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(PC) Research: Signal Transduction and Receptor-Inhibitor in the
Progress of PC

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13. ABSTRACT (Maximum 200 Words)

This program aims to help eradicate prostate cancer (PC) disparity in African Americans through educational and research programs. Our hypothesis is that through PC education, exposure to PC issues, and participation in PC research, a meaningful number of African Americans will be able to contribute to the elimination of disparity in PC. Our program comprises three Specific Aims. (1) To develop, promote, and sustain independent, competitive research and training programs at Xavier University. This Specific Aim has already proceeded significantly toward its goal: each major research project has either presented or is scheduled to present its research at symposia, and for each project several African American students have contributed to the original work. (2) To increase the number of Xavier University investigators focused on PC research. This Aim has also met with early success: one Junior Faculty has been identified and matched with an experienced researcher from the mentoring Tulane Cancer Center (TCC). (3) To establish a long-term collaborative relationship between Xavier University and the TCC in PC research. In this area progress has developed slowly beyond the three focused partnerships mentioned in the first two Aims.

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Annual Report on Grant #W81XWH-04-1-0252
Training HBCU Faculty and Students in Prostate Cancer (PC) Research:
Signal Transduction and Receptor-Inhibitor
Interactions in the Progress of PC
Elizabeth A. Barron, Ph.D.

INTRODUCTION

As stated in the original Technical Abstract, we intend to help eradicate prostate cancer (PC) disparity in African Americans by targeting this population through educational and research programs. Our hypothesis is that through PC education, exposure to PC issues, and participation in PC research, a meaningful number of African Americans will acquire the knowledge to contribute to the elimination of disparity in PC. Our program comprises three Specific Aims. The first Aim develops, promotes, and sustains independent, competitive research and training programs at Xavier University. This Specific Aim has already proceeded significantly toward its goal: each major research project has either presented or is scheduled to present its research at symposia, and for each project several African American students have contributed to the original work. The second Aim increases the number of Xavier University investigators focused on PC research. This Aim has also met with early success, although the pace of progress has been slower: one Junior Faculty has been identified and matched with an experienced researcher from the mentoring institution, the Tulane Cancer Center (TCC). Research plans are at an early stage of development. The third Aim establishes a long-term collaborative relationship between Xavier University and the TCC in PC research. In this area progress has developed slowly beyond the four focused partnerships mentioned in the first two Aims.

BODY

The grant funding period began on March 1, 2004. Until existing teaching obligations had been fulfilled (mid-May 2004), however, the Xavier University faculty could not begin to claim release time for their projects. The Grants Manager at the DOD (Dr. Julie Wilberding) was informed of this situation and raised no objections.

Dr. Elizabeth A. Barron, the PI, is on sabbatical for the spring semester of 2005. Her role is being capably filled by Dr. Marguerite Giguette, who has previously served as the chair of the Department of Computer Sciences at Xavier University. Dr. Barron has remained in touch with Drs. Giguette and Klassen (the Co-PI) and has thereby continued to fulfill her obligations to the grant. This includes making herself available for the site visit scheduled for April 27, 2005.

SPECIFIC AIM I

Develop, promote, and sustain independent, competitive research and training programs at Xavier University.

A. Ireland/Abdel-Mageed project: “Genetic Basis of Prostate Cancer: Factors influencing the mortality rates of minorities” (Former title “Cross-Talk between Apoptotic Signaling Pathways: Role in a Biracial Population in Prostate Cancer”)

There has been a change in personnel. Dr. Nitsa Rosenzweig has left Xavier University. With the consent of the DOD Grant Manager, we substituted Dr. Shubha Kale Ireland. Dr. Ireland is ideal for several reasons: she has experience working with cultured cells and already knows several members of the Tulane Cancer Center (TCC), including Dr. Asim Abdel-Mageed. She stepped immediately into this project without changing the SOW and with only little loss of time. Dr. Ireland reports, “Dr. Mageed and I meet as often as needed and sometimes more than once a week. I also attend the Mageed weekly lab meetings as often as I can... [Our] experiments are conducted on both campuses, although any experiments involving radiation have to be conducted in Dr. Mageed's lab. I am myself learning a lot under Dr. Mageed's mentorship.” She and Dr. Abdel-Mageed separately report an excellent rapport.

1. Hire technician to assist in project (Month 1)

Dr. Qiuyun Yang has been hired to assist with the design and performance of the experiments involving cultured cells described in Specific Aim I.A. (Dr. Yang's biosketch is attached as Appendix A-1.)

2. Identify student to assist in project (Month 3)

Dr. Ireland has already involved five students in this work at different levels of participation. One of these, Danielle Haney, is an NIH Minority Access to Research Careers (MARC) Fellow and is committed to participate in this research project for two years. Dr. Ireland reports that her students travel frequently to the Tulane University Health Sciences Center (TUHSC) and “are getting an excellent training and learning a lot under Dr. Mageed's mentorship.” The other four students are Jenais Miller, Javay Ross, Dung Dinh, and Larry Shuler.

3. Establish a connection between androgen-receptor presence and activity, and drug resistance (Months 1-9)

a. *Establish prostate cancer cell lines (Month 1)*

Dr. Ireland has established three prostate cancer (CaP) cell lines at Xavier University's cell culture facility. They include PC-3, DU-145 (both androgen independent) and LNCaP (androgen dependent).

b. *Test the effect of radiation and chemotherapy on apoptosis (Months 2-5)*
(See I.A.4)

c. *Test the effect of radiation and chemotherapy on growth (Months 6-9)*
(See I.A.4)

4. Identify the relation between NF- μ B and p53 in establishing drug-resistance phenotype in CaP cells (Months 10-21).

A key element in the development of this project is demonstrating that NF- μ B activation is affected by levels of p53 expression (i.e., that cross-talk exists between NF- μ B—a transcription factor and an antiapoptotic agent—and p53, a tumor suppressor gene). Expression of NF- μ B was measured by the luciferase assay using the PC-3 and

DU-145 cell lines as model systems. While both cell lines constitutively express NF- μ B, PC-3 cells are p53-deficient and DU-145 cells express mutant p53. Thus, the introduction of exogenous wild-type p53 might reveal cross-talk between p53 and NF- μ B. To accomplish this, CaP cells were co-transfected with appropriate plasmids (the NF- μ B-luc plasmid and either a control CMV plasmid or a p53 wild type plasmid). The assay conditions were optimized and conducted in triplicates. Further, the assays were repeated in the presence and absence of TNF- α , a known inducer of endogenous NF- μ B. RESULTS: After confirming that luciferase activity (measured by a fluorometer) was equivalent to the level of NF- μ B expression (detected by Western blot analysis), the researchers found that the level of NF- μ B activation differed significantly between cells transfected with wild-type p53 and those transfected with the control plasmid (Appendix A-2). PC-3 and DU-145 cells responded similarly in all experiments. The data suggest that wild-type p53 inhibits NF- μ B activation by an average of 50% irrespective of the presence of TNF- α . These reproducible results demonstrate that cross-talk exists between the p53 (tumor suppressor) and NF- μ B (antiapoptotic) genes. ONGOING EXPERIMENTS AND FUTURE PLANS: This section of the SOW, including its three subordinate aims, remains unchanged. Drs. Ireland and Abdel-Mageed are engaged in several activities that meet the stated objectives. (1) They are determining if the inhibitory effect of p53 on NF- μ B activation is limited to androgen-independent CaP cells and if this relationship has any correlation with the androgen receptor. To this end, they are repeating the assays described above in two androgen-dependent CaP cell lines, LNCaP and 22rv1. (2) They are examining possible mechanisms for the NF- μ B and p53 interactions. To this end, they are conducting experiments (luciferase assays and Western blot analysis) using wild-type and mutant CBP, a coactivator for both NF- μ B and p53-dependent transactivation. (3) In the near future, they will study the effect and mechanisms of NF- μ B (p65 subunit) on p53 transactivation in both androgen-dependent and androgen-independent CaP cell lines. (4) They have begun studying the influence of radiation (in the dose ranges used in hospitals, typically 200-800 Rads) on the NF- μ B/p53 interactions. Specifically, initial cell proliferation assays have begun to determine the highest non-lethal dose to be used in the lab experiments.

- a. *Measure p53 in sensitive and resistant cell lines and PC tissues from biracial populations (Months 10-15).*
 - b. *Measure NF- κ B in sensitive and resistant cell lines and PC tissues from biracial populations (Months 10-15)*
 - c. *Measurement of NF- κ B-induced expression of anti-apoptotic proteins (Months 16-21)*
5. Test if chemoresistance is mediated via crosstalk between NF- μ B and p53 (Months 22-36)
 Plans for Aim I.A.5 are unchanged.
- a. *Test p53 level and activity after blocking NF- κ B (Months 22-27)*
 - b. *Test p53 level and activity after activating NF- κ B. (Months 28-30)*
 - c. *Test if changes in NF- κ B status affect p53 status in PC cell lines. (Months 31-36)*
6. Deliverables/measurable outcomes: Dr. Ireland will prepare or oversee the following:

Dr. Ireland has submitted one report to date. The Co-Project Director has paid several visits to her office and maintained frequent contact to monitor progress and identify problems. A high level of activity, and especially the continuing involvement of African American students in her projects, has been noted.

- b. *One abstract will be submitted to a professional conference each year (Months 12, 24, 36)*

No off-campus presentations have yet been made. Dr. Ireland expects to make a presentation at a national conference in Year 2.

- c. *Students involved in the research will present a poster at the annual research workshop (Months 12, 24, 36)*

On March 31-April 1, 2005, Xavier University hosts its 2nd Annual Festival of Scholars. Dr. Ireland's NIH-sponsored MARC student (see I.A.2. above), Danielle Haney, will be making an oral presentation entitled "Prostate Cancer and Genetics." (A copy of the preliminary program, with the appropriate section highlighted, is included as Appendix A-3) The Festival of Scholars is an especially appropriate forum for addressing one explicit aim noted in our Technical Abstract, the exposure of a meaningful number of African Americans to information concerning PC disparity issues: the event includes presentations by students across all disciplines and hundreds of students, faculty, administrators, and visitors from the community, primarily African Americans, attend.

- d. *One competitive grant application will be submitted by end of Month 24*
Plans are unchanged.

- e. *One paper will be submitted by end of Month 36*

Dr. Ireland hopes to have a publication during Year 2.

B. Stevens/Jones project: "The Search for Tyrosine Kinase Inhibitors (TKIs) of Prostate Cancer Cell Growth"

1. Hire technician to assist in project (Month 1)

Tracy Kirksey was hired within one month of initiating this project. She has a B.S. degree in Chemistry from Xavier University. Her primary responsibilities are (1) to grow and maintain the prostate cancer cell lines and (2) to test compounds as prospective inhibitors of ErbB2 tyrosine kinase activity. Because Dr. Stevens has just been elected chair of the Department of Chemistry at Xavier University, she plans to hire another technician to work alongside Ms. Kirksey, performing the molecular modeling studies.

2. Identify student to assist in project (Month 3)

Joseen Bryant, a chemistry major, was hired within one month of the start of this project. Her primary responsibilities are to use the SYBYL molecular modeling software package to develop a pharmacophore that can be used to search databases for new compounds to be tested for tyrosine kinase inhibition. Dr. Stevens is adding three additional students to this project. The first, Nicole Bell, is a Graduate Alliance for Education in Louisiana (GAELA)/American Chemical Society Scholar. She has already contributed significantly to this project, solving the two x-ray crystal structures described in Aim I.B.3. Ms. Bell will be supported in part as a Slayton Evans Summer

Research Scholar. The other two students are Nyote Oliver, who is a MARC Scholar, and Shelley Schmidt.

3. Identify novel small molecules that inhibit ErbB2 activity related to among quinazoline, pyrimidine, and quinoline derivatives (Months 1-18)

a. *Determine X-ray crystal structures of known tyrosine kinase inhibitors (quinazoline, pyrimidine, and quinoline derivatives) (Months 1-12)*

The x-ray crystal structures of two tyrosine kinase inhibitors have been completed. These are a dimethylamino benzylindazolyl pyrimidine derivative and a trihydroxy dicyano tyrphostin derivative. In both cases, crystals were obtained by slow evaporation of solvent and x-ray diffraction data were collected at room temperature on an Enraf-Nonius CAD4 diffractometer with a molybdenum target x-ray tube. Data were refined on a LINUX workstation with the maXus software package (Bruker Instruments). The structures are included as Appendix A-4. The Stevens group continues to recrystallize available tyrosine kinase inhibitors. As crystals are obtained, they will collect x-ray diffraction data for these compounds. These x-ray crystal structures will be used as reference structures for the molecular modeling results.

b. *Identify detailed pharmacophore and determine geometric, electronic, and lipophilic characteristics required for tyrosine kinase inhibition using Comparative Molecular Field Analysis quantitative structure activity relationships (Months 1-12)*

An extensive literature search was used to identify small molecules that are known to inhibit ErbB2 activity. These molecules are related to quinazolines, pyrimidines, quinolines, and tyrphostins. Dr. Stevens has collected a library of molecules that have been divided into two sets. One set includes 31 tyrphostins whose EGFR and ErbB2 activities were measured by Gazit and Levitzki.¹⁻³ The second set includes 71 quinazolines and similar molecules whose activities were measured by Wissner.⁴⁻⁶ Using the molecules with the highest EGFR inhibition, ErbB2 inhibition, and known activity against prostate cancers cell lines, two pharmacophore models have been developed (see Appendix A-5). These pharmacophore models identify the geometric relationship between structural features of a set of molecules known to act as tyrosine kinase inhibitors.

c. *Identify new compounds to be tested for tyrosine kinase inhibition with conformationally flexible searches of compound databases using detailed pharmacophore and CoMFA QSAR results (Months 9-18)*

The geometric models noted in I.A.3.b will be used to search databases for new compounds having the same geometric arrangement of structural features but not yet been identified as tyrosine kinase inhibitors. These databases have been installed and are available for searching at Xavier University. They include the Available Compounds Database (Molecular Design Limited), the National Cancer Institute Database, the EPA Toxic Chemicals Database, and the SMILE CAS Database. Software available for database searching includes HiVol and Unity (part of the SYBYL software package). A Comparative Molecular Field Analysis (CoMFA) QSAR study of the quinazoline derivatives has begun. Dr. Stevens plans to complete this study

by the end of the summer. The database has been created with nearly 80 molecules. The structures of all molecules have been optimized and MOPAC charges have been calculated. The most difficult part of the study has been to obtain an appropriate alignment of all molecules in the database. Dr. Stevens plans to begin the database search using the pharmacophore models developed during the past year. She anticipates that this initial searching will take until the end of the summer to yield preliminary results. At that point, she will use the CoMFA model results to search for additional compounds that might be active as tyrosine kinase inhibitors.

4. Explore the mechanism of ErbB2 tyrosine kinase inhibition (Months 13-24).

Plans are unchanged.

- a. *Build a homology model of the ErbB2 tyrosine kinase ATP binding site. (Months 13-18)*
- b. *Dock proven and proposed TKIs into the tyrosine kinase ATP binding site using multiple poses, and score results (Months 18-24)*

5. Determine activity and specificity of novel ErbB2-targeting molecules: specifically, the ability of each small molecule to ablate ErbB2 activation (Months 18-36)

Plans are unchanged.

6. Determine the impact of novel ErbB2-targeting molecules on PC cell growth: specifically, determine the effect of the ErbB2 inhibitor on the growth of LNCaP-ErbB2 cells using several standard cellular growth assays. (Months 18-36)

Plans are unchanged.

7. Deliverables/measurable outcomes: Dr. Stevens will prepare or oversee the following:

- a. *Semiannual reports will be submitted to the Co-PI*
Dr. Stevens has submitted one report to date. The Co-Project Director works across the hall from her and has daily contact with her, monitoring progress and identifying problems. A high level of activity, and especially the continuing involvement of African American students in her projects, has been noted.
- b. *One abstract will be submitted to a professional conference each year (Months 12, 24, 36)*
No off-campus presentations have yet been made by Dr. Stevens. Her generation of computational data and of completed x-ray crystal structures, however, leaves little doubt that significant publishable progress has been made.
- c. *Students involved in the research will present a poster at the annual research workshop (Months 12, 24, 36)*
Dr. Stevens' research student, Joseen Bryant, has already presented her work in two separate venues during the past year. Her poster, titled "A Molecular Modeling Study of ErbB2 Tyrosine Kinase Inhibitors", is included as Appendix A-6. These presentations included the following:
 - 1 Joseen Bryant and Cheryl L. Klein Stevens, "A Molecular Modeling Study of ErbB2 Tyrosine Kinase Inhibitors", American Chemical Society, Louisiana Section, October 2004.
 - 1 Joseen Bryant and Cheryl L. Klein Stevens, "A Molecular Modeling Study of ErbB2 Tyrosine Kinase Inhibitors", Annual

Biomedical Research Conference for Minority Students
(ABRCMS), Dallas, Texas, November 2004.

In addition, Ms. Bryant will also present her poster on March 31-April 1, 2005, at Xavier University's 2nd Annual Festival of Scholars (see Appendix A-3). As noted in I.A.6.c, the Festival of Scholars is an especially appropriate forum for addressing one explicit aim noted in our Technical Abstract.

- d. *One competitive grant application will be submitted by end of Month 24*
Plans are unchanged.
- e. *One paper will be submitted by end of Month 36*
Plans are unchanged.

SPECIFIC AIM II

Increase number of Xavier University investigators focused on PC research.

A. The Co-Project Director will identify two additional Junior Faculty that express an interest in PC research in order to include them in the group activities (Month 1)

One Xavier University faculty member, Dr. Gurdial Arora of the Department of Mathematics, has been partnered with a Dr. Suresh Sikka, a TCC prostate cancer researcher. Dr. Arora will use his expertise in statistics to analyze data collected by Dr. Sikka. A fuller description of the research to be performed by Dr. Arora is included as Appendix A-7. This partnership is strengthened by existing ties between Drs. Sikka and Dr. Abdel-Mageed, who hold joint lab meetings. A second partnership between Xavier University and TCC faculty has not yet been established, but the Co-Project Director is facilitating ongoing discussions between faculties at the two institutions. Two Xavier University faculty, Drs. Mary Carmichael (from the Department of Biology) and Maryam Foroozesh (from the Department of Chemistry), have expressed interest. Dr. Asim Abdel-Mageed is exploring the possibility of working with one of them. One difficulty has been that we did not allocate much money for the Junior Faculty to develop their research. This is partly offset by Xavier University's provision for up to one-quarter release time to faculty developing competitive grant proposals. In her role as the Associate Vice President for Academic Affairs, the Project Director has promised to give full consideration to faculty requests for such release time. In addition to the participation of two Junior Faculty envisioned by the SOW, the Co-Project Director hopes to initiate a research project through DOD Funding Opportunity #PC05-HDR. He has also engaged one student to assist with the background literature survey in preparing to submit a grant proposal in June 2005.

B. Establish participation of the selected Junior Faculty in Tulane Cancer Center seminars (Months 4-11)

The Co-Project Director regularly reviews the seminar listings at the Tulane Cancer Center website and informs the Xavier University researchers of relevant events. Few seminars specifically related to prostate cancer have occurred during the past year. Two Xavier University faculty and both Tulane mentors attended the 2nd annual Tulane University Mauvernay Research Excellence Award Seminar & Poster session on Friday, October 8, 2004. Two Xavier University faculty and one research staffer also attended the joint LSU-Tulane Cancer Seminar on January 20, 2005, which featured Dr. Oliver Sartor (LSU) speaking on the topic "Prostate Cancer Update." On a related note, the Co-Project Director has also tried to keep Xavier University researchers abreast of conferences and symposia

related to their research. In addition to reviewing online announcements at scheduled intervals and distributing announcements from organizations such as the American Association for Cancer Research, the Co-Project Director scrutinizes listings of featured talks to identify those specifically related to the research of participating faculty.

- C. **Subscribe to cancer- and/or prostate-related journals (Month 1)**
We have not yet selected the journals to which we will subscribe. *Cancer Research*, *Prostate*, and *Cancer* have been considered. This activity has been overlooked and the situation will be corrected.
- D. **Establish information-flow from the Office of Sponsored Programs about funding opportunities in PC (Month 9)**
Xavier University's Senior Vice President for Resource Development, who heads the university's Office of Sponsored Programs, regularly forwards information about new funding opportunities to the Co-PI, who passes it on as appropriate to the Xavier University researchers.
- E. **Determine Tulane Cancer Center mentors for the Junior Faculty (Month 6)**
See II.A.
- F. **Junior Faculty collect preliminary data (Months 7-24)**
The first established partnership (Drs. Arora of Xavier University and Sikka of the TCC) has developed a research plan, as noted in II.A. The Co-Project Director has requested that they present a budget before initiating their joint studies.
- G. **Host a workshop on grant preparation and how to identify proper funding opportunities (Month 24)**
Plans are unchanged.
- H. **Junior Faculty develop grant proposal (Months 25-36)**
Plans are unchanged.

SPECIFIC AIM III

Establish long-term collaborative relationship between Xavier University and Tulane University Cancer Center.

- A. **Grant membership in the Tulane Cancer Center to Xavier University researchers including Junior Faculty (Month 1)**
Xavier University faculty have not yet become members in the TCC. They must first become adjunct faculty members at Tulane University, after which they will be eligible for membership in the TCC. Adjunct faculty must be appointed by individual departments within the TUHSC. To this end, Dr. Prescott Deininger, the Associate Director of Tulane Cancer Center for Basic Science Programs, has kindly offered to shepherd the Co-Project Director through the process of obtaining appointments for Drs. Stevens and Ireland to the Biochemistry department. This task is not being neglected as it will provide them with fuller and speedy access to the relevant scientific literature through Tulane's online journal collection.
- B. **Include Xavier University researchers (including Junior Faculty) in the Tulane Cancer Center programs/working groups/task forces, which focus on a particular organ such as the prostate or on a specific class of phenomena such as signal transduction (Months 1, 7)**

On January 22, 2005, the newly established Louisiana Cancer Research Consortium (LCRC) met for the first time, holding a faculty retreat at Loyola University of New Orleans. Several DOD HBCU prostate cancer researchers attended, including the Co-Project Director and the Tulane University faculty mentors, Drs. Jones and Abdel-Mageed. At this meeting several working groups were established. One of these is Molecular Signaling, which is especially relevant to the both of the projects compassed by our program. This working group has begun regular meetings. Members of this working group in turn provide the membership for three focus groups: Cell Signal Integration in Breast Cancer, Prostate Cancer, and Ovarian Cancer; Steroid Receptors and Coactivation in Cancer; and Apoptosis and Cell Survival. At the faculty retreat, the Co-Project Director established contacts with other members within the LCRC. He also learned that adjunct faculty status within the TCC will facilitate participation in LCRC activities, providing another impetus for speeding these appointments described in Aim III.A. Finally, he inquired about various core facilities contained within the Louisiana State University Health Sciences Center and the TUHSC that might be made available to the Xavier University faculty involved in this PC program. Of special interest are core facilities for proteomics, immunology, and cell analysis core.

C. Include Xavier University researchers (including Junior Faculty) in the Tulane Cancer Center PC journal club (Month 1)

Faculty have not participated in the journal club.

D. Grant access to core research facilities at the Tulane Cancer Center (Month 1)

Access to the core facilities has to this point posed no problem: Drs. Ireland and Abdel-Mageed share a technician who has access to any of the facilities available at the TUHSC. Dr. Stevens has conducted all of her research to date at Xavier University, while Dr. Jones has normal access to the TUHSC facilities. The Co-Project Director is aware that this issue may need to be revisited as projects involving the Junior Faculty unfold.

E. Establish external advisory board (LSU-Tulane Cancer Research Consortium) for the purpose of reviewing program progress, offering solutions to identified problems, and providing an ongoing mechanism for planning improved collaboration (Month 3, 12, 24, 36)

One of the activities of the LCRC's Molecular Signaling working group is to provide feedback to its members on research in progress and to review grant proposals. After Xavier University faculty become members of the LCRC, they plan to avail themselves of the working group's expertise.

F. Invite Tulane University researchers to give seminars at Xavier University (Months 3, 7, 15, 19, 27, 31)

One seminar has been given at Xavier University by a Tulane University researcher. On March 11, 2004, Dr. Frank Jones gave a seminar at Xavier University titled "ErbB4: Guardian of the Breast." The talk was attended by approximately 40 Xavier University faculty and students, including most of the students actively engaged in prostate cancer research. The Co-Project Director plans to arrange for a visit by Dr. Asim Abdel-Mageed and to invite Dr. Suresh Sikka to present his work. Other prostate cancer researchers belonging to the Cell Signal Integration focus group (see III.B. above) will also be invited to give talks at Xavier University.

G. Invite Xavier University faculty-at-large to attend seminars related to PC research (Months 3, 7, 15, 19, 27, 31)

The talk by Dr. Frank Jones (noted in III.F.) was advertised to the Xavier University community at large. Xavier University has begun to publish its seminar schedules online regularly to ensure the widest possible exposure for talks of interest to the community. The Co-Project Director will ensure that the prostate cancer program's future listings are included in online announcements. The Co-Project Director has regularly reviewed the seminar listings at the Tulane Cancer Center and other local institutions, disseminating this information among the various researchers at Xavier University. Among the events attended by some Xavier University faculty are the following:

- 1 The 2nd Annual Tulane University Mauvernay Research Excellence Award Seminar & Poster session on October 8, 2004
- 1 An interdisciplinary cancer research workshop at the University of New Orleans on November 6, 2004
- 1 The LSU-Tulane Cancer Center joint seminar on Jan 20, 2005 (delivered by Dr. Oliver Sartor on the subject "Prostate Cancer Update.")
- 1 A tutorial-format seminar entitled "Small interfering RNA (siRNA) application for gene silencing", organized by Dr. Abdel-Mageed. This seminar has helped Dr. Ireland to design, execute, and troubleshoot experiments using siRNA strategies. Dr. Ireland and her lab have frequently attended other relevant seminars at the TUHSC.

H. Hold annual workshop, open to all in the Xavier University and Tulane communities, for all PC participants to present results of the preceding year. Faculty, students, and staff will attend and at least one person from each group will present a talk; students will present posters (Months 12, 24, 36)

The first annual workshop/minisymposium was originally scheduled for April 22, 2005. It has been postponed until summer for the purpose of accommodating the schedules of highly desired speakers, and also to allow a synergetic combined meeting with researchers of the DOD HBCU breast cancer program grant. Consulting with participating faculty, the Co-Project Director targeted several speakers whose expertise is recognized in areas closely related to the research described in the SOW. Those selected have been contacted and a gratifying number of them expressed interest, although they were unable to accommodate our original date.

I. Report the activities of the PC program to the presidential-level Tulane-Xavier University Partnership Committee (Months 12, 24, 36)

Our presentation to this committee was originally scheduled for March 31, 2005. Changes have been made to the agenda of this particular meeting, however. Instead, our presentation will occur at a later date per the request of Xavier University's Senior Vice President for Resource Development.

J. Ad-hoc committee will explore feasibility of academic course on cancer biology or cancer chemistry taught jointly by Xavier University and Tulane faculty (Month 13)
Plans are unchanged.

K. Submit competitive grant proposal for renewal and expansion of Xavier University-Tulane collaboration in PC (Month 24)

Plans are unchanged.

KEY RESEARCH ACCOMPLISHMENTS

- 1 Establishment at an HBCU of a cluster of 4 scientists, 2 technicians, 1 administrator, and 7 students engaged in PC research at varying levels of contribution
- 1 Establishment of prostate cancer cell lines at Xavier University's Cell Culture Facility (Ireland/Abdel-Mageed)
- 1 Demonstration of cross-talk between p53 and NF- μ B, two significant agents associated with prostate cancer (Ireland/Abdel-Mageed)
- 1 X-ray crystal structures of two tyrosine kinase inhibitors (Stevens/Jones)
- 1 Development of two distinct pharmacophore models for molecules having ErbB2 and EGFR activity (Stevens/Jones)

REPORTABLE OUTCOMES

- 1 2 Poster presentations (and 1 abstract accepted for presentation) by Ms. Joseen Bryant (Stevens/Jones)
- 1 1 Oral presentation accepted for presentation by Ms. Danielle Haney (Ireland/Abdel-Mageed)

CONCLUSIONS

A prostate cancer research program has started at Xavier University, an HBCU, and it has at an early date:

- 1 produced scientific results
- 1 increased the number of researchers focusing on prostate cancer research
- 1 incorporated African-Americans and Africans in the research at all levels, including one of the faculty at the mentoring institution and one laboratory technician
- 1 engaged more African-American students in prostate cancer research than promised in the original proposal
- 1 used or adapted existing resources to support its African-American student researchers (such as NIH or American Chemical Society scholarships)
- 1 used or adapted existing scientific resources to support its research program (such as an onsite cell culture facility)
- 1 received public recognition in the form of an article in the campus press
- 1 received visible support from the administration, in the form of acknowledgement at faculty meetings and the offering of release time to participating Junior Faculty
- 1 established a meaningful collaboration, at an administrative level, with a Breast Cancer Research Program funded by the DOD at approximately the same time, which will allow for the sharing/pooling of certain resources (see below).

The Peer Review Panel Summary Statement (July 23-25, 2003) noted several strengths and weaknesses of our proposal. The panel cited as strengths the following:

- 1 "each research project will include one student training at all times;"
- 1 "Xavier University faculty ...[will] continue to enlarge upon their work through competitive grants;"
- 1 "the leadership at Xavier University has demonstrated a commitment to prostate cancer research and is aware of the importance of having African-American scholars engaging in such research."

The panel also expressed the following concerns:

- 1 “no clear plans [have been] delineated for the continuation of the partnership after the grant period;”
- 1 “the small amount of time the Project Director [and Co-Project Director] will spend on this proposal is a concern.”

We believe that on the whole we have justified the faith of the reviewers and the DOD in our original proposal and have addressed their concerns. Some of the tasks and goals have already been exceeded; others are underway and on schedule. The Co-Project Director acknowledges that he will need to be more aggressive in tackling some of the tasks in the SOW, particularly in shepherding Junior Faculty to start on their projects and integrating the Xavier researchers more fully into the local cancer research community.

A collaborative program joining Xavier University and the TCC for the purpose of studying breast cancer was funded just after the prostate cancer program was funded. The two programs work together when justified, and the PDs and the Co-PD have excellent rapport. Problems arising with either grant can be discussed by a larger group of interested people and working solutions shared. In particular, the two programs plan to hold joint symposia, strengthening and broadening them. Resources, costs, and organizational planning for the symposia will be shared, while public interest will be deepened. Information received by one group is passed to the other, where justified, through the use of a common email “listserv”.

One of the investigators, Dr. Stevens, has just been elected chair of the Department of Chemistry and will serve for at least three years. Therefore, this department will continue to promote the development of our program, perhaps even more vigorously than before.

(“So-what section”) In terms of the scientific research now underway, both collaborations have made discoveries that should prove meaningful to race-related disparity issues. The first collaboration has already established the existence of cross-talk between two very important agents involved in the development and progression of cancer. The existence of race-based differences in the interaction between NF- μ B and p53 has never before been examined, but is now possible. The second collaboration has already made substantial progress in modeling tyrosine kinase inhibitors. Receptor tyrosine kinases have been implicated in the progression of high-grade prostatic intraepithelial neoplasia (HGPIN) to metastatic cancer, and HGPIN is in turn much more prevalent in African-Americans than in other populations. Thus, this work should contribute to our understanding of a phase that definitely affects African-Americans disproportionately and thereby address disparity issues directly.

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2. Levitzki, A.; Gazit, A. "Tyrosine Kinase Inhibition: An Approach to Drug Development", *Science*, **267**, 1782-1788 (1995).
3. Osherov, N.; Gazit, A.; Gilon, C.; Levitzki, A. "Selective Inhibition of the Epidermal Growth Factor and HER2/Neu Receptors by Tyrophostins", *J. Bio. Chem.*, **268**(15), 11134-11142 (1993).
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6. Wissner, A.; Floyd, M.B.; Rabindran, S.K.; Nilakantan, R.; Greenberger, L.M.; Shen, R.; Wang, Y.-F.; Tsou, H.-R. "Synthesis and EGFR and HER-2 Kinase Inhibitory Activities of 4-Anilinoquinoline-3-carbonitriles: Analogues of Three Important 4-Anilinoquinazolines Currently Undergoing Clinical Evaluation as Therapeutic Antitumor Agents", *Bioorg. & Med. Chem. Lett.*, **12**, 2893-2897 (2002).

APPENDICES

- A-1. Biosketch of Qiuyun Yang, M.D.
- A-2. NF- μ B and p53 cross-talk in CaP cells in the presence or absence of TNF- .
- A-3. Program for Xavier University of Louisiana's 2nd Annual Festival of Scholars, highlighting the participation of Ms. Danielle Haney and Ms. Joseen Bryant.
- A-4. X-ray crystal structures of two tyrosine kinase inhibitors.
- A-5. Pharmacophore models created using molecules having ErbB2 and EGFR activity
- A-6. Poster presented by Ms. Joseen Bryant at two conferences/meetings.
- A-7. "Biomarker Laboratory Project": Proposed research of Dr. Gurdial Arora (Xavier University) with Dr. Suresh Sikka (TCC)

Qiuyun Yang, MD

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Sex: Female

Education:

1982-1987 Hunan Medical University (Changsha), Clinical Medicine

Degree:

1987 MD, Hunan Medical University

Work Experience:

2001.7-present Medical Research technician in Tulane University Health Sciences Center(Full-time)
1999.8-2001.7 Medical Research technician in Tulane University Health Sciences Center (Part-time)
1993.6-1999.4 Attending physician (Nephrologist) and Lecturer in Department of Nephrology, the First Teaching Hospital of Henan Medical University
1988.7-1993.5 Resident in Department of Nephrology and Hematology, the First Teaching Hospital of Henan Medical University
1987.7-1988.7 Interne in the First Teaching Hospital of Henan Medical University

Publications:

1. Yang Q, Zhang M. Renal symptoms of 118 cases of polymyositis / dermatomyositis, Journal of Henan Medicine. 1991;15(4):145-147
2. Yang Q, Liu Z, Zhang M. Cardiac symptoms of 118 cases of polymyositis / dermatomyositis Journal of Zhongyuan Medicine. 1992; 5(3): 178-180
3. Yang Q, Li Y. Liu Z. The death cause analysis of 50 cases of systemic lupus erythematosus (SLE). Journal of Henan Medical University. 1995;30(1):17

4. Yang Q, Li Q, Liu Z. The evaluation of platelet count in the diagnosis of nephrotic syndrome. *Journal of Henan Medicine*. 1998;22(4):74-77
5. Yang Q, Li Q, Liu Z. The evaluation of plasma and urine D-dimer in the diagnosis of nephrotic syndrome. *Journal of Chinese Modern Medicine*,. 1999;5(4):74-77

Experimental Experience:

1 Clinical tests

- Qualitative and quantitative determination of human urine protein
- Morphological assortment and count of urine red blood cell
- Determination of plasma BUN and creatinine (Cr)
- Determination of MMS
- Determination of ENA polypeptide enzyme spectrum
Anti-Sm Ab, Anti-RNP Ab, Anti-SS-A Ab, Anti-SS-B Ab, Anti-Scl-10 Ab, Anti-PM Ab, Anti-JO-1 Ab

2. Diagnostic tests

Renal biopsy, ECG, etc.

3. Biological and Molecular biological experiments.

Cell Culture. Plasmid Transfection using electroporation method.
Luciferase and B-Galactosidase Assay. DNA Fragmentation.

Processing of *Xenopus* oocytes, micro-injection of cRNA into *Xenopus* oocyte and check the expression of cRNA in the *Xenopus* oocytes, and some related electro-physiological experiments.

Restriction enzyme digestion of DNA fragment, DNA ligation and transformation into *E. coli*.

Southern hybridization and the isotope manipulation techniques(including ^{125}I , ^{32}P and ^{99}Tc).

In-situ Hybridization and gene typing.

Labeling active peptides and proteins with ^{125}I .

PCR and RT-PCR.

Western Blot.

Good experience with animal handling including rat, mouse and *Xenopus*.

4. Surgical experience

Surgical operations on rabbits, mice and *Xenopus* had been experienced. Accurate surgery like mouse spinal cord injury model establishment was performed successfully.

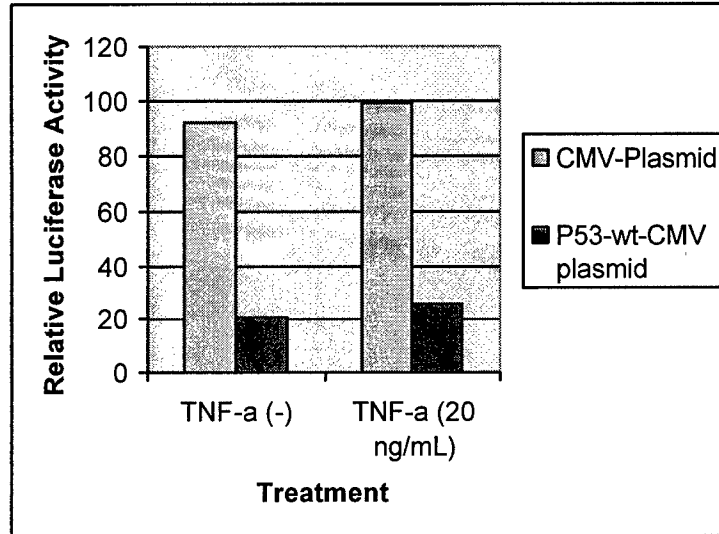
Teaching Experience

Subject	Audience	Hours	Duration
Diagnostics	undergraduates	120	1994-98
Diagnostic Experiment	undergraduates	580	1994-98
Nephrology	undergraduates	70	1997
ECG	undergraduates	120	1996-98

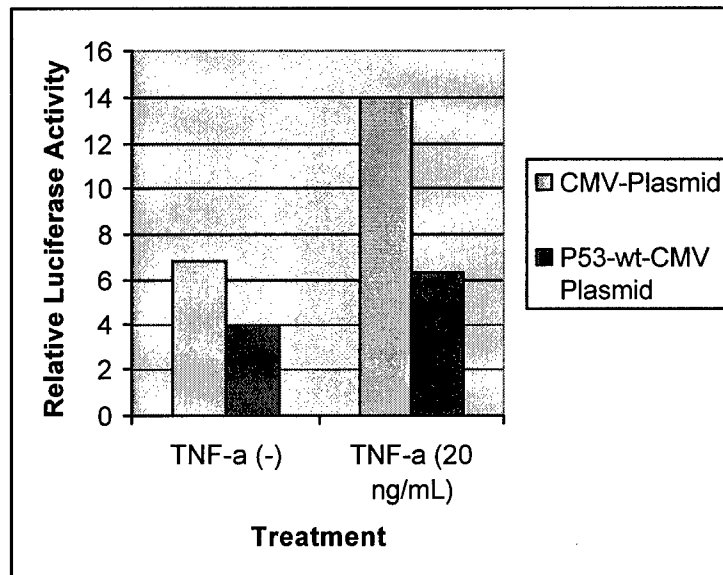
NF- κ B and p53 cross-talk in CaP cells in the presence or absence of TNF- α .

(a) In PC-3 cells, expression of wild-type p53 reduces NF- μ B expression even in the presence of TNF- . (b) In DU-145 cells, expression of wild-type p53 reduces NF- μ B expression even in the presence of TNF- .

(a)



(b)



Program for the 2nd Annual

March 31 - April 1 2005

FESTIVAL OF SCHOLARS

Highlighting Research

&

Creativity of Xavier Students

Science

Art

Poetry

Math

Humanities

Sponsored by the Center for
Undergraduate Research

Xavier University of Louisiana

Dr. Bryan Klassen
Chemistry Department
Campus Box: 114

ORAL PRESENTATIONS

Each oral presentation will last 15 minutes, 12 minutes for the presentation and 3 minutes for questions. Oral presentations will be held in UC 218, UC 205 and UC 205C. Please see the schedule at the end of this program for times of each oral presentation

African American Females: Sexuality Stereotyping

By: Sarah Ann Anderson, Sociology Major
Faculty Mentor: Beverly Mason

Sedimentation of Particles in a Viscous Fluid

By: Aviva Baird, Physics and
Dual Degree Engineering
Faculty Mentor: Kathleen McCloud

Ageism and the Measurement of it

By: Eboni Davis, Psychology
Faculty Mentor: Cecile Brookover

Professional Black Women & Nonverbal Communication

By: Yolanda Davis, Communications
Faculty Mentor: Rockell Brown

Jonah in Biblical Hebrew: Puns and Word Play

By: Roy DuBose III and Whitney Davis, Theology
Faculty Mentor: Michael M. Homan

My Family History: An Examination of Culture

By: Dana Foster, History
Faculty Mentor: Edie Ambrose

Neighborhood Transition

By: Mikhail Frazier, History
Faculty Mentor: Edie Ambrose

Service Learning and Resegregation of LA Schools

By: Nichole Guillory, Communications
Faculty Mentor: Ross Louis

Prostate Cancer and Genetic Factors

By: Danielle Haney, Biology
Faculty Mentor: Shubha Kale-Ireland

Language Development in Children

By: Alina Harris, Sociology
Faculty Mentor: Beverly Mason

Teen Girls and Pregnancy Resolution

By: Tasany Lazard, Sociology
Faculty Mentor: Beverly Mason

Is Desegregation Complete?

By: Jenais Means, Communications
Faculty Mentor: Ross Louis

The Option to Resist: the Preferential Option for the Poor and Xavier Students

By: Malik Muhammed, Theology
Faculty Mentor: Gerald BooDoo

Party Identification in the Modern Era

By: Terry Richards, Political Science
Faculty Mentor: Pamela Waldon-Moore

Abortion Attitudes

By: Tachera Tomes, Political Science
Faculty Mentor: Pamela Waldon-Moore

A Historical Study of Paper and Pulpmill Effluents and their Affects on Altered Gonadal Development in longear sunfish *Lepomis megalotis*

By: LeShay Wesson, Biology
Faculty Mentor: Jeanine Burse

Abstract Accumulation

By: Melvin Brown, Art
Faculty Mentor: MaPo' Kinnord-Payton

A Questionnaire on Gender Roles

By: Tiffany Black, Theron Exum, and
Derrick Williams, Psychology
Faculty Mentor: Lisa Schulte

**Alu Insertion: Non-Human Primate,
Human Divergence**

By: Wayne Borders, Chemistry
Faculty Mentor: Marion L. Carroll

**A Molecular Modeling Study of ErbB2
Tyrosine Kinase**

By: Joseen Bryant, Chemistry
Faculty Mentor: Cheryl Stevens

Actively Learning Computer Graphics Using 3DSM

By: Jermaine Coston, Computer Science and
Computer Engineering
Faculty Mentor: Andrea Edwards

Diabetic Education: Pharmacist Involvement

By: Rashida Daily, Pharmacy
Faculty Mentor: Adrienne Allen

Investigation of the MuKB Antiparallel Coiled Coil

By: Shameka Darisaw, Chemistry
Faculty Mentor: Guangdi Wang

Investigation into Communication Studies

By: Laketa Entzminger, Robyn J. Peebles,
Angel Bradford, Communications
Faculty Mentors: Melissa Sparks, Dominique Gendrin,
Ross Louis and Sharon Thomson

The Effects of Absent Fathers on the Black Family

By: Sharita Farmer, Sociology
Faculty Mentor: Beverly Mason

College Student's Attitudes About Older Adult Love

By: Dana Foster and Lakasha Barksdale, Psychology
Faculty Mentor: Cecile Brookover

Color-Color Plots to Identify Objects

By: Jessica Fuselier, Physics
Faculty Mentor: Robert Benjamin

Media Exposure and Global Self-Esteem

By: Tiffany Haynes, Psychology
Faculty Mentor: Elliott Hammer

Pharmacophore Determination of Butyrophenones

By: Ayanna Jackson, Chemistry
Faculty Mentor: Cheryl Stevens

The Synthesis of Water Soluble Phosphine Catalysts

By: Gregory Jean-Noel, Chemistry
Faculty Mentor: Ralph Isovitsch

A Smarter BLAST Search Tool

By: Derrick Johnson, Computer Science and
Computer Engineering

Faculty Mentor: Andrea Edwards

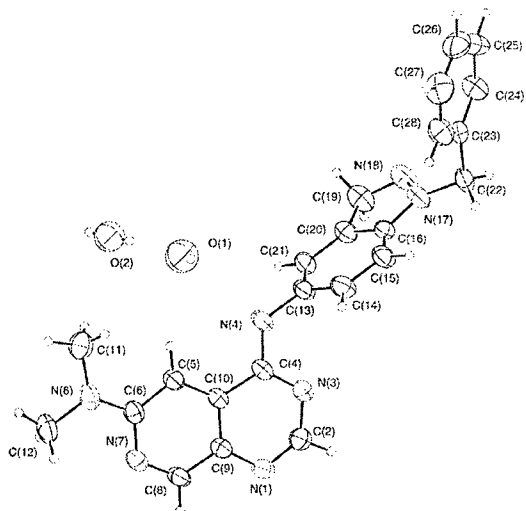
The Role of ATPys in Cochlear Potentials

By: Mallory Johnson
Faculty Mentor: Derrick Rovaris

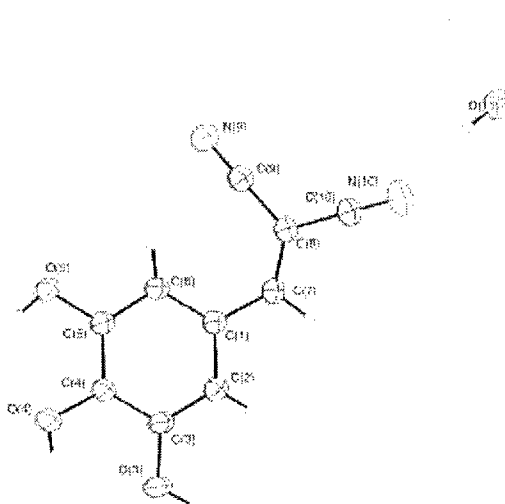
Should Two Wrongs Make a Right?

By: Marlinda Johnson
Faculty Mentor: Dereck Rovaris

X-ray crystal structures of two tyrosine kinase inhibitors



ORTEP diagram of the dimethylamino benzylindazolyl pyrimidine derivative



ORTEP diagram of the trihydroxy dicyano typhostin derivative

Pharmacophore models created using molecules having ErbB2 and EGFR activity

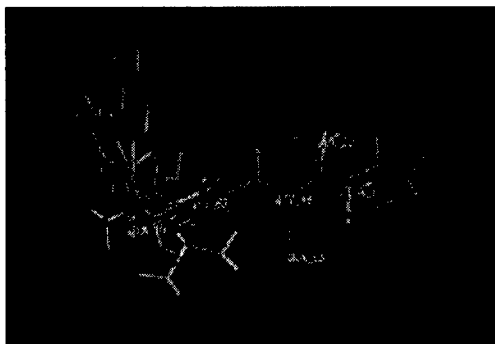


Diagram showing the 6 point pharmacophore model derived from the alignment of the structural features of 6 tyrphostin derivatives.

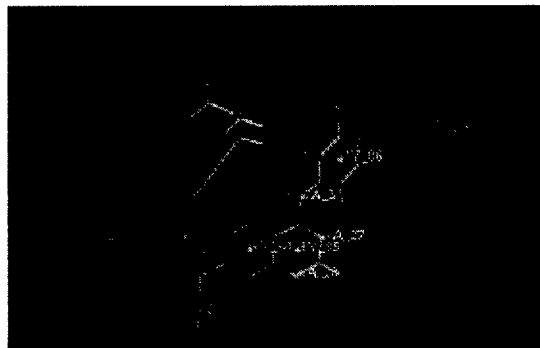


Diagram showing the 6 point pharmacophore model derived from the alignment of the structural features of 6 quinazoline derivatives.

A Molecular Modeling Study of ErbB2 Tyrosine Kinase Inhibitors



Joseen A. Bryant & Cheryl L. Klein Stevens, PhD
Department of Chemistry, Xavier University of Louisiana, New Orleans, LA, USA 70125

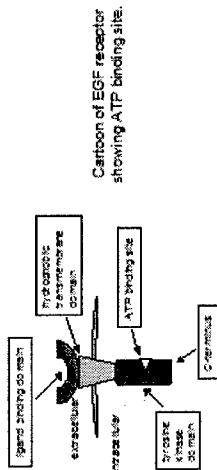
Introduction

Growth factor receptors have been implicated in human cancers. These receptors belong to the tyrosine kinase (TK) family which includes the EGF receptor (EGFR, erbB1) and HER2/Neu receptor (erbB2). These receptors are involved in the regulation of reactions involving cell function and signaling pathways. In cancer cells, uncontrolled signaling results in receptor overexpression and deregulated cell growth.

Small molecule inhibitors that bind to the ATP binding site of the tyrosine kinase domain of the receptor disrupt the uncontrolled signaling reactions by inhibiting tyrosine phosphorylation. Our interest is in determining the structural features of these molecules that make them optimal candidates for use as tyrosine kinase inhibitors. Using molecular modeling techniques, we have developed a pharmacophore model for two series of molecules known to have significant ErbB2 tyrosine kinase inhibitory activity. These molecules belong to the tyrosinostin and quinazoline classes of compounds.



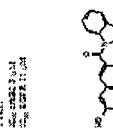
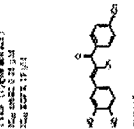
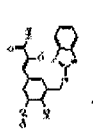
*The traditional medicinal chemistry definition of a pharmacophore is the minimum functionality a molecule has to contain in order to exhibit activity.



Experimental

- Identify molecules to be used in pharmacophore development using the following criteria.
 - All of the chosen compounds are known to be ErbB2 tyrosine kinase inhibitors (according to references).
 - All compounds have IC_{50} values that imply significant ErbB2 activity ($IC_{50} \leq 1 \mu M$).
 - Compounds show a significant amount of structural diversity.
- Molecules are drawn and energy minimized using the Sybyl 6.9.2 (Tripos) software package on an Octane3 SGI workstation.
- Conformational searches are performed to generate alternative molecular conformations.
- Pharmacophoric features are identified in the molecules.
- Molecular alignment of these features results in pharmacophore models that describe the relative location of important structural features within a series of molecules.

Tyrosine TKIs of ErbB2



Quinazoline TKIs of ErbB2

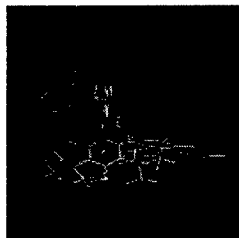
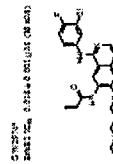
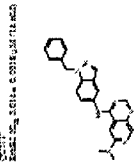
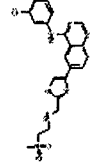


Diagram showing a view of the 6-point pharmacophore model derived from the alignment of the structural features of 6 quinazoline derivatives (GW2947 blue, C11033 green, QUN1 magenta, QUN2 orange, QUN3 yellow).

Results

- There is excellent alignment of the features of the quinazoline ring system and melononitile portion of the tyrosinostin series of chosen molecules.
- In both series, there are floppy substituents on the periphery of the molecules. This implies that the binding pocket must be able to accommodate this steric bulk.
- The technique used here for developing a pharmacophore model was useful for identifying the important pharmacophoric features to be used in proposing a binding site model for these inhibitors and the tyrosine kinase enzyme.

Support for this research from the Department of Defense (PC-030845) is gratefully acknowledged.

Biomarker Laboratory Project

Development of Biomarker for Early Detection and Prevention of Benign and Malignant Prostate Disease in Aging Men

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Department of Urology, Tulane University Health Sciences Center, New Orleans, LA.

Gurdial Arora, PH.D

Department of Mathematics, Xavier University of Louisiana, New Orleans, LA

As men age, environment, diet, and genetics play a significant role in the development of benign prostatic hyperplasia (BPH) or prostate cancer (PC). How these etiologic factors interact in prostate growth and differentiation leading to cancer is not known. Altered redox mechanisms affecting cellular oxidative insult leading to specific gene mutations is now considered to be a key hypothesis in this respect. Oxidative insult or stress is a condition caused by increased generation of free radicals and/or decreased antioxidant capacity in associated cells and tissues. By far, the preventative and early therapeutic options available to men prone to BPH and/or PC are limited - mainly due to the lack of markers for early detection of these conditions. In addition, there is no clear understanding of cellular and molecular mechanisms that are responsible for genetic mutations due to altered oxidative stress in the aging prostate.

Dr. Sikka recently demonstrated a differential growth and inhibition pattern in benign and normal prostate epithelial cells in response to oxidative insult. BPH cells unlike normal prostate cells showed significant proliferative response over control under very low oxidative stress. By induction of apoptotic stimuli (caspase activation), this selective BPH proliferation could be prevented by antioxidants. Dr. Sikka hypothesizes that low oxidative stress is responsible for inducing genetic and physiological events in cells most prone to such altered responses.

In this context Dr. Sikka has observed that intracellular calcium ($[Ca^{2+}]_i$) plays a vital role that may regulate the differential growth patterns that exists between BPH and normal prostate epithelial cells under conditions of induced oxidative stress. The mRNA expression of T-type Ca^{2+} channel was observed only in BPH cells at low oxidative stress (even near the resting membrane potential) resulting in the elevation of basal $[Ca^{2+}]_i$ concentration. Dr. Sikka is currently evaluating other specific Ca^{2+} channels in normal and cancer cell lines established in our cell culture facilities. In addition, the preliminary results have shown that the expression levels of gene and/or protein of cytochrome c oxidase II and III subunits are extremely low in BPH cells but relatively higher in normal prostate epithelial cells. Since the mitochondrial potential and activities of such key redox enzymes play an important role in activation of caspase cascade leading to induction of apoptosis, Dr. Sikka plans to evaluate the expression of these subunits II, III at both gene and protein levels. Dr. Sikka hypothesizes that this differential response is due to induction of specific mutations and/or deletions in these key mitochondrial enzymes. Evaluation of such mutations/deletions with functional expression of selective calcium channels can be **used as biomarkers for early detection and prevention of benign and malignant prostate disease in aging men**. Dr. Sikka plans to specifically use their patient resources (biopsy tissue, EPS and blood samples) from men of various age groups who attend their Urology clinics and focus on evaluating these biomarkers.

Dr. Arora's participation related to this project is multifold. First he wants to learn the terminology and specific problems (epidemiological, pathophysiological, genetic linking, early diagnosis, prevention and treatment) related to this area, which are of paramount importance. He would also like to use his expertise in the area of mathematical modeling and applied biostatistics to analyze the data so far generated by Dr. Sikka's research in this area. The goal is to collaborate with Dr. Sikka's group on this project, learn from their expertise and hope to continue working with Dr. Sikka and submit grant for funding in the near future.