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TITLE: Predictive Biomarkers of Response to Bcl-2 Biomodulation by G3139 and Docetaxel in Hormone-Refractory Prostate Cancer

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Predictive Biomarkers of Response to Bcl-2 Biomodulation by G3139 and Docetaxel in Hormone-Refractory Prostate Cancer

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The central hypothesis is that bcl-2 protein over expression confers intrinsic resistance to chemotherapy in patients with hormone-refractory prostate cancer (HRPC), therefore downregulation of bcl-2 protein by the antisense oligonucleotide directed to Bcl-2, G3139, will enhance the antitumor activity of docetaxel in patients HRPC. To address this hypothesis and determine the predictive biomarkers for response to this therapy a phase II clinical study of G3139 combined with docetaxel was initiated in men with HRPC. The specific aims of the current grant (PC010504) are to demonstrate that bcl-2 over expression in prostate cancer specimens, the degree of bcl-2 downregulation in normal peripheral blood mononuclear cells (MNCs), and the pharmacokinetic parameters of G3139 and docetaxel will predict prostate cancer responsiveness to G3139 and docetaxel. Preliminary Results: thirty-one patients have been entered on the clinical study. Original tumor specimens have been obtained in 30/31 patients and immunohistochemical Bcl-2, Bax, Bcl-X, expression will be determined in year 2. The mean G3139 steady-state concentrations were 5.51±1.63 μg/mL with moderate interpatient variability whereas MNC Bcl-2 protein expression declined by a median of 58% following 5 days of G3139 therapy. In year 2 and 3 of the grant the predictive biomarker and pharmacokinetic relationships will be determined.
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PC010504: Predictive Biomarkers of Response to \textit{bcl-2} Biomodulation by G3139 (antisense oligonucleotide to bcl-2) with Docetaxel in Hormone-Refractory Prostate Cancer

\textbf{INTRODUCTION}

The central hypothesis is that \textit{bcl-2} protein overexpression confers intrinsic resistance to chemotherapy in patients with hormone-refractory prostate cancer (HRPC), therefore downregulation of \textit{bcl-2} protein by G3139 will enhance the antitumor activity of docetaxel in patients HRPC. We further hypothesize and the focus of the current grant application is that patients whose tumors utilize \textit{bcl-2} overexpression as a mechanism to escape the apoptotic events of chemotherapy will benefit from G3139 biomodulation, whereas tumors that exhibit other mechanisms for impaired apoptosis, such as diminished Bax expression, will fail to respond or fail to have a durable response. The specific aims of this project are to demonstrate (1) that \textit{bcl-2} overexpression in prostate cancer specimens is a predictive biomarker for enhanced responsiveness to G3139 and docetaxel therapy; (2) that the degree of \textit{bcl-2} downregulation in normal tissue surrogate (peripheral blood mononuclear cells) will predict prostate cancer responsiveness to G3139 and docetaxel; and (3) whether the pharmacokinetic parameters of G3139 and docetaxel are predictive of \textit{bcl-2} biomodulation and antitumor activity, respectively.
Annual Report Year 1 (0-12 months)

A total of 31 patients have been entered into the clinical study entitled A Phase I/II pharmacokinetic and biologic correlative study of G3139 (antisense oligonucleotide directed to bcl-2) and docetaxel in patients with hormone-refractory prostate cancer and accrual of new patients is now complete. This accrual is somewhat faster than predicted and has permitted the correlative biologic studies to proceed moderately ahead of schedule. The clinical study has been overseen by Dr. Anthony Tolcher, the principal investigator of the clinical study and the biologic correlative grant, and Dr. Eric Rowinsky (co-investigator). The correlative biologic and pharmacokinetic studies are the companion to the clinical study and funded by the current Department of Defense Grant (PC010504). The accomplishments of year 1 are described below in order of the Tasks described in the original grant application.

Task 1: Immunohistochemical Detection of bcl-2, bcl-Xl, and Bax from patients entered onto phase II study of G3139 and docetaxel for HRPC

a. Obtain primary tissue blocks (paraffin embedded) from each patient entered on Phase II study (30 patients total) for banking, section paraffin blocks for representative tumor, and perform immunohistochemical staining for bcl-2, bcl-Xl, and Bax staining. 24 months.

b. Pathologic scoring of all immunohistochemical stained specimens will be complete by end of year 2. (24 months).

To date, paraffin blocks or unstained slides representitive of the original biopsy specimens have been obtained from 30 of the 31 patients entered. One specimen cannot be obtained and will not be pursued further. These specimens have been batched for uniformity in staining technique and will undergo immunohistochemical staining for Bcl-2, bcl-Xl, Bax staining in the next 6 months. Personnel changes for Task 1 include the
addition of Dr. Jeffrey Kreisberg (CV attached in appendix), an associate member of the Institute for Drug Development, who will be substituted for Dr. Shan Wu. Dr. Kreisberg brings to the project his expertise in immunohistochemical detection of predictive biomarkers (see attached publications). Furthermore, the important work by Kreisberg and others have examined in prostate cancer specimens apoptotic regulatory pathways mediated by the PTEN/Akt signal transduction pathway. We will therefore examine, in addition to the stated biomarkers in Task 1, upstream regulators of the proapoptotic protein BAD including phospho-Akt and PTEN. This work is being performed by J. Kreisberg (co-investigator), and Dean Troyer (co-investigator).

Task 2: Quantification of G3139 mediated bcl-2 downregulation in peripheral blood mononuclear cells.

a. Obtain isolate blood mononuclear cells (MNCs) from all patients (30 patients) at the two time points (prior to G3139 therapy and on day 5), isolate protein 18-24 months.

b. Perform western assay for bcl-2 protein. 18-24 months.

Paired collections of peripheral blood mononuclear cells (MNC) have been obtained on 27 of 31 patients to date. Bcl-2 has been quantified by western blot methods on all 27 patients and the values have been normalized using quantification of actin expression. The majority of patients have marked decrements in Bcl-2 protein expression at day 6 compared to pretreatment values although a minority of patients have either no change in Bcl-2 expression or an increase (Figure 1). The median change in MNC Bcl-2 protein levels for the entire patient population to date was a 58% decrement (range +444% to –99.5%). The median decrement in bcl-2 protein by day 6 for the 19 patients who had declines was –71%. The differences in the magnitude of decline in Bcl-2 protein expression will be analyzed with respect to G3139 steady-state concentrations and patient’s clinical response to therapy and the relationships determined (Task 4 Year 3).
Figure 1: Distribution of percent change in Bcl-2 protein expression in 27 patients treated with G3139 (antisense oligonucleotide directed to Bcl-2). A single patient had a 444% rise in Bcl-2 expression with data point not shown in this figure. This work and analysis was performed by E. Izbicka (co-investigator), Gilbert Carrizales (research associate), Eric Rowinsky (co-investigator) and Anthony Tolcher (Principal Investigator).

Task 3: Pharmacokinetic Sampling of G3139 and docetaxel from patients entered on Phase II study.

a. Collect G3139 and docetaxel plasma samples for pharmacokinetic analysis from all 30 patients (18-24 months).

b. Perform high-performance liquid chromatography to determine plasma concentrations of each agent (18-24 months).

The analysis for G3139 is complete for 31 of 31 patients. Steady-state concentrations were reached by 24-hours following the start of the infusion and the mean steady-state concentration of G3139 is 5.51 (± 1.63) μg/mL. Figure 2 illustrates the mean values at each time point for the population as a whole. Overall there is only modest inter-patient
variability at 7 mg/kg/day of G3139 in this large patient sample although there are some outlier results that may have implications for the effectiveness of Bcl-2 downregulation measured in the surrogate normal mononuclear cells. This work has been performed by Dr. John Kuhn (co-investigator).

Figure 2: Mean values for G3139 plasma concentrations at 2, 24, 48, and 168-hours in patients receiving 7 mg/kg/day G3139 CIVI for 7 days

In years 2 and 3 the relationship of G3139 steady-state concentrations to Bcl-2 protein downregulation in peripheral blood mononuclear cells (MNCs) (Task 4b) will be explored.

Docetaxel pharmacokinetic parameters will be examined during year 2.

Task 4: Examine the predictive pharmacokinetic and biomarkers for response to bcl-2 biomodulation by G3139 and docetaxel.

a. Examine relationships between the biomarkers of bcl-2, bcl-Xₐ, and Bax expression and clinical outcome (6-12 months, Year 3).
b. Examine relationships between MNCs and G3139 steady-state concentrations, and patient clinical outcome (e.g. response rate, time to progression, survival). (6-12 months, Year 3).

c. Model docetaxel pharmacokinetic parameters, and determine relationships with clinical outcome (6-12 months, Year 3)

The examination of the relationships of the pharmacokinetic, predictive biomarkers and clinical outcome and analysis will occur in year 3 of the grant year.
Key Research Accomplishments (Year 1)

- Quantified MNC Bcl-2 protein decrements (and increments) following antisense Bcl-2 therapy and documented median decrement following 5 days of therapy.
- Quantified G3139 steady-state concentrations for 31 patients

Reportable Outcomes:
The data is too preliminary after 12 months to report outcomes

Conclusions:
The data is too preliminary after 12 months to draw conclusions. However, to more thoroughly address the predictive markers of apoptosis, we will expand the examination of pathways that regulated apoptosis and the original tumor blocks will also be subjected to immunohistochemistry for address the status of Akt activation which regulates the Bcl-2 family member BAD and we will complete the tasks of year 2 and 3 in the time allotted.
REFERENCES

Immunohistochemical Demonstration of Phospho-Akt in High Gleason Grade Prostate Cancer

Shazli N. Malik, Michael Brattain, Paramita M. Ghosh, Dean A. Troyer, Thomas Prihoda, Roble Bedolla, and Jeffrey I. Kreisberg

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ABSTRACT

Purpose: Whereas the early stage of prostate cancer is marked by excessive proliferation, in advanced stages of the disease, a decreased apoptotic death rate (increased cell survival) also contributes to net tumor growth. Altered regulation of the mitogen-activated protein kinase (MAPK)-regulated cell proliferation and Akt-regulated cell survival pathways are suspected causes. In this study, we wanted to determine: (a) whether the degree of Akt activation can be assessed by immunohistochemical staining of paraffin-embedded human prostate cancer biopsies with an antibody to phospho-Akt (Ser473); and (b) whether phospho-MAPK/Erk1/2 and phospho-Akt expression are altered in prostate cancer.

Experimental design: To examine the activation status of MAPK/Erk1/2 and Akt, archival paraffin-embedded sections from 74 cases of resected prostate cancer were immunostained with antibodies to phospho-MAPK/Erk1/2 (Thr202/Tyr204) and phospho-Akt (Ser473).

Results: The staining intensity for phospho-Akt was significantly greater in Gleason grades 8–10 (92% of such cases staining strongly) compared with prostatic intraepithelial neoplasia and all other grades of prostate cancer (only 10% of these cases staining strongly; P = 0.001). The staining intensity for phospho-MAPK/Erk, on the other hand, was significantly greater for normal, hyperplastic, and prostatic intraepithelial neoplasia lesions but declined with disease progression, reaching its lowest level of expression in high Gleason grades 8–10 (P < 0.0001).

Conclusion: The activation state of the cell survival protein Akt can be analyzed in human prostate cancer by immunohistochemical staining of paraffin-embedded tissue with a phospho-specific Akt (Ser473) antibody. Advanced disease is accompanied by activation of Akt and inactivation of Erk.

INTRODUCTION

Prostate cancer causes more than 41,000 deaths annually in the United States and is the second leading cause of cancer deaths in men (1). Although prostate cancer is initially dependent on androgens for growth and, thus, is responsive to androgen ablation, progression to an androgen-insensitive state generally ensues (1). When this occurs, the prognosis is poor, because no systemic therapy is effective. Therefore, there is an urgent need for targeted nonhormonal treatment that inhibits prostatic cancer cells. In normal prostate epithelium, cell proliferation is balanced by an equal rate of apoptosis, such that there is neither involution nor overgrowth (1). In prostate cancer this balance is altered. Whereas the early stage of the disease is marked by excessive proliferation, in advanced stages of the disease, net growth of the tumor results from a decreased apoptotic death rate (cell survival) in addition to increased proliferation (1).

Activation of the PI3Kα/serine-threonine kinase Akt signaling pathway promotes cell survival by inhibiting apoptosis through phosphorylation of the proapoptotic protein BAD and other proteins (2–5), whereas activation of the MAPK signaling pathway is accompanied by increased cellular proliferation (6, 7). PTEN is a tumor suppressor gene that is altered and inactive in many types of tumors, including prostate cancer (2, 3). Among its substrates are the lipid products of PI3k, phosphatidylinositol 3,4,5-trisphosphate, and phosphatidylinositol 3,4,5-trisphosphate, which mediate the activation of Akt (4, 5). It was demonstrated recently by IHC that high Gleason-grade prostate cancer displays loss of tumor suppressor phosphatase PTEN (2). This suggests that increased activation of Akt in poorly differentiated prostatic carcinoma results from the loss of PTEN.

In this paper, with phospho-specific antibodies we demonstrate by IHC that advanced prostate cancer is accompanied by the expression of the activated (phosphorylated) form of Akt and decreased expression of activated MAPK/Erk1/2. These results may provide the molecular basis for the observed activation of a cell survival pathway that has been reported to

1 Supported by a Merit Review from the Office of Research and Development, Medical Research Service, Department of Veterans Affairs (to J. I. K.), and a grant from the San Antonio Cancer Institute. J. I. K. is a Career Scientist with the Department of Veterans Affairs.

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The abbreviations used are: PI3k, phosphatidylinositol 3'-kinase; MAPK, mitogen-activated protein kinase; IHC, immunohistochemistry; PIN, prostatic intraepithelial neoplasia; Erk, extracellular-regulated kinase; TBS-T, tris-buffered saline-tween.
Fig. 1 Immunoperoxidase staining for phspho-Akt using a phospho-specific Akt (Ser 473) antibody. A, negative control. B, PIN showing weak cytoplasmic staining for phospho-Akt. C, well-differentiated adenocarcinoma showing weak cytoplasmic staining. D, poorly differentiated carcinoma showing strong membrane staining for phospho-Akt (×40, original magnification).

Contribute significantly to the progression of prostate cancer growth (1).

MATERIALS AND METHODS

Primary Antibodies. Rabbit polyclonal phospho-Akt (Ser 473; Cell Signaling Technology, Beverly, MA, Cat. No. 9277, IHC specific) was used at a 1:50 dilution; rabbit polyclonal phospho-p44/42 MAP kinase (Thr 202/Tyr 204; Cell Signaling Technology; IHC-specific) at a 1:50 dilution; rabbit polyclonal Akt (Santa Cruz Biotechnology Inc., Santa Cruz, CA) at a 1:50 dilution; and rabbit polyclonal Erk1 (Santa Cruz Biotechnology) at a 1:100 dilution.

Analysis of Human Tissues. A total of 74 formalin-fixed, paraffin-embedded human primary prostate cancer specimens were studied from the archival files of Audie Murphy Veterans Medical Center. Fifty-three samples were obtained from radical prostatectomies, and 22 samples were obtained from transurethral resections. H&E-stained slides were reviewed for Gleason score. In a majority of the cases, adjacent areas of normal prostatic epithelium, benign prostatic hyperplasia, and PIN were also available for review along with infiltrating carcinoma.

IHC. Sections were heated to 60°C, and rehydrated in xylene and graded alcohols. Antigen retrieval was performed with 0.01 M citrate buffer at pH 6.0 for 20 min in a 95% water bath. Slides were allowed to cool for another 20 min, followed by sequential rinsing in PBS and 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, Tween 20 (0.1%; TBS-T). Endogenous peroxidase activity was quenched by incubation in TBS-T containing 3% hydrogen peroxide. Each incubation step was carried out at room temperature and was followed by three sequential washes (5 min each) in TBS-T. Sections were incubated in primary antibody diluted in TBS-T containing 1% ovalbumin and 1 mg/ml sodium azide (12 h) followed by incubations with biotinylated secondary antibody for 15 min, peroxidase-labeled streptavidin for 15 min (LSAB-2; Dako Corp., Carpinteria, CA), and diaminobenzidine and hydrogen peroxide chromogen substrate (Dako Corp.) along with 3,3’diaminobenzidine enhancer (Signet) for 10 min. Slides were counterstained with hematoxylin and mounted. The negative controls were incubated with nonimmune rabbit IgG in place of primary antibody.

One representative slide per case was evaluated with the above antibodies. The proportion of carcinoma and PIN staining, and the intensity of staining seen in different areas of the same slide were analyzed according to criteria described previously in the literature (8). The intensity is designated as 0 when no tumor cells stain, 1+ when 10–20% of cells stain (weak), 2+...
Table 2  Relationship between phospho-MAPK/Erk1/2 staining intensity vs. Gleason score

<table>
<thead>
<tr>
<th>Gleason score</th>
<th>Number of cases</th>
<th>Weak staining intensity</th>
<th>Strong staining intensity</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>60</td>
<td>17</td>
<td>43</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>53</td>
<td>20</td>
<td>33</td>
</tr>
<tr>
<td>PIN</td>
<td>51</td>
<td>3</td>
<td>48</td>
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<tr>
<td>2–4</td>
<td>9</td>
<td>3</td>
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<td>5–6</td>
<td>26</td>
<td>17</td>
<td>9</td>
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<td>7</td>
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<td>1</td>
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<tr>
<td>8–10</td>
<td>25</td>
<td>21</td>
<td>4</td>
</tr>
</tbody>
</table>

when 20–50% of cells stain (moderate), and 3+ when >50% of cells stain (strong).

**Imaging.** Digital images for photomicroscopy were acquired with a Cool Snap camera from Nikon. Minor adjustments in the captured images were performed identically and in parallel for the images presented using Adobe Photoshop 5.5. Composite images were made using Microsoft PowerPoint and printed on a Phaser 780 plus printer (Tetronics Co., Westborough, MA).

**Statistics.** For statistical analyses, groups scored 0 and 1+ were combined ("weak staining") as were groups scored 2+ and 3+ ("strong staining"). Statistical analysis was performed by using 2 analyses with Kappa and McNemar statistics in contingency tables for agreement and disagreement of specific comparisons (9). Normality of residuals was assessed for phospho-Akt and phospho-MAPK Erk levels each analyzed separately to assure valid analyses. The analyses were performed using a statistical analysis system on a PC-compatible computer with SAS 6.12 software (SAS Institute, Cary, NC).

**RESULTS**

**Increased Expression of Phospho-Akt (Ser473) in Paraffin-embedded Poorly Differentiated Prostate Cancer.**

The PI3K dependent serine threonine kinase Akt (also known as protein kinase B) has been implicated in mediating cell survival in various prostate cancer cells (2, 3, 10, 11). Therefore, we examined human prostate cancer tissues by IHC to determine whether the expression of the activated (phosphorylated) form of the cell survival protein Akt correlated with prostate cancer differentiation. Ninety percent of PIN, well to moderately differentiated adenocarcinomas, and Gleason score 7 carcinomas were either completely negative or showed weak staining (intensity score of 0 to 1+) for phospho-Akt (P ≤ 0.001; Fig. 1; Table 1). Phospho-Akt staining intensity progressed as the disease progressed with strongest staining observed in the highest Gleason scores. That is, >90% of poorly differentiated adenocarcinomas (Gleason score 8–10) exhibited strong staining for phospho-Akt (intensity score of 2 to 3+), (P ≤ 0.001; Fig. 1; Table 1). Interestingly, the staining appeared to be localized to the membrane where Akt has been shown to be active (4, 5). Total Akt levels were expressed in all of the tissues with no change in the degree of expression during disease progression.

**Decreased Expression of Phospho-MAPK/Erk1/2 in Paraffin-embedded Poorly Differentiated Prostate Cancer.**

In contrast to the PI3K/Akt signaling pathway, the MAPK signaling pathway is well recognized for mediating cell prolifera-
tion. As a measure of MAPK activation, we used an antibody that recognized phosphorylated MAPK/Erk1/2. More than 75% of normal, hyperplastic, and PIN displayed strong staining (2+ to 3+) for phospho-MAPK/Erk1/2 (Fig. 2; Table 2). Phospho-
MAPK/Erk1/2 staining was significantly greater in PIN versus
hyperplasia (P = 0.03) and normal (P = 0.001). The intensity of
staining decreased as the disease progressed to carcinoma,
with only 27% of the tumor cells showing strong staining for
phospho-MAPK/Erk1/2 (P < 0.0001). The weakest staining
was observed in poorly differentiated cancers (Fig. 2; Table 2).
The staining appeared to be localized to the nucleus. Total Erk
levels were expressed in all of the tissues with no change in the
degree of expression during disease progression.

DISCUSSION

Immunohistochemical examination of paraffin-embedded human prostate cancer showed that 92% of the poorly differentiated adenocarcinomas of the prostate stained strongly for phos-
pho-Akt in a membrane location. In other grades of prostate
cancer as well as in PIN, only 10% stained for phospho-Akt. On
the other hand, >75% of normal, hyperplastic, and PIN lesions
showed a high level of expression of phospho-MAPK/Erk1/2
that significantly decreased in adenocarcinoma.

This is the first report of the immunohistochemical detec-
tion of phospho-(active) Akt using a phospho-specific antibody in paraffin-embedded human prostate cancer. Similar to our
observations, Pawelcz et al. (11) showed by reverse-phase
protein microarrays that cancer progression was associated with
increased phosphorylation of Akt and suppression of apoptotic
pathways as measured using antibodies to cleaved caspase 7 and
poly(ADP-ribose) polymerase.

Advanced prostate cancer is often accompanied by andro-
gen independence, and growth of the tumor becomes dependent
on activation of cell survival pathways as well as cell prolif-
eration pathways. Graff et al. (10) showed that Akt activation
was markedly increased in an androgen-independent LNCaP cell
line that was isolated from LNCaP xenografts. In addition to
increased Akt activation, there was increased phosphorylation
and inactivation of the proapoptotic protein BAD, a target protein
of Akt, and decreased expression of the cyclin inhibitor,
p27Kip1 (10). These results would explain the emergence of an
antiapoptotic pathway in androgen-independent prostate cancer
as well as explain the enhanced proliferation observed in ad-
vanced prostate cancers. In human prostate cancer, the tumor suppressor phosphatase PTEN is mutated and inactive (2, 3, 10,
11). This phosphatase normally negatively regulates compo-
nents of the PI3K pathway such as the cell survival protein Akt.
Loss of PTEN activity is accompanied by increased expression of
the activated form of Akt and activation of cell survival pathways.
Similar to our findings by IHC, Pawelcz et al. (11)
demonstrated in protein microarrays that prostate tumor pro-
gression is accompanied by increased expression of phospho-
Akt. Importantly, this coincided with suppression of apoptosis.
Also similar to our findings, they showed that expression of
phospho-Erk was suppressed with progression of disease. These
findings are in contrast to the IHC studies by Gioeli et al. (12)
who showed increased expression of phospho-MAPK with in-
creasing Gleason score. Studies by Zimmerman and Moeling
(13) may explain our observations of high phospho-Akt expres-
sion accompanied by low levels of phospho-MAPK/Erk; namely,
they showed that phospho-Akt inactivates Raf by direct phos-
phorylation on Ser259, resulting in inhibition of the Raf-
MEK-Erk signaling pathway. In conclusion, we show by IHC
on paraffin-embedded tissue that progression of prostate cancer
is accompanied by increased levels of phospho-Akt and de-
creased levels of phospho-MAPK/Erk. Understanding the me-
chanisms of prostate tumor growth could prove critical to de-
veloping new effective therapies for prostate cancer.

REFERENCES

1. Denmeade, S. R., Xiaohui, S. L., and Isaacs, J. T. Role of pro-
grammed (apoptotic) cell death during the progression and therapy for
2. McMenamin, M. E., Soung, P., Perera, S., Kaplan, I., Loda, M., and
Sellers, R. Loss of PTEN expression in paraffin-embedded primary
prostate cancer correlates with high Gleason score and advanced stage.
3. Persad, S., Attwell, S., Gray, V., Delanchenne, M., Troussard, A.,
Sanghera, J., and Dedhar, S. Inhibition of integrin-linked kinase (ILK)
suppresses activation of protein kinase B/Akt and induces cell cycle
arrest and apoptosis of PTEN mutant prostate cancer cells. Proc Natl
4. Kandel, E. S., and Hay, N. The regulation and activities of the
multifunctional serine/threonine kinase Akt/PKB. Exp. Cell Res., 253:
5. Burgess, B. M. T., and Coffor, P. Protein kinase B (c-Akt) in
phosphatidylinositol 3-OH kinase signal transduction. Nature (Lond.),
6. Lowenstein, E. J., Daly, R. J., Batzer, A. G., Li, W., Margolis, B.,
Lammers, R., Ulreich, A., Skolnik, E. Y., and Bar-Sagi, D. The SH2 and
SH3 domains containing proteins GRB2 links receptor tyrosine kinases
7. Halberg, B., and Rayter, S. I., Downward, J. Interaction of Ras and
Raf in intact mammalian cells upon extracellular stimulation. J. Biol.
8. Allred, D. C., Clark, F. M., Elllidge, R., Fuqua, S. A. W., Brown,
R. W., Chambers, G. C., Osborne, A. K., and McGuire, W. L. Associa-
tion of p53 protein expression with tumor cell proliferation rate and
10. Graff, J. R., Konieck, B. W., McNulty, A. M., Wang, Z., Houck, K.,
Allen, S., Paul, J. D., Hbuau, A., Goode, R. G., Sandusky, G. E.,
Vessella, R. L., and Neubauer, B. L. Increased Akt activity contributes
to prostate cancer progression by dramatically accelerating prostate
zyme growth and diminishing p27kip1 expression. J. Biol. Chem., 273:
11. Pawelcz, C. P., Charboneau, L., Richel, V. E., Simone, N. L.,
Chen, T., Gillespie, J. W., Emmert-Buck, M. R., Roth, M. J., Petricoin,
E. F., Ii, and Liotta, L. A. Reverse phase protein microarrays which
capture disease progression show activation of pro-survival pathways at
12. Gioeli, D., Mandell, J. W., Petroni, G. R., Frierson, H. F., and
Weber, M. J. Activation of mitogen associated protein kinase associated
13. Zimmerman, S., and Moelling, K. Phosphorylation and regulation of
Raf by Akt (Protein kinase B). Science (Wash. DC), 286: 1741–1744,
1999.
Curriculum Vitae

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1967- City University of New York, Queens College
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III. Postgraduate Training:

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IV. Academic Appointments:

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Sep 1992-present Professor Department of Veterans Affairs
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Jul 1978-1980 Assistant Professor Department of Pathology, Harvard Medical
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Jul 1977-1978 Instructor Department of Pathology, Harvard Medical
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V. Administrative Responsibilities and Teaching:

A. Teaching

1980-1999 Lecturer, Cell Injury and Environmental Pathology, Sophomore Pathology Course, Medical School UTHSCSA, 3 hours/yr.
1980-1999 Laboratory Instructor, Sophomore Pathology Course, Medical School UTHSCSA, 30 hours/yr.
1980-1999 Lecturer, Cell Injury, University of Texas School of Pharmacy, Austin, Texas. 8 hours/yr.
1988-1999 **Course Master**, General Concepts of Pathology, Sophomore Pathology Course, Medical School, UTHSCSA
1998-1999 Lecturer, Cell Injury and Adaptation, Dental School, UTHSCSA, 3 hours/yr.
1998-1999 Lecturer, Cell Injury and Adaptation, Dental Hygiene, UTHSCSA, 3 hours/yr.
1998-1999 Lecturer, Cell Injury and Adaptation, Occupational Therapy, UTHSCSA, 3 hours/yr.

B. Grants

1980-pres Principal Investigator of grants from the National Institutes of Health and Department of Veterans Affairs totalling over $2,500,000 in direct costs (see pp 19-21).

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1981-1982 William Stewart, Ph.D.
1981-1983 Dorie Swertz, Ph.D.
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1988-1993 Suzanne H. Ayo, M.D.
1994-1995 Victoria Magnusson, Ph.D.
1996-pres Paramita Ghosh, Ph.D.
1997-1998 Nandini Ghosh-Choudhury, Ph.D.
1999 Magarita Mikhailova, Ph.D.
1999 Shazli Malik, M.D.

D. Administrative Courses Taken

VI. Professional Affiliations:

a) Current professional and scientific organizations and societies:

* indicates election or examination required for membership

1975 American Association for the Advancement of Science
*1978 American Society of Nephrology
*1978 American Association of Pathologists
*1980 The American Society for Cell Biology
1980 American Diabetes Association
1982 The Tissue Culture Association
*1985 International Society of Nephrology
1986 American Heart Association
   (Council on the Kidney in Cardiovascular Disease)
*1994 American Physiological Society
*1997 San Antonio Cancer Institute
*1998 American Association of Cancer Research

b) Past and current committees:

Department:

1999-2000 Tenure and Promotions Committee
1997-1998 Tenure and Promotions Committee
1988-1997 Seminars in Pathology, Chair
1988-1996 Education Committee, Member
1986-1987 Seminars in Pathology, Member

School:

2000-pres Selection subcommittee, Medical School
1999-2000 Post-Tenure Evaluation Committee, Department of Medicine
1996-1998 Interview Subcommittee, Medical School Admissions Committee
1997 Nominating Committee, Graduate Assembly
1995-1996 Finance Sub-Committee of the LCME Self Study Committee

University:

2001-2002 Faculty Senate
1999-1999 Ad hoc Committee to Review Tenure and Promotion Guidelines
1998-1999 Faculty Tenure and Promotions Committee, Chair
1996-1998 Faculty Tenure and Promotions Committee
1996-1998 Committee on Committees
1991-1992 Preclinical Promotions Committee, Chair
1990-1992 Preclinical Promotions Committee
1989-1990 Educational Resources Committee, Chair
1988-1990 Educational Resources Committee
1985-1986 Energy Conservations Committee, Member
1984-1985 Subcommittee on Physical Safety, Member

**Audie Murphy Veterans Administration Hospital:**
2001-pres Research and Development Committee
1996-1999 Research and Development Committee
1998-1999 Research and Development Committee, Chair
1998-1999 VISN 17 Research Committee

e) Other professional activities:

2001-pres Grass Roots Committee, American Heart Association, Texas Affiliate
2001-pres Grass Roots Committee, American Society of Nephrology
1998 Ad hoc Member, General Medicine B Study Section, National Institutes of Health
1995 Member, grant review committee. National Institutes of Health. (August 9).
1994-pres Member, Study Section, American Heart Association, Cardiorenal
1994 Abstract Reviewer, American Society of Nephrology
1994-pres Member, Study Section, Texas Affiliate, American Heart Association
1994 Member, pre-site visit team (University of Colorado School of Medicine, June 15-16).
1992 Member, National Institutes of Health site visit team (Washington University School of Medicine, December 16-18).
1992 Member, National Institutes of Health pre-site visit team (University of Colorado, July 9-10).
1991 Member, Special Study Section, National Institutes of Health, Chicago Illinois, October 15-17.
1990 Member, Department of Veteran's Affairs site visit team (San Francisco VA, September 10, 1990).
1990 Member, National Institutes of Health site visit team (New York Medical College, January 31-February 2, 1990).
1990 Member, National Institutes of Health Pathology A Study Section, Taos, New Mexico, February 28-March 5, 1990.
1988-1989 Member, 1989 Program Committee, The American Society of Nephrology
1988-1992 Member, Texas Affiliate, American Heart Association Central Research Review Committee
1987 Program Chairman, Tissue Cell Culture, American Society of Nephrology Meetings
1987-present  Member, External Review Committee, The University of Colorado Health Sciences Center (Dr. Robert W. Schrier)
1987  Member, Special Study Section, National Institutes of Health, February 26, 1987
1986-1991  Member, Outside Advisory Group, Cystic Fibrosis Core Center, Case Western University
1986-1991  Consulting for Cystic Fibrosis Core Center, Case Western University (Dr. Pam Davis)
1986  Member of the National Institutes of Health site visit team (University of Minnesota Medical School, August 27-28, 1986)
1985-1988  Member, Veterans Administration Merit Review Board, Nephrology
1985  Ad hoc member, National Institutes of Health Pathology A Study Section
1985  Member, Special Planning Committee, NIADDK, National
1984  Member of the National Institutes of Health site visit team (University of Minnesota Medical School, November 12-15, 1984)
1984  Member of the National Institutes of Health site visit team (Michael Reese Hospital, Chicago, IL, October 25-29, 1984)
1984  Member, Special Study Section, National Institutes of Health, Bethesda, MD, April
1983  Chairman, National Institutes of Health site visit team (Harvard Medical School, Boston, MA, July 27-29, 1983)
1982  Member, National Institutes of Health site visit team (St. Louis, MO, December 9-10)
1982  Member, National Institutes of Health site visit team (St. Louis, MO, September 8-10)
1979  Consultant in Pathology, Kidney Disease Institute, New York Department of Health, Albany, New York (Dr. Peter Burkholder)
1979  Consultant in Pathology, Yale University School of Medicine (Laboratory of Dr. Michael Kashgarian)

**Journal Reviewer**

- New England Journal of Medicine
- Anatomical Record
- American Journal of Anatomy
- Kidney International
- Journal of Clinical Investigation
- Laboratory Investigation
- American Journal of Physiology (member of Editorial Board)
- Hypertension
- In Vitro
- Diabetes
- American Journal of Pathology
- Journal of Laboratory and Clinical Investigation

**d) Community activities:**
1984-present Science lectures/arrangements for HSC field trips; elementary school health classes
1984-present Arrange Third Form St. Mary's Hall field trips to HSC

VII. Honors and Awards:

1969-1971 New York Regents Incentive Award
1971-1975 NIH Predoctoral Training Grant
1989-2003 Department of Veterans Affairs, Career Scientist Award

VIII. Bibliography:

Books and/or Book Chapters:


Papers published or in press

* indicates those papers that are refereed


23.*


28.*


33.*


Abstracts:


42. Troyer DA, Kreisberg JI and Glass WF: Release of urokinase-type plasminogen activator (u-PA) from mesangial (MS) cells treated with phospholipase C. American Society of Nephrology Annual Meetings, Nov. 17-20, 1991.


44. Kreisberg J, Radnik RA, Garoni J, Ayo S: Role of proteolytically activated protein kinase C in the stimulation of mRNA levels of extracellular matrix proteins by


47. Kreisberg JI, Radnik RA, Garoni J, and Ayo SH: Role of proteolytically activated protein kinase C in the stimulation of mRNA levels of extracellular matrix proteins by high glucose in mesangial cell cultures. JASN 4:491a, 1993.


Invited Reviews and Editorials:


IX. *Invited Lectures and Workshops:*


4. Lecture: *Properties of glomerular cells in culture*. Yale University School of Medicine, Department of Pathology, March 6, 1979.

5. Seminar: *Contractile and phagocytic cells in the glomerular mesangium*. Yale University School of Medicine, Department of Pathology, March 7, 1979.


13. Invited Lecture: Contractile properties of the glomerular mesangium. The Department of Physiolgy, University of Massachusetts Medical School, November 26, 1984.


15. Invited Lecture: Glomerular cells in culture. The University of Utah School of Medicine, February 10, 1986.


19. Invited Lecture: Biology of mesangial cell contraction. Department of Medicine, Case Western University, Cleveland, Ohio, November 12, 1986.


22. Invited Lecture: Synthetic atrial peptide (APII) fails to inhibit inositol trisphosphate (IP3) release and contraction induced by vasopressin (V) in cultured mesangial (MS) cells. American Society of Nephrology, December 7-10, 1986, Washington, D.C.


24. Invited Lecture: Biology of contractile mesangial cells. Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia, March 5, 1987.


27. Invited Lecture: The cell biology of cultured renal mesangial cells. Department of Pathology, Rhode Island Hospital, Brown University, Providence, Rhode Island, April 21, 1987.


33. Invited Lecture: Vasoactive agents that affect mesangial (MS) cell adhesion and shape change alter plasminogen activators (PA) located in cellular adhesion


43. Invited Lecture: The cell biology and adhesion of cultured mesangial cells. The Finsen Institute, Copenhagen, Denmark, October 10, 1989.


53. Invited lecturer: Mechanisms of mesangial cell shape change and adhesion loss following cAMP elevation. Department of Medical Physiology, Texas A and M University Health Sciences Center, College Station, TX. February 22, 1995.


X. Current Projects

(1) Biomarkers for poor clinical outcome in prostate cancer

(2) Mechanisms by which RhoA affect prostate tumor cell growth.

XI. Research Support

NATIONAL

National Institutes of Health
Pathobiology of Diabetic Glomerulosclerosis
DK 29787-09
08/01/81 thru 12/31/96
$1,500,000 direct costs
Principal Investigator

National Institutes of Health
Pathobiology of Occlusive Vascular Disease
2T32HL07446-16
1996-2001
$523,600 direct costs
Preceptor

National Institutes of Health
“Metastatic potential of colorectal carcinoma-APRC Supplement”
Type: Supplement Period: 2000-2002
$54,000
Principal Investigator
National Institutes of Health
“Biological evaluation of CCI-779 in brain tumors”
Type: Quick Trials for Cancer Therapies
06/02-06/04
$160,000 direct costs
Co-investigator

National Institutes of Health
“Biological evaluation of ZD1839 in colon cancer”
Type: Quick Trials for Cancer Therapies
06/02-06/04
$160,000 direct costs
Co-investigator

National Institutes of Health
“ErbB1 and ErbB2 Blockade in Advanced Breast Cancer”
Type: Quick Trials for Cancer Therapies
10/02-9/04
$250,000 direct costs
Co-investigator

Kronos Foundation
“Modulation of age related changes in plasma membrane signal transduction by dietary fatty acids”
04/01-03/03
$250,000
Co-investigator

Veterans Administration
Career Scientist Award
04/01/89 thru 03/31/03
$1,000,000
Principal Investigator

American Diabetes Association
Mechanisms by which High Glucose Increases Fibronectin Transcription
01/01/96 thru 12/31/97
$100,000
Principal Investigator

Veteran's Administration-Merit Review
Pathobiology of Diabetic Glomerulosclerosis
04/01/96 thru 03/31/01
$500,000 direct costs
Principal Investigator

Veteran’s Administration-Shared Equipment Grant
Confocal Microscope
1998
$240,000
Principal Investigator
Pending

Veteran's Administration
"RhoA dependent and independent pathways of prostate cancer growth"
04/03-03/08
$750,000 direct costs
Principal Investigator

LOCAL

San Antonio Cancer Institute
Efficacy of Protein Prenylation Inhibitors on Apoptotic Cell Death in Adenocarcinoma of the Prostate
02/01/97 thru 01/31/98
$20,000

Fellows' Support:

William Glass II, M.D., Ph.D.:

National Kidney Foundation Fellowship
Molecular basis of mesangial cell adhesion
Dr. Jeffrey Kreisberg, Mentor
July 1, 1987 through June 30, 1988
$19,000

Veterans Administration
Research Advisory Group Summary Statement
Dynamics of extracellular matrix structure in mesangial cell function
Principal Investigator
Dr. Jeffrey Kreisberg, Mentor
July 1, 1990 through June 30, 1992
$60,842

American Heart Association, Texas Affiliate, Inc.
Grant-in-Aid Award
Nonenzymatic glycosylation and mesangial cell matrix accumulation
Principal Investigator
Dr. Jeffrey Kreisberg, Mentor
July 1, 1989 through June 30, 1991
$55,000
National Institutes of Health
First Award
Alteration of mesangial cell function by thrombin
Principal Investigator
Dr. Jeffrey Kreisberg, Mentor
July 1, 1991 through June 30, 1996
$331,124

Suzanne H. Ayo, M.D.

National Kidney Foundation Fellowship
Mechanisms of increased mesangial matrix deposition in diabetic nephropathy
Dr. Jeffrey Kreisberg, Mentor
July 1, 1989 to June 30, 1990
$21,000

American Heart Association, Texas Affiliate, Inc.
Grant-In-Aid Award
Molecular mechanism of extracellular matrix accumulation in mesangial cells
Principal Investigator
Dr. Jeffrey Kreisberg, Mentor
July 1, 1993 to June 30, 1995
$83,600

Nandini Ghosh-Choudhury, Ph.D.

Institutional Grant
Molecular mechanisms of breast cancer cell growth
Principal Investigator
Dr. Jeffrey Kreisberg, Mentor
June 1, 1998 to May 31, 1999
$15,000.