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

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| 6. AUTHOR(S)<br>Georgi Guruli, M.D., Ph.D.<br>Mark L. Jordan, M.D.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                  |                          |                               | 5d. PROJECT NUMBER                            |                                              |
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| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)<br><br>University of Medicine and Dentistry of<br>New Jersey<br>Newark, NJ 07107-3001                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                  |                          |                               | 8. PERFORMING ORGANIZATION REPORT<br>NUMBER   |                                              |
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| 13. SUPPLEMENTARY NOTES                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                  |                          |                               |                                               |                                              |
| 14. ABSTRACT<br>First annual report for this award. Experiments were conducted as was scheduled in the Statement of Work. So far studies have demonstrated for the first time the presence of endothelin receptors on murine DC, and the fact of endothelin-1 production by murine DC upon stimulation with TNF•. Phenotyping of dendritic cells stimulated with TNF• and treated with endothelin receptor inhibitors demonstrated decreased expression of pro-inflammatory co-stimulatory molecules (CD40, VD80, CD86, CD205, MHC class II) with the blockade of ETA receptors, and no change or mild increase in the expression of co-stimulatory molecules with the blockade of ETB receptors. In vivo administration of endothelin A receptor inhibitor abolished the effect of infectious stimulus to mobilize dendritic cells to draining lymph nodes. Functional studies are under way to further characterize the role of endothelin receptors in the biology of dendritic cells, as well as to study the interaction of dendritic cells and prostate cancer cells, and develop the means of active cell therapy for murine prostate cancer model. |                  |                          |                               |                                               |                                              |
| 15. SUBJECT TERMS<br>Prostate cancer, Dendritic cells, Endothelin receptors, Immunotherapy                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                  |                          |                               |                                               |                                              |
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| a. REPORT<br>U                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | b. ABSTRACT<br>U | c. THIS PAGE<br>U        |                               |                                               | USAMRMC                                      |
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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 first year of the award. Experiments are progressing according to the statement of the work so far.

**Body of the Report:**

First task was to proceed with the characterization of role of endothelin axis in DC. For this purpose, DC were grown from C57BL/6 mice bone marrow, as was described earlier<sup>1</sup>. 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 ( $\alpha$ CD4,  $\alpha$ CD8a, 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<sub>2</sub>) in six-well plates (Falcon, Franklin Lakes, NJ) at a concentration of 10<sup>6</sup> 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<sup>5</sup> 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<sub>2</sub>. Nonadherent DC are collected by gentle pipetting, counted, characterized as described previously<sup>2</sup>, and used for further studies.

For the characterization of the general impact of endothelin receptors, dendritic cells were stimulated with TNF $\alpha$  for the endothelin production and the expression of endothelin receptors, since our preliminary data indicated increased expression of endothelin receptors upon stimulation in mice (unpublished data). We have previously demonstrated as well increased production of endothelin-1 (ET-1) by human DC, and increased expression of endothelin receptors<sup>3</sup>.

For the characterization of ET-1 production, DC were cultured as described above, and stimulated with TNF $\alpha$  (10ng/ml, added on Day 5)) for 48 hours. After that, supernatant was collected, and ET-1 was measured using ET-1 ELISA kit as described<sup>3</sup>. Stimulated cells produced 1044.2  $\pm$  118.6 pg/ml/mln cells, while nonstimulated cells produced 351.9  $\pm$  131.9 pg/ml/mln cells. The difference was statistically significant (P=0.008).

In preliminary data, we have reported increased expression of the endothelin receptors by murine DC upon stimulation using immunohistochemistry. We were not able to repeat these experiments so far because were not able to obtain endothelin receptor antibodies from Abbott Labs. Recently, antibodies has been found and ordered from Alomone Labs, and these confirmatory experiments will be carried out. We will perform RNA studies as well (gene arrays, and RT-PCR).

Next, changes in phenotype has been evaluated. Murine DC has been stimulated with TNF $\alpha$  on day 5 for 48 hours. Stimulated DC were treated with endothelin receptor

inhibitors BQ-123 (Selective ET<sub>A</sub> receptor inhibitor, American Peptide Company), at a final concentration of  $10^{-6}$  M, for the last 48 hours, and with BQ-788 (Selective ET<sub>B</sub> receptor inhibitor, American Peptide Company), at a final concentration of  $10^{-6}$  M, for the last 48 hours as well. After that, cells were collected, washed, counted and stained for flow cytometry. We have evaluated cells for the expression of CD40, CD80, CD86, MHC class II antigen, and CD205. Results are presented in figures 1-3 (Appendices). Briefly, stimulation with TNF $\alpha$  resulted in the increased expression of these costimulatory molecules (as expected). The blockade of ET<sub>A</sub> receptor with BQ-123 induced in general decreased expression of the costimulatory molecules, which was especially significant for CD40 and CD205 (figures 2-3, difference was statistically significant by chi-square test,  $P < 0.001$ ). On the other hand, the blockade of ET<sub>B</sub> receptor with BQ-788 resulted in no change or increased expression of costimulatory molecules, especially CD40 and CD205 (figures 2-3, difference was statistically significant by chi-square test,  $P < 0.001$ ).

One experiment was carried out in the in vivo model: C57BL/6 mice were given ET<sub>A</sub> receptor inhibitor, and after 5 days were given foot pad injection with LPS. Mice were sacrificed anesthetic overdose, and lymph nodes were recovered. Nontreated mice, and mice treated with foot injection only served as controls. Collected nodes were crushed. Crushed tissue was resuspended in 20 ml medium, cells collected and passed through the cell strainer. After the lysis of red blood cells, the remaining cells were counted, and labeled with MACS CD11c magnetic beads (Miltenyi Biotech, Auburn, CA). After 15 min of labeling, cell suspension was passed through columns with metallic beads inserted into magnets. Negative cells (washed through) were discarded, columns removed from magnets and CD11c+ cells were collected. Obtained cells were characterized for the expression of costimulatory molecules. Results of this preliminary experiment are presented in figure 4. As it can be seen, LPS induced increased expression of costimulatory molecules, while pretreatment with ET<sub>A</sub> receptor inhibitor abolished the effect. Further experiments are needed to confirm or clarify these results.

One gene array experiment was performed, to assess the influence of prostate cancer cells on DC. Briefly, 7-day-old cultured DC were harvested and co-incubated with the murine prostate cancer cell line RM-1 in six-well plates. DC and tumor cells were separated using membrane inserts with 0.4- $\mu$ m pore size, which exclude direct cell-to-cell contact, but allow free exchange of soluble factors. Specifically,  $5 \times 10^5$  DC will be placed in six-well plates in 3 ml of medium. One million prostate cancer cells resuspended in 2 ml of medium were placed into the inserts on the top of each well. As controls, DC were co-incubated with murine splenocytes. DC were harvested 48 h later, washed, RNA was extracted using RNA extraction minikit, and used for gene arrays. We used mouse 22K Oligo Arrays (Center for Applied Genomics) which is composed of fifteen-thousand 70 mer oligonucleotides corresponding to specific mouse transcripts. The oligonucleotides were spotted onto poly-lysine-coated glass microscope slides by using a Gene Machines Omnigrid 100 arrayer (Genomic Solutions, Ann Arbor, Mich.) and SMP3 pins (Telechem, Sunnyvale, Calif.). RNA labeling and hybridization was performed using the 3DNA Array detection Array 350 Kit (Genisphere Inc.) according to the manufacturer's instructions. We used "comparison design" for this experiment, where RNA's were compared to each other directly, without standard (results can be only preliminary). Raw labeled image is presented in figure 5. Preliminary analyze of data demonstrated so far decreased

expression of receptors for IL-12 and interferon gamma in DC incubated with RM-1 cells. More experiments with “reference settings” are scheduled.

**Key research Accomplishments:**

- Production of ET-1 by murine DC has been documented first time
- The presence of endothelin receptors on murine DC has been shown for the first time
- The influence of endothelin receptor inhibitors on DC phenotype was demonstrated. Other functional experiments (MLR) are on the way, but it seems that ET<sub>A</sub> receptors are involved in the activation of DC, driving them towards TH1 response.
- It seems that ET<sub>B</sub> receptor stimulation might drive DC toward tolerance, with decreased expression of co-stimulatory molecules. Further studies are needed to clarify the exact role these receptors in DC biology. Functional studies are under way.

**Conclusion:**

So far experiments have demonstrated the presence of the endothelin receptors on murine DC, which is a novel finding. In addition, the production of ET-1 by murine DC has been demonstrated as well. Experiments suggested the possible role of endothelin receptor inhibitors in the function of DC, which can be useful in the treatment of different diseases, ranging from cancer to transplantation. More in vitro and in vivo experiments are under way to clarify this role.

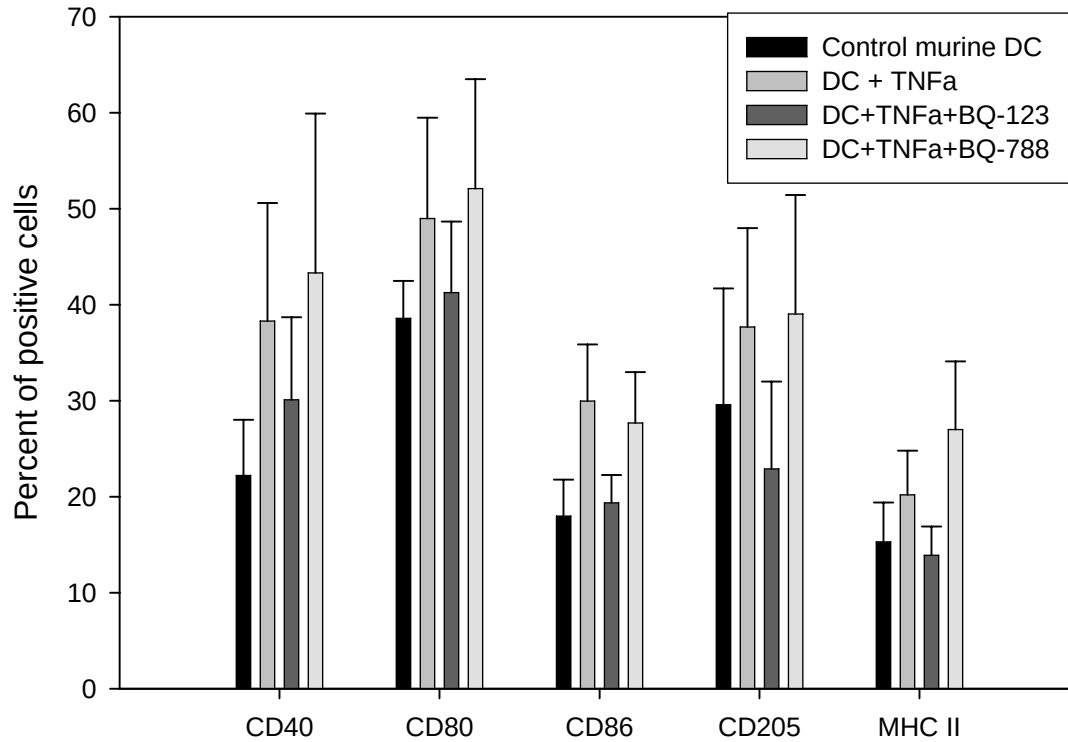
**References:**

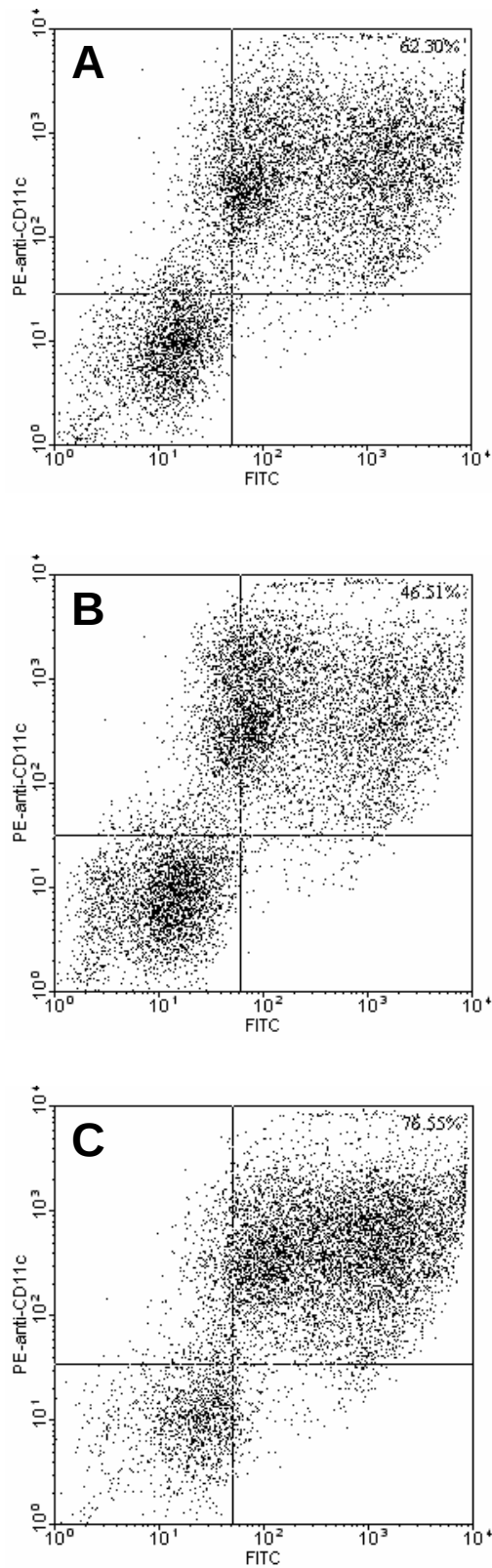
1. Pirtskhalaishvili G, Shurin GV, Gambotto A, Esche C, Wahl M, Yurkovetsky ZR, Robbins PD, Shurin MR. Transduction of dendritic cells with Bcl-xL increases their resistance to prostate cancer-induced apoptosis and antitumor effect in mice. *Journal of Immunology*. 2000;165:1956-1964
2. Shurin MR, Pandharipande 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. Guruli G, Pflug BR, Pecher S, Makarenkova V, Shurin MR, Nelson JB. Function and survival of dendritic cells depend on endothelin-1 and endothelin receptor autocrine loops. *Blood*. 2004;104:2107-2115

## Appendices:

**Figure 1.**

Phenotyping of murine dendritic cells (DC) stimulated with  $\text{TNF}\alpha$  and treated either with  $\text{ET}_A$  receptor inhibitor (BQ-123) or  $\text{ET}_B$  receptor inhibitor (BQ-788). Blockade of  $\text{ET}_A$  receptors resulted in the decrease of the costimulatory molecule expression, while  $\text{ET}_B$  receptor blockade was accompanied by mild increase in the expression of costimulatory molecules.

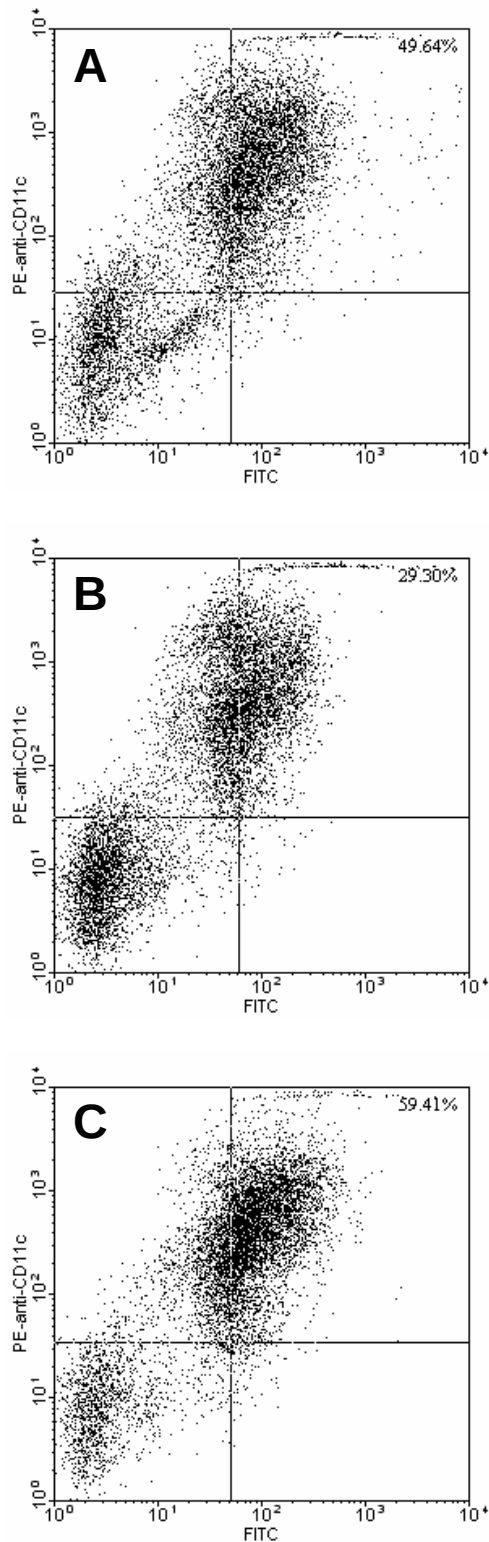


**Figure 2.**

Expression of CD40 molecules on murine dendritic cells after different treatment (double stained cells – CD11c and CD205).

- A. Murine DC stimulated by TNF $\alpha$ .
- B. Murine DC stimulated by TNF $\alpha$  and ETA receptor antagonist BQ-123.
- C. Murine DC stimulated by TNF $\alpha$  and ETB receptor BQ-788.



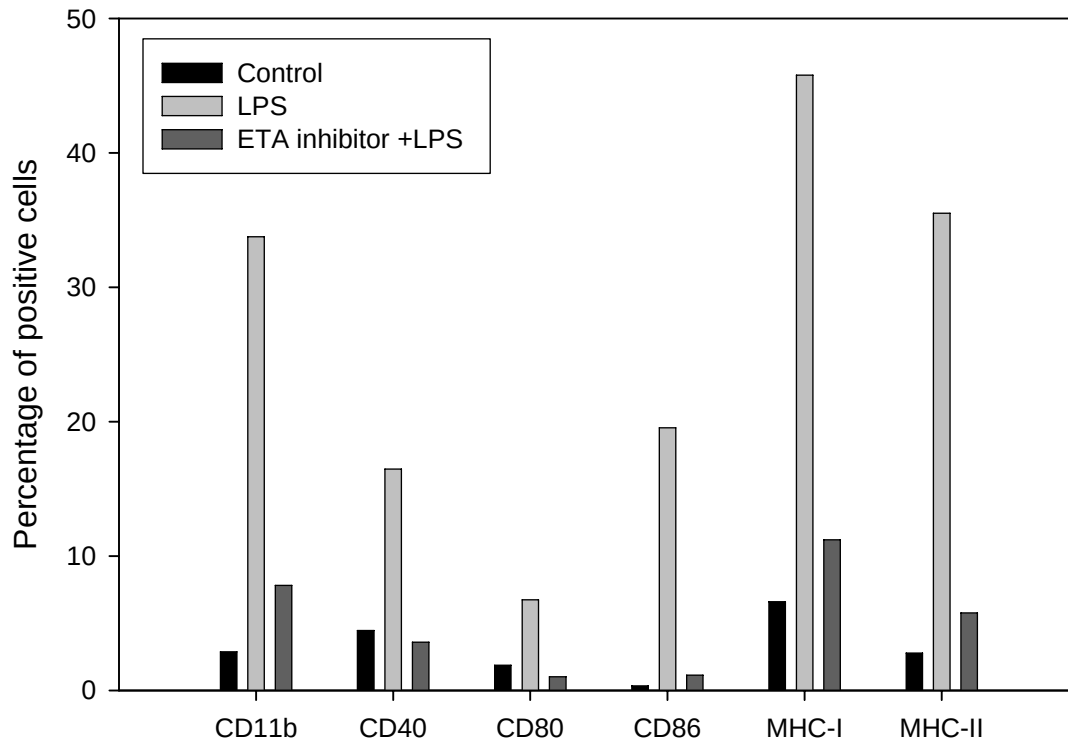
**Figure 3.**

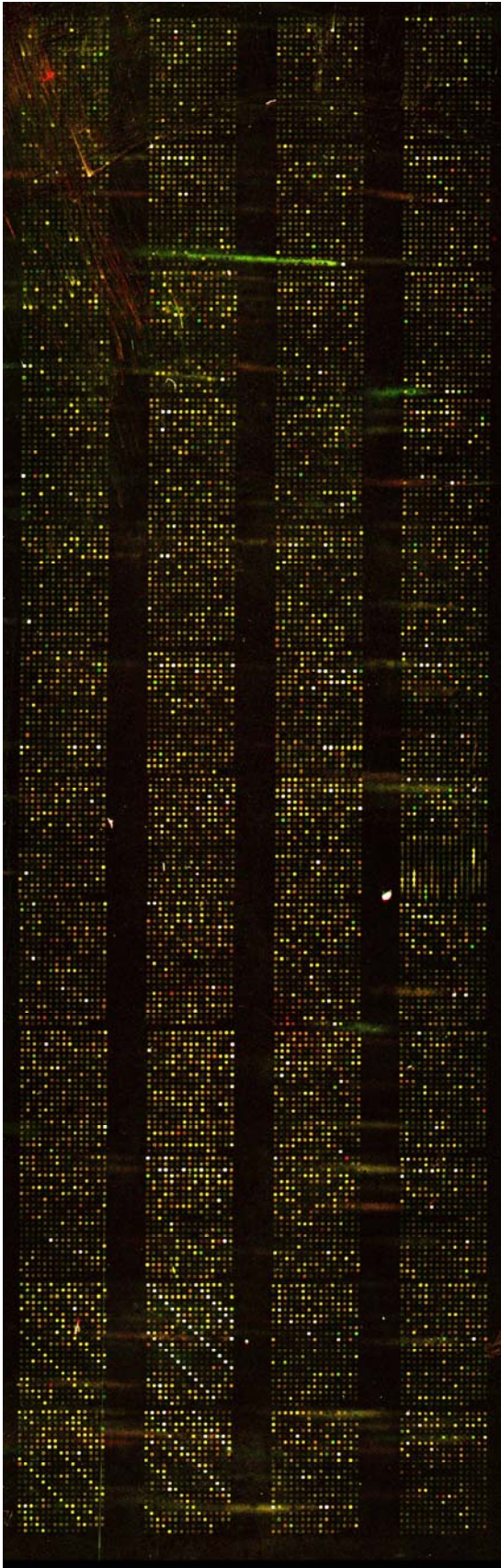
Expression of CD205 molecules on murine dendritic cells after different treatment (double stained cells – CD11c and CD205).

- A. Murine DC stimulated by  $\text{TNF}\alpha$ .
- B. Murine DC stimulated by  $\text{TNF}\alpha$  and ETA receptor antagonist BQ-123.
- C. Murine DC stimulated by  $\text{TNF}\alpha$  and ETB receptor BQ-788.

**Figure 4.**

Phenotyping of dendritic cells (CD11c positive) isolated from lymph nodes of the mice (untreated – control, treated with LPS injection, and treated with LPS injection after the pretreatment with ET<sub>A</sub> receptor inhibitor). LPS induced increased mobilization of CD11c cells, as was expected. Pretreatment with ET<sub>A</sub> inhibitor resulted in decreased mobilization of CD11c positive cells in the lymph nodes.



**Figure 5.**

Spot arrays comparing gene expression in dendritic cells incubated with splenocytes to dendritic cells incubated with murine prostate cancer cells (RM1). Preliminary experiments.

### **Curriculum Vitae**

Name: Georgi Guruli (Pirtskhalaishvili)

Office Address: Division of Urology  
Department of Surgery  
UMDNJ – New Jersey Medical School  
MSB G-528  
185 South Orange Avenue  
Newark, NJ 07103  
Tel: (973) 972-4097  
Fax: (973) 972-3892  
Email: [gurulige@umdnj.edu](mailto:gurulige@umdnj.edu),

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 ordinatoria (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,  
MD 067593 L, Initial License Date: 03/05/1999. Expiration date:  
12/31/2004.
  - b. State of New Jersey – Medical Doctor, License # 25MA07655400,  
Initial license date: 09/08/2003. Expiration date: 06/30/2007
- 4. University Appointments:
  - Department: Urology  
University of Pittsburgh School of Medicine  
Title: Research Fellow  
Inclusive dates: 07/2001 – 11/2003.
  - Department: Surgery, Division of Urology  
UMDNJ – New Jersey Medical School  
Title: Assistant Professor  
Inclusive dates: 12/2003 – present.
- 5. Hospital Appointments
  - Department of Surgery, Division of Urology  
Hospital Name: Georgian Oncological Research Center  
Title: Ward physician  
Inclusive dates: 1985-1995
  - Department of Surgery, Division of Urology  
University Hospital, Newark  
Title: Attending physician  
Inclusive dates: 12/2003 – present.
- 6. Awards and Honors
  - 1977                      Gold Medal (Highest Honors), High School #1, Tbilisi, Georgia

|                 |                                                                                                            |
|-----------------|------------------------------------------------------------------------------------------------------------|
| 1983<br>Georgia | Highest Honors (“Red Diploma”), Tbilisi State Medical Institute,                                           |
| 1998            | Second Prize, Clinical Section, Pittsburgh Urological Society Meeting, Pittsburgh, Pennsylvania            |
| 1999            | Pfizer Scholars in Urology Award.                                                                          |
| 1999            | Best Basic Science Paper Award, 51 <sup>st</sup> 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, 52 <sup>nd</sup> Annual Meeting of Northeastern Section, AUA. Pittsburgh, USA. |
| 2002            | Sylvia Sorkin Greenfield Award, for the best paper published in <i>Medical Physics</i> .                   |
| 2004            | AUA travel Award to attend NIDDK Clinical Research Meeting                                                 |

#### 7. 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.

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.

Inclusive Dates: 12/2003 – present.

#### 8. Ad Hoc Reviewer:

International Journal of Cancer

American Cancer Society

Medical Science Monitor

Grant reviewer for NIH

#### 9. Memberships, Offices and Committee Assignments in professional Societies

- i. European Association of Urology  
Active Member  
1996 – 2000.
  - ii. American Urological Association  
Candidate Member  
Dates: 1997 – 2001.  
Associate Member  
Dates: 2002 – Present.
  - iii. American Association for Cancer Research  
Associate Member  
Dates: 1999 – 2004.  
Active Member – since 2005.
- 10. Major Research Interests: Brief Narrative Description in full sentences
  - b. 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.
  - c. 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.
- 11. Grant History (No proposed or pending funding, only full awards)
  - 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.  
Direct costs awarded: \$44,000.  
Total amount awarded: \$44,000.
    - ii. Funding Organization: Department of Defence, Physician  
Research Training Grant  
Title of Award: Activation and Protection of Dendritic Cells in  
the Prostate Cancer Environment.,  
Inclusive dates of Funding: 2005 – 2009.  
Direct Costs awarded: \$449,668  
Total amount awarded: \$699,232.

## 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.  
 Total amount awarded: \$10,000.
- ii. Funding Organization: The Pittsburgh Foundation Program for Medical Research  
 Title of Award: New Approach for Prostate Cancer Therapy: Dendritic Cells Protected from Tumor-Induced Death.  
 Inclusive dates of Funding: 1999-2002.  
 Total amount awarded: \$148,132.
- iii. Funding Organization: Department of Defense (DAMD17-00-1-0099 P1832735).  
 Title of Award: Immune Gene Therapeutic Correction and Protection of Disordered Dendritic Cells in Prostate Cancer.  
 Inclusive dates of Funding: 1999-2002.  
 Total amount awarded: \$471,339.

## 12. 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.
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