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TITLE: Activation and Protection of Dendritic Cells in the Prostate Cancer Environment

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CONTRACTING ORGANIZATION: University of Medicine and Dentistry of New Jersey
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Fourth annual report for this award. Experiments were conducted as was scheduled in the Statement of Work. In vivo experiments were carried out, in which mice with prostate cancer (RM-1 cells) were treated with modified dendritic cells (DC). These cells were treated TNFa and ETB receptor inhibitor. Unlike experiments performed in previous years of this award, DC were delivered not intratumorally, but in subcutaneously in the flank opposite to the tumor injection side. Because of that, these DC were stimulated with RM-1 cells lysates, to provide target antigen for DC. Translating to human population, if these experiments are successful, they should provide the ability to treat patients who have no easily injectable tumor site. This treatment resulted in reduction of prostate cancer growth in mice in the experimental group, in comparison to untreated control mice. No statistically significant difference was found due to low number of treated mice, but more experiments are scheduled. RNA was extracted from different cells (murine prostate, murine prostate cancer cells, dendritic cells after different stimulation), and comparison gene arrays are underway. We hope that these arrays will provide us with directions for further more detailed studies to elucidate the mechanisms of prostate cancer-induced apoptosis of DC, and the role of endothelin receptors in the functioning of dendritic cells.

15. SUBJECT TERMS
Prostate cancer, Dendritic cells, Endothelin receptors, Immunotherapy

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Introduction:

This study is being conducted for the (i) characterization of the prostate cancer and dendritic cells (DC) interaction; (ii) defining the role of endothelin axis in the maturation of DC, (iii) elucidating the role of endothelin axis in the prostate cancer-DC interaction, and (iv) modification of dendritic cells to be used in the treatment of prostate cancer. Mouse model will be used. This is the report for the 4th year of the award, from (2008), covering the work done at the New Jersey Medical School. In August of 2008, I have accepted a position of the associate professor at the Virginia Commonweakth University (VCU) School of Medicine, and was able to move lab there by the end of the year, and continue working on this project. Experiments are progressing according to the plan so far.

Report:

For most of the experiments, mice bone-marrow derived DC were needed. For this purpose, DC were grown from C57BL/6 mice bone marrow, as was described earlier [1]. Briefly, bone marrow cells were first depleted of RBC with lysing buffer for 2–3 min. The single-cell suspensions then was incubated with a cocktail of Abs (αCD4, αCD8a, and B220) for 1 h at 4°C, followed by incubation with rabbit complement for 30 min at 37°C to deplete cells expressing lymphocyte Ags B220, CD4, and CD8. Cells were then incubated overnight (37°C, 5% CO_2) in six-well plates (Falcon, Franklin Lakes, NJ) at a concentration of 10^6 cells/ml in complete medium, consisting of RPMI 1640, 2 mM L-glutamine, 50 μg/ml gentamicin sulfate, 10 mM HEPES, 10% FBS, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate (Life Technologies). The nonadherent cells were collected by gentle pipetting and resuspended at a concentration of 2.5 x 10^5 cells/ml in complete medium supplemented with 1000 U/ml recombinant murine GM-CSF and recombinant murine IL-4 (R&D system). Cells were cultured in six-well plates (4 ml/well) for 7 days at 37°C in 5% CO_2. Nonadherent DC are collected by gentle pipetting, counted, characterized as described previously [2], and used for further studies.

In previous experiments, as it was described in the annual reports, we demonstrated the ability of dendritic cells to produce endothelin-1 (ET-1) after the stimulation with either TNFα (20 ng/ml final concentration) or the lipopolysaccharide (LPS, Sigma-Aldrich). We have also demonstrated the increased expression of endothelin receptors on DC after their stimulation.

We also evaluated the effect of the endothelin receptors on the expression of co-stimulatory molecules. We used BQ-123 (Selective ET_A receptor inhibitor, American Peptide Company), at a final concentration of 10^{-6} *M*, for the last 48 hours, and BQ-788 (Selective ET_B receptor inhibitor, American Peptide Company), at a final concentration of 10^{-6} *M*, for the last 48 hours as well, for the selective blockade of endothelin receptors. The blockade of ET_A receptor with BQ-123 induced in general decreased expression of the costimulatory molecules, which was especially significant for CD40 and CD205. Functional studies (T-cell proliferation experiments) also demonstrated that the addition

of BQ-123 resulted in decreased ability of DC to stimulate T cells, in comparison to DC treated with TNFα alone. These results lead us to speculate that the stimulation of ET_A receptors may lead to the activation of DC, and that their blockade might abolish or lessen immune response.

During the previous years, we also demonstrated the influence of prostate cancer on DC, and the role of endothelin receptors in the prostate cancer-DC interaction. We performed the incubation of murine DC with RM-1 cells (murine prostate cancer cells), which resulted in DC apoptosis. Pretreatment of DC with TNFα lowered their apoptotic rate. Blockade of ET_A receptors with BQ123 increased prostate cancer-induced DC apoptosis, while the blockade of ET_B receptors with BQ788 improved DC resistance to prostate cancer-induced apoptosis and dropped DC apoptosis rate.

In our previous in vivo experiments, we assessed the effectiveness of the dendritic cells in stimulating the immune system after direct injection of dendritic cells into the developed tumors (please see previous annual reports for this grant). Direct injection of DC into the tumor proved successful. Translating into human trials, that will mean the injection of modified dendritic cells into the tumor (in our case, in the prostate), which is feasible in most cases, but might not be possible in patients who progressed after radical prostatectomy (in which prostate is removed). To address the need of these patients as well, we scheduled a new set of experiments, which includes the modification of DC by manipulating the endothelin receptors, and DC stimulation with tumor antigens. TNFα was used for DC maturation and increased expression of endothelin receptors, while ET_B receptors where blocked by BQ788. RM-1 cell lysates were used as antigens to stimulate DC. Lysates were obtained by repeated freeze/thaw cycles of the RM-1 cells (total of 5 cycles). Tumors were induced by subcutaneous injection of 25,000 RM-1 murine prostate cancer cells into groups of the C57BL/6 mice. Date of tumor injection was considered as day 0. There were 5 mice per group, and a total of 5 groups. Treatment was started on day 3, and repeated on days 7 and 10. Injections were performed in the flank opposite to tumor cells injection.

Group 1 received Hank's solution (control); Group 2 – unmodified DC; Group 3 – DC stimulated with RM-1 cell lysates (20 μg/ml) during the last 48 hours; Group 4 – DC treated with TNF-α (20ng/ml) and ET_B receptor antagonist BQ-788 (final concentration – 10^{-6} M) during the last 48 hours (our previous studies have shown the increased expression of endothelin receptors after the stimulation of DCs with TNFα, and improved DC survival with the blockade of ET_B receptors); Group 5 – DC treated with TNF-α and BQ-788 and stimulated with RM-1 cell lysates for the final 48 hours. Tumor size was assessed by measuring the perpendicular tumor diameters with a Vernier caliper (Electron microscopy Sciences, Ft. Washington, PA). Tumor volume was calculated using the formula of rotational ellipsoid: m_1^2 X m_2 X 0.5236, where m_1 represents the shorter axis and m_2 the longer axis[3]. Treatment groups consisted of five mice per group. Mice were sacrificed when they exhibited signs of distress or when total tumor volume exceeded 3000 mm^3. By day 28, mean tumor size reached 2158.67±984.19 mm^3 in the Group 1 (control), 1041.34±456.64 mm^3 in the Group 2, 1722.37±246.16 mm^3 in the Group 3, 1181.02 mm^3 in Group 4, and 779.06±644.69 mm^3 in Group 5. As it can be

seen from figure 1, mice treated with modified and stimulated DC (Group 5), had the smallest tumors by the end of experiment. There is no statistically significant difference in tumor sizes among groups, probably because of the low number of the mice in each group so far (experiment was performed only once). We are planning to repeat experiment 2 more times, and evaluate the combined data after that.

Several groups of cells were prepared, collected and RNA was extracted for the further gene array studies. Groups for comparison include (i) murine prostate and murine prostate cancer cells (RM-1); (ii) murine DC, DC stimulated with TNFα, DC stimulated with TNFα and treated with either BQ123 or BQ788; (iii) murine DC incubated with splenocytes and DC incubated with prostate cancer cells (RM-1). RNA extracted from murine splenocytes will provide control for these studies. We hope that gene array data will provide us with directions for further more detailed studies to elucidate the mechanisms of prostate cancer-induced apoptosis of DC, and the role of endothelin receptors in the functioning of dendritic cells.

Training

In December of 2003 I accepted the position of assistant professor at the division of urology of the New Jersey Medical Center. In 2004 I was awarded Physician Research Training Grant (Mentor – Dr. Mark L. Jordan) from the Department of Defense. Physician research training grant relieved me from some clinical duties, and allowed me to devote more than 50% of my time to prostate cancer research. Time at New Jersey Medical School was very productive for me, and I was able to proceed with my research as planned. Obtained data are very promising, and they resulted in several presentations at the national meetings (and are bases for the pending manuscript). Because of my success as physician-scientist, I was offered a position of an associate professor at the division of urology of the Virginia Commonwealth University (VCU) School of Medicine, which I accepted. My choice was determined in large by the offered opportunity to continue research in prostate cancer area, and by the commitment of VCU's leadership to my growth as a physician-scientist. With the research experience and theoretical knowledge (which I acquired while conducting the research and training, supported by the Physician Resarch Training Award from DOD), I hope I will be well-positioned to apply and get federal and other peer-reviewed grants to continue my work in the immunology of prostate cancer and, hopefully, to make an impact on the course of this formidable disease, which claims almost 30,000 lives every year in the United States alone.

My mentor on this project, Dr. Mark L. Jordan, is an experienced surgeon and researcher. I worked with Dr. Jordan in Pittsburgh as a resident, and he recruited me at the New Jersey Medical School after the completion of my AFUD fellowship. Main areas of Dr. Jordan's research are immunology and transplantation, and his research is concentrated on the modification of DC to alter their function. Since the study of the DC function and altering of their activity so that they can be used for the treatment of prostate cancer is the area I am most interested in, Dr. Jordan was an ideal mentor for me. Though I moved to the VCU school of Medicine in August of 2008, we continued our scientific

relationship through emails and frequent phone calls, and Dr. Jordan still provides valuable help and guidance in planning the experiments and interpreting the scientific data, as well as providing his help in preparing the manuscripts and grant submissions. VCU School of Medicine has other prostate cancer research projects as well, directed by Ph.D.-s, and they are already providing help with the planning and continuing of my project at the new place. I have guaranteed institutional start-up money as well as lab space at the VCU Massey Cancer Center, to successfully complete my entry as an independent scientist in the prostate cancer research area. To allow me the opportunity to continue my basic research, only 50% of my time will be devoted to the clinical duties.

Despite my move to the VCU, there will be only minimal interruption in both the training and research programs. Every step of the research program will be discussed with the mentor and consultants, and next step will be scheduled after thorough analysis of the obtained results. There are weekly research programs at the Department of Surgery of the VCU School of Medicine, and monthly genitourinary cancer conferences, conducted together by the division of Urology and Massey Cancer Center. In addition, I will be presenting the results of the ongoing research to the Department of Surgery research conference, and to the Division of Urology Grand Rounds. Results of the research project will be presented at the national meetings (Annual meetings of the American Association of Cancer Research and American Urological Association), with the benefit of feedback from world-known experts in the field. I hope that obtained results will allow me to apply for federal and other grants as an independent investigator, and that obtained results will be published in the peer-reviewed journals. Mentor and co-investigators will be assisting in accomplishing these goals.

Key research Accomplishments:

- Production of ET-1 by murine DC has been documented first time, as well as the presence of endothelin receptors on murine DC.
- Treatment of DC with prostate cancer cells supernatants induced decreased expression of some co-stimulatory molecules.
- We have demonstrated for the first time that the modification of endothelin axis on dendritic cells may result in increased resistance and improved survival against prostate cancer cells.
- Treatment of murine prostate cancer by the modified dendritic cells resulted in the reduction of the tumor growth. These data may provide basis for the development of clinical trials protocol.

Reportable outcomes:

Research experience gained by Dr. Guruli during his training as a physician-scientist, supported by this award, allowed him to apply and receive new employment at the Virginia Commonwealth University School of Medicine. Dr. Guruli received the position of associate professor (he had the position of assistant professor at the New Jersey Medical School), and increased institutional support (both start-up funds and lab space) to continue his research in the area of prostate cancer immunology.

Conclusion:

So far experiments have demonstrated the possible role of endothelin receptor inhibitors in the function of DC. Our in vivo experiments showed the possible role of endothelin receptors modification on DC in the treatment of prostate cancer in mice. More experiments are underway, and clinical trials protocol is being planned for patients with advanced prostate cancer, using modified autologous DC.

References:

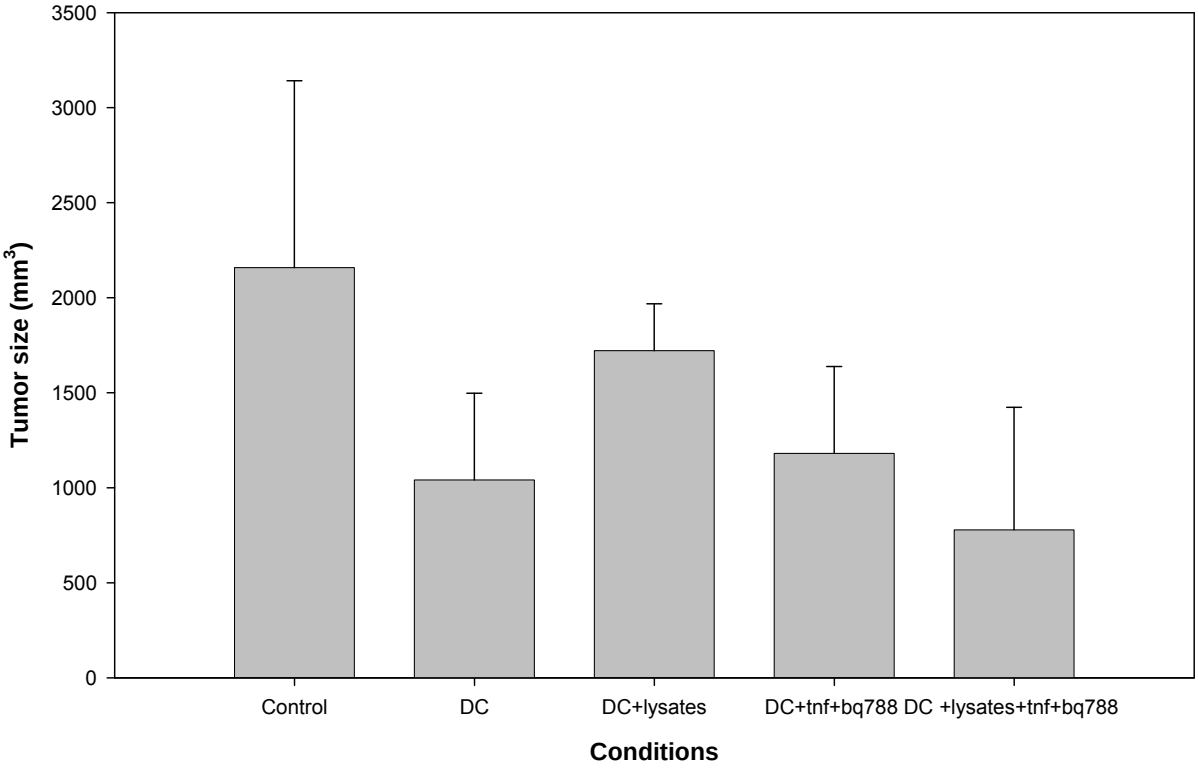
1. Pirtskhalaishvili G, Shurin GV, Gambotto A, Esche C, Wahl M, Yurkovetsky ZR, Robbins PD, Shurin MR. Transduction of dendritic cells with Bcl-xL increases their resistance to prostate cancer-induced apoptosis and antitumor effect in mice. Journal of Immunology. 2000;165:1956-1964

2. Shurin MR, Pandharipande PP, Zorina TD, Haluszczak C, Subbotin VM, Hunter O, Brumfield A, Storkus WJ, Maraskovsky E, Lotze MT. FLT3 ligand induces the generation of functionally active dendritic cells in mice. Cell Immunol. 1997;179:174-184

3. Janik P, Briand P, Hartmann NR. (1975). The effect of estrone-progesterone treatment on cell proliferation kinetics of hormone-dependent GR mouse mammary tumors. Cancer Res. 1975; 35: 3698-3704.

Appendices:

Figure 1.
Mice were injected with RM-1 murine prostate cancer cells in the flank area. Treatment was started on day 3 and injections were delivered in the flank opposite to tumor. Group 1 (control) mice were injected with the vehicle (HBSS), Group 2 – unmodified DC, Group 3 – DC stimulated with RM-1 cell lysates, Group 4 – DC treated with TNF-α and ET_B receptor antagonist BQ-788, Group 5 – DC treated with TNF-α and BQ-788 and stimulated with RM-1 cell lysates.



Curriculum Vitae

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1. Education
   a. Undergraduate N/A
   b. Graduate

      Tbilisi State Medical Institute, Tbilisi, Georgia (former USSR)
      Degree: **M.D.,** Date Awarded: June 26, 1983

2. Post Doctoral Training
   a. Internships and Residencies
      i. Clinical ordinatura (residency):
         Location: Georgian Oncological Research Center, Tbilisi, Georgia
         Discipline: Surgical Oncology
         Inclusive Dates: 09/1983 – 09/1985.
      ii. Internship (PGY-I):
         Location: University of Pittsburgh Medical Center, Pittsburgh, PA
         Discipline: Surgery
         Inclusive dates: 07/1995 – 06/1996.
      iii. Residency (PGY-II):
         Location: University of Pittsburgh Medical Center, Pittsburgh, PA
         Discipline: Surgery
         Inclusive dates: 07/1996 – 06/1997.
      iv. Residency (PGY-III – PGY-VI):
         Location: University of Pittsburgh Medical Center, Pittsburgh, PA
         Discipline: Urology

Inclusive dates: 07/1997 – 06/2001.

b. Research Fellowships

i. Research Fellow:
National Oncological Research Center, Moscow, USSR
Discipline: Urologic Oncology
Inclusive Dates: 09/1987 – 12/1990.
Ph.D. Degree awarded on 12/08/1990.

ii. Research Fellow:
University of Pittsburgh School of Medicine, Pittsburgh, PA
Discipline: Urologic Oncology
Inclusive Dates: 07/2001 – 11/2003.

3. Licensure (state, specialty, issue date, expiration date)
   a. Commonwealth of Pennsylvania – Medical Physician and Surgeon,
      Initial License Date: 03/05/1999.
   b. State of New Jersey – Medical Doctor,
      Initial license date: 09/08/2003.
   c. Commonwealth of Virginia – License to practice Medicine and Surgery
      Initial license date: 07/11/2008.

4. Narcotics Certification (state, dates)
   CDS registration (NJ), Date issued – 08/25/2006.
   DEA registration, Date issued – 03/17/2005.

5. University Appointments:
   Department: Surgery, Division of Urology
   UMDNJ – New Jersey Medical School
   Title: Assistant Professor
   Inclusive dates: 12/2003 – 08/2008

   Department of Surgery, Division of Urology
   VCU School of Medicine
   Title – Associate Professor
   Inclusive dates – 08/2008 - present

6. Hospital Appointments
   Department of Surgery, Division of Urology
   Hospital Name: Georgian Oncological Research Center
   Title: Staff physician
   Inclusive dates: 1985-1995

   Department of Surgery, Division of Urology
   University Hospital, Newark, NJ
   Title: Attending physician

Inclusive dates: 12/2003 – 08/2008

Department of Surgery, Division of Urology
MCV Hospitals, Richmond, VA
Title: Attending physician
Inclusive dates: 08/2008 – present.

7. Awards and Honors

1977	Gold Medal (Highest Honors), High School #1, Tbilisi, Georgia
1983	Highest Honors ("Red Diploma"), Tbilisi State Medical Institute, Georgia
1998	Second Prize, Clinical Section, Pittsburgh Urological Society Meeting, Pittsburgh, Pennsylvania
1999	Pfizer Scholars in Urology Award.
1999	Best Basic Science Paper Award, 51st Annual Meeting of Northeastern Section, AUA. Bermuda, UK.
1999	First Prize, Basic Research Section, Pittsburgh Urological Society Meeting, Pittsburgh, Pennsylvania.
2000	Resident Prize Essay Award, 52nd Annual Meeting of Northeastern Section, AUA. Pittsburgh, USA.
2002	Sylvia Sorkin Greenfield Award, for the best paper published in *Medical Physics.*
2004	AUA travel Award to attend NIDDK Clinical Research Meeting

8. Board Certification – February 2006 – By the **American Board of Urology**.

9. Principal Clinical and Hospital Service Responsibilities:

Hospital Name: Georgian Oncological Research Center, Tbilisi, Georgia
Department or Service: Urology
Responsibilities – Admission of patients in the hospital, preoperative evaluation and designing of treatment plan, administration of treatment (surgical or medical), postoperative care in the hospital, analyzing treatment outcomes, designing new treatment methods and schemas.
Inclusive Dates: 1985 – 1995.

Hospital Name: University Hospital, Newark, NJ
Department or Service: Surgery (Urology)
Responsibilities – Admission of patients in the hospital, evaluation and elaboration of treatment plan, administration of treatment, post-treatment follow-up, analyzing treatment outcomes, designing and participating in clinical trials.
Inclusive Dates: 12/2003 – 08/2008.

Hospital Name: MCV Hospitals, Richmond, VA
Department or Service: Surgery (Urology)
Responsibilities – Admission of patients in the hospital, evaluation and elaboration of treatment plan, administration of treatment, post-treatment follow-up, analyzing treatment outcomes, designing and participating in clinical trials.
Inclusive Dates: 08/2008 – present.

Hospital Name: Hunter Holmes McGuire VA Medical Center, Richmond, VA
Department or Service: Surgery (Urology)
Responsibilities – Admission of patients in the hospital, evaluation and elaboration of treatment plan, administration of treatment, post-treatment follow-up, analyzing treatment outcomes.
Inclusive Dates: 08/2008 – present.

10. Ad Hoc Reviewer:
International Journal of Cancer
American Cancer Society
Cytokine
Medical Science Monitor
Grant reviewer for NIH

11. Memberships, Offices and Committee Assignments in professional Societies
    i. European Association of Urology
        Active Member
        1996 – 2000.
    ii. American Urological Association
        Candidate Member
        1997 – 2001
        Associate Member
        2002 – 2006
        Active Member – since 2006
    iii. American Association for Cancer Research
        Associate Member
        Dates: 1999 – 2004.
        Active Member – since 2005.

12. Major Research Interests:
    i. Prostate cancer:

Relationship and interaction between prostate cancer and dendritic cells (DC), the major antigen-presenting cells. To study the mechanisms of prostate cancer-induced DC suppression, and design the ways of protecting DC from apoptosis. Development of DC-based therapies of advanced prostate cancer.

ii. Immunomodulation and the role of endothelin-1 (ET-1) and its receptors in the generation of immune response, in particular, the role of endothelin axis in affecting of DC function.

13. Grant History

a. Principal Investigator

i. Funding Organization: American Foundation for Urologic Disease / American Urological Association Research Scholar Program
Title of Award: The Endothelin Axis: Signaling Pathways and Maximizing Efficacy in the Treatment of Advanced Prostate Cancer
Inclusive dates of Funding: 07/2001 – 06/2003.

ii. Funding Organization: Department of Defense, Physician Research Training Grant
Title of Award: Activation and Protection of Dendritic Cells in the Prostate Cancer Environment.
Inclusive dates of Funding: 2005 – 2009

b. Co-Investigator

i. Funding Organization: University of Pittsburgh Prostate and Urologic Cancer Center Pilot Project (Co-PI)
Title of Award: Effective Protection of Human Dendritic Cells from Prostate Cancer Induced Cell Death.
Inclusive dates of Funding: 1999-2000.

ii. Funding Organization: The Pittsburgh Foundation Program for Medical Research (Co-PI)
Title of Award: New Approach for Prostate Cancer Therapy: Dendritic Cells Protected from Tumor-Induced Death.
Inclusive dates of Funding: 1999-2002.

iii. Funding Organization: Department of Defense (DAMD17-00-1-0099 P1832735, Co-Investigator).

Title of Award: Immune Gene Therapeutic Correction and Protection of Disordered Dendritic Cells in Prostate Cancer.
Inclusive dates of Funding: 1999-2002.

14. Clinical Trial

Cell Genesys, Inc. **VITAL-1 clinical trial,** Phase 3, for the patients with metastatic hormone-refractory prostate cancer.
Role: P.I. for the University Hospital of the NJMS, Newark, NJ.

15. Articles

1. Gotsadze, D. T. & **Pirtskhalaishvili, G. G**. (1988). [Diagnosis and treatment of regional metastasis of penile cancer] [Russian]. ***Urologiia i Nefrologiia***, 48-51.

2. Gotsadze, D. T., Daneliia, E. V. & **Pirtskhalaishvili, G. G.** (1988). [Lymphogenic metastasis in penile cancer] [Russian]. ***Voprosy Onkologii* 34**, 1501-1504.

3. Gotsadze, D. T., Nemsadze, G. G., Chigogidze, T. G., Daneliia, E. V., **Pirtsakhalaishvili, G. G.** & Chovelidze Sh, G. (1990). [A method for forming a large-intestine reservoir for the urine] [Russian]. ***Urologiia i Nefrologiia***, 35-39.

4. Gotsadze, D., Mosidze, B., Chigogidze, T., Nemsadze, G., Chovelidze, S. & **Pirtskhalaishvili, G.** (1990). [Surgical aspects for the construction of colonic urinary reservoirs] [Georgian]. ***Sakartvelos Sameditsino Moambe***, 36-41.

5. Matveev, B. P., Shipilov, V. I., Gotsadze, D. T., Abdushelishvili, K. O. & **Pirtskhalaishvili, G. G**. (1990). [The incidence of bladder tumor recurrences after transurethral resection during combined treatment] [Russian]. ***Urologiia i Nefrologiia***, 53-56.

6. Shipilov, V. I. & **Pirtskhalaishvili, G. G**. (1990). [Transurethral resection in the treatment of locally advanced cancer of the bladder] [Russian]. ***Voprosy Onkologii* 36**, 1369-1371.

7. Gotsadze, B. T., Nemsadze, G. G., Mosidze, B. A., **Pirtskhalashvili, G. G**., Chovelidze Sh, G. & Daneliia, E. V. (1991). [A "dry" abdominal urinostoma] [Russian]. ***Vestnik Khirurgii*** *Imeni i - i - Grekova* **146**, 120-122.

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12. Gotsadze, D. T. & **Pirtskhalaishvili, G. G.** (1992). [The quality of life of patients after cystectomy for cancer] [Russian]. ***Voprosy Onkologii* 38**, 489-493.

13. Matveev, B. P., Gotsadze, D. T. & **Pirtskhalaishvili, G. G.** (1993). [The causes of mortality following cystectomy for bladder tumor] [Russian]. ***Urologiia i Nefrologiia***, 20-22.

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18. Patents held:

Title: **"Endothelin Axis and the Action of Dendritic Cells"**
University of Pittsburgh Case No. 00743
U.S. Patent Number: **U.S. Patent Application No. 60/513,729**
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19. Languages spoken: Georgian, English, Russian