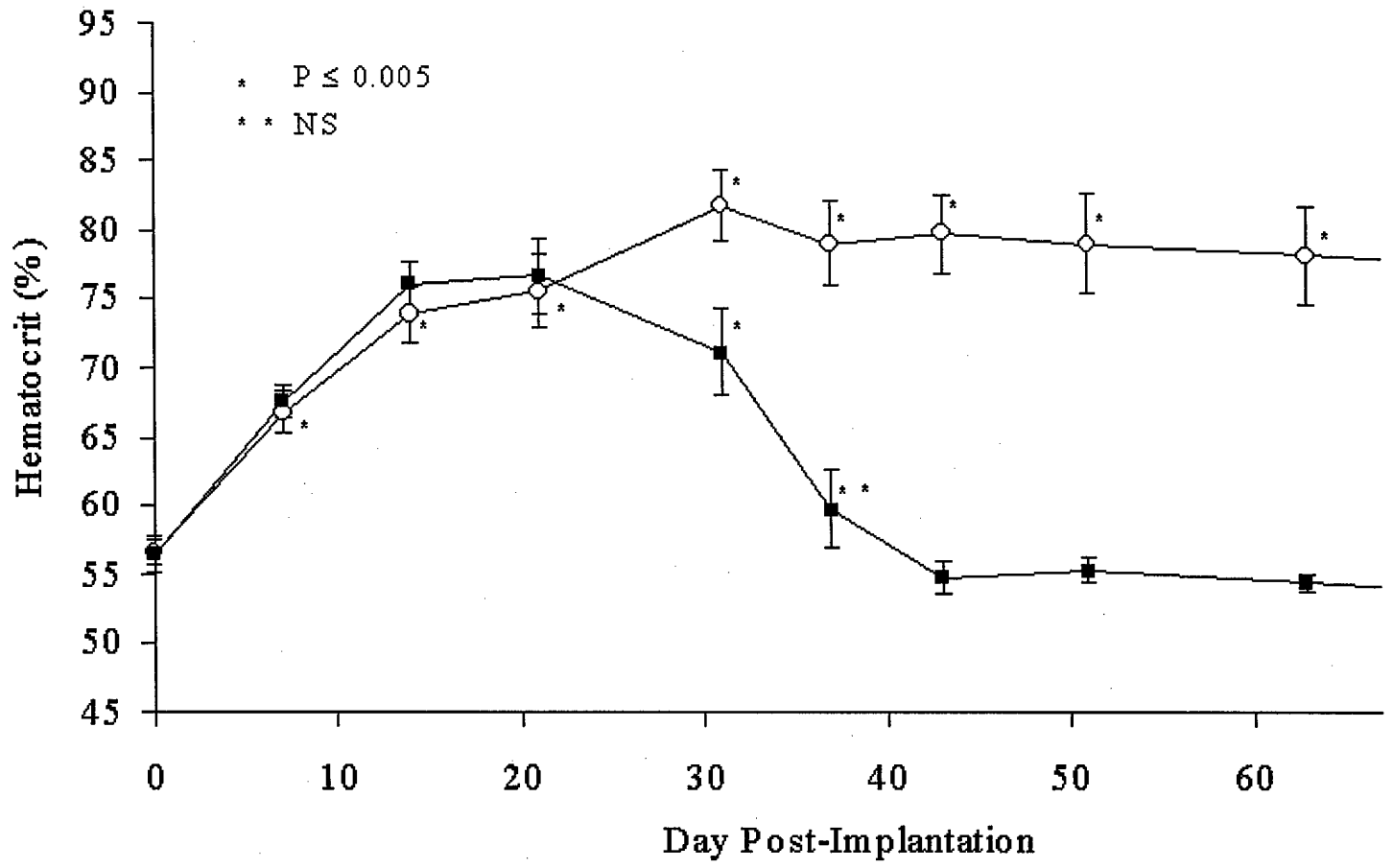


Figure 6

A.



B.

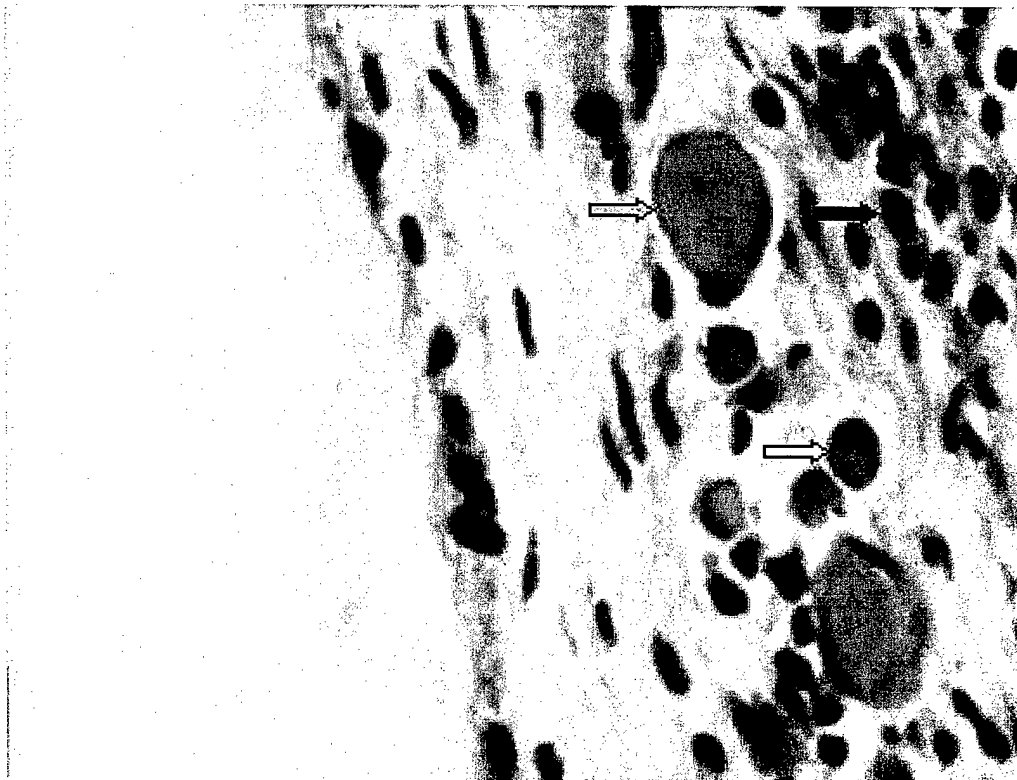
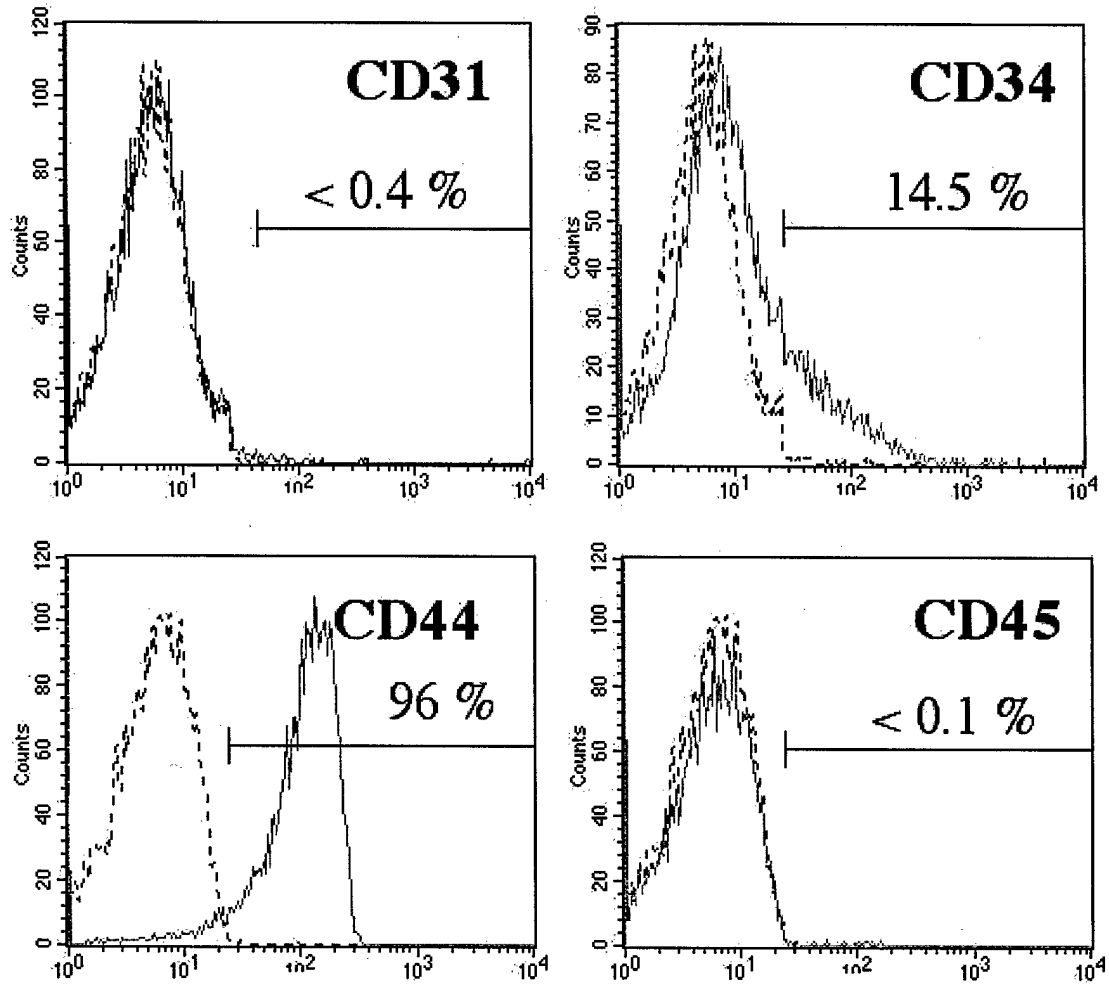


Figure 7



FALL 2002

American Association for

Summer Workshop Teaches Students About the Pathobiology of Cancer



The majestic backdrop of the Rocky Mountains framed the Edward A. Smuckler Memorial Workshop on the pathobiology of cancer. The July 14-21, 2002, workshop at the Keystone Resort in Keystone, Colorado, provided intensive training through laboratory exercises and lectures to enhance attendees' knowledge of current concepts and research in pathology and oncology. Of more than 175 applicants, 99 students participated, including six senior scientists. The workshop is funded through a grant from the National Cancer Institute.

Taught by leading pathologists, the workshop is designed for predoctoral students and postdoctoral fellows engaged in research in areas such as molecular biology, genetics, biochemistry, cellular biology, immunology, experimental therapeutics, and virology.

"These individuals work in molecular and cellular biology, but really don't have the opportunity to experience what human cancer is all about," said Dr. John M. Lehman, the Workshop Director and Professor of Pathobiology and

Microbiology at Albany Medical College. "This is an excellent way of training future scientists about the pathobiology of cancer. All students go away with a learning curve; in a sense, they bring information to the course, but take so much more away."

Students were assigned to lab groups and heard an introduction to histology followed by a rotation of labs that dealt with malignancies of specific organ sites: breast, uterus, cervix, ovaries; male genitourinary tract; hematopoietic tissues and AIDS; lung and skin; gastrointestinal tract; and childhood developmental neoplasms and primary brain tumors. Correlations were made with known genetic alterations in specific types of human cancer and with animal models, particularly transgenic and gene deletion mouse models.

"The labs enabled students to become familiar with the pathogenesis of human cancer with actual case presentation," said Dr. Lehman. "We also try to highlight the labs with discussions from faculty about the cutting-edge research that's going on

Other AACR Educational Workshops

Pathobiology of Cancer will be held July 13-20, 2003 at the Keystone Resort in Keystone, CO.

Molecular Biology in Clinical Oncology will be held June 27-July 3, 2003, at the Given Institute of the University of Colorado in Aspen, CO.

Methods in Clinical Cancer Research will be held July 26-August 1, 2003, at the Vail Cascade Hotel & Club in Vail, CO.

Please check in the coming months for information on the AACR Website at www.aacr.org.

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Suggestions and news items are welcomed.

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A Message from AACR President, Susan Band Horwitz



It is truly an honor for me to serve as the President of the American Association for Cancer Research. I met many Associate Members during the recent Annual Meeting, and your enthusiasm and interest in AACR programs encourages me in my belief that working together, we can both strengthen the AACR, and help to develop your professional careers. Your participation at the Annual Meeting provides you with the opportunity to meet and network with established scientists who are committed to helping you achieve your career goals. The Associate Members represent the future of the AACR, and I personally am very proud of your many accomplishments. Your dedication to the cure and prevention of cancer through your basic and translational research projects is impressive and ensures the future of our organization.

We are fortunate to be working at a time when there are enormous opportunities to make meaningful discoveries in cancer etiology and progression, and also to translate these into better patient care. The most important thing you can do to enhance your career is to perform the highest quality research in whatever area you choose to work. It is our responsibility to educate ourselves and the public on the significant advances that are being made in cancer research and treatment. I look forward to working with you and your leadership body, the Associate Member Council, in the coming year and addressing your specific needs, which will strengthen the AACR and the next generation of cancer researchers.

Susan Band Horwitz, Ph.D.
Falkenstein Professor of Cancer Research
Albert Einstein College of Medicine
Bronx, New York
AACR President, 2002-2003

A Message from the Associate Member Council Chairperson, Cynthia Wetmore



It is a privilege and honor to serve as Chairperson of the Associate Member Council (AMC). The AMC is the leadership body representing the interests of all Associate Members of the AACR. We hope to continue to grow in strength, diversity, and effectiveness and to foster the development of early career investigators involved in cancer research.

Recently, the AMC submitted a Development Plan to the Board of Directors of the AACR, which outlines goals and objectives of the AMC for the next five years. A new AMC Committee, International Issues, has been formed to address the specific concerns of investigators working outside of the United States. We are developing programs to foster more interaction among basic, clinical, and translational investigators.

We are in the midst of a very exciting time in cancer research with advances in biology and technology making it possible to address questions that were not approachable a decade ago. We are the future of cancer research, and as Associate Members, are in a fortunate position to be recognized by the governing body of the AACR and to have financial support and the enthusiastic collegial support of the senior membership. The AMC asks for your help in developing relationships and programs to further our understanding of this disease and to work together to improve cancer treatment.

I encourage you to take advantage of the programs the AACR offers and consider participating in some of the programs, workshops, discussions, or social events sponsored by the Associate Member Council. It may be one of the best investments you can make in your future success in cancer research. We look forward to seeing you at the 2003 Annual Meeting in Toronto and hearing from you in the intervening months.

Cynthia Wetmore, M.D., Ph.D.
Associate Professor and Senior Associate Consultant
Division of Pediatric Hematology/Oncology
Mayo Clinic and Cancer Center
Rochester, Minnesota
AMC Chairperson, 2002-2003

Pathobiology Workshop...

(Continued from page 1)

in their labs, or by having invited guests talk about their research."

This year, the laboratories were equipped with a "virtual microscope" that allowed students to study magnified images of human tissues and cells on digitized slides via a computer. They were provided by Dr. Fred R. Dee, a workshop faculty member and Professor of Pathology at the University of Iowa, who is Principal Investigator on the Virtual Microscope Project at the University of Iowa.

The learning experience is also enriched by lectures on the pathophysiology of cancer given by experts in diverse fields. This year's speakers and topics included:

An Introduction to Neoplasia

John M. Lehman, Albany Medical College

Molecular and Cellular Genetics

Frederic M. Waldman, University of California, San Francisco Cancer Center

Human Papillomaviruses and Cervical Cancer

Karl Münger, Harvard Medical School

The Use of the erbB-2 Transgenic Mouse Model To Study Epigenetic Factors Associated with Breast Morphogenesis and Neoplasia

Ann D. Thor, University of Oklahoma Health Sciences Center

The Therapeutic and Diagnostic Implication of HER-2 Alteration in Human Breast Cancer

Dennis J. Slamon, University of California, Los Angeles

Modeling Cancer Pathobiology Using Transgenic and Knockout Mice

Jeffrey M. Arbeit, University of California, Los Angeles

Navigating the NIH

Marianna J. Bledsoe, National Cancer Institute

Stem Cells and Cancer

Stewart Sell, Albany Medical College

Dr. Carlyne Cool, Assistant Professor in the Department of Pathology at the University of Colorado Health Sciences Center presents the specimens to be used during the Skin and Lungs Laboratory.



Students and faculty participate in lunchtime roundtable discussions.

Critical Determinants of Metastasis

Isaiah J. Fidler, UT M.D. Anderson Cancer Center

Students also had the opportunity to interact with faculty during roundtable discussions on topics such as centrosomes and genomic instability, prostate cancer research, gene therapy, and women in science.

"My graduate program does not deal a lot with histology or pathology courses, so I came to the conference with a big knowledge gap in this area," said Kelly K. Andringa, a graduate student at the University of Iowa. "Some of the research I do deals with tissue samples, therefore having an understanding of what the pathologist is seeing and reading will greatly enhance not only my knowledge but also my understanding of my results."

Each participant was given the opportunity to present one poster of ongoing or proposed research projects at two evening poster sessions. This year, two

awards were given to students for poster abstracts in basic and clinical research. Dr. Nicoletta Eliopoulos of the Lady Davis Institute for Medical Research at McGill University received the 2002 Edward A. Smuckler Award for Basic Science. Dr. Susan H. Wei of the University of Missouri School of Medicine's Ellis Fischel Cancer Center received the 2002 Rodger C. Haggitt Award for Translational Science.

Awards were also presented to faculty members. Dr. Stewart Sell received the 2nd Annual Gary J. Miller Award, named for Dr. Gary J. Miller, a distinguished faculty member who died shortly before the 2001 workshop. Dr. Isaiah J. Fidler received a Special Recognition Award for outstanding lectureship.

For more information on the Pathobiology of Cancer Workshop, contact Ms. Nadine Lomakin, AACR Program Administrator, at lomakin@aacr.org.

Dr. Brian J. Druker: A Career in Research

Brian J. Druker, M.D., is Director of the Leukemia Center at the Oregon Health & Science University Cancer Institute in Portland, OR. Dr. Druker earned his medical degree from the University of California, San Diego School of Medicine; served his internship and residency in internal medicine at Barnes Hospital of Washington University School of Medicine in St. Louis, MO; and completed his medical oncology fellowship at Dana-Farber Cancer Institute, Harvard Medical School, in Boston. He is also a member of the AACR Board of Directors.

Dr. Druker is world renowned for his breakthrough in leukemia treatment, imatinib (Gleevec), a tyrosine kinase inhibitor. Dr. Druker played a key role in shepherding imatinib through development—from early laboratory work to large-scale clinical trials in patients with chronic myelogenous leukemia (CML). Imatinib has also worked in clinical trials on gastrointestinal stromal tumors (GIST), a previously untreatable intestinal cancer.

Imatinib, which was approved for treatment of all phases of CML by the Food and Drug Administration in the United States, has been hailed as the first of a new class of molecularly targeted therapies against cancer.

Among many other awards and honors, Dr. Druker received the AACR-Richard and Hinda Rosenthal Foundation Award in 2001.

In this interview, Dr. Druker provides insight into such issues as choosing a mentor, developing collaborations, honing career skills outside of research, and the factors that led to his success as a cancer scientist.

AACR: To what do you attribute your success in cancer research?

Druker: I believe the most important aspect was getting good training—both in medicine and basic research. In 1985, after completing my medical training, I started

working in a lab and continued there until my first faculty appointment nine years later. With any training, the broader your base is, the higher your peak can be.

Having completed training it was time to make a decision: should I do pure basic science and contribute to the cancer community by gaining a greater understanding of cancer, or combine basic science and clinical training by focusing on a specific human disease and use this as a springboard to developing therapies for cancer. This was a transition I wanted to make, and for me, the ideal combination of my medical and basic science training was to focus on CML.

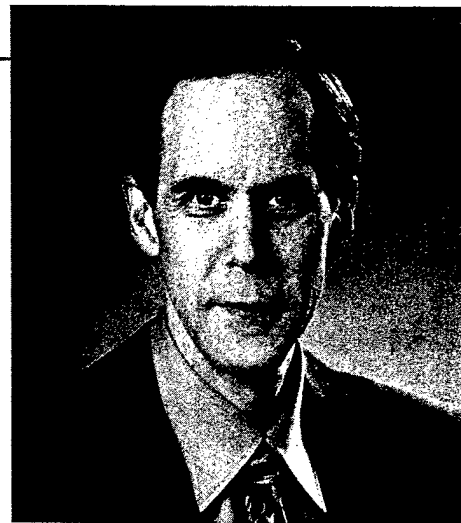
AACR: What is the best way for early-career scientists to progress in their field?

Druker: For me, it was a matter of being patient and getting solid training. Research and training take time and patience. If getting ahead is your entire focus, you won't take the time to get properly trained.

AACR: Who are your mentors? How do you choose a mentor?

Druker: I've had many mentors. There has not been one person. What I have tried to do is to combine qualities or strengths from many different people. My first research experience was as an undergraduate student with Dr. John Abelson, George Beadle Professor of Biology at California Institute of Technology, in 1976. At the time, he was on the cusp of becoming a household name for his work in molecular biology. It was a very exciting time in his laboratory. He shared with me that his success had come in his late 30s, early 40s—which, for a Ph.D., is relatively late in his career. I remember that it gave me hope. I could be unfocused early on in my career and still become successful.

During medical school, I worked on tumor immunology with Dr. Terry Wepsic. Terry has an incredible love of science: he



lived, ate, and breathed science. It was amazing to see his enthusiasm, and he instilled this enthusiasm in those who surrounded him.

Dr. Thomas Roberts of the Dana-Farber Cancer Institute is also a mentor. I worked with him on tyrosine kinases. He's an incredible scientist. He always thought big, and had ideas that were often years ahead of their time. He could synthesize fields and see beyond where we are. As a young scientist, these ideas were attractive. But as with many ideas that come before their time, progress can be slow and you can feel that you're not getting anywhere.

Dr. Jim Griffin, of Dana-Farber, helped me get started on the leukemia project. Jim brought tremendous focus to his work, and as a result was highly successful. The combination of working for Tom, who taught me to think big, and Jim, who taught me to focus, has served me well.

Lastly, Dr. Grover Bagby, Director of the Oregon Health & Science University Cancer Institute, helped get me set up in a lab with my first faculty appointment. I admired his honesty and his belief in me. At some point in your career, you need someone who believes in you and who understands what it takes for you to become successful. For me, it was Grover.

AACR: How do you develop collaborations?

Druker: In 1988, I developed an antiphosphotyrosine antibody to detect substrates of tyrosine kinases. In those days, only a couple antibodies were available and ours outperformed the others. At that time, identifi-

cation of tyrosine phosphorylated proteins was the focus of hundreds of laboratories, and I was contacted by many of them for assistance with their work.

Generally, I tend to be accessible—you never know who you're talking to. It's just as easy to be friendly as unfriendly. By collaborating with others, you open up so many doors. I had something people wanted, and in turn, my collaborators gave me access to their agents and advice.

AACR: Are there career development skills that early-career scientists should hone outside of research?

Druker: The most important skill an early-career scientist can learn is the ability to write grants. Seeking out someone who has received a grant doesn't necessarily mean they're a good person to help you. It is far better to get help from someone with significant experience on one of the NIH study sections. They will be the most helpful in terms of knowing when, how, and what to write.

I was very fortunate—when I moved to Oregon, I had my first grants looked at by several OHSU faculty with study section experience and expertise in my field. Their

advice was critical to securing funding for me, at a time when less than 10% of grants were being funded. As I now have my own study section experience, I have learned even more about the components of a good grant.

I also feel that it's our responsibility as scientists to educate the public about what we do. Ultimately, the public pays for our research, and, more importantly, votes for the representatives who set the NIH budget. It's very difficult to talk to people in a way that's accessible and understandable. My wife, who is a writer, has helped me with analogies to help accomplish this goal, and I have again been extremely fortunate to have this assistance.

AACR: What career advice do you wish someone had given you 10 years ago? 20 years ago?

Druker: When I was applying to medical school, people would ask me what I wanted to do in 10 years. I told them that I wanted to do research, teaching, and patient care. I was told that that was unrealistic—it was unusual to do well at one, and certainly not possible to do well at all three, and that I should seriously consider revising my goals.



It's easy to be discouraging; it's much more difficult to encourage people. I would much rather have been told that my goals would be difficult to reach, but if I was committed to achieving them, I wish someone had said, 'Here's what you need to do.'

Dr. Lucile Adams-Campbell on Seeking a Post-Doctoral Position

By Cherie L. Butts, M.S.

Lucile L. Adams-Campbell, Ph.D., currently serves as the AACR-MICR Chairperson and is Director of the Howard University Cancer Center in Washington, DC, the only African-American female Cancer Center Director in the country. She received her Ph.D. in Epidemiology from the University of Pittsburgh in 1983, and continued there to do her post-doctoral work in cardiovascular disease. She is also a Professor of Medicine (tenured), Graduate Professor of Psychology, and Associate Professor of Physiology and Biophysics at Howard University.

Dr. Adams-Campbell was recently nominated to the Board of Directors of the Association of American Cancer Institutes.

Dr. Adams-Campbell is a fellow of the American College of Epidemiology and has received numerous awards, including the Distinguished Alumni Fellows Award from the University of Pittsburgh; the Sigma Xi National Lecturer Award; the FDA Deputy Commissioner Community Service Award; the Searle Distinguished Graduate Award; as well as a host of others. Dr. Adams-Campbell's research focuses on women's health issues, clinical trials, and cancer prevention and control in the African Diaspora. She has over 90 peer-reviewed journal publications reflecting her research focus.

In this article, Dr. Adams-Campbell offers her thoughts and advice about vari-



ous aspects of seeking a post-doctoral position in today's scientific environment.

According to Dr. Adams-Campbell, the most influential aspects of her post-doctoral training experience were her working environment and her mentor. "The relationship with my mentor was critical," she said.

"Also, the environment was key, which was conducive to working in research. The people whom I worked with had a com-

(Continued on next page)

Dr. Adams-Campbell...

(Continued from page 5)

mon interest in major research, and the expectancy of individuals was similar among the group. Other aspects, including teaching, were considered secondary."

In selecting the appropriate laboratory for her post-doctoral training, Dr. Adams-Campbell decided to remain at the University of Pittsburgh; however, she generally recommends that her students train at other institutions. "Although I was highly recruited by other institutions, I decided to stay with my mentor because he had been strongly supportive of me," said Dr. Adams-Campbell. "I knew that he would open doors for me by sending me to meetings and introducing me to other scientists. I knew that I wouldn't be treated as an extra 'pair of hands.'"

If you're wondering whether you would be better off doing a post-doctoral fellowship in a "big name" laboratory versus choosing a less well-known mentor who would be more readily available, Dr. Adams-Campbell said that it depends upon you, the individual. "At this stage, everyone must become independent. Although it is much easier to obtain a junior faculty position when coming from a laboratory with a big name, an individual should not rely on the fame of the mentor. Whichever type of mentor one chooses, setting up an individual's own reputation is important, and developing networks and lines of communication should be done independently and with the help of the mentor."

Dr. Adams-Campbell not only stressed the importance of post-doctoral training in pursuing a faculty position, but also the need for post-doctoral trainees to establish themselves as researchers. "It is imperative to establish oneself as a researcher, which is done through successful grant-writing and publications," she said. "But the first years of a junior faculty position include heavy teaching and other responsibilities that

take away from time to do research. The time spent as a post-doctoral trainee should be used to develop a record for writing grants and publishing work on research."

It is clear Dr. Adams-Campbell believes that writing is the most important skill one should develop during the post-doctoral experience, but she said individuals should also develop skills to be able to make presentations at national meetings.

On the same topic, Dr. Adams-Campbell said she recognizes that in basic science it is becoming increasingly common for people to do two post-docs, and believes that a minimum of three years in a fellowship is necessary to have sufficient time to write grants and publish. "It is important to be highly successful in the first post-doc in order to avoid needing to do additional ones, and if necessary, an individual should stay longer in the first one to ensure the success of the experience," said Dr. Adams-Campbell.

In terms of the importance of choosing a field for your post-doc that is very different from your pre-doctoral training experience, Dr. Adams-Campbell said, "Pre-doctoral training is usually done in laboratories that have sufficient funding to complete a project. The post-doctoral experience should be used to establish a reputation in the field you hope to study throughout your scientific career."

She added that the post-doctoral experience doesn't necessarily have to be different from your pre-doctoral training if you're seeking an industry position as opposed to an academic position, because industry representatives usually recruit from academia to select the best candidates.

The following are Dr. Adams-Campbell's thoughts on what steps are necessary in obtaining a post-doctoral fellowship position:

- Identify what you want out of your career.
- Search the Internet or speak with your mentor to identify institutions and individuals moving in the appropriate directions.
- Make contacts with individuals.
- Research the publication record of individuals in your field and examine what research is being conducted.
- Contact junior faculty at the institution to ask about their experiences and assessments of the work environment.
- Do not choose an institution or person based on name only.

"It is mandatory to obtain your own funding for your post-doctoral experience," said Dr. Adams-Campbell. "It is important to at least go through the process of grant submission. Follow-ups to the critiques made in initial grant submissions and revisions to submissions are also just as important. This will help in building a reputation, which is necessary to move forward."

In closing, Dr. Adams-Campbell offered her "formula" for a successful post-doctoral experience:

- Come in with a plan and strategy of goals.
- Negotiate your project and make a clear road map of the path for completing it.
- Publish, publish, publish to show productivity.
- Work on securing grants.

Ms. Butts is a graduate student in the department of gynecologic oncology at UT M.D. Anderson Cancer Center, Houston, TX.

Mentoring: Professional Support for Career Success

By Jean-Philippe Spano, M.D.

Pursuing a career in cancer research is a difficult challenge; however, getting advice and skills from other students, researchers, supervisors, or a mentor can enhance your training and make the road less arduous.

Although the choice of your institution, research discipline, and work remains an important aspect of your doctoral education, the choice of your mentor may be the most important decision you will make. The role of scientific mentors is to guide students while providing them with optimal instruction and direction for their research. Good mentors will provide their students with enough freedom to pursue their work and also ample time for discussion.

In addition to providing scientific guidance, mentors can help to locate research funding, advise on proper writing techniques to establish a strong publications record, or introduce students to the leading scientists in their field. In short, a mentor should arm a student with the tools necessary for achieving success and instill the attitude that he or she can be successful.

The student in turn should become a good mentor. To help develop their own mentoring skills, students should help oth-

ers meet deadlines, and emotional support and encouragement are especially important. Students can also learn from other students or researchers who can provide different feedback than their mentor.

Forming relationships and partnerships is essential during this long scientific process. Early-career scientists are faced with changing situations. Enthusiasm will be high at the beginning of a project due to the new experience and the desire to achieve results quickly. However, science requires a lot of time and a lot of research, so enthusiasm may diminish and students may feel like dropping out. They often feel that they won't ever get results and won't meet deadlines. The increased concentration that is necessary, along with working long hours, can create frustration and exhaustion.

It is during this challenging phase that the support of mentors, colleagues, and fellow students will help students re-focus and organize their work; manage their time; set goals; and become inspired and energized by the potential of the work. With this collegial support, feelings of frustration are replaced by feelings of euphoria. Where stu-



dents may have once felt overwhelmed, they now feel powerful and in complete control of their work. Step by step, the process appears easier; results appear; and motivation helps students carry through.

Early-career scientists should be open to mentoring relationships and develop their own mentoring skills. Partnership and mentorship play a crucial role in helping students reach their goals, to ensure they're in the best position to develop their skills, and to afford the opportunity to be independent researchers in their field. In time, the mentée will become the mentor.

Dr. Spano is a clinical fellow at the Department of Oncology, Hospital Avicenne, Bobigny, France.

Associate Members are encouraged to:

A. **Review** membership information as it appears on the AACR Online Directory and forward any revisions by e-mail to membership@aacr.org.

C. **Apply** for a transfer in status from Associate to Active if you are no longer in training and qualify for this membership category.

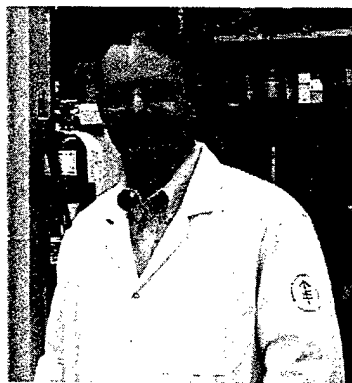
B. **Submit** Associate Member Dues payments ASAP.

D. **Tell** a colleague so that he or she can take advantage of membership in the AACR.

AACR Fellowships Support Young Cancer Research Scientists

Since 1996, the AACR has provided more than \$1.5 million to support 41 outstanding young cancer research scientists through highly competitive Research Fellowships. The fellowships, which have terms from one to three years, foster the basic, translational, clinical, and prevention research of clinical and postdoctoral fellows by providing \$30,000 of salary support per year.

If you are interested in applying for an AACR Research Fellowship, visit the AACR Website at www.aacr.org. The application deadline is November 15, 2002.



Loren S. Michel, M.D., Research Fellow at Memorial Sloan Kettering Cancer Institute, New York, NY, recently completed the 2001-2002 AACR-Sidney Kimmel Foundation for Cancer Research Fellowship in Basic Research for his study on the role of

Mad2, a mitotic checkpoint protein, in tumorigenesis, cell cycle control, and drug sensitivity. The data suggest that the mitotic checkpoint could be a potential target of cancer therapy and may enhance the utility of spindle-disrupting agents such as the taxanes and vinca alkaloids. Dr. Michel, a medical oncologist by training, began working in cancer research after a clinical fellowship in medical oncology convinced him that breakthroughs in treatment would ultimately originate from the laboratory. For him, the AACR Research Fellowship "has been an excellent bridge for additional longer-term grants" as he pursues his new career path.

Stacy Moulder, M.D., Clinical Fellow at Vanderbilt University Medical Center, Nashville, TN, received the 2001-2002 AACR-Amgen, Inc. Research Fellowship in Clinical or Translational Research. Her research project involved determining the effects of

inhibition of the flk-1 receptor on tumor vasculogenesis. As a clinical oncology fellow, Dr. Moulder first realized that the future of cancer therapy would involve molecular strategies that targeted pathways unique to cancer cells. "Through this fellowship, I learned basic laboratory techniques, how to interpret results, and how to critically review the basic science literature," she states. "In addition, with the protected research time provided by the fellowship, I was able to establish a basic science knowledge base that will be invaluable to me as I pursue an academic career in cancer research. The future of cancer therapy lies in the explosion of basic science research supported through the AACR. The organization has benefited me not only through financial support, but also by providing a forum for the presentation and discussion of novel scientific data."



The 2001-2002 AACR-Anna D. Barker Fellowship in Basic Cancer Research was presented last year to **Sheila A. Stewart, Ph.D.** Dr. Stewart, a Postdoctoral Fellow at the Whitehead Institute for Biomedical Research, Cambridge, MA, has been interested

in understanding the role of telomerase in tumorigenesis. "Telomerase is known to elongate telomeres and it was assumed that this was its sole contribution to tumorigenesis. Since I received my AACR grant, I was able to complete experiments that demonstrated that the role of telomerase in tumorigenesis goes beyond its ability to elongate telomeres. This work will soon be published in the *Proceedings of the National Academy of Sciences.*" Dr. Stewart noted that AACR funding also allowed her to look for an independent position.

AACR Scholar-in-Training Awards

AACR offers a variety of awards that enhance the education and training of early career scientists by providing financial support for their attendance at the AACR Annual Meeting.

Abstract Submission Deadline: November 14, 2002.

ELIGIBILITY

- Eligible candidates are graduate students, medical students and residents, clinical fellows or equivalent, and postdoctoral fellows.
- Eligible candidates must be first authors on abstracts.
- Eligible candidates may be traveling within the U.S. or from abroad.
Special awards are available for those traveling from Asia and Europe.
- AACR Associate Members and those who are not yet members are eligible.

APPLICATION

Qualified persons who want to be considered for an AACR Scholar-in-Training Award should follow the instructions included in the abstract submission materials for the AACR Annual Meeting and must submit their abstracts by November 14, 2002.

NOTIFICATION

Selection is based upon the abstract rating of the proffered paper; AACR staff are not able to provide information about specific abstract ratings. Winners of AACR Scholar-in-Training Awards will receive notification separate from abstract acceptance and scheduling information. Note that selection for a poster session, poster discussion session, or minisymposium does not automatically mean that the presenter will receive an AACR Scholar-in-Training Award.

SUPPORT

A stipend (in an amount between US\$400–\$2,000) will be presented to winners on-site. If an award winner is unable to attend the Annual Meeting, or will not be the presenter of the paper, the award must be forfeited.

AWARDS

Eligible candidates are automatically considered for an unrestricted award and any restricted award(s) for which they qualify. For details about specific awards, visit the AACR Website at www.aacr.org. AACR Scholar-in-Training Awards are generously sponsored by AACR, AFLAC, Inc., AstraZeneca, Aventis, Bristol-Myers Squibb Oncology, Busch Family Travel Fund, California Department of Health Services–Cancer Research Section, Genentech, GlaxoSmithKline, ILEX, Inglenook Vineyards, ITO EN, Ltd., Novartis, Ortho Biotech, Inc., Pezcoller Foundation, Pfizer, Inc., Pharmacia Corporation, and others.

Complete details about the nomination process can be viewed at the AACR Website: www.aacr.org.

AACR *American Association for Cancer Research*

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Looking Ahead to the 94th Annual Meeting...

**Abstract Deadline
November 14, 2002**

Associate Member Resource Center (ARC)

New! All Associate Members and those who qualify for Associate Membership will have access to the center, which will be located at the Crowne Plaza Ballroom B (adjacent to the Metro Toronto Convention Centre).

Town Meeting for Early Career Scientists and Associate Members

Sunday, April 6, 2003

Metro Toronto Convention Centre, Room 716

Announcing the Professional Advancement Series

The AACR, through the programs of the Associate Member Council, Women in Cancer Research Council, Minorities in Cancer Research Council, and other committees, is launching the Professional Advancement Series (PAS) to be held at the 2003 Annual Meeting in Toronto.

The PAS sessions will feature leading AACR scientists discussing issues important to a successful career in cancer research. Please look for future announcements about the complete series schedule on the AACR Website (www.aacr.org) and by e-mail.

The Associate Member Council is pleased to announce its PAS sessions for the 2003 Annual Meeting:

Sixth Annual Grant Writing Workshop

Saturday, April 5, 2003 12:00 noon – 5:00 p.m.

Westin Hotel, Harbor Ballroom

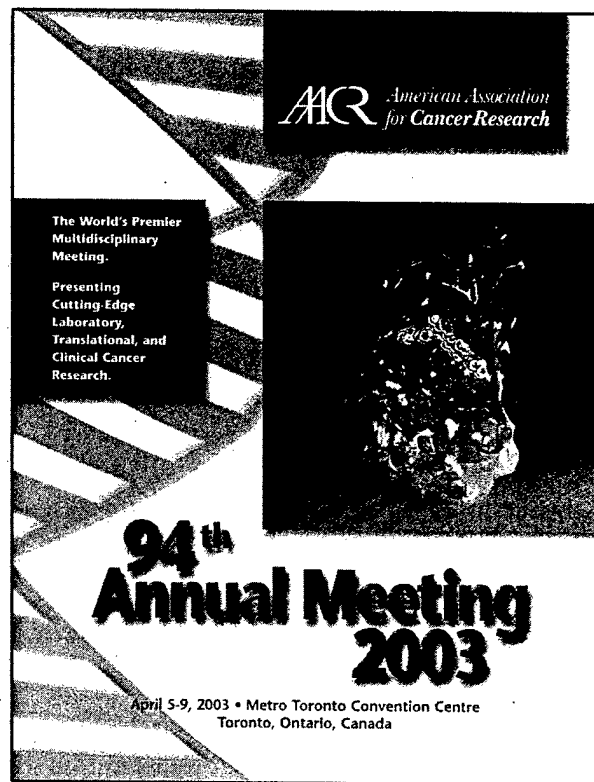
Advance registration required – Registration form to be provided in early 2003

Workshop open only to AACR Associate Members

How to Publish Papers in the Best Scientific Journals: The Insiders' View from the Editors

Monday, April 7, 2003, Sunset Session 6:45 p.m. – 8:00 p.m.

Metro Toronto Convention Centre, Auditorium Theatre Centre



How to Plan, Set-up, and Run Your First Lab

Tuesday, April 8, 2003, Sunset Session 6:45 p.m. – 8:00 p.m.

Metro Toronto Convention Centre, Room 201

The Three C's of Abstract Writing: How to Make It Concise, Clear, and Compelling

Tuesday, April 8, 2003, Sunrise Session 6:30 a.m. – 8:00 a.m.

Crowne Plaza, Ballroom B

Late-Breaking Session News

Dr. Mary L. Disis of the University of Washington has agreed to reprise her role as Chairperson of the Grant Writing Session. Thanks to the former attendees, mentors, and speakers who provide their assessment and suggestions each year, this program continues to evolve and improve. We anticipate another excellent Workshop this year.

Dr. Michael B. Kastan of St. Jude Children's Research Hospital has accepted the Council's invitation to be the keynote speaker of this year's "How to Publish Papers in the Best Scientific Journals: The Insiders' View from the Editors" Session. Dr. Kastan was recently appointed Editor-in-Chief of the newly re-launched *Molecular Cancer Research* (formerly *Cell Growth & Differentiation*).

More speakers and details on Associate Member Council-sponsored Annual Meeting events will be announced early in 2003.

2002-2003 Associate Member Council

Mission Statement of the Associate Member Council

The Council supports AACR's mission to prevent and cure cancer by promoting the professional development of early career scientists throughout the world. The goal of the Council is to foster excellence in cancer research through programs and initiatives related to communications, education and training, and collaboration.

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International Issues Committee Formed

The Associate Member Council announces the establishment of an International Issues Committee (AMC-IIC), which has been formed to address the unique needs, challenges, and opportunities facing AACR Associate Members both from and working in countries outside the United States. The AMC-IIC seeks to increase the number of AACR Associate Members in non-U.S. countries and to encourage participation of all non-U.S. scientists in the programs of the AACR.

The AMC-IIC is being led by Co-Chairpersons, Dr. Monique den Boer, a postdoctoral fellow at Sophia Children's Hospital in Rotterdam, the Netherlands, and Dr. Jean-Philippe Spano, a clinical fellow at Hôpital Avicenne, Bobigny, France. According to Dr. Spano, "It is a common misconception among scientists in non-U.S. countries that AACR membership is open only to those in the United States. This new Committee will work to show that AACR membership benefits all cancer researchers around the world."

Approximately 983 of the 3,747 Associate Members reside outside of the U.S., including members in:

Argentina, Austria, Australia, Belgium, Bulgaria, Brazil, Canada, China, Cuba, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, India, Iran, Ireland, Israel, Italy, Japan, Macedonia, New Zealand, Nigeria, Norway, Poland, Portugal, Russia, Saudi Arabia, Singapore, South Africa, South Korea, Spain, Sweden, Switzerland, Thailand, The Netherlands, Turkey, United Kingdom, Uzbekistan, Taiwan, and Yugoslavia. However, the majority of non-U.S. Associate Members reside in the United Kingdom, Canada, Japan, and Germany.

The AMC-IIC asks all Associate Members to assist in their efforts to communicate with cancer researchers around the world. The Committee seeks information on membership organizations for cancer scientists in non-U.S. countries, particularly those for early-career scientists such as graduate students, medical students and residents, and clinical and postdoctoral fellows. Please forward names and contact information including Websites to Dr. den Boer at denboer@kgk.fgg.eur.nl, Dr. Spano at aerio@noos.fr, or the AACR at waskey@aacr.org.

**Pathobiology
Workshop
Coverage**

ACR American Association
for Cancer Research

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**ASSOCIATE MEMBER
NEWS**

American Association for

**Summer Workshop
Teaches Students
About the Pathobiology
of Cancer**



The student body of the Rocky Mountain School of Health Sciences (RMSHS) gathered for the Summer Memorial Workshop on the Pathobiology of Cancer. The July 14-21, 2002 workshop at the Fox Chase Research Institute, Philadelphia, provided intensive training through laboratory exercises and lectures to enhance students' knowledge of cancer concepts and research in pathology and oncology. Of more than 175 applicants, 79 students participated, including 15 cancer survivors. The workshop is funded through a grant from the National Cancer Institute.

Taught by leading pathologists, the workshop is designed for postdoctoral fellows and postgraduate fellows engaged in research in areas such as molecular biology, genetics, biochemistry, cellular biology, immunology, experimental therapeutics, and virology.

"These individuals work in molecular and cellular biology, but until now they haven't had the opportunity to experience what human cancer is all about," said Dr. John M. Lehman, the Workshop Director and Professor of Pathobiology and

Microbiology at Rocky Mountain College. "This is an excellent use of training future scientists about the pathobiology of cancer. All students go away with a training course in a sense, they bring information to the course, but take so much more away."

Students were assigned to lab groups and had an introduction to histology followed by a session of lab that dealt with malignancies of specific organ sites: breast, stomach, colon, and esophagus. They also learned about hematopoietic tissues and AIDS. Lung and skin gastrointestinal tract and childhood developmental neoplasms and primary brain tumors.

Contributions were made with known genetic alterations in specific types of human cancer and with animal models, particularly transgenic and gene deletion mouse models.

The lab student students to become familiar with the pathogenesis of human cancer with small case presentations," said Dr. Lehman. "We also try to highlight the lab with discussions from faculty about the cutting edge research that's going on."

(Continued on page 2)

**Other AACR
Educational
Workshops**

Pathobiology of Cancer
will be held July 15-20, 2003
at the Fox Chase Research
Institute, Philadelphia, PA.

**Molecular Biology in Clinical
Oncology** will be held June 27-
July 3, 2003, at the Green Institute
of the University of Colorado in
Denver, CO.

National in Clinical Cancer Research
will be held July 28-August 1, 2003,
at the Vail Grande Hotel & Club
in Vail, CO.

For more details and information for
space information on the AACR
website at www.aacr.org.

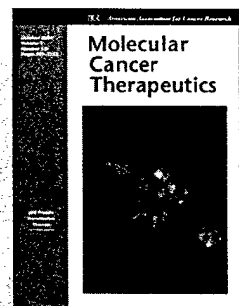
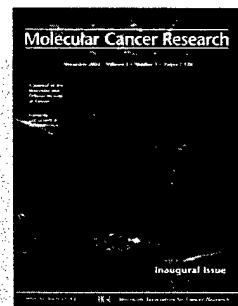
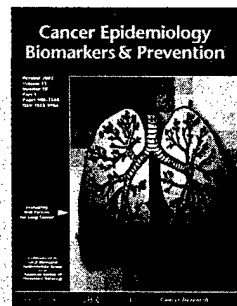
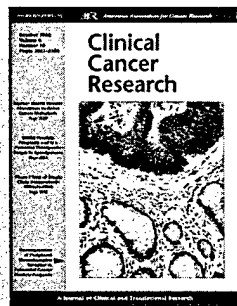
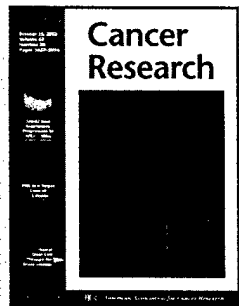
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AACR Associate Member News

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**ACR ASSOCIATE MEMBER
NEWS**



AACR Journals to be Offered at Special "Online-Only" Rates for Associate Members

The AACR's prestigious and highly-cited journals, *Cancer Research*, *Clinical Cancer Research*, *Molecular Cancer Therapeutics*, *Molecular Cancer Research* (formerly *Cell Growth & Differentiation*), and *Cancer Epidemiology, Biomarkers & Prevention*, will be available to Associate Members at special discounted rates for online-only access.

Beginning in January 2003, Associate Members will have immediate, personal access to the journals through the AACR's journals Website, www.aacrjournals.org. Subscribers can view the journals at any time, thus bringing cutting-edge scientific information to desktops whenever and wherever it's needed.


"The new e-journals initiative makes the AACR journals uniquely affordable and accessible for our Associate Members,"

said Jeremy Thompson, AACR's Director of Marketing. "I hope that many of the Associate Members will take advantage of this opportunity to subscribe."

Online-only journal rates for Associate Members for 2003 are as follows:

<i>Cancer Research</i>	\$57
<i>Clinical Cancer Research</i>	\$47
<i>Cancer Epidemiology, Biomarkers & Prevention</i>	\$37
<i>*Molecular Cancer Research</i>	\$33
<i>Molecular Cancer Therapeutics</i>	\$33
*(Formerly <i>Cell Growth & Differentiation</i> ; launching in November 2002)	

Full details about how to subscribe will be on the dues notice and will be e-mailed to all Associate Members in November.



ACR *American Association
for Cancer Research*

**The 2002 Edward A. Smuckler Award
for an outstanding abstract in basic science**

is presented to

Nicoletta Eliopoulos

**The 2002 Edward A. Smuckler Memorial
Pathobiology of Cancer Workshop**

July 14 - 21, 2002
Keystone Resort
Keystone, Colorado



**Pathobiology
of Cancer**
*The Edward A. Smuckler
Memorial Workshop*