PROSTATE CANCER PROGNOSTIC MARKERS

Terrence P McGarty
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by

Terrence P McGarty

<u>NOTICE</u>

Contents

1   INTRODUCTION

We have examined many studies looking at genetic prognostic markers from a causative basis. Namely we look at genes in specific pathways which are altered result in malignant conditions for cell growth and proliferation. In this note we look at a recent article examining blood borne proteins which have some putative prognostic value.

Both reports we discuss herein are prognostic in their approach. They are prognostic, however, for androgen resistant PCa. Although it is always good to understand what the prognosis is, even if you cannot do anything about it, it does raise the concern of what benefit is this to either the physician or patient. The results seem to say that the prognosis is that one has 9-10 months versus 3-4 years of expected life. There is nothing that can be done, and even care of the patient is in question. No matter what it is palliative. Although the results are interesting the question is are they beneficial, to anyone. One may ask why waste the money to find out something that you can do nothing about. That is both an ethical and an economic issue.

A summary reported in the press states[1]:

The first study, demonstrating that a nine-gene signature could distinguish between lower and higher risk castration-resistant prostate cancer (CRPC), was led by Johann de Bono, MD, of the drug development unit at the Royal Marsden NHS Foundation Trust in Sutton, United Kingdom, and was conducted with colleagues in both Europe and the United States.

The second study, which found a six-gene signature that also stratified CRPC into different risk groups, was led by William K. Oh, MD, of the division of hematology/oncology at the Tisch Cancer Institute at Mount Sinai School of Medicine in New York.

One of the findings was related to immune system genes not those normally thought of in pathway control. The authors state[2]:

The result that immune function is key to prostate cancer outcome is very surprising, said Dr. de Bono.

"The biggest surprise of this study was that the most significant six genes which predicted survival were not primarily cancer-related genes, but were involved in immune function," said Dr. Oh. "In some ways, this is not a surprise, since it suggests that the patient's innate immune response to cancer may be a strong predictor of the impact of the cancer." Dr. Oh added that the function of the identified genes in the immune system is not yet understood. Nor is it understood how the genes may interact and lead to a difference in survival for patients.

Both authors of the studies see the RNA analysis as highly applicable for the clinic. Dr. Oh said that the particular six-gene signature his study identified "could be translated fairly easily to the

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[1] http://www.cancernetwork.com/prostate-cancer/content/article/10165/2106848

[2] http://www.cancernetwork.com/prostate-cancer/content/article/10165/2106848

clinic, since it uses simple technologies such as PCR to identify the genes of interest." Dr. Oh and colleagues collected the RNA in blood using a special preservation tube (PAXgene), which are widely available. Dr. de Bono and colleagues are currently testing whether a DNA analysis could provide the same information.

Dr. Oh highlighted the different approaches of the two teams: "What is interesting about the Royal Marsden paper is that they took a very different analytic approach, which in fact looked at more genes and was thus potentially more unbiased, and found that the most prognostic genes were again driving immune function in patients." Both teams ended up with a similar result: "The blood contains a molecular signature in patients with advanced prostate cancer which predicts survival based on the functioning of the immune system."

Now it must be emphasized that these studies examined prognostic factors and not diagnostic and that further they examined patients who were already androgen resistant, namely the PCa had progressed extensively. Thus the implication of immune system elements is not unexpected. Also this analysis is not diagnostic in any way and further is not prognostic in any manner related to a watchful waiting strategy. As the authors suggest survival in his risk is about 8 months and in "low" risk is about 35 months. In either case the patient is terminal.

2   RECENT FINDING

There are two recent papers regarding this issue. The first is a recent Lancet article by Ross et al, entitled, *A whole-blood RNA transcript-based prognostic model in men with castration-resistant prostate cancer: a prospective study*[3], the authors state:

Survival for patients with castration-resistant prostate cancer is highly variable. We assessed the effectiveness of a whole-blood RNA transcript-based model as a prognostic biomarker in castration-resistant prostate cancer. Peripheral blood was prospectively collected from 62 men with castration-resistant prostate cancer on various treatment regimens …

A six-gene model (consisting of ABL2, SEMA4D, ITGAL, and C1QA, TIMP1, CDKN1A) separated patients with castration-resistant prostate cancer into two risk groups: a low-risk group with a median survival of more than 34·9 months (median survival was not reached) and a high-risk group with a median survival of 7·8 months (95% CI 1·8—13·9; p<0·0001). The prognostic utility of the six-gene model was validated in an independent cohort. This model was associated with a significantly higher area under the curve compared with a clinicopathological model (0·90 [95% CI 0·78—0·96] vs 0·65 [0·52—0·78]; p=0·0067).

Transcriptional profiling of whole blood yields crucial prognostic information about men with castration-resistant prostate cancer. The six-gene model suggests possible dysregulation of the immune system, a finding that warrants further study.

We wish to examine this in some detail. There are several issues we wish to look at.

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[3] http://www.thelancet.com/journals/lanonc/article/PIIS1470-2045%2812%2970263-2/fulltext?_eventId=login

First, what pathways do these genes participate in and thus how do they play a role in the management of the homeostasis of the cell. Why would one want to consider these genes?

Second, are these genes causative or reflective of a cancer state? If reflective are there causative genes related thereto which may merit more detailed examination.

Third from a prognostic perspective, why are these expressed as they are?

Fourth from a treatment perspective are these markers useful in targeting gene aberrations so as to mitigate further uncontrolled growth and in fact reduce what is present.

Fifth, is there a holistic picture of how most likely metastatic growth is identified by such expression and how one may ascertain the spread of the metastatic cells?

There is also a second paper entitled, *Prognostic value of blood mRNA expression signatures in castration-resistant prostate cancer: a prospective, two-stage study* by Olmos et al which notes[4]:

Biomarkers are urgently needed to dissect the heterogeneity of prostate cancer between patients to improve treatment and accelerate drug development. We analysed blood mRNA expression arrays to identify patients with metastatic castration-resistant prostate cancer with poorer outcome.

Whole blood was collected into PAXgene tubes from patients with castration-resistant prostate cancer and patients with prostate cancer selected for active surveillance. In stage I (derivation set), patients with castration-resistant prostate cancer were used as cases and patients under active surveillance were used as controls. These patients were recruited from The Royal Marsden Hospital NHS Foundation Trust (Sutton, UK) and The Beatson West of Scotland Cancer Centre (Glasgow, UK).

In stage II (validation-set), patients with castration-resistant prostate cancer recruited from the Memorial Sloan-Kettering Cancer Center (New York, USA) were assessed. Whole-blood RNA was hybridised to Affymetrix U133plus2 microarrays. Expression profiles were analysed with Bayesian latent process decomposition (LPD) to identify RNA expression profiles associated with castration-resistant prostate cancer subgroups; these profiles were then confirmed by quantative reverse transcriptase (qRT) PCR studies and correlated with overall survival in both the test-set and validation-set.

LPD analyses of the mRNA expression data divided the evaluable patients in stage I (n=94) into four groups. All patients in LPD1 (14 of 14) and most in LPD2 (17 of 18) had castration-resistant prostate cancer. Patients with castration-resistant prostate cancer and those under active surveillance comprised LPD3 (15 of 31 castration-resistant prostate cancer) and LDP4 (12 of 21 castration-resistant prostate cancer).

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[4] http://www.thelancet.com/journals/lanonc/article/PIIS1470-2045%2812%2970372-8/fulltext

Patients with castration-resistant prostate cancer in the LPD1 subgroup had features associated with worse prognosis and poorer overall survival than patients with castration-resistant prostate cancer in other LPD subgroups (LPD1 overall survival 10·7 months [95% CI 4·1—17·2] vs non-LPD1 25·6 months [18·0—33·4]; p<0·0001).

A nine-gene signature verified by qRT-PCR classified patients into this LPD1 subgroup with a very low percentage of misclassification (1·2%). The ten patients who were initially unclassifiable by the LPD analyses were subclassified by this signature. We confirmed the prognostic utility of this nine-gene signature in the validation castration-resistant prostate cancer cohort, where LPD1 membership was also associated with worse overall survival (LPD1 9·2 months [95% CI 2·1—16·4] vs non-LPD1 21·6 months [7·5—35·6]; p=0·001), and remained an independent prognostic factor in multivariable analyses for both cohorts.

Our results suggest that whole-blood gene profiling could identify gene-expression signatures that stratify patients with castration-resistant prostate cancer into distinct prognostic groups.

3 SUMMARY OF PROGNOSTIC GENE MARKERS

The following Table is a summary of the prognostic gene markers.

Gene	Description[5]	Location
ABL2	This gene encodes a member of the Abelson family of nonreceptor tyrosine protein kinases. The protein is highly similar to the c-abl oncogene 1 protein, including the tyrosine kinase, SH2 and SH3 domains, and it plays a role in cytoskeletal rearrangements through its C-terminal F-actin- and microtubule-binding sequences. This gene is expressed in both normal and tumor cells, and is involved in translocation with the ets variant 6 gene in leukemia. Multiple alternatively spliced transcript variants encoding different protein isoforms have been found for this gene.	1q25.2
SEMA4D	CD100; SEMAJ; coll-4; C9orf164; M-sema-G. Semaphorin 4D (Sema 4D) is an axon guidance molecule which is secreted by oligodendrocytes and induces growth cone collapse in the central nervous system. By binding plexin B1 receptor it functions as an R-Ras GTPase-activating protein (GAP) and repels axon growth cones in both the mature central nervous system. In the immune system, CD100 binds CD72 to activate B cells and dendritic cells, though much about this interaction is still under investigation. During skin damage repairs, SEMA4D interacts with Plexin B2 on gamma delta T cells to play a role in the healing process	9q22.2
ITGAL	ITGAL encodes the integrin alpha L chain. Integrins are heterodimeric integral membrane proteins composed of an alpha chain and a beta chain. This I-domain containing alpha integrin combines with the beta 2 chain (ITGB2) to form the integrin lymphocyte function-associated antigen-1 (LFA-1), which is expressed on all leukocytes. LFA-1 plays a central role in leukocyte intercellular adhesion through interactions with its ligands, ICAMs 1-3 (intercellular adhesion molecules 1 through 3), and also functions in lymphocyte costimulatory signaling. Two transcript variants encoding different isoforms have been found for this gene.	16p11.2
C1QA	This gene encodes a major constituent of the human complement subcomponent C1q. C1q associates with C1r and C1s in order to yield the first component of the serum complement system. Deficiency of C1q has been associated with lupus erythematosus and glomerulonephritis. C1q is composed of 18 polypeptide chains: six A-chains, six B-chains, and six C-chains. Each chain contains a collagen-like region located near the N terminus and a C-terminal globular region. The A-, B-, and C-chains are arranged in the order A-C-B on chromosome 1. This gene encodes the A-chain polypeptide of human complement subcomponent C1q.	1p36.12

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[5] http://www.ncbi.nlm.nih.gov/gene

Gene	Description[5]	Location
TIMP1	This gene belongs to the TIMP gene family. The proteins encoded by this gene family are natural inhibitors of the matrix metalloproteinases (MMPs), a group of peptidases involved in degradation of the extracellular matrix. In addition to its inhibitory role against most of the known MMPs, the encoded protein is able to promote cell proliferation in a wide range of cell types, and may also have an anti-apoptotic function. Transcription of this gene is highly inducible in response to many cytokines and hormones. In addition, the expression from some but not all inactive X chromosomes suggests that this gene inactivation is polymorphic in human females. This gene is located within intron 6 of the synapsin I gene and is transcribed in the opposite direction.	Xp11.3
CDKN1A	This gene encodes a potent cyclin-dependent kinase inhibitor. The encoded protein binds to and inhibits the activity of cyclin-CDK2 or -CDK4 complexes, and thus functions as a regulator of cell cycle progression at G1. The expression of this gene is tightly controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli. This protein can interact with proliferating cell nuclear antigen (PCNA), a DNA polymerase accessory factor, and plays a regulatory role in S phase DNA replication and DNA damage repair. This protein was reported to be specifically cleaved by CASP3-like caspases, which thus leads to a dramatic activation of CDK2, and may be instrumental in the execution of apoptosis following caspase activation. Multiple alternatively spliced variants have been found for this gene.	6q21.2

4 TIMP-1

TIMP-1 is a tissue inhibitor of metalloproteinases. Metalloproteinases (matrix metalloproteinases, MMP) are zinc dependent proteases which have the ability to cleave cell walls[6]. Transcription of this gene and thus increase in its product are activated by cytokines and various hormones. However, in this analysis, it is most likely the excitation from the immune system cytokines which activate the response.

There has been extensive work performed analyzing TIMP-1 recently in various other cancers. The work of Wang et al examines Gastric cancers, Lee examines Colorectal cancers, and Bloomston looks at pancreatic cancers. Other detailed analyses have been done by Vaghooti et al as well as Wang. Thus is should be no surprise as to the use of TIMP-1 in this specific case as well.

In addition as per Marks et al[7], The TIMP, tissue inhibitors of metalloproteases, MMP, are within the class of ADAM proteins which are membrane bound.

The following is a summary by Bigelow et al and although it focuses on breast cancer issues it does provide a reasonable summary as applied to this case:

TIMP-1 (Tissue inhibitor of matrix metalloproteinase-1) is typically associated with inhibition of matrix metalloproteinases (MMP) induced invasion. However, TIMP-1 is overexpressed in many malignancies and is associated with poor prognosis in breast cancer.

The mechanisms by which TIMP-1 promotes tumorigenesis are unclear. Reduced levels of TIMP-1 mediated by shRNA in MDA-MB-231 breast cancer cells had no effect on cellular physiology in vitro or tumor growth in SCID mice compared to vector control MDA-MB-231 cells.

However, overexpression of TIMP-1 in MDA-MB-231 cells resulted in inhibition of cell invasion and enhanced phosphorylation of p38 MAPK and AKT in vitro. Additionally, treatment of parental MDA-MB-231 cells with purified TIMP-1 protein led to activation of p38 MAPK and MKK 3/6. cDNA array analysis demonstrated that high expression of TIMP-1 in MDA-MB-231 cells resulted in alterations in expression of approximately 200 genes, 1.5 fold or greater compared to vector control cells (P < 0.1).

Real-time RT-PCR confirmed changes in expression of several genes associated with cancer progression including DAPK1, FGFR4 and MAPK13.

In vivo, high TIMP-1 expression induced tumor growth in SCID mice compared to vector control cells and increased tumor vessel density. Affymetrix array analysis of vector control and TIMP-1

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[6] For example the use of doxycycline as a suppressor of MMP at low doses is used to treat corneal abrasions and certain types of dental erosions.

[7] See Marks et al, pp. 455-459.

MDA-MB-231 xenograft tumors revealed that TIMP-1 altered expression of approximately 600 genes in vivo, including MMP1, MMP13, S100A14, S100P, Rab25 and ID4.

These combined observations suggest that the effects of TIMP-1 differ significantly in a 2-D environment compared to the 3-D environment and that TIMP-1 stimulates tumor growth.[8]

Thus we have the question that TIMP-1 at an inhibitor of MMP is thus increased in response to cytokines which may themselves be increased as a result of the PCa metastatic expansion. The question then becomes; is this just a natural and expected result, is this just consistent with PCa evolution, or is there something special here.

5   ABL2

BCR and ABL are genes closely related to CML. In a 2002 paper in NEJM by Katarjian et al we have:

Chronic myelogenous leukemia (CML) accounts for about 20 percent of newly diagnosed cases of leukemia in adults. The course of the disease is characteristically triphasic: a chronic phase lasting three to six years is followed by transformation to accelerated and then blast phases of short duration. The cause of CML is the translocation of regions of the BCR and ABL genes to form a BCR-ABL fusion gene. In at least 90 percent of cases, this event is a reciprocal translocation termed t(9;22), which forms the Philadelphia (Ph) chromosome. The product of the BCR-ABL gene, the BCR-ABL protein, is a constitutively active protein tyrosine kinase with an important role in the regulation of cell growth.

Thus this fusion product has been found to result in a cancerous growth of the immune system. ABL2 is a product which is a tyrosine kinase resident in the cytoplasm.

Considerable work has been done on ABL and reference is made to that of Wong and Witte as well as O'Hare. Also there is the recent work of Sirvent et al examining Abl in normal and cancer cells.

In the work by O'Hare et al the authors note:

The BCR-ABL signaling network and ABL kinase inhibition.

A, BCR-ABL signaling pathways activated in CML. Dimerization of BCR-ABL triggers autophosphorylation events that activate the kinase and generate docking sites for intermediary adapter proteins such as GRB2. BCR-ABL─ dependent signaling facilitates activation of multiple downstream pathways that enforce enhanced survival, inhibition of apoptosis, and perturbation of cell adhesion and migration.

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[8] TIMP-1 overexpression promotes tumorigenesis of MDA-MB-231 breast cancer cells and alters expression of a subset of cancer promoting genes in vivo distinct from those observed in vitro, Rebecca L. H. Bigelow, Briana J. Williams, Jennifer L. Carroll, Lisa K. Daves and James A. Cardelli, Breast Cancer Research and Treatment Volume 117, Number 1 (2009), 31-44, DOI: 10.1007/s10549-008-0170-7. http://www.springerlink.com/content/a61k12012441l672/

A subset of these pathways and their constituent transcription factors, serine/threonine-specific kinases, and apoptosis related proteins are shown. A few pathways that were more recently implicated in CML stem cell maintenance and BCR-ABL–mediated disease transformation are shown.

Of note, this is a simplified diagram and many more associations between BCR-ABL and signaling proteins have been reported. BCR-ABL is unstable upon disruption of primary CML cells; therefore, pharmacodynamic evaluation of BCR-ABL activity is performed by monitoring the tyrosine phosphorylation status of either CRKL or STAT5, with CRKL phosphorylation considered the most specific readout.

B, Predicted effectiveness of ABL kinase inhibitors in three therapeutic scenarios: to inhibit native BCR-ABL , to inhibit mutated BCR-ABL, and as a component in the control of CML involving a BCR-ABL–independent alternate lesion[9].

Now ABL by itself has certain control mechanisms. They are well known and reviewed extensively, refer to Wong and Witte.

6 SEMA4D

SEMA4D is also known as CD100. The CD or cluster of determination molecules often are receptors and frequently found on immune system sourced cells. CD100 specifically is characterized as one of Mono migration; with T and B activation; T cell-B cell and T cell-DC interaction. Thus SEMA4D is another immune cell related marker and not one of internal pathway control.

From the work of Gelfand et al we have:[10]

(a) Sema4D signaling in the nervous system. Proteins in the R-Ras pathway are shown in red: in the presence of Sema4D, Rnd1 is recruited to Plexin-B1. Plexin-B1 R-RasGAP activity is activated and downregulates the active form of R-Ras. The decrease of active R-Ras inhibits PI3K–Akt activity, decreasing GSK3β phosphorylation and, thus, activating it. GSK3β then phosphorylates and deactivates CRMP2 and causes microtubule disassembly. Proteins in the RhoA pathway are shown in blue: in the presence of Sema4D, receptor tyrosine kinase ErbB2 binds and subsequently phosphorylates Plexin-B1 (as indicated by the double-headed arrow) and then activates PDZ–RhoGEF and LARG, which associate with Plexin-B1. PDZ–RhoGEF and LARG activate RhoA, causing actin depolymerization through ROCK. Proteins in the Rac1 pathway are shown in green. Upon Sema4D binding, activated Plexin-B1 competes for active Rac1 with PAK. The shift in the equilibrium between Plexin-B1- and PAK-bound Rac1 results in decrease of PAK activity, LIMK activity and Cofilin phosphorylation, thus, causing actin depolymerization. So far, this pathway has only been shown in heterologous cells, as indicated

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9 http://clincancerres.aacrjournals.org/content/17/2/212.full.pdf+html

10 http://www.sciencedirect.com/science/article/pii/S096289240900018X

by the dashed box. Both the actin depolymerization and microtubule disassembly lead to axon growth-cone collapse.

*(**b**) Sema4D signaling in the vascular system. Proteins in the RhoA pathway are shown in blue: in the presence of Sema4D, the receptor tyrosine kinase Met binds and phosphorylates Plexin-B1 (as indicated by the double-headed arrow) and then activates PDZ–RhoGEF and LARG, which activates RhoA and leads to endothelial cell migration through the ROCK, Pyk2 and PI3K pathway. It is not clear how this pathway affects actin dynamics or microtubule dynamics in vascular system.*

*(**c**) Sema3A signaling in the nervous system. Rac1-regulating proteins are shown in green: in the presence of Sema3A, FARP2 is released from Plexin-A1 and actives Rac1. Rac1 then activates PAK and LIMK and, as a result, phosphorylates Cofilin, which finally causes actin depolymerization. R-Ras-regulating proteins are shown in red: in the presence of Sema3A, Rac1 facilitates Rnd1 recruitment to Plexin-A1, which induces the R-RasGAP activity of Plexin-A1 and downregulates active R-Ras. A decrease in active R-Ras downregulates PI3K–AKT activity and leads to axon growth-cone collapse through three different pathways: reduced phosphorylation of GSK3β, reduced phosphorylation of ERM and activation of myosin II. Kinases are shown in blue: in the presence of Sema3A, FARP2 inhibits PIPKIγ661 and suppresses integrin-induced adhesion. Fer and Fes are activated upon Sema3A binding to Plexin-A1 and phosphorylate and inactivate CRMP2, which leads to microtubule disassembly. Fyn is also activated after its binding to Plexin-A1 and inactivates CRMP2 by phosphorylating and activating Cdk5. Both actin depolymerization and microtubule disassembly lead to axon growth-cone collapse.*

*(**d**) Sema3A signaling in the vascular system. Sema3A, through an unknown mechanism (possibly through Npn-1 and/or a co-receptor, shown as a dashed line and '?'), inhibits VEGF-induced activation of Src and FAK and contributes to angiogenesis. Sema3A might also function through Npn-1 to inhibit integrin-mediated adhesion of endothelial cells to the ECM. Sema3A can induce VE-cadherin phosphorylation and causes vascular permeability through unknown mechanisms (indicated by '?'), in which PI3K–Akt is involved.*

In the work of Neufeld and Kessler we have:

The main signal transduction pathways by which SEMA3A and SEMA4D activate plexin A1 (PLEXA1) or PLEXB1…[11]. The information is derived mainly from the study of neuronal cells. The activation of PLEXA1 by SEMA3A (left side) or PLEXB1 by SEMA4D (right side) induces activation and sequestration of RAC1 and RND1 by the plexins.

Sequestration of RAC1 results in reduced phosphorylation of p21-activated kinase 1 (PAK), inhibition of LIM domain kinase 1 (LIMK1) activity and activation of cofilin, which causes actin depolymerization.

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[11] http://www.nature.com/nrc/journal/v8/n8/fig_tab/nrc2404_F5.html

Activation of PLEXA1 by SEMA3A also results in the activation of the tyrosine kinases FYN, FES and FER, which is followed by the recruitment and activation of cyclin-dependent kinase 5 (CDK5), which in turn inactivates collapsin response mediator proteins (CRMPs) such as CRMP2. CRMPs affect microtubule dynamics and the organization of the actin cytoskeleton.

The activation of PLEXA1 also leads to the activation of MICALs (molecules interacting with CasL), which form complexes with CRMPs and are also essential for the effects of SEMA3A on the cytoskeleton.

In the case of SEMA4D, activation of PLEXB1 can also lead to the inactivation of CRMPs through inhibition of phosphoinositide 3-kinase (PI3K) and AKT activation that leads to GSK3β activation and as a result to the inactivation of CRMPs. The activation of PLEXA1 and PLEXB1 by their respective semaphorins also activates the p190^{RHOGAP} enzyme, which inactivates RHOA and thus contributes to the activation of cofilin by Rho-associated coiled-coil-containing kinase (ROCK) and LIMK1, thereby promoting cofilin activation and actin fibre disassembly.

In contrast with PLEXA1, activated PLEXB1 can also induce the activation of the guanyl nucleotide exchange factors (GEFs) LARG and RHOGEF, thereby counteracting the activity of p190^{RHOGAP}, promoting activation of RHOA and ROCK and leading to increased actin polymerization.

In addition to these short-term effects there are also long-term effects. In the case of SEMA3A, activation of PLEXA1 induces apoptosis of neuronal and endothelial cells, which is manifested by inhibition of extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2) phosphorylation and activation of caspase 3 (indicated in purple). The insert shows the effects of SEMA3A on the actin cytoskeleton of endothelial cells.

We depict below a modified version of their pathway description.
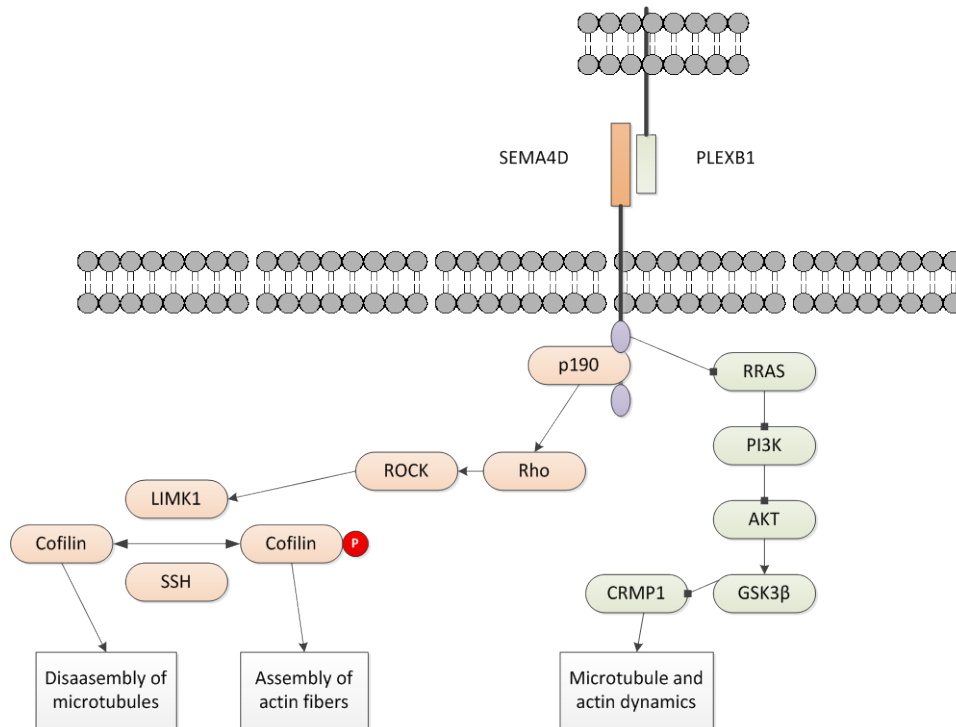
Also below we have from the work of Siderovski and Willard the following discussion of pathway involvement[12]:

Membrane targeting strategies employed by multi-domain RGS proteins.

*(**A**) The R7 RGS proteins form obligate heterodimers with Gβ5 via a Gγ-like sequence (the "GGL" domain) N-terminal to the RGS-box. This GGL/Gβ5 interaction could allow R7 RGS proteins to act as conventional Gβγ subunits in coupling Gα subunits to 7TM receptors, thereby localizing RGS-box-mediated GAP activity to particular receptors. The DEP domain of RGS9-1 interacts with a membrane-anchoring protein (R9AP) analogous interactors may exist for the DEP domains of other R7 subfamily members.*

*(**B**) The PDZ domain of RGS12 is able to bind the C-terminus of the IL-8 receptor CXCR2 (at least in vitro). The RGS12 PTB domain binds the synprint ("synaptic protein interaction") region of the N-type calcium channel ($Ca_v2.2$); this interaction is dependent on neurotransmitter-mediated phosphorylation of the channel by Src.*

*(**C**) The AtRGS1 protein of Arabidopsis thaliana (thale cress) has a unique structure for an RGS protein: an N-terminus resembling a 7TM receptor and a C-terminal RGS-box. Although a ligand is not known for the 7TM portion of AtRGS1, a simple sugar is most likely.*

*(**D**) The transmembrane receptor Plexin-B1 couples binding of the membrane-bound semaphorin Sema4D to RhoA activation via an interaction with the PDZ domain of PDZ-RhoGEF (and of the related RGS-RhoGEF LARG). Domain abbreviations: IPT, immunoglobulin-like fold found in*

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[12] http://www.biolsci.org/v01p0051.pdf

plexins, Met and Ron tyrosine kinase receptors, and intracellular transcription factors; PSI, domain found in plexins, semaphorins, and integrins; Sema, semaphorin domain.

The pathway involvement is similar to what we have depicted above.

7   ITGAL

ITGAL is integrin alpha L and is also known as CD11, another CD protein and thus another immune response marker and not a pathway marker.

From the KEGG database we have the following additional information[13]:

Gene name	ITGAL, CD11A, LFA-1, LFA1A	
Definition	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)	
Orthology	K05718 integrin alpha L	
Organism	hsa Homo sapiens (human)	
Pathway	hsa04514	Cell adhesion molecules (CAMs)
	hsa04650	Natural killer cell mediated cytotoxicity
	hsa04670	Leukocyte transendothelial migration
	hsa04810	Regulation of actin cytoskeleton
	hsa05144	Malaria
	hsa05150	Staphylococcus aureus infection
	hsa05166	HTLV-I infection
	hsa05169	Epstein-Barr virus infection
	hsa05323	Rheumatoid arthritis
	hsa05416	Viral myocarditis

From KEGG we have the following pathway[14]:

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[13] http://www.genome.jp/dbget-bin/www_bget?hsa:3683

[14] http://www.genome.jp/kegg-bin/show_pathway?hsa04650+3683
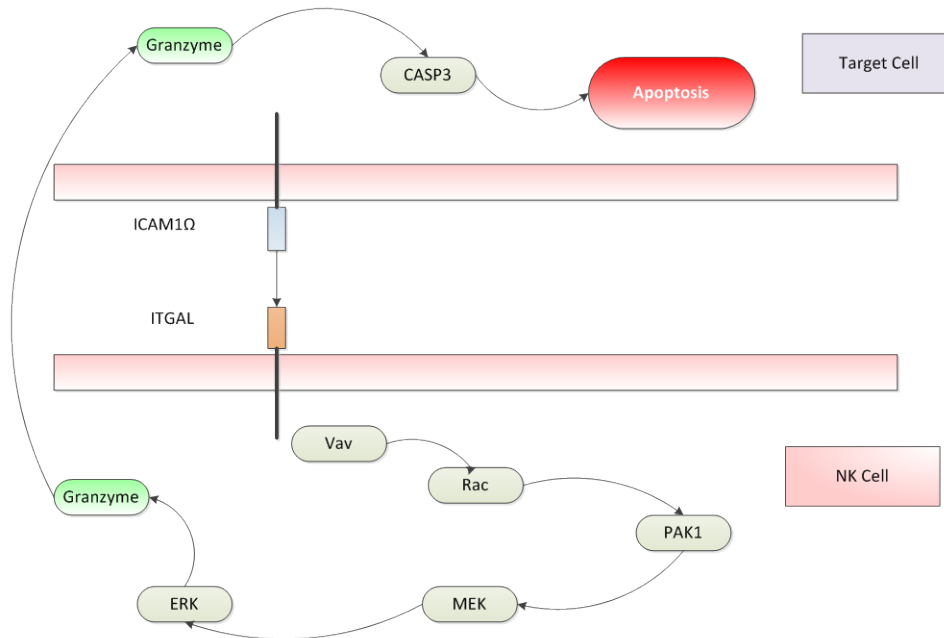
Note the connection between the target cell and the NK or Natural Killer sell from the immune system. ITGAL facilitates the apoptosis of the cell. If ITGAL is defective then we have a loss of natural apoptosis.

This then is another step in the immune system failing to manage the cell status.

8   CDKN1A

CDKN1A is controlled by SAD4. SMAD4 is an element in the TGF-β signalling chain. TGF is a cytokine, specifically a transforming growth factor cytokine. Like the Wnt-Apc pathway, the TGF pathway links defective development to cancer. The pathway is shown in part below (from Bunz p 199). Normal TGF signalling down-regulates the growth of most normal cells. Several of the genes in the TGF/SMAD pathway activation suppress growth. Specifically the genes CDKN1A and CDKN2B encode the cyclin dependent kinase inhibitors which suppress growth. Activated SMAD pathways also appear to suppress the transcription of other genes including c-Myc.

Kibel et al have recently examined CDKN1A and CDKN1B specifically in prostate cancers with extensive insight.

We show some of the TGF SMAD signalling below along with its control over the CDKN1A element. We will elaborate this later. Note here that CDKN1A controls apoptosis as well.
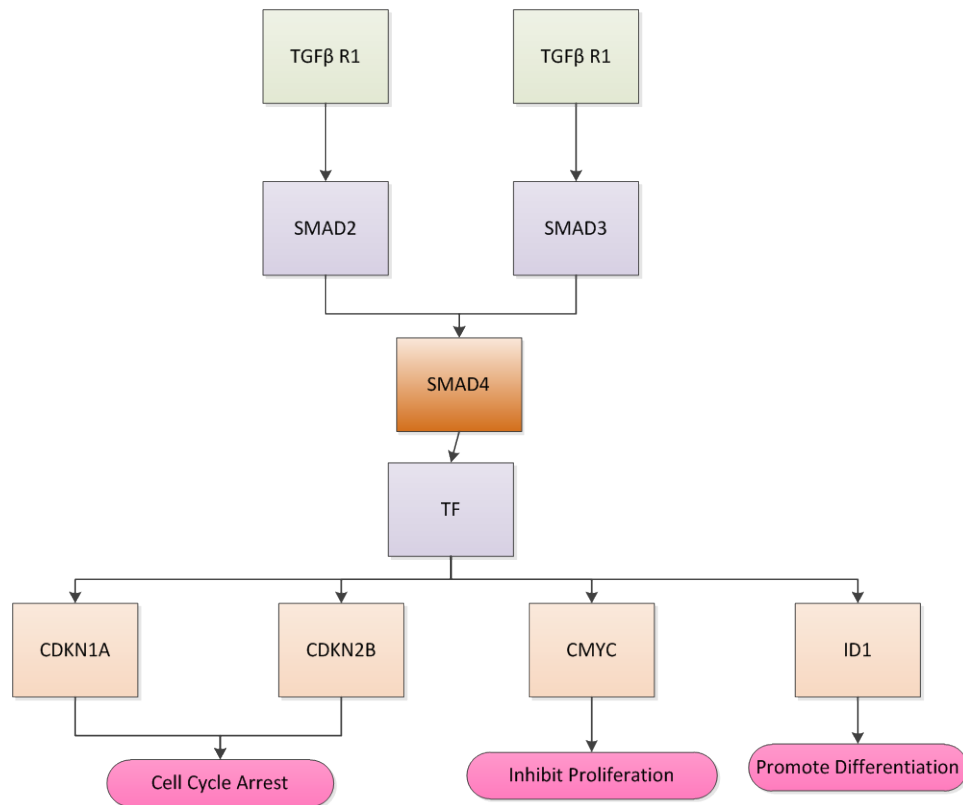
SMAD4 controls the G1 to S transition. As stated in NCBI[15]:

This gene encodes a member of the Smad family of signal transduction proteins. Smad proteins are phosphorylated and activated by transmembrane serine-threonine receptor kinases in response to TGF-beta signaling. The product of this gene forms homomeric complexes and heteromeric complexes with other activated Smad proteins, which then accumulate in the nucleus and regulate the transcription of target genes.

This protein binds to DNA and recognizes an 8-bp palindromic sequence (GTCTAGAC) called the Smad-binding element (SBE). The Smad proteins are subject to complex regulation by post-translational modifications. Mutations or deletions in this gene have been shown to result in pancreatic cancer, juvenile polyposis syndrome, and hereditary hemorrhagic telangiectasia syndrome.

We use the NCI data set for its pathway[16]:

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15 http://www.ncbi.nlm.nih.gov/gene/4089

16
http://pid.nci.nih.gov/search/pathway_landing.shtml?pathway_id=100160&source=BioCarta&genes_a=4089&genes_b=&what=graphic&jpg=on&ppage=1
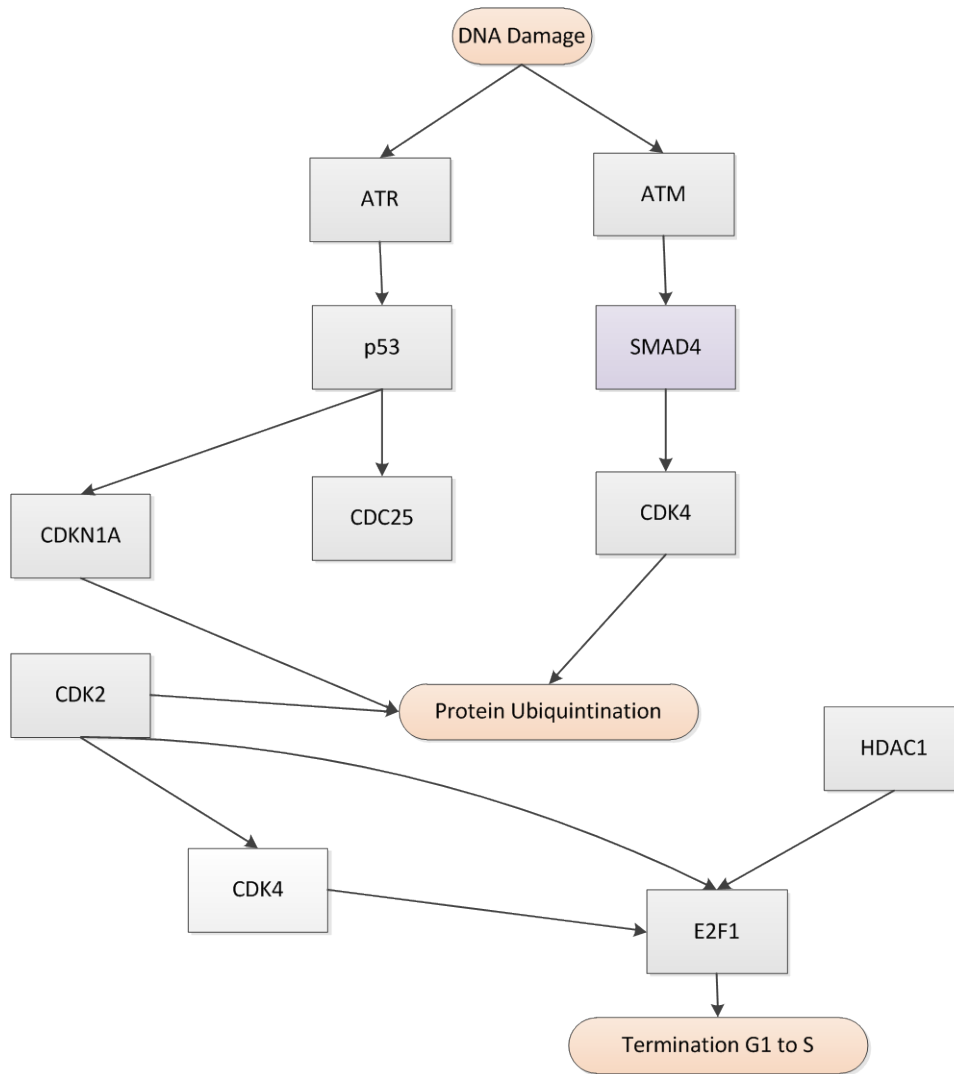
The SMAD pathway is also detailed by NCI and one is referred to that source for further detail.

From Weinberg (p 291) we also have the SMAD4 pathway showing its immediate control of the DNA transcription.
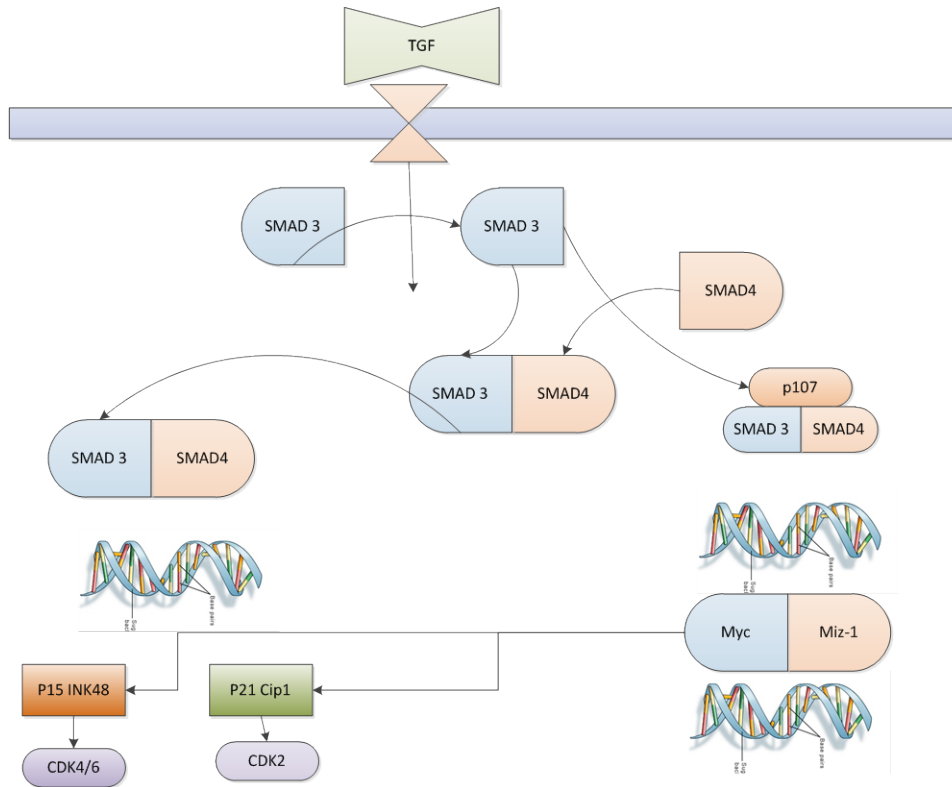
As Weinberg states (p 292):

"… Half of all pancreatic carcinomas and more than a quarter of all colon carcinomas carry mutant inactivated Smad4 proteins. Without the presence of Smad4 neither Smad2-Smad4 norr Smad3-Smad4 complexes can form. These two complexes are the chief agents dispatched by the TGF-β receptor to the nucleus with the important assignment to shut down proliferation."

This control mechanism is shown above.

9   C1QA

As NCBI states[17]:

This gene encodes a major constituent of the human complement subcomponent C1q. C1q associates with C1r and C1s in order to yield the first component of the serum complement system. Deficiency of C1q has been associated with lupus erythematosus and glomerulonephritis. C1q is composed of 18 polypeptide chains: six A-chains, six B-chains, and six C-chains. Each chain contains a collagen-like region located near the N terminus and a C-terminal globular region. The A-, B-, and C-chains are arranged in the order A-C-B on chromosome 1. This gene encodes the A-chain polypeptide of human complement subcomponent C1q.

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17 http://www.ncbi.nlm.nih.gov/gene/712

Azzato et al have examined C1QA in breast cancer and they discuss it broadly based presence. They state:

Complement is involved in the primary defence against intravascular microorganisms and has been reported to be involved in the clearance of tumour…. Recently, we have reported an association between expression of C1QA and prognosis in oestrogen receptor (ER)-negative breast cancer… in more than one cohort. We found that ER-negative tumours with overexpression of gene C1QA were associated with a better prognosis. The C1QA gene, located on chromosome 1p36.12, encodes for one of the components of the C1q complex. There are seven single nucleotide polymorphisms (SNPs) catalogued for C1QA on the NCBI database, of which there is only one common SNP (minor allele frequency 45%) located in an exon rs172378 is a synonymous SNP characterised by a G for A substitution at position 361 (A361G).

Thus we have another element from the immune system. It is part of the complement system, not the adaptive part and thus has primitive roots.

Now we depict a selection of its pathway as below (modified from KEGG)[18]:
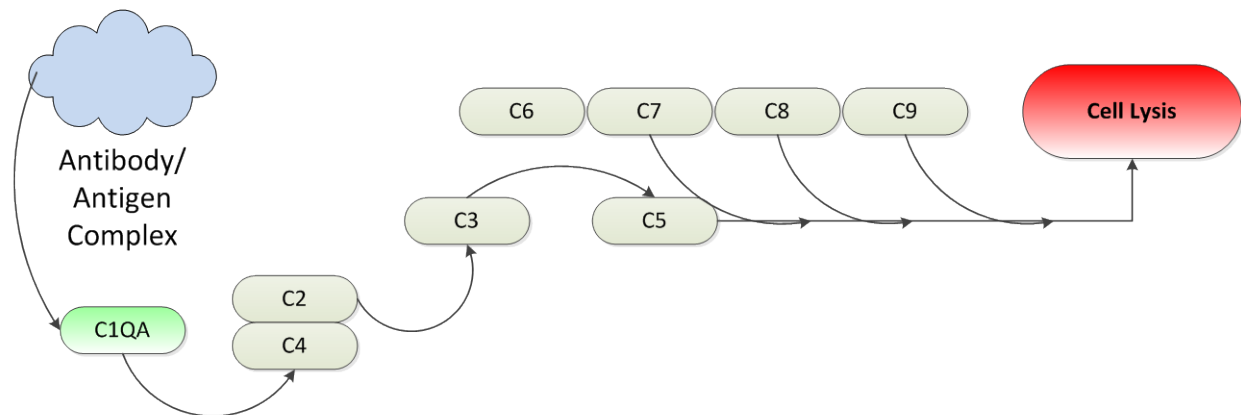
Note that the expression of C1QA is controlling the chain of complement factors which result in cell destruction. Suppression of C1QA then results in loss of this function. C1QA is thus just another factor in the overall control of cell proliferation.

10 OBSERVATIONS

There is a seemingly endless progression of genes identified as related to various cancers. All too often they are just noted as almost an incidental finding and as we have discussed before they are often putatively posed with no detailed pathway implications cited.

In this case we see a preponderance of immune system genes expressed albeit in a late stage of cancer. As indicated it is expected that all of these patients are terminal and that we are arguing

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[18] http://www.genome.jp/kegg-bin/show_pathway?hsa04610+712

of how soon. The range is from 10 to 40 months. Survival is not an end point; we seem to be arguing over when death occurs. As we had indicated above although it has some prognostic capability it has de minimis quality of care capacity. Thus one wonders why even attempt it other than having some scientific value.

On the other hand we can always view this in a Rosenberg manner and see the immune system kicking in in all manners and fashions. Its failure may then result in metastatic results and rapid death. An interesting question for treatment would be if one could re-stimulate or activate these broken elements and see if they can restore a protective barrier against metastatic results. Rosenberg sought this path in his years of melanoma research. Perhaps this is a means to rejuvenate that to but a later stage of the cancer. Namely we are seeing multiple immune elements failing so what can we achieve to mediate that result.

The problem seen in analyses of this type is that the press all too often exploits its ramifications. This is quite unfortunate for the patients in that they may somehow infer that this discovery may add hope to their plight when in reality it does nothing more than better estimate their demise.

For example there is a quote which states[19]:

"There is an urgent need for predictive models that help assess how aggressive the disease is in prostate cancer patients, as survival can vary greatly," said lead investigator William K. Oh, MD, Chief of the Division of Hematology and Medical Oncology of The Tisch Cancer Institute at The Mount Sinai Medical Center. "Our six-gene model, delivered in a simple blood test, will allow clinicians to better determine the course of action for their patients, determine clinical trial eligibility, and lead to more targeted studies in late-stage disease."

This set of tests is not what is desired. We are really desirable of tests which can predict the aggressive nature when the Gleason score is at 6 or less, namely when do we allow, with some sense of safety, for watchful waiting. This report is only for ultimately terminal patients, not those who could survive. This is a classic problem when results like this hit the media, even the professional media. In fact the reports get more exaggerated when we see the results in the popular media.

In summary we may pose the following:

1. There are many of these markers which are immune system related. Is this a common cancer response in the late stages, as much of the literature suggests. If so is the immune system attempting to isolate and defend the body.

2. How does this progress. Somehow one sees snapshots, namely patient A has such and such a profile and we then know when they reach that point the prognosis is bad or very bad. But what are the details of the evolution, do they all follow the same trajectory and if not why not and if so why and what does that mean.

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[19] http://www.onclive.com/web-exclusives/Blood-Test-May-Stratify-Risk-in-Prostate-Cancer

3. Is there an interaction between the pathways and the immune system or is this just a normal, in the case of cancers, immune response. How much of this is prostate specific and how much is common across a wide variety of malignancies.

4. What does this tell us about potential treatment paths? Can we activate the immune system, can we target it, and is the complement system of special interest. Is this a call to further focus on immune system alternatives?

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