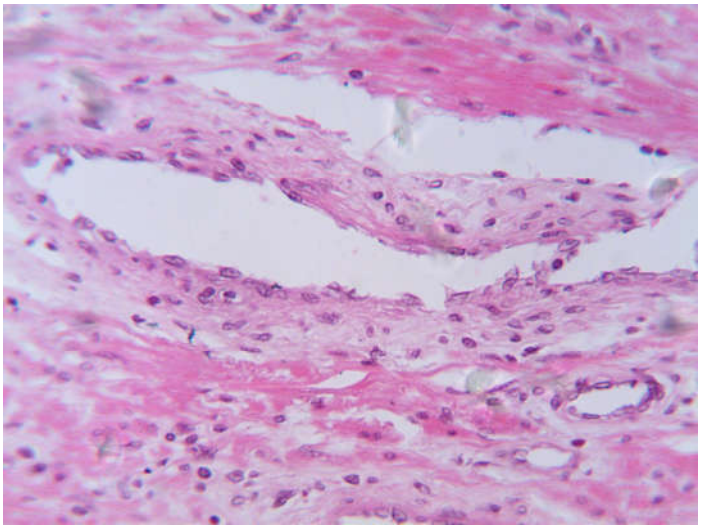

PCa Receptors: Targets for Therapeutics

November 2024

ABSTRACT

Prostate Cancer has been a challenging malignancy to treat. Surface molecules that uniquely identify PCa have been examined and combined with the multiplicity of targetable antibody therapies one may suspect several viable approaches may be evolving. We examine these in this Note.

Terrence McGarty

TGL 210

NOTICE

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Furthermore, this document contains references to and quotes and modified charts and figures from papers and documents under the premise of "Fair Use" in order to present ideas and understandings in context. The Author has attempted to make any and all references to such material separate from those of the author per se and has referenced the source expressly in all cases. These documents are for the dissemination of ideas and have no commercial intent. Our approach herein is to take elements of what is recent in the literature focused on a specific topic and attempt to develop a tapestry image of these connectable elements. We do not necessarily provide any new or fundamental results but merely attempt to assemble elements in a systematic and holistic manner.

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Access to prior Technical Notes can be made via Research Gate,
https://www.researchgate.net/profile/Terrence-Mcgarty/research

Contents

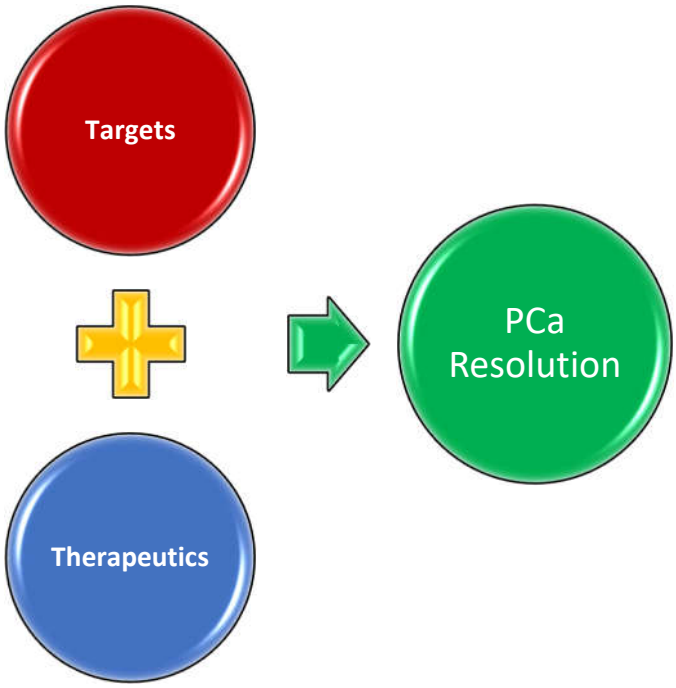
1 INTRODUCTION

Over the past decade, immunotherapeutic and targeted therapies have exploded in application. Many of the approaches rely on understanding the targeting of malignant cell surface markers that uniquely attack the malignant cell. For example in HER2+ breast cancer, BCa, targeting HER2 surface proteins with an antibody, Ab, and attaching to that Ab a molecule to kill the cell, created a complemented Ab targeting the HER2 cells. This approach seems to have been a dramatic addition to the therapeutics for a once highly lethal cancer.

PCa, the male equivalent to BCa, has had less successful approaches. The current favorite target seems to be PSMA[1]. Yet the literature presents many other targets. We examine many of these known targets herein.

1.1 PARADIGM

The therapeutic paradigm is to identify surface molecules on the malignant cells, and then using Abs, target these molecules using a conjugate type approach or other types of approaches facilitated by the immune system, such as ADC, CAR cells, BITES and the like.



Thus understanding both targets and therapeutics dependent upon targets allows for more efficious approaches. In addition multiple targets simultaneously may increase efficacy as well as reduce harmful effects to other cells.

[1] https://www.researchgate.net/publication/352554812_PSMA_A_Prostate_Cancer_Target

1.2 WHY PCA

We have studied PCa extensively over the past two decades, Unlike many others the use of immunotherapy in dealing with PCa has had limited at best results. The reason seems to be that it is a highly heterogenous cancer, namely many locations with disparate genetic drivers. Unlike some other cancers, such as breast cancer, BCa, having strong HER2 expression, there is no such focus for PCa. The strong HER2 PCa has been argued to have the potential for a cure give the strong well defined surface target. PCa, not so much.

A large percentage of PCa is slow growing and not the cause of death amongst men. However there is an appreciable segment that show aggressive growth and high lethality. Many studies have tried to isolate the causes and seek therapeutic approaches.

1.3 OUTLINE

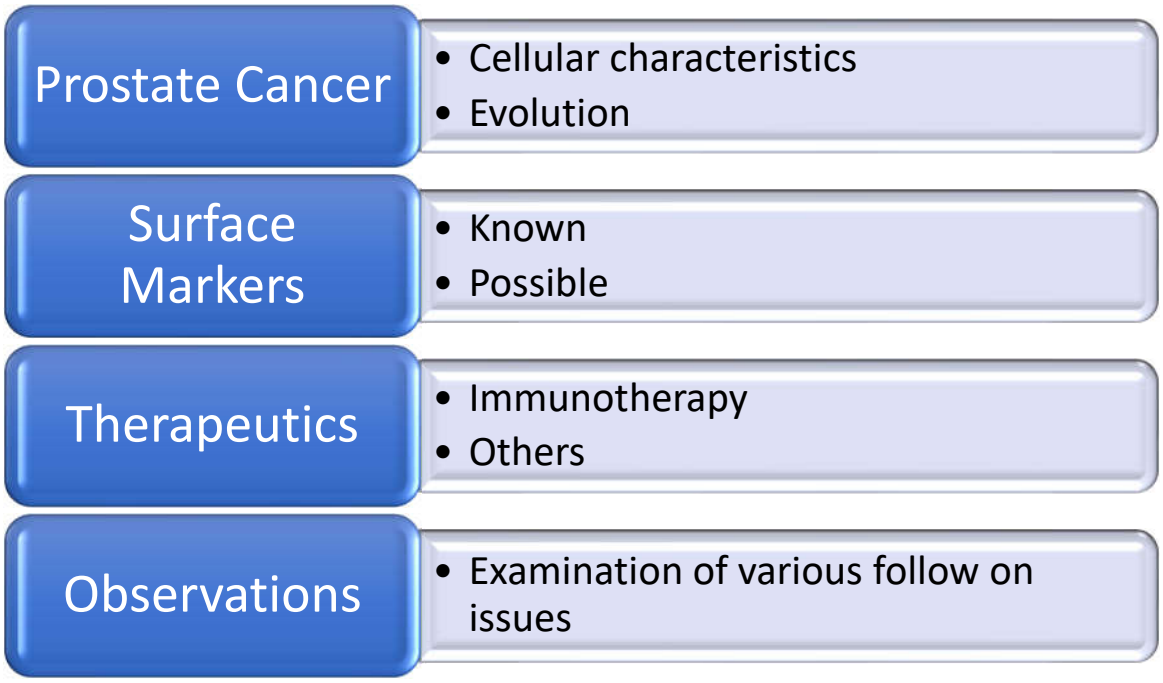
Our approach in this not is somewhat simple.

1. We briefly review PCa. This is a simple high order precis.

2. We then examine the putative surface targets as discussed in the literature. Many of these have associated therapeutic alternatives.

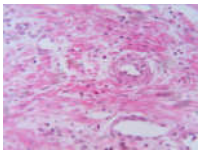
3. We review the therapeutic options available and those that could have potential.

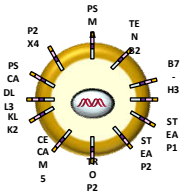
4. Finally we pose a set of issues based upon the analysis which need some consideration.

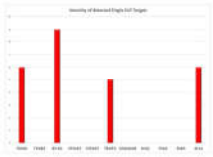


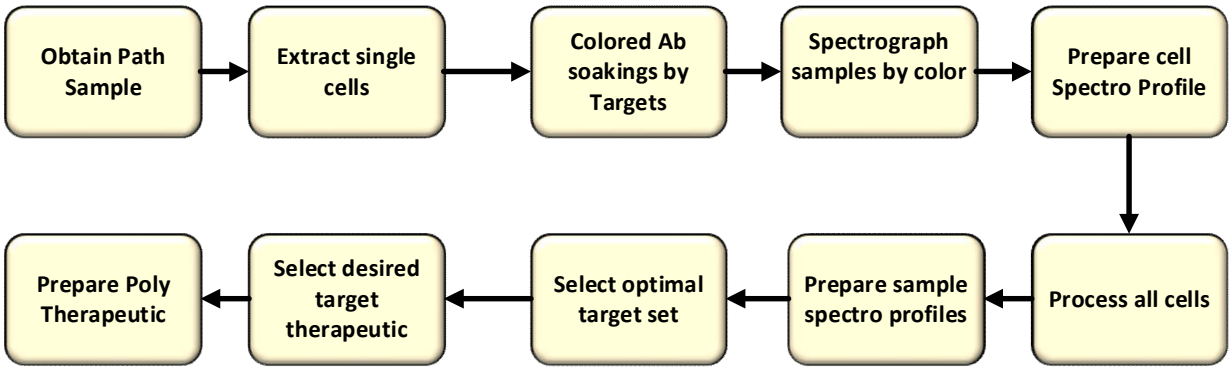
1.4 A PROPOSAL

We now make a proposal as how to select targets and prepare therapeutics. The following is the process proposed:

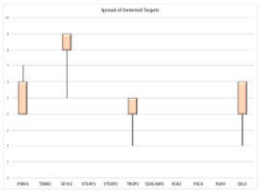








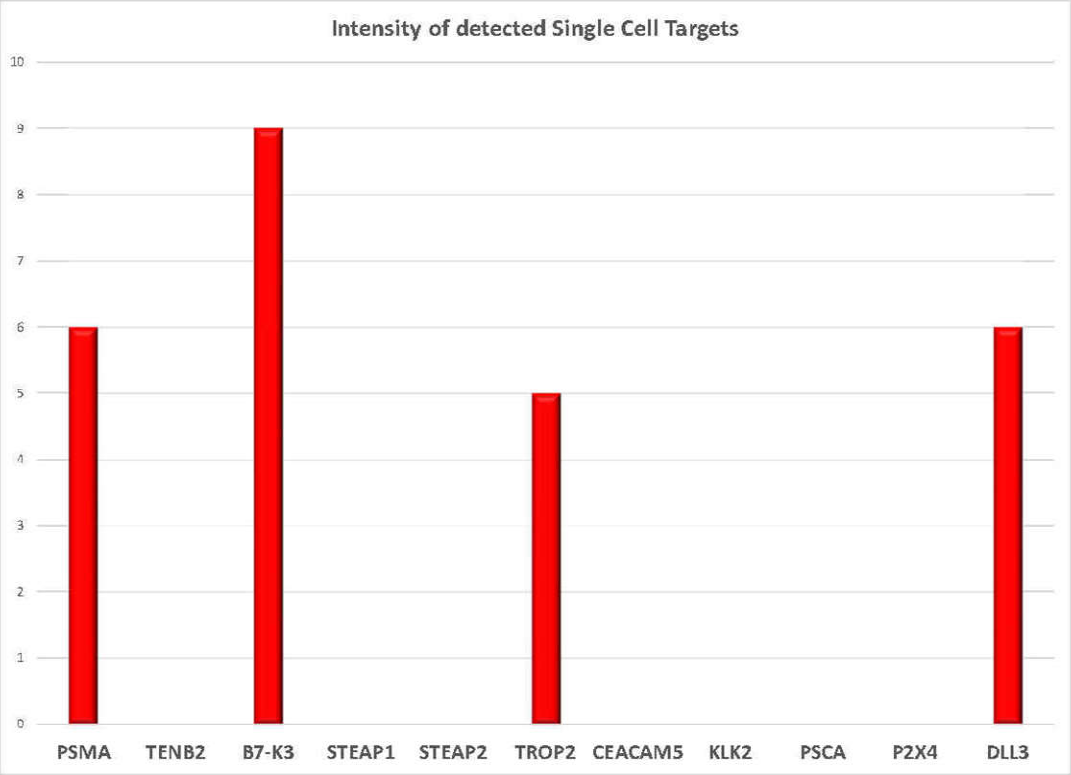
PSMA, B7-H3, DLL3



We now follow through the steps:

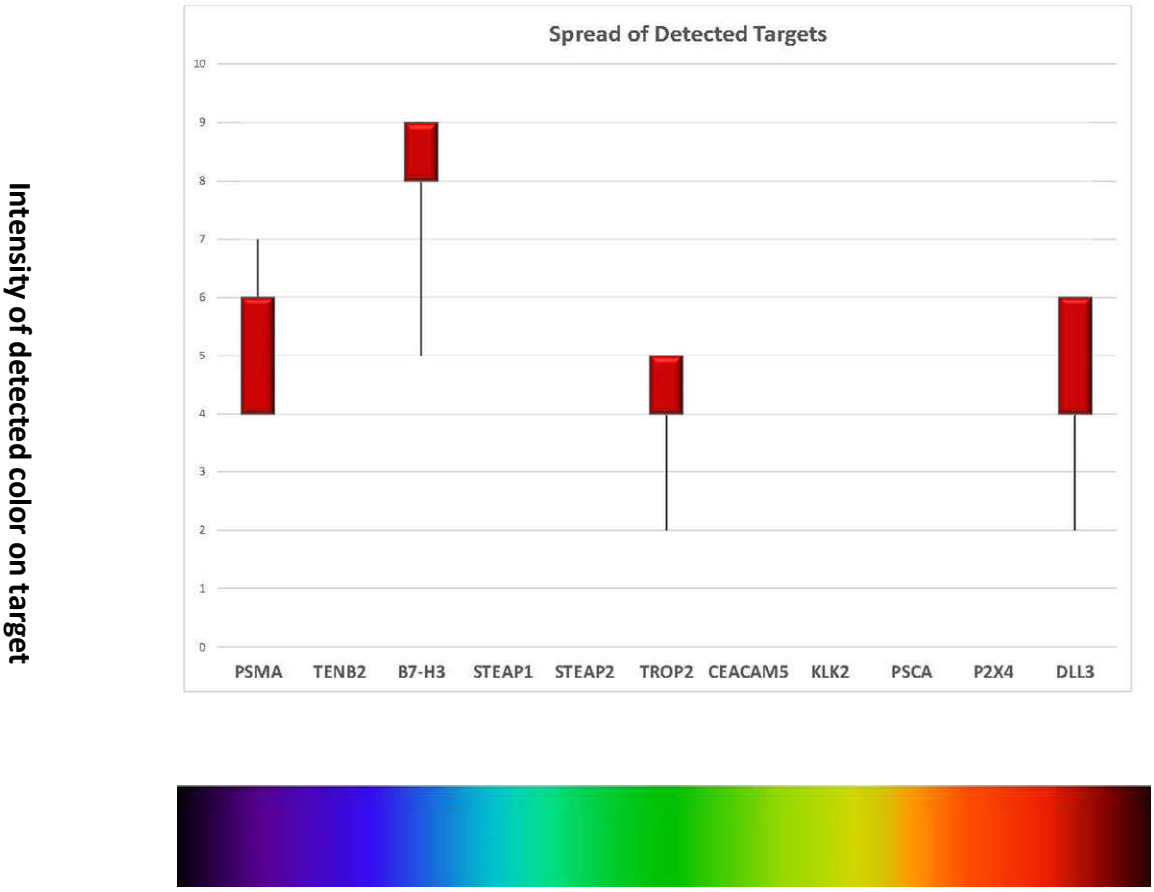
1. First obtain a path sample
2. The select a cell by cell from the sample. This allows a detection of the targets
3. Then using colorable Abs for each target select a specific color which can be determined by spectrographic means
4. Scan the cell to obtain spectrographic intensity

5. Prepare the cell spectrographic intensity as follows: Note that we see only 4 targets.



6. Then continue for all cells examining the targets spectrographically.
7. Process the cells

8. Prepare combined spectrographic data by spread analysis as shown below:



9. Select the optimal set of targets and then cull to a desired set. Here we show three selected targets
10. Prepare a polyspecific therapeutic based on procedures outline later.

This proposal, protocol, allows individualized targeting for a specific malignancy. In fact, based upon collected clinical data these therapeutic polys can have been pre-prepared and used in a timely and cost effective manner.

Furthermore, we examine this for PCa herein but extensions to other malignancies is readily extended.

2 PROSTATE CANCER

We have examined PCa extensively over the past decade plus[2]. The prostate is a glandular organ and as such upon microscopic examination displays the glands, their structure of basal and lamina cells and the cells that surround the glands establishing the structure. It is a heavily vasculated organ and in a constant state or renewal. When the cells become malignant they begin to lose their well-established glandular appearance and become an overgrown complex of cells. Like so many cancer cells the internal appearance of the glandular cells becomes exaggerated and often excessive.

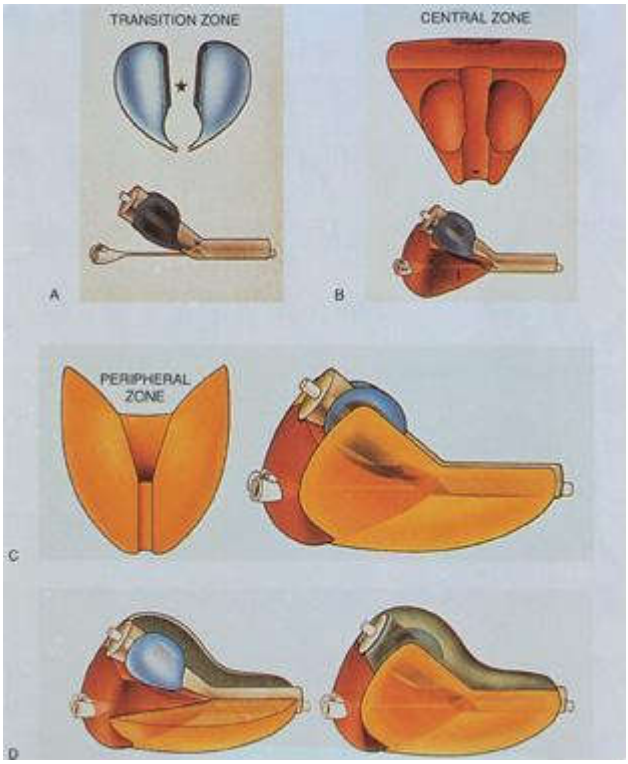
In this section we examine first the normal histology of the prostate and then we examine various types of dysplasia and malignancies. The intent here is not to become expert in the histological specificities of the prostate in both benign and malignant state but to have a fundamental understanding of how on a microscopic scale a malignancy develops and progresses. This will then allow us to, on the one hand look deeper into the genetic mechanism, and on the other hand, be able to look upward to cancer as a system level disease. The ultimate objective is to develop that system model for prostate cancer which aligns with the genetic underpinnings as well as being reflective of the histological development.

2.1 THE NORMAL PROSTATE

We first examine the normal prostate. The prostate is normally about 40 cc in dimension with the prostate surrounding the urethra below the bladder.

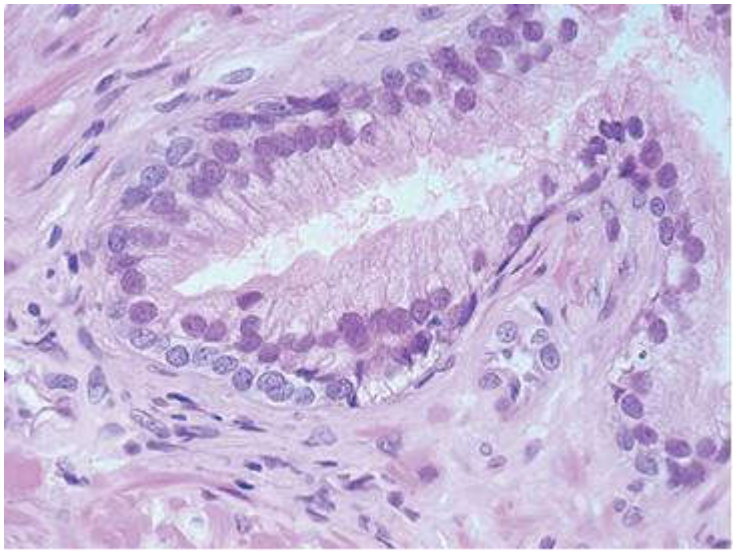
The basic structure of the prostate is shown below. It consists of three major zones; peripheral (dominant zone), central zone which is around the urethra), and the transition zone.

[2] https://www.researchgate.net/publication/264960277_Prostate_Cancer_A_Systems_Approach

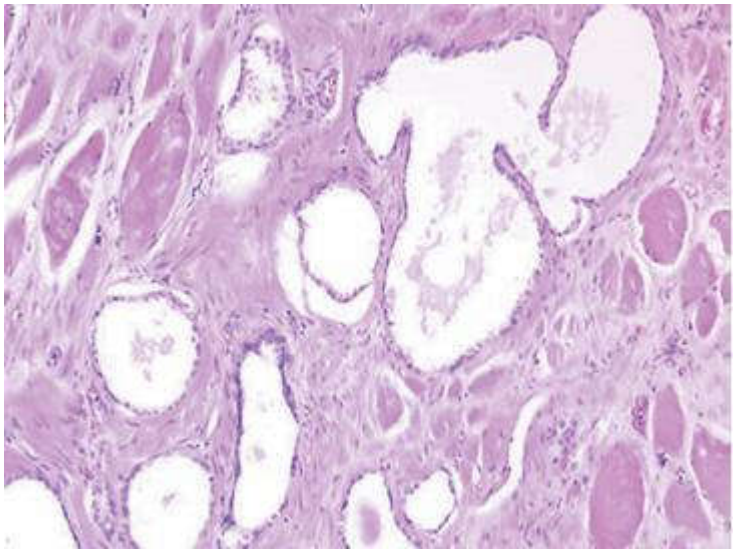


The cellular structure is depicted below. There are approximately 35-50 glands in the prostate, mostly in the peripheral zone and the glands have a lumen in which the prostatic secretions flow and the glands have basal cells and luminal cells as shown below. The basal cells are dark and the luminal cells are somewhat lighter.

Between the cells is the stroma which includes the blood flow from veins and arteries, the muscle and other stroma elements. Simply stated, the prostate is a collection of the basal/luminal glands scattered about veins, arteries, muscles and nerves.



The figure below depicts a second view of the prostate glands. Again this is with HE stain and under low magnification. The basal cells are clearly seen with their dark stains and the luminal stand above them. The stroma is fairly well articulated in this slide.



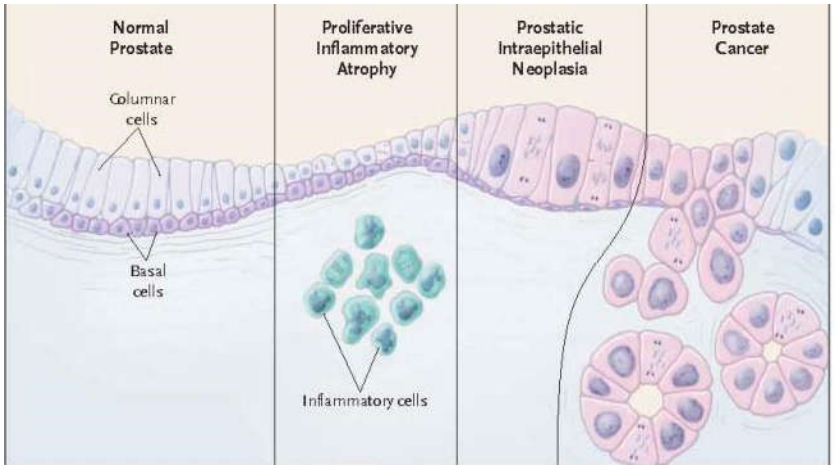
The normal prostate then is merely a collection of glands, glands composed of basal and luminal cells, with open glandular portions, the white areas above. As we noted before these glands emit various proteins and are an integral part of the male reproductive system.

2.2 Summary of Prostate States

We now provide a high level summary of the changes in the prostate histologically as PCa is developed. We do this to lay out the various changes we will examine and to better understand what we may be looking for when developing pathways. We believe that it is essential that we always go back and forth between abstractions of pathways, and the reality of the cell histology.

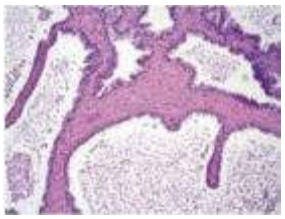
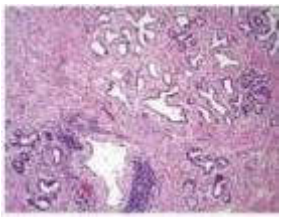
There is a general agreement, with of course many exceptions, as to the progression of prostate pathology and its related causes. A graphic from a recent NEJM article is shown below[3]:

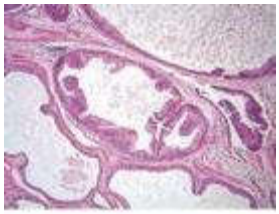
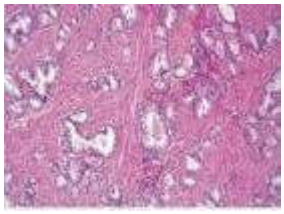
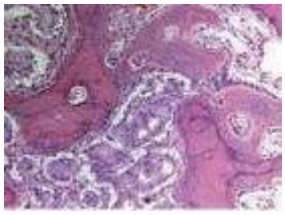
[3] See Nelson et al, Prostate Cancer, NEJM, July 24, 2003. p 376.



Not the progression from normal prostate with basal and luminal cells and then through PIA and then PIN and finally PCa. The PIN demonstrates a complex but contained development of cells. As one moves o PCa, that is when the cells move away from the existing gland, and they are for the most part luminal cells establishing de novo glandular like structures.

An excellent tabular summary from Taichman et al follows:

Disease State	*Histology*	*Details*
Normal Prostate		Large glands with papillary infoldings that are lined with a 2-cell layer consisting of basal and columnary secretory epithelial cells (luminal) with pale cytoplasm and uniform nuclei. Susceptibility genes or events related to hereditary PCa: RNASEL: regulates cell proliferation through the interferon regulated 2-5 oligoadenylate pathway ELAC2/HPC2: Loss of function of tRNA-3 processing endoribonuclease MSR1: Macrophage scavenger receptors process negatively charged macromolecules.
PIA		Atrophic glands have scant cytoplasm, hyperchromic nuclei and occasional nucleoli and are associated with inflammation Susceptibility genes or events: NKX3: Allelic loss of homeobox protein allowing growth of prostate epithelial cells PTEN: Allelic loss of phosphatase and tensin homolog allowing decreased apoptosis and increased cell proliferation. CDKN1B: Allelic loss of cyclin dependent kinase inhibitor p27 allowing increased cell proliferation

Disease State	*Histology*	*Details*
PIN		Intermediate to large size glands with proliferation changes contained within the gland and having nuclear abnormalities that resemble invasive carcinoma. Susceptibility genes or events: GSTP1: Hypermethylation of the upstream regulatory region inactivates the Pi class gluthionine S transferase enzyme which detoxifies carcinogens. Hepsin: Increased expression of this serine protease leads to increased invasiveness and disruption of the basement membrane. AMACR: Increased expression results in increased peroxisomal b-oxidation of branched chain fatty acids from red meat thereby increasing carcinogen exposure. TMPRSS2: Fusion of this androgen regulated gene with ETS family of transcription factors in late stages of PIN results in in increased breakdown of the extracellular matrix. Telomerase: Activation leads to maintenance of telomere length and immortalization of cells.
Prostate Cancer		Small irregular glands with cells having abnormal nuclei and nucleoli and lacking basal cells. Susceptibility genes or events: MYC: Overexpression leads to cell proliferation and transformation RB: Loss of expression leads to cell proliferation and transformation
Metastatic PCa		Nests of cancer cells within the bone Susceptibility genes or events: TP53: Mutation results in loss of multiple tumor suppressor functions E-cadherin: Aberrant expression leads to increased invasive and metastatic phenotype NM23: loss of this NDP kinase leads to increased metastasis EZH2: Histone methyltransferase PcG protein whose activation causes repression of genes that suppress invasion and metastasis
AR PCa		Cancer cells that grow in androgen depleted environment Susceptibility genes or events: AR: may remain active through amplification, phosphorylation by other steroids or non-androgen growth factors BCL2 Increased expression leads to protection from apoptosis Stem cells: potential repopulation by progenitor cells

Note in the above, Taichman et al make mention of the separate gene elements that are putatively assumed to have caused the subsequent event. These genetic changes then will become a key factor in how we view PIN transitions.

Also note in the above, it implies a set of sequences of genetic changes that moves from benign to malignant. The question then is; if a genetic change is necessary for a morphological change,

then is the genetic change reversible or are the genetically changed cells killed off by some other process, and if so what process?

To understand this question, and hopefully set a path to answering it, we lay out the known elements in the path towards malignancy, look at the gene maps and dynamics, and then attempt to establish a model for examining the dynamic processes which move the cell forward to malignancy or backwards towards a benign state.

We shall now examine each of these in some detail.

2.3 Prostatic Intraepithelial Neoplasia

Prostatic Intraepithelial Neoplasia, PIN, is considered a precursor to PCa. High Grade PIN, HGPIN, is often considered almost certainly a precursor. However as we shall discuss this is at times not the case and HGPIN is known to regress. One must be careful, however, since we are generally discussing biopsy samples which may be subject to substantial sampling deficiencies as we have already discussed.

Let us now provide a simple overview of the development of models. We develop it in the following manner:

First, we look at the histological structure of PIN and PCa. Cell changes occur and the changes morphologically are dependent upon the expression of or lack thereof of certain genes. The linking of morphology and gene expressions seems to fall short at this stage. Thus the nexus is missing.

Second, we look at some simple models for the development of HGPIN. As we have stated, the reason for this is twofold. First HGPIN is often assumed to be a natural precursor of PCa and as such one can assume that genetic changes necessary for PCa are first seen in HGPIN. Second we know that HGPIN can suddenly regress and the cells revert to benign state. If that is the case and indeed it is one may ask if the genetic changes were the cause also of the regression or was there some exogenous cause. We focus primarily on the Goldstein et al model because it demonstrates both HGPIN and PCa and the relationship to morphological and genetic changes.

Third, we examine the cancer stem cell, CSC, model. The CSC is an interesting paradigm which may explain the less than rapid growth of certain cancers. PCa may be dominated in many cases by indolent slow reproducing CSC. Understanding the dynamics of the CSC is therefore essential.

Fourth, we look at the many specific genetic drivers such as PTEN and the other first and second order products in the pathway chain. This is an extensive discussion which we will rely upon to build pathway models.

Fifth, we examine the epigenetic factors such as miRNA and methylation. These may be the most significant factors in cell change and genetic expression alteration that we see in PCa progression.

Sixth, we present and examine in some high level detail the many complex pathway models currently presented.

Seventh, we examine the various models for reaction kinetics. This will be essential when we attempt to model the dynamics. The classic approaches are significant and their simplifications are useful. By looking at linear models we often can find reasonable insight but it is often by examining the nonlinear models that we can ascertain the tipping points with more clarity.

Eighth, we examine pathway controls that are what components such as PTEN play the most significant role.

Ninth, we look at the three dominant modeling techniques; Boolean, Bayesian, and System model using reaction rates and complex time varying differential equations. We do not in this analysis examine the spatial models (as initially developed by Turing and detailed by Murray).

Tenth, we examine how the constants in these models may be obtained by means of system identification methods. We have accomplished this in other pathway systems and we believe it is directly applicable here as well.

2.3.1 HGPIN Characterization

HGPIN is represented by morphological changes in prostate cells in the acinar or glandular locations. It generally is a complex set of growth patterns of new cells whose morphological appearance is similar to but not identical to the existing cells in the gland. The new cells clearly have form and shape that demonstrates pre-malignant morphology, with enlarge and prominent nucleoli.

From the paper by Putzi and DeMarzo we have:

The high-grade form of prostatic intraepithelial neoplasia (PIN) has been postulated to be the precursor to peripheral zone carcinoma of the prostate. This is based on zonal co-localization, morphologic transitions, and phenotypic and molecular genetic similarities between high-grade PIN and carcinoma. Although high-grade PIN is thought to arise from low-grade PIN, which in turn is thought to arise in normal or "active" epithelium, little is known whether truly normal epithelium gives rise to PIN or whether some other lesion may be involved.

Focal atrophy of the prostate, which includes both simple atrophy and postatrophic hyperplasia, is often associated with chronic, and less frequently, acute inflammation. Unlike the type of prostatic atrophy associated with androgen withdrawal/ blockade (hormonal atrophy), epithelial cells in simple atrophy/postatrophic hyperplasia have a low frequency of apoptosis and are highly proliferative. In addition, hormonal atrophy occurs diffusely throughout the gland and is not usually associated with inflammation.

To simplify terminology and to account for the frequent association with inflammation and a high proliferative index in focal atrophy of the prostate, we introduced the term "proliferative inflammatory atrophy" (PIA).

In a similar manner in a review paper by O'Shaughnessy et al on multiple intraepithelial neoplasia the authors state the following regarding HGPIN:

The evidence that PIN is a morphological and genetic precursor to prostate cancer is extensive and conclusive...

When examined microscopically, PIN lesions are characterized by collections of proliferative prostatic epithelial cells confined within prostatic ducts that exhibit many morphological features of prostate cancer cells, including architectural disorganization, enlarged cell nuclei and nucleoli. ...

In addition to the similarity of the cellular morphologies of HGPIN and invasive lesions, evidence that HGPIN is a precursor of prostatic adenocarcinoma includes the multifocality of both lesions and the presence of carcinoma in foci of PIN; among older men, foci of PIN are found in 82% of prostates with carcinoma but in only 43% of normal prostates.

PIN is frequently located in the peripheral zone of the prostate, the site at which 70% of prostatic carcinomas occur. Additional similarities include enhanced proliferative activity of both PIN and carcinoma (3-fold that of benign tissue), cytokeratin immunoreactivity, lectin binding, and loss of blood group antigen with both PIN and carcinoma.

Prevalence of PIN and its temporal association with invasive cancer are illustrated by the known 40–50% PIN incidence in men 40–60 years of age, evolving into the 40–50% incidence of prostate cancer in men 80 years of age. Autopsy data reveal that PIN lesions appear in the prostates of men in their 20s and 30s in the United States, preceding the appearance of prostate cancer lesions by as many as 10 years ...

African-American men, who are at higher risk of prostate cancer mortality, appear to have a greater extent of PIN at any given age. PIN and prostate cancer lesions share a number of somatic genome abnormalities, including loss of DNA sequences at 8p and increased GSTP1 CpG island DNA methylation, among others.

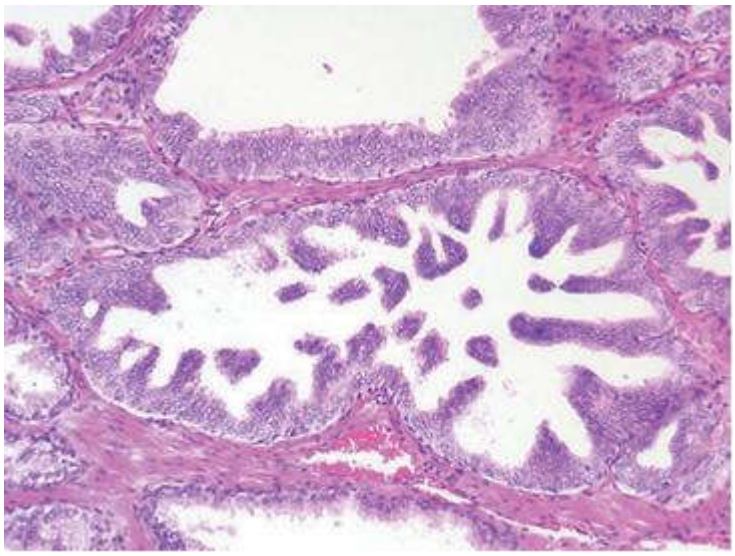
Finally, transgenic mouse strains prone to developing prostate cancers typically develop PIN lesions in advance of the appearance of invasive cancer. PIN lesions are always asymptomatic and cannot currently be diagnosed or detected by any reliable means other than examination of prostate tissue histologically. In autopsy studies, the incidence and extent of PIN increases with age, as does the incidence of prostate cancer.

Notwithstanding the correlation, there does not seem to be causality. In addition, the authors do indicate that HGPIN can be reduced but they seem to fail to speak to the issue of total remission without any treatment. The question is therefore, is PIN a precursor of PCa? If it is or is not, is PIN the result of a genetic change as has been postulated by many? It would seem clear that the

existence of remission of PIN would imply that it is not at all necessarily a precursor and furthermore that it is not necessarily a genetic change for all PIN. That is can there be a morphological PIN that is genetic and not remissionable and one which is remissionable. Remissionable implies the existence of apoptosis that is a natural cell death or perhaps a cell death due to some immune response.

2.3.2 PIN Morphology

Prostatic Intraepithelial Neoplasia, PIN, is a growth within the normal glands of more cells than should normally be there. The slide below depicts high grade PIN, HGPIN. Note the PIN in the center shows significant cell growth in the existing gland as compared to the gland at the bottom which shows normal thinner growth.



The PIN shows papillae which are shooting out within the gland and there is also significant basophillic staining of the papilla cells whereas the normal gland has limited staining of the luminal cells. The key question is one of whether PIN is a precursor to PCa. Many articles state that it is but when one looks at the data there is still a significant area of doubt.

2.3.3 Some HGPIN Models

There has been an extensive amount of work in trying to create HGPIN from normal prostate cells. There are questions as to what cells the HGPIN derives from, for example basal or luminal, and then there are questions as to what genetic changes result in PIN. As with so many parts of the puzzle there are no single set of answers. We start with the recent Goldstein model and use it as a basis. Then we look at other models and specific genes expressed. We defer until later the issue of pathways.

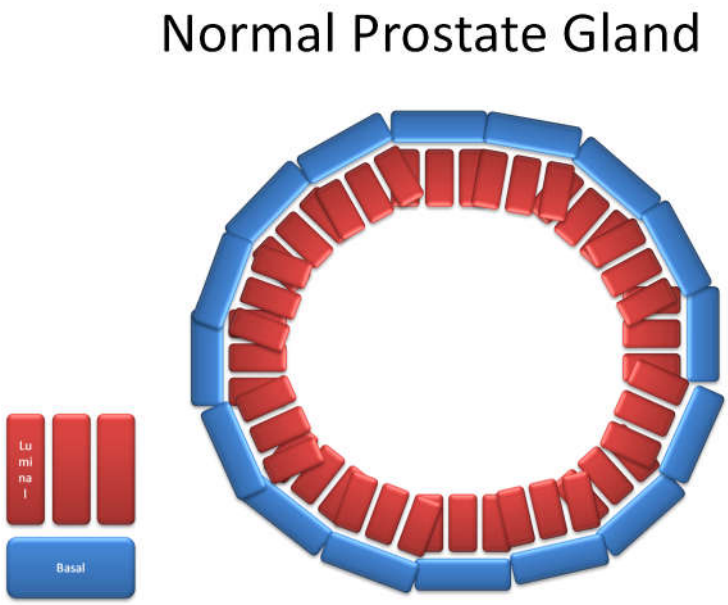
1.1.1.1 The Goldstein Model

A novel set of experiments on prostate cancer were based on the work by Goldstein et al at UCLA. Understanding this work is useful in understanding both HGPIN and PCa. Goldstein et al demonstrate that one set of elements in the intracellular pathways if disturbed in a certain manner can result in morphological changes that first become HGPIN and then mode to PCa. The essential usefulness of this work is that it allows for a demonstrable relationship first between genetic change and histological change and second that changes in pathway elements lead to progression.

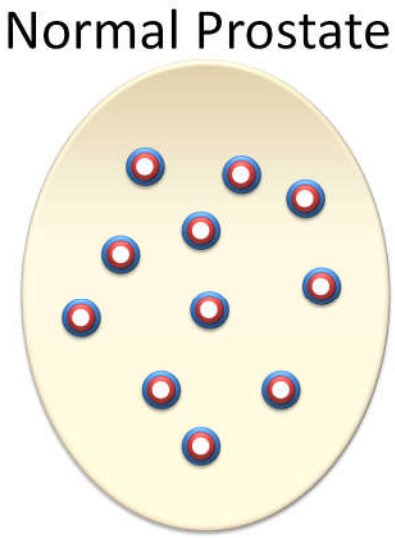
Simply what they did was to take two types of prostate cells, the basal and the luminal, tag them with surface tags, inject them into a mouse, and saw that only the basal cells grew, then they added two genes encoding for putative cancer pathways, and they saw that the basal cells grew to basal and luminal, like PIN, and then finally they added an AR, androgen receptor gene, and voila, prostate cancer. Result, showing how a specific pathway can generate cancer.

Let us go back and look at this a bit more.

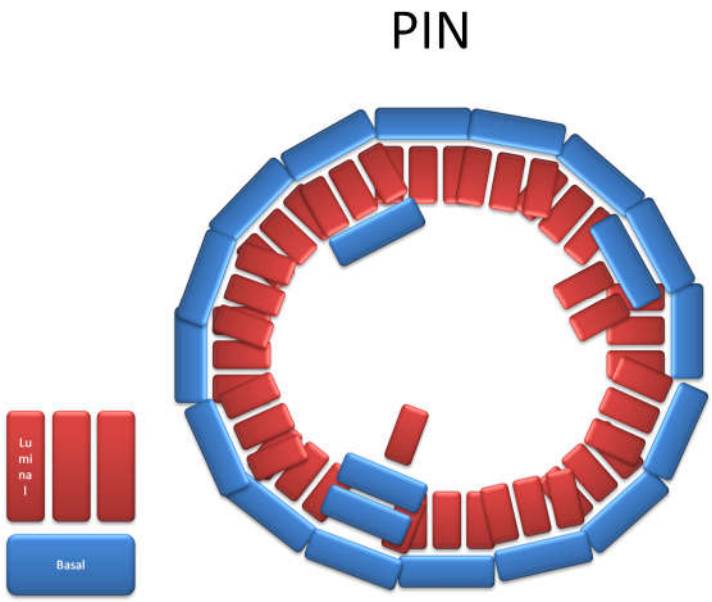
1. First the prostate has cell collections which act as glands with basal cells at the base and luminal cells on top. The luminal cells secret to the gland, the luminal space. This we show below.



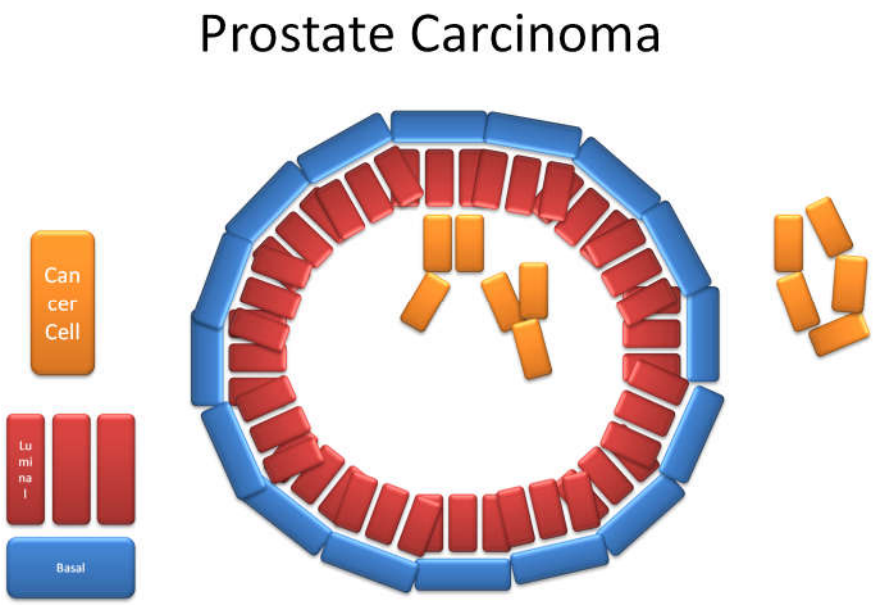
2. The normal prostate looks like what we show below, about 35-50 of these glands, and then surrounding material of muscle, blood supply, nerves, and lymphatics. The glands stand apart and they secret fluids into the lumen, the open parts of the gland. In between is the stroma composed of nerves, blood vessels and other connective tissues.



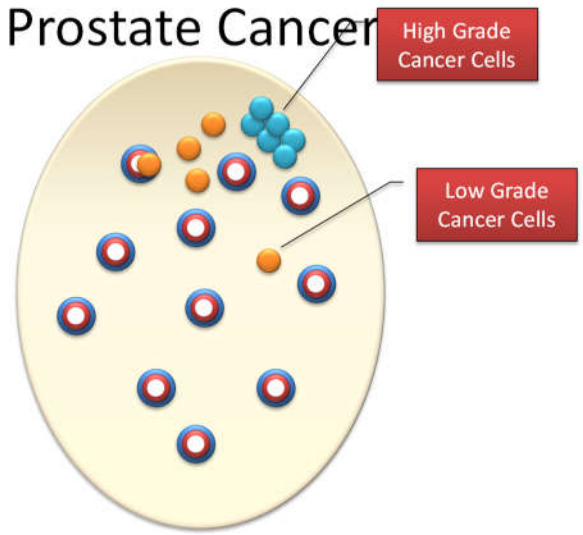
3. Now sometimes we see PIN, prostatic intraepithelial neoplasia, which is a growth of normal cells but not where they are to be. We may see the basal cells growing outwards and even some more luminal cells as well. The sign may be an increase in PSA since we have more luminal cells but the percent free PSA may stay high since the luminal cells are health ones. We show this below:



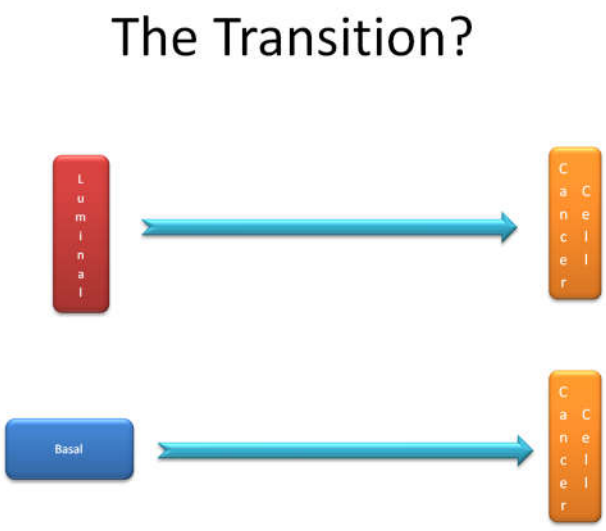
4. Then we may get prostate cancer, PCa, where the luminal cells types start to appear and grow without bound. The question is, where did these cells come from, other luminal cells or basal cells, or what. This is the question that the authors addressed with this elegant experiment. There is also the key question of whether it is just one cell that starts it or if the changed basal cells grow and if the environment switches many on over time. The latter effect is similar to that which has been observed in melanoma. Below we show what happens next,



Looking at the prostate as a whole we then may see what appears below. Namely we may see low grade cancer cells and then clusters of high grade cancer cells, this leads to the Gleason grading system.



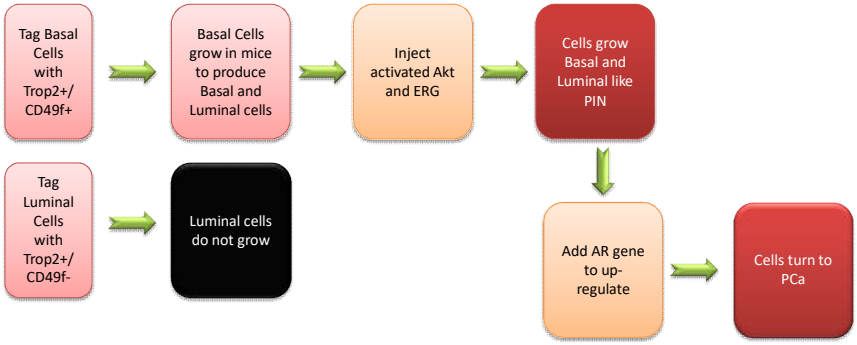
5. Thus the question posed by the authors was the one which asks from what cell does cancer begin? Their answer suggests the basal cell.



6. Pathways have been studied for PCa extensively and we shall discuss them in some detail.

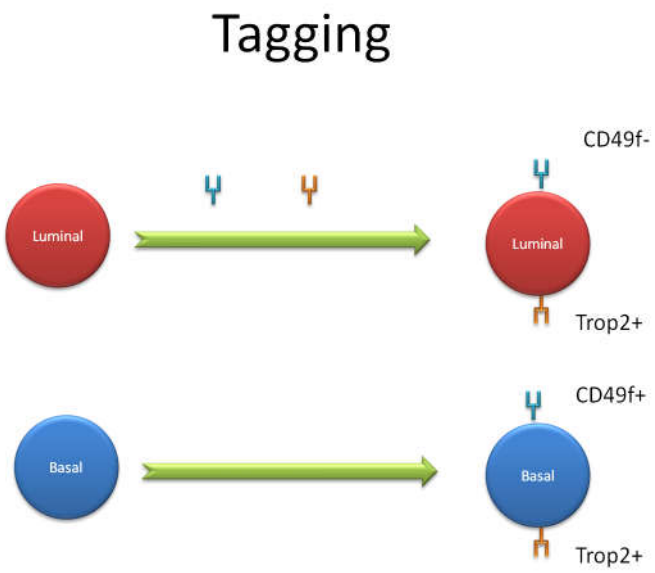
But the authors took a simple approach and looked at three genes in the putative pathway process. This is shown below:

Goldstein Process



First they showed that only basal cell proliferate into both basal and luminal. Then they added ERG and Akt genes known as key in the pathways, and they obtained PIN, and then they added AR, the androgen receptor to drive the previous two genes and the result was PCa.

They were able to keep track of basal and luminal cells by tagging them with cell surface markers, as shown below. Basal was positive for both and luminal positive for one and negative for another, a good example of tracking the cells as the transform.



As to the two initial genes we have:

(i) Akt: There are in humans three genes in the "Akt family": Akt1, Akt2, and Akt3. These genes code for enzymes that are members of the serine/threonine-specific protein kinase family. Akt1 is involved in cellular survival pathways, by inhibiting apoptotic processes. Akt1 is also able to induce protein synthesis pathways, and is therefore a key signaling protein in the cellular pathways that lead to skeletal muscle hypertrophy, and general tissue growth. Since it can block apoptosis, and thereby promote cell survival, Akt1 has been implicated as a major factor in many types of cancer.

(ii) ERK: Extracellular signal regulated kinases, ERK, are protein kinase signaling molecules involved in the regulation of meiosis, mitosis, and postmitotic functions in cells.

This study still leaves several open questions:

1. Is the clonal theory of cancer still standing or can a single cell transform and then induce other cells via chemical signaling.

2. Is the basal cell the only one. There appears to be some issues here and the review article looks at these.

3. Is PIN an artifact or a precursor. Clinically men with PIN have a slightly higher risk of PCa but not a substantially higher as would be argued in this model. In fact men with PCa do not always have PIN and men with PIN do not always get PCa.

4. Is this just an artifact pathway, the true pathway, one of many pathways.

5. If we can duplicate pathways can we than better control the disease.

6. What does this tell us about detection and staging.

1.1.1.2 Other Models

The Goldstein et al model is but one of several which have taken this approach. There are others and the results are not always consistent. Two of them are discussed as follows:

1. Yen et al (2003) have reported on a murine model which demonstrated that by implanting c-Myc genes into a mouse that it resulted in murine PIN and then shortly thereafter PCa. Yen et al also shown loss of NKX3.1, a tumor suppressor gene, which is putatively involved in PCa as well as PIN. NKX3.1 is a 8p21 gene whose function is to generate the Homeobox protein[4]. It is known to be suppressed in familiar prostate cancer and in the case of Yen it is reduced in its expression as well.

2. Lawton and Witte discuss the generation of PIN by means of lentivirus infection via an siRNA which is a knock out for PTEN.

2.3.4 HGPIN, A Precursor of PCa?

There has been an extensive amount of literature claiming that high grade prostatic intraepithelial neoplasia, HGPIN, is a precursor to prostate cancer, PCa. The research has gone as far as delineating genetic changes which ultimately lead to metastatic PCa. However, at the same time it is not uncommon for HGPIN to regress and totally disappear. This would seem to counter the theory of genetic change and resulting morphological change of the prostate acini cells.

Moreover there have been many murine models of HGPIN which have been induced with specific genetic changes in specific pathways which lead inexorably to PIN and then to PCa. Likewise there have been many microarray analyses of HGPIN demonstrating the presence or absence, enhancement or deactivation, of the same or similar genes. Yet again there is at time spontaneous remission.

Thus it begs the question; what causes the remission of HGPIN? Is it possibly akin to the remission seen in certain cancers, a remission generated by an immune response effect, as discussed by Rosenberg? Or is it a pathway apoptosis that occurs as a natural course of having aberrant genes?

1.1.1.3 Key Questions

Let us begin with what we assume is known:

[4] Pecorino, Cancer, p 114.

1. HGPIN is driven by underlying progressive and non-changeable changes in the genetic structure of benign cells in the prostate glands.

2. There is a putative association between HGPIN and PCa, reflected in an increased incidence of PCa when HGPIN is present.

3. PCa like most other cancers is characterized by the clonal model, namely one cell becomes aberrant and all other cancers cells are daughter cells of the aberrant clone.

4. PCa is known to result via a set of genetic changes resulting in the cell growth outside of the gland and the creation of malignant glandular structures wherein additional genetic changes occur and result in a less structured morphology and then metastasis.

5. HGPIN regression is seen. This means that the HGPIN cells totally disappear resulting in a purely benign appearance of the prostate glands. It begs the question of; do they cells die or are they attacked and destroyed or is there some reversion mechanism? PIN is a proliferation, so any continuation of cell existence would imply at best a morphological change of say the nucleus and nucleoli but not the total cell count, namely the clustering of many cells in the gland. Thus in regression we do not know what happens or how.

Thus these observations pose the following questions:

1. What causes the disappearance of multiple clusters of HGPIN? Is it apoptosis of some form, an immune response, a genetic switch, or something else?

2. Has there been any extensive studies of HGPIN regression to understand how it arises?

3. If HGPIN regression is based upon some to-be-understood mechanism, can that same mechanism be applied in some form to PCa?

4. Does HGPIN, which is regressionable, have certain cell surface markers which are presentable to the immune system and thus enable enhanced immune responses?

5. Is there a stem cell created when PCa evolves and is PIN lacking in such a stem cell?

The literature demonstrates how to create PIN. There are a few presentations on how to regress PIN[5]. However the nexus of forward PIN progression and backward PIN regression is not complete. We attempt herein to review this in some detail and then to place it in a structure for further analysis and study.

As a natural extension to these questions we can then ask similar ones regarding PCa. How does PCa progress and what are the pathway dynamics related to that progression.

[5] Narayanan et al using NSAID.

1.1.1.4 An Example

Let us begin with a simple example. A 68 year old male is examined due to an increase in PSA from 1.5 to 2.3 in a one year period. The DRE is normal but there is a family history of a first degree relative who died from an aggressive PCa, at 79 years of age. Re-measuring the PSA from two independent sources yields values of 1.8 and 1.9 two months after the raised PSA.

A 20 core biopsy is performed and the results are as follows:

A. Prostate, right apex, biopsy: Benign prostatic glands and stroma.
B. Prostate, left apex, biopsy: Prostatic intraepithelial neoplasia, high grade, focal. Glandular hyperplasia of prostate.
C. Prostate left peripheral zone, biopsy: Prostatic intraepithelial neoplasia, high grade, focal, Glandular hyperplasia of prostate.
D. Prostate, right peripheral zone, biopsy: Benign prostatic glands and stroma.
E. Prostate, transition zone, biopsy: Prostatic intraepithelial neoplasia, high grade, focal Glandular hyperplasia of prostate.

After an eight month period PSA was measured again and this time it was 2.0. A second biopsy was performed using 24 cores. The results are:

A. Prostate, right apex, needle core biopsy: Benign prostatic tissue with very focal and mild acute inflammation.
B. Prostate, left apex, needle core biopsy: Benign prostatic tissue.
C. Prostate, right mid, needle core biopsy: Benign prostatic tissue.
D. Prostate, left mid, needle core biopsy: Benign prostatic tissue.
E. Prostate, right base, needle core biopsy: Benign prostatic tissue.
F. Prostate, left base, needle core biopsy: Benign prostatic tissue.
G. Prostate, transition zone, needle core biopsy: Benign prostatic tissue.

This is a clear case of total HGPIN regression. The question then is, how common is this and what is its cause, and if regression can be obtained how it might be achieved clinically?

2.4 PCa Histology and Grading

In this section we provide more detail on grading of PCa. The emphasis here is upon histological change and does not reflect any changes in specific gene pathways.

Prostate Cancer is simply the growth of abnormal glandular like structures outside of the normal prostate glands the resulting continued growth of the cells making up those structures both within and without the prostate. The PCa cells take over the stroma, pushing aside the normal stromal cells and then migrate in a metastatic fashion throughout the body.

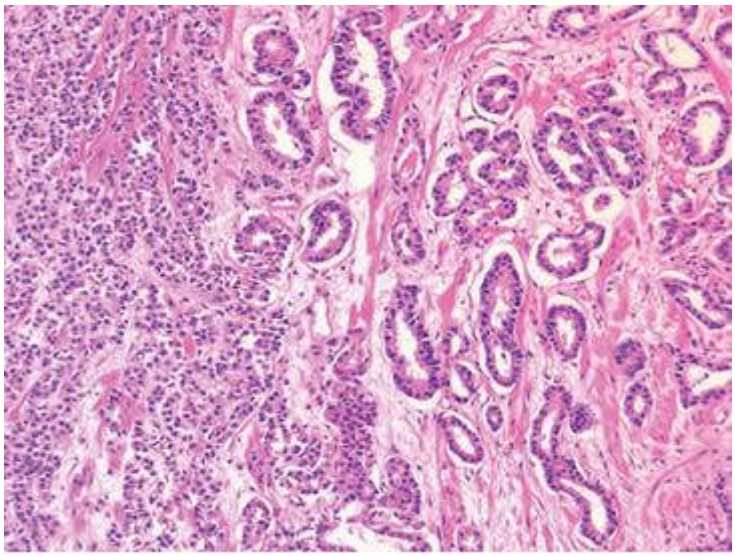
We will use the Gleason grading score as a means to characterize the level of cancer progression within the prostate.

2.4.1 Grading

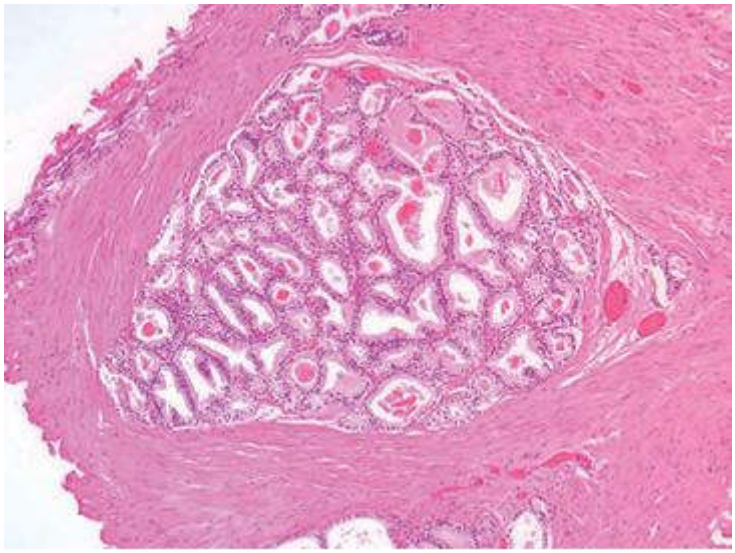
We present the grading system developed by Gleason. On the one hand this has been used as a gold standard for ascertaining future progress and yet it is still just a morphological tool. It fails to determine the pathways and regulators in a cell by cell basis.

2.4.1.1 Gleason 1

The following is a Gleason 1 grade tumor. Note that there are a proliferation of small glandular like clusters with dark basophillic stains and they are separate and have clear luminal areas. Gleason 1 is generally composed of many single and separate and closely packed glands of well circumscribed uniforms glands. One rarely sees Gleason 1 grade tumors, and they are often found as incidental findings when examining for other issues.



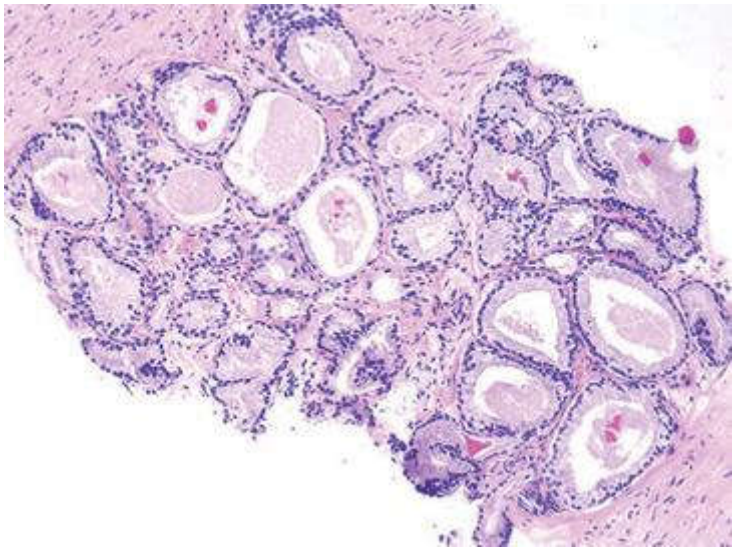
We show another view of a Gleason 1 below. This is especially descriptive of such a form because it appears almost as a single and isolated structure. The interesting question will be if this is PCa then if PCa is clonal is this cluster an aberrant outgrowth of a normal cells, if so which one, and if so is this just one cell growing. It appears that at this stage the intercellular signaling is still trying to function. However the clarity of cell form is being degraded.



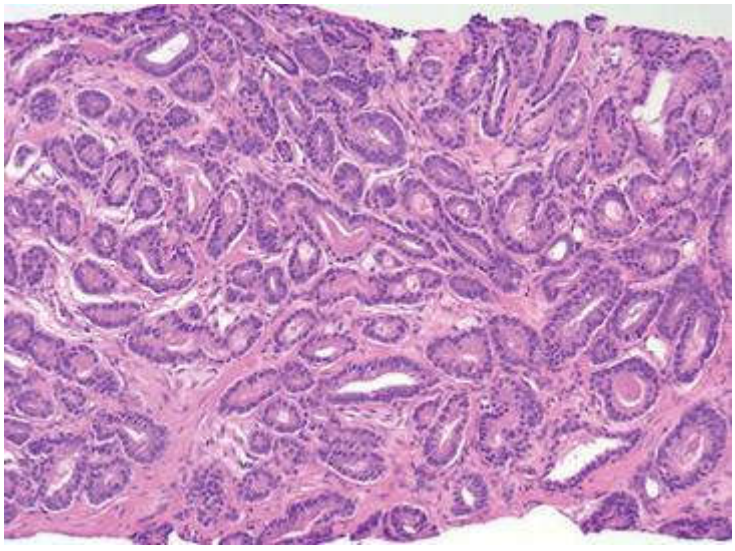
2.4.1.2 Gleason 2 and 3

Gleason 2 shows many newer glandular like cells but now of varying larger sizes. As Epstein notes: "*Grade 2 … is still fairly circumscribed, at the edge of the tumor nodule there can be minimal extension by neoplastic glands into the surrounding non-neoplastic prostate. The glands are more loosely arranged and not as uniform as Gleason 1*." We see those in the figure below which combines Gleason 2 and 3.

Gleason 3 is often composed of single glands. The Gleason 3 infiltrates in and amongst the non-neoplastic glands. Gleason 3 still can be seen as a separate gland and there are no single cells starting to proliferate. In Gleason 3 we still have some semblance of intercellular communications and coordination, albeit with uncontrolled intracellular growth. Again in the figure below we see both the smaller 2 and the larger 3 with gland structure being preserved and no separate cells proliferating.

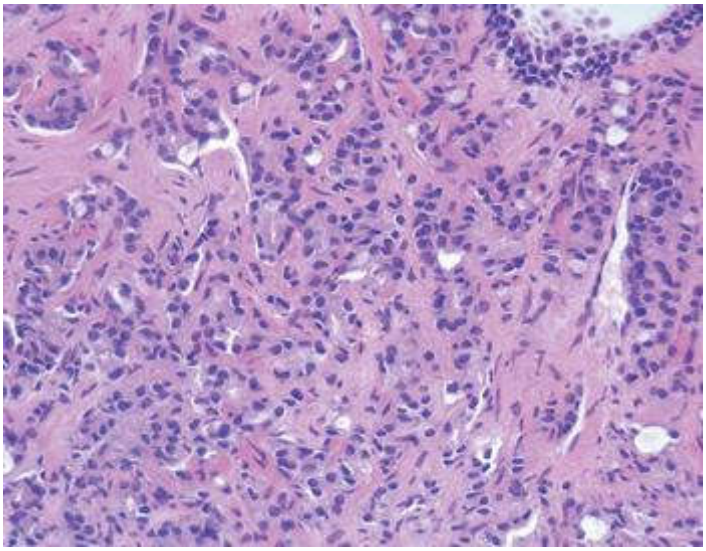


A Gleason 3 throughout is shown below.

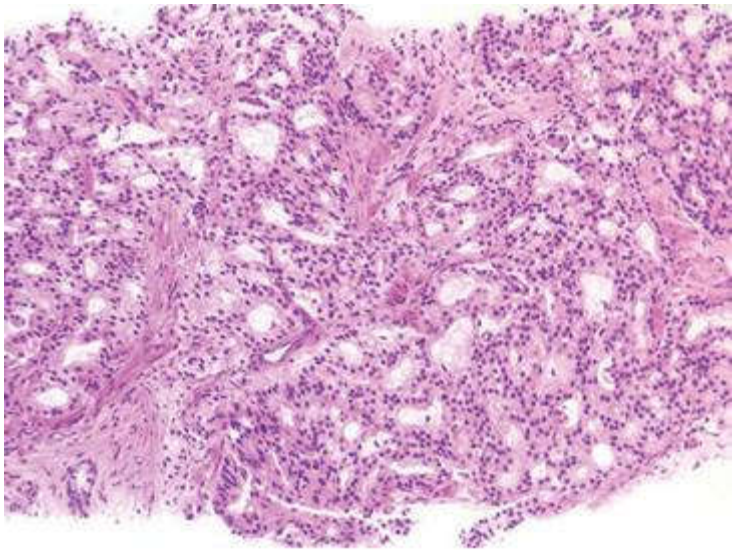


2.4.1.3 Gleason 4

Gleason 4 consists mostly of cribiform cells (perforated like a sieve) or fused and ill-defined glands with poorly formed glandular lumina. The glands appear to start to "stick" together. A Gleason 4 with a Gleason 3 is shown below. Note the sieve like structure and the closing of the glands.

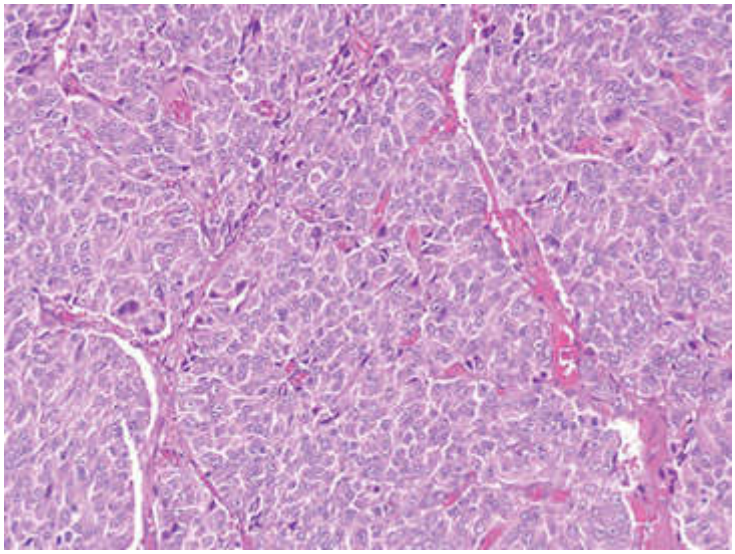


A Gleason all 4 is shown below. Note that the cells are sticking closed and the entire mass appears as a sieve like mass.



2.4.1.4 Gleason 5

Gleason 5 is a complete conversion to independent malignant cells. They have lost all intercellular coordination. As shown below it is a mass or mat or sheet of independent cancer cells and it has lost any of the sieve like structures. There may also appear to be some necrosis



2.4.2 Gleason Summary

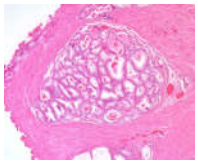
The Gleason scores are then determined by taking the predominant type and adding it to the secondary type. Thus a 4+3 yields a Gleason combined 7 but it is 4+3 and that is more aggressive than say a 3+4 with the same total score.

We repeat the grading commentary below.

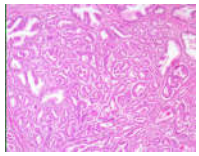
Gleason 1	Gleason 2	Gleason 3	Gleason 4	Gleason 5
Many acini with no basal layers and large nucleoli. Closely packed clumps of acini.	Many very small single separate glands (acini) with no basal layer and large nucleoli . Glands, acini, are more loosely arranged and not close packed.	Many small microglands extending throughout the stroma and out of the normal gland structure	Glands are now spread out and fused to one another throughout the stroma.	No gland structure seen, all luminal cells throughout the stroma with large nucleoli.

The following chart is a summary of the progression.

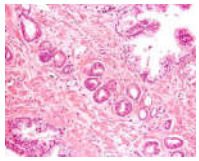
Gleason Grades



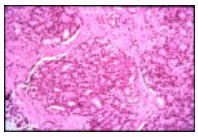
Gleason 1



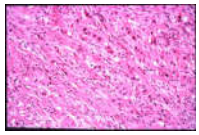
Gleason 2



Gleason 3



Gleason 4



Gleason 5

2.4.3 Models From Grading

In looking at the grading one may also hypothesize a possible path of progression. The steps appear to be:

1. Movement from existing benign gland to a separate but glandular like proliferation. Cells which would normally remain dormant go through a replication cycle, apoptosis and cell proliferation control seems lost. New glands appear clustered but appear separate.

2. Growth of the new glands makes them expand but remain morphologically glandular. They close packing begins to disappear and glands start to stand on their own. It is as if they are expanding and growing and the basal layer begins to disappear. Luminal like cancer cells start to be predominant.

3. Many small micro-glands start expanding and cell growth accelerates and the cells appear more cancer like but there is still some morphological glandular structure left.

4. The many glands have dramatically different shaped and start closing in one another and appear sieve like with small openings. They look as if they are losing any intercellular communications resulting is a common mat of cells.

5. Cells have lost any morphological form related to glands and appear as a mat of cancer cells replacing the stroma totally. No intercellular communications is left and cellular growth control has been eliminated totally.

These five steps are consistent with the Gleason grading but they also parallel the way the intracellular and intercellular controls are lost. We will look at these mechanism later.

2.5 REGRESSION

There has been some discussion of regression in the literature. We examine briefly three possible means here. However, there does not seem to have been any detailed clinical study or models, murine or otherwise, which have been used to ascertain the details which surround the regression issue. As we have seen above the current general understanding is that HGPIN is a clear and unambiguous predecessor of HGPIN, albeit regression is evident.

2.5.1 NSAID Regression

An interesting paper by Narayanan et al describes their work using NSAIDs as a means to reduce and in some cases eliminate PIN. They used specifically celecoxib and exisulind as the NSAID and they demonstrated that the use of these drugs did reduce PIN lesions. Now exactly why this happened one cannot determine. The authors present the factual results without any further interpretation. In addition there would not seem to be any rational explanation based upon the above overviews.

2.5.2 Androgen Deprivation Therapy Regression

In the paper by Kang et al they indicate that ADT, androgen deprivation therapy does reduce PIN[6]. They state:

Our results demonstrate that ADT does cause PIN regression, and that there is heterogeneity in this effect with respect to hormonal duration. We propose for future prospective, multi-centered, randomized trials in which ADT impact on PIN is characterized further….However PIN response to ADT was not uniform as 16% of patients with ADT longer than 6 months had residual PIN, suggesting variable sensitivity of PIN to ADT.

Kang et al also noted in another paper:

Eighteen patients initially diagnosed with PIN who had no ADT were identified, and 28 with PIN who had ADT were also assessed. All patients who had had no ADT had residual PIN, whereas seven of 28 receiving ADT had no residual PIN (P=0.043). The evaluation of ADT between responders and nonresponders showed a statistically significant association between PIN regression and the duration of ADT (P<0.001).

However, the response of PIN to ADT was not uniform, as 16% of patients on ADT for >6 months had residual PIN, suggesting variable sensitivity of PIN to ADT.

[6] http://meeting.ascopubs.org/cgi/content/abstract/24/18_suppl/4648

2.5.3 mTOR Inhibition

The mTOR gene can be activated by the Akt gene which in turn can be activated by the suppression of the PTEN gene. This is but a small segment of a pathway. mTOR then

Thus there seems to be an ability to eliminate PIN via ADT. In this case there is some clear pathway dependence. mTOR is short for "mammalian target for rapamycin"[7]. mTOR when positively enhanced by activation can result in cell growth by the up-regulation of protein synthesis. Akt regulates mTOR via the negative regulation of an intermediate pathway element the gene product TSC2 which inhibits mTOR.

By inhibiting TSC2 the inhibition of mTOR is reduced and in fact mTOR expression and actions are increased. It is this change which Majumder et al used to create PIN.

Majumder et al state that they were able to revers PIN in murine models by managing mTOR pathways. The use of rapamycin was e reasonable approach for pathway control. Akt induced PIN was totally controlled by mTOR and reversal allowed regression of the PIN.

The above three are a few of the known mechanisms related to regression. There may be many others yet to be determined but the existence of these may assist in understanding the possible options.

2.6 SUMMARY

One can gain some insight into PCa and its evolution by understanding the histological changes. PCa starts out with a simple glandular structure, and then as a result of many changes begins to have within the gland excess growth, thus the PIN, and then the growth of new quasi glands, small and somewhat poorly shaped ones, which become the early signs of PCa. Then the differentiation of gland and stroma begins to disappear until the glandular elements are almost blocked from any possible view. The malignant cells have taken over the prostate and at this stage metastasis may very well have begun as well.

It will be useful to maintain a reasonably high level understanding of these cellular changes. They will be the driver of any model. We will now move on to understanding the genetic factors related to these changes.

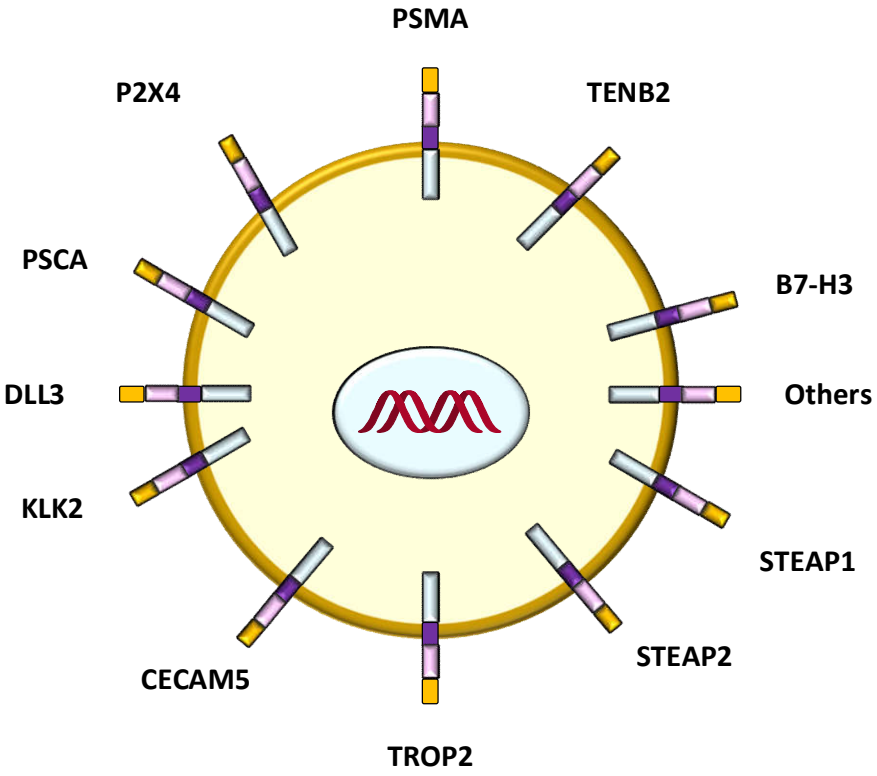
[7] Bunz, pp 192-194.

3 SURFACE TARGETS

There has been extensive studies attempting to determine PCa cell surface markers. We examine a few of the most examined herein. There are several question that should be addressed in each. Namely:

1. What causes the surface marker to be generated
2. What is the function of the surface marker internally in the cell
3. What drives the surface marker externally
4. What are the immune system responses to this surface marker
5. How specific is this surface marker
6. What other cells does this marker appear
7. What is the correlation between surface markers
8. Is the surface marker an active entity in the malignant state or merely a target for attack

Many of these questions have yet to be answered for the markers shown below.



3.1 PSMA

PSMA is also known as the Prostate Specific Membrane Antigen, is a surface protein which is highly expressed on prostate cells, especially those that are androgen resistant and metastatic. It has been used as a target for PET scans and somewhat for prognostic evaluation. The effects of PSMA have been understood to some degree whereas its expression control does not yet seem to be fully understood. PSMA is both correlative and causative of PCa metastatic growth. It also presents an interesting cell target for a variety of therapeutic strategies.

3.1.1 Gene and Protein

We begin with the NCBI definition which notes[8]:

This gene encodes a type II transmembrane glycoprotein belonging to the M28 peptidase family. The protein acts as a glutamate carboxypeptidase on different alternative substrates, including the nutrient folate and the neuropeptide N-acetyl-l-aspartyl-l-glutamate and is expressed in a number of tissues such as prostate, central and peripheral nervous system and kidney.

A mutation in this gene may be associated with impaired intestinal absorption of dietary folates, resulting in low blood folate levels and consequent hyperhomocysteinemia. Expression of this protein in the brain may be involved in a number of pathological conditions associated with glutamate excitotoxicity.

In the prostate the protein is up-regulated in cancerous cells and is used as an effective diagnostic and prognostic indicator of prostate cancer. This gene likely arose from a duplication event of a nearby chromosomal region. Alternative splicing gives rise to multiple transcript variants encoding several different isoforms.

We now present a summary of what is understood about PSMA. As Caromile et al note:

*PSMA is a **750–amino acid type II transmembrane peptidase enzyme** that is encoded by the folate hydrolase 1 (FOLH1) gene. Although PSMA is also known as glutamate carboxypeptidase II, N-acetyl-Laspartyl-L-glutamate peptidase I, and N-acetylaspartylglutamate peptidase, those studying PCa or general oncology commonly use the term PSMA, which will be used here.*

It has been shown that PSMA is present in low amounts on prostate epithelial cells and is progressively up-regulated during disease progression in prostate tumors, in which it correlates negatively with prognosis and consequently may be a promising tool for the diagnosis, detection, localization, and treatment of PCa.

Currently, PSMA is used as an immunoscintigraphic target in the clinic to direct therapy to androgen-independent prostate tumors. RNA aptamers selectively targeting PSMA enzymatic activity have also been successful in slowing primary tumor growth in murine models.

[8] https://www.ncbi.nlm.nih.gov/gene/2346

Although we have previously shown that endothelial-expressed PSMA regulates angiogenesis and retinal neovascularization primarily via b1 integrin–mediated cell adhesion, an important functional role for PSMA in PCa has not been demonstrated.

Caromile et al continue:

Here, we report that expression of PSMA in prostatic epithelial cells directly underlies prostate tumor progression in vivo. We found that tumors in wild-type animals were larger and of higher grade with a higher microvessel density as compared to tumors in the PSMA knockout animals, which is consistent with our previous results implicating PSMA as an angiogenic regulator.

In addition, PSMA-positive tumor cells were viable at greater distances from the vasculature than their PSMA knockout counterparts, suggesting that cell-intrinsic survival components also contribute to tumor growth.

Accordingly, wild-type tumors expressed relatively greater amounts of IGF-1R and exhibited greater activation of the phosphatidylinositol 3-kinase (PI3K)–AKT pathway, whereas tumors lacking PSMA not only had decreased IGF-1R expression but also had diverted signaling downstream of PI3K-AKT to the mitogen-activated protein kinase (MAPK)–extracellular signal–regulated kinases 1 and 2 (ERK1/2) pathway, consistent with a PSMA-dependent signaling switch.

Moreover, manipulation of PSMA expression in mouse TRAMP-C1 cell lines and human PCa cell lines recapitulated this change in signaling. Analysis of publicly available gene expression data sets from PCa samples confirmed that high PSMA expression was predictive of a high Gleason score.

In addition, patient samples with high PSMA expression and high Gleason scores displayed a prosurvival gene expression signature with increased expression of the antiapoptotic marker survivin and IGF-1R, consistent with a role for PSMA in the regulation of signal transduction in human PCa disease as well. Therefore, in addition to its role as a PCa marker and target, our results indicate that increasing amounts of PSMA in prostate tumor epithelium serve to drive prosurvival mechanisms and thus identify it as a functional regulator of prostate tumor progression. These findings also suggest that PSMA-positive tumors may be more sensitive to PI3K pathway inhibitors and less sensitive to MAPK pathway inhibitors.

3.1.2 Functions

What function does PSMA play? Science Signalling notes Conway et al who observe:

Prostate-specific membrane antigen (PSMA) is so-named because its expression is enhanced in advanced prostate carcinomas, where its increased presence correlates with a poor prognosis. The protein is also called glutamate carboxypeptidase II and is a transmembrane protein with peptidase activity.

PSMA has been found in endothelial cells in tumor vasculature. Given roles of other peptidases in angiogenesis*, Conway et al. explored the possibility of such a role for PSMA. They used an in vivo angiogenesis assay in knockout mice lacking PSMA to show that loss of the PSMA protein inhibited formation of new blood vessels.*

Proteolysis contributes to remodeling of the extracellular matrix that is necessary for angiogenesis, but further studies by the authors suggest that PSMA may instead be part of a complex regulatory loop that controls integrin signaling and activation of the p21-activated kinase 1 (PAK1). In vitro cell invasion studies with PSMA-null cells or with inhibitors of the enzyme showed that PSMA has an important role in cell invasion and in signaling from β1 integrins to focal adhesion kinase (FAK) and PAK1.

The authors confirmed that PSMA interacts with the actin-binding protein filamin A. Disruption of this interaction with a peptide designed to compete with PMSA for binding to filamin A decreased the peptidase activity of PMSA and decreased phosphorylation of PAK1 in cultured cells. PAK1 also interacts with filamin A, and the authors propose that it may compete with PMSA for binding to filamin A.

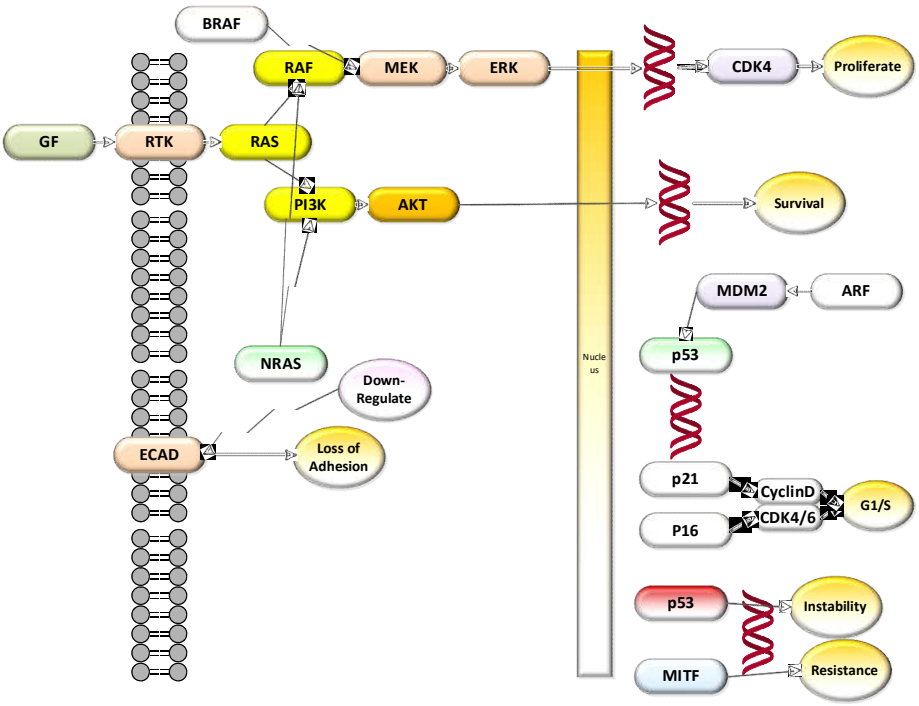
The interaction of PMSA and the cytoskeletal protein filamin A may allow a feedback signal from integrin β1 and PAK to keep PMSA activity in check. Inhibition of PAK by expression of a peptide corresponding to its autoinhibitory domain enhanced association of PMSA with filamin A, increasing its peptidase activity. Further understanding of PMSA's roles in control of angiogenesis may allow new strategies to inhibit angiogenesis in cancers and other diseases to which it contributes.

Thus PSMA can become a significant driver of a multiplicity of downstream proteins and genes.

3.1.3 Downstream Pathways

PSMA is also known as the Prostate Specific Membrane Antigen. It is a putative target for attacking malignant prostate cancer cells. There has been recent interest in this transmembrane protein as a target for various imaging modalities. Moreover, it has an interest as a target for a multiplicity of therapeutic modalities. We examine this marker as a means for several of these therapeutic modalities. The objective is to consider how we can “engineer” a therapeutic strategy using the many tools now available.

One of the targets for PSMA is AKT. We show below a generic flow on actions with AKT at a central role.



As Kaittanis et al have noted:

Prostate-specific membrane antigen (PSMA) or folate hydrolase 1 (FoLH1) is highly expressed on prostate cancer. Its expression correlates inversely with survival and increases with tumor grade. However, the biological role of PSMA has not been explored, and its role in prostate cancer remained elusive. Filling this gap, we demonstrate that in prostate cancer,

PSMA initiates signaling upstream of PI3K through G protein–coupled receptors, specifically via the metabotropic glutamate receptor (mGlur)*.*

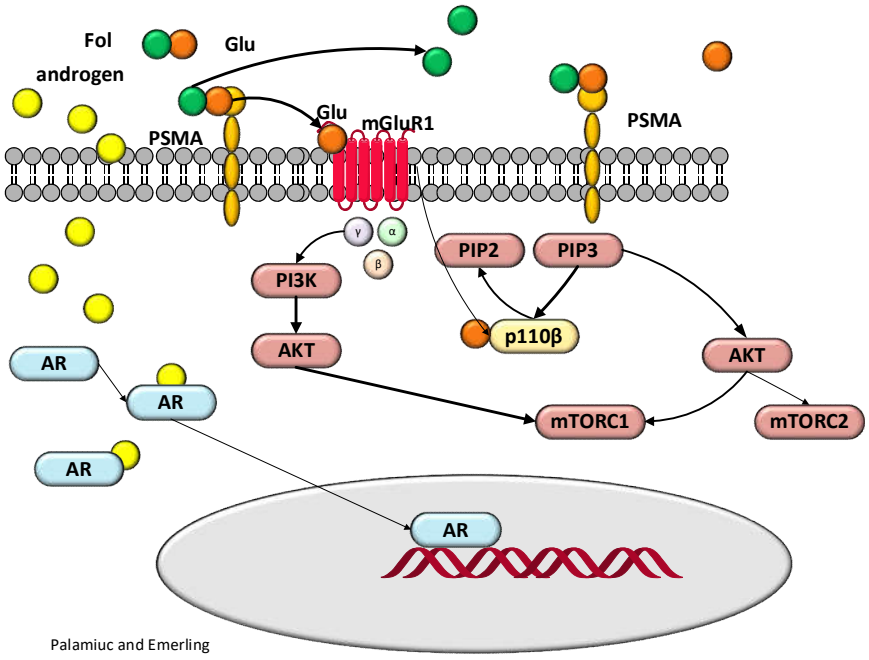
PSMA's carboxypeptidase activity releases glutamate from vitamin B9 and other glutamated substrates, which activate mGlur I. Activated mGlur I subsequently induces activation of phosphoinositide 3-kinase (PI3K) through phosphorylation of p110β independent of PtEn loss. the p110β isoform of PI3K plays a particularly important role in the pathogenesis of prostate cancer, but the origin of its activation was so far unknown.

PSMA expression correlated with PI3K–Akt signaling in cells, animal models, and patients. We interrogated the activity of the PSMA–PI3K axis through positron emission tomography and magnetic resonance imaging. Inhibition of PSMA in preclinical models inhibited PI3K signaling and promoted tumor regression. our data present a novel oncogenic signaling role of PSMA that can be exploited for therapy *and interrogated with imaging*

What is attractive in this case is that there appears in PSMA to be a cell surface marker targetable in malignant cells. We examine this marker in some detail and then examine ways in which it can be utilized in a therapeutic manner. For example, we can use PSMA as an epitope for immunotherapeutic attack. We could also use it as a target for viral insertion. Thirdly we could

use bi-specific antibodies for the delivery of cancer attacking therapeutics, in short it becomes a useful target for a variety of therapeutic application.

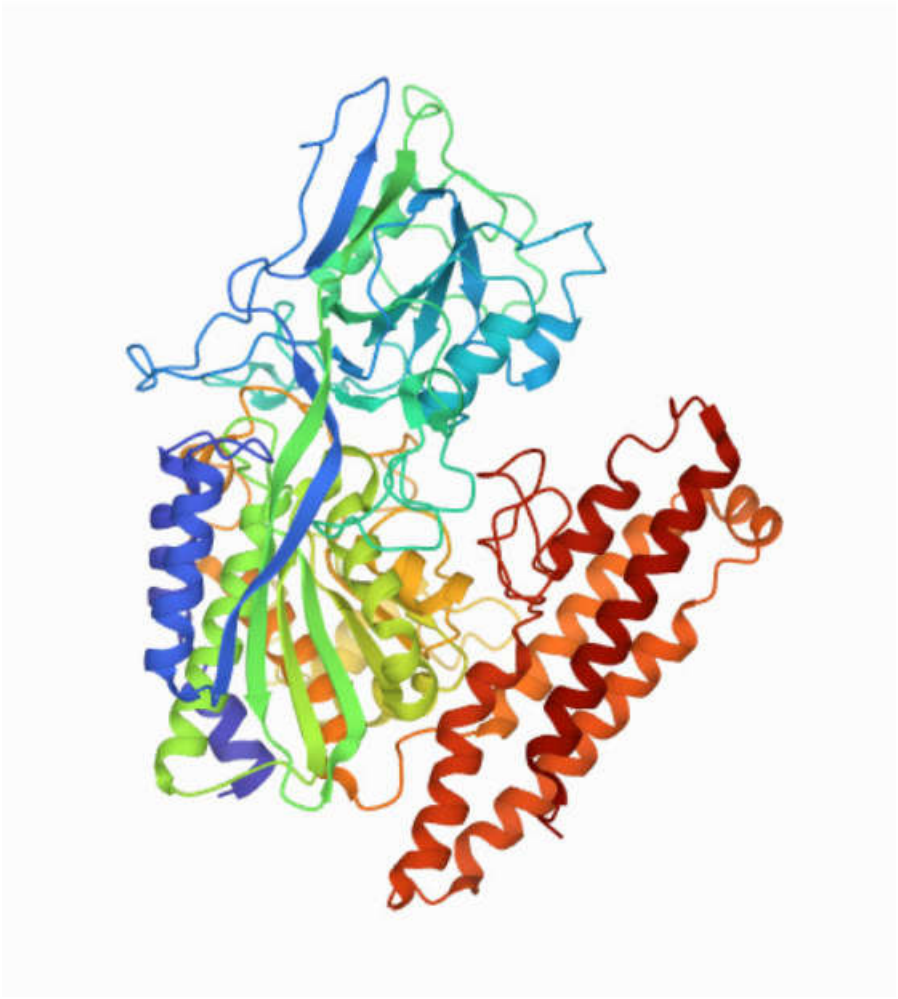
The authors, Palamiuc and Emrling, also note the dynamics using the PSMA work of Kaittanis et al, as follows:



Note the PSMA releases glutamate from the bound form and this glutamate then binds to the receptor and activates Akt. We shall examine this in detail in the next section. Now PSMA protein[9] containing 695 nucleic acids is shown below.

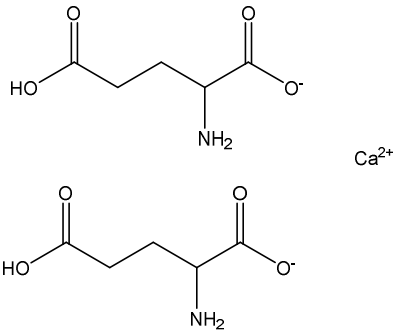
Details of the PSMA protein structure are shown in the following.

[9] https://www.rcsb.org/structure/1Z8L

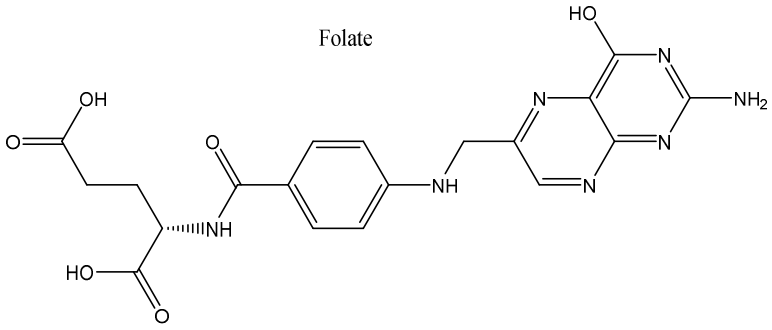


We also provide below the chemical forms of Glu and Fol as are effective in this model.

Glutamate



Folate



3.2 TENB2

TENB2 is also known as TMEFF2 as well as other names[10]. As NCBI notes:

This gene encodes a member of the tomoregulin family of transmembrane proteins. This protein has been shown to ***function as both an oncogene and a tumor suppressor depending on the cellular context and may regulate prostate cancer cell invasion****. Multiple soluble forms of this protein have been identified that arise from both an alternative splice variant and ectodomain shedding. Additionally, this gene has been found to be hypermethylated in multiple cancer types.*

Now Boswell et al have reported on TENB2:

TENB2, a transmembrane proteoglycan protein, is a promising target for antibody drug conjugate (ADC) therapy due to overexpression in human prostate tumors and rapid internalization.

We previously characterized how predosing with parental antiTENB2 monoclonal antibody (mAb) at 1 mg/kg in a patient-derived LuCap77 explant model with high (3+) TENB2 expression could (i) block target-mediated intestinal uptake of tracer (< 0.1 mg/kg) levels of radiolabeled

[10] https://www.ncbi.nlm.nih.gov/gene/?term=tenb2+homo

anti-TENB2-monomethyl auristatin E ADC while preserving tumor uptake, and (ii) maintain efficacy relative to ADC alone.

Here, we systematically revisit this strategy to evaluate the effects of predosing on tumor uptake and efficacy in LuCap96.1, a low TENB2-expressing (1+) patient-derived model that is more responsive to ADC therapy than LuCap77. Importantly, rather than using tracer (< 0.1 mg/kg) levels, radiolabeled ADC tumor uptake was assessed at 1 mg/kg – one of the doses evaluated in the tumor growth inhibition study – in an effort to bridge tissue distribution (PK) with efficacy (PD).

Predosing with mAb up to 1 mg/kg had no effect on efficacy. These findings warrant further investigations to determine whether predosing prior to ADC therapy might improve therapeutic index by preventing ADC disposition and possible toxicological liabilities in antigen-expressing healthy tissues.

Georgescu et al have examined this gene extensively. They note:

MEFF2 is an androgen regulated transmembrane protein mainly restricted to brain and prostate. Our studies in PCa demonstrate a role of TMEFF2 as a tumor suppressor. Furthermore, studies using limited numbers of clinical samples, reveal changes in the expression of Tmeff2 with disease stage in PCa and gliomas, supporting an important role of Tmeff2 in these diseases. ...

Boswell et al also note:

The development of novel ADC therapies represents a promising strategy in the treatment of prostate cancer.

However, target expression in normal tissue continues to be challenging for the clinical development of ADCs. ***We previously tested the hypothesis that predosing of unconjugated anti-TENB2 mAb at an optimal dose will saturate specific binding sites for the antibody in normal tissue sinks while retaining sufficient tumor upta****ke [20]. This approach relies upon a similar biodistribution, but very different toxicity profiles between mAb and ADC. It is assumed that the level of predose must be fine-tuned in order to avoid saturating tumor receptors.*

However, it is also plausible that a predose may have a differential ability to block low level antigen expression in a highly perfused tissue sink, while leaving the majority of antigens in a less readily accessible solid tumor microenvironment available for subsequent ADC therapy. Various impediments to the delivery of antibodies to solid tumors have been widely discussed and studied, especially in the context of microspatial distribution [25, 26].

More recent work has suggested that administering a cocktail of mAb and ADC can have better tumor penetration and efficacy than the ADC alone [27]. These data seem to support the original concept of a 'binding site barrier' proposed by Weinstein nearly three decades ago [28, 29]. Weinstein postulated that, in cases wherein (i) tumor cells express receptors in very high copy numbers and/or (ii) an antibody binds tumor receptors very tightly, it is plausible that an

antibody therapeutic may have limited spatial penetration throughout a tumor due to its 'consumption' by the first few layers of tumor cells proximal to the blood vessel from which it extravasated.

It is possible that such a binding site barrier could explain the anomalously poor efficacious response of LuCaP77 (3+) to ADC therapy despite high target expression. In contrast, the LuCaP96.1 (1+) might have better response to ADC therapy since the ADC would achieve better penetration throughout the tumor allowing the cytotoxic payload to reach a greater number of overall cells due to lower expression levels.

However, besides target expression, differences in other factors like multi-drug resistance [9], sensitivity to MMAE, levels of antigen shedding, and/or tumor explant vascularization could also contribute to these observations. Although our results do not preclude any of the above mechanisms, the much lower tumor uptake of radiolabeled ADC in LuCaP96.1 (~ 30%ID/g at 72 h), relative to previous results in LuCaP77 (> 300%ID/g at 72 h), confirm that the superior efficacy in the 1+ expressing explant model cannot be explained by superior uptake on a whole tumor basis. We have no experimental evidence that significant levels of antigen shedding occur for either of these patient-derived explant models. Furthermore, we confirmed that the considerable tumor uptake of radiolabeled ADC observed in both LuCap77 and LuCap96.1 models was roughly proportional to antigen expression level, suggesting that antigen shedding likely does not play a major role in ADC disposition.

3.3 B7-H3

B7-H3 has recently become a putative target for PCa. In a recent conference, work reported by Carmichael has discussed this possibility[11]. It is noted:

B7-H3 (CD276) is a member of the B7 family of immunomodulatory glycoproteins, which includes PD-L1/B7-H1. Notably, PD-L1 is not expressed in most prostate cancers. B7-H3 is expressed in human placenta, but few other normal tissues. It is commonly overexpressed in prostate and other cancers, including ovarian cancer and its overexpression is correlated with worse survival outcomes. B7-HR's functions include immune stimulation/suppression (context-dependent), tumor growth, survival, and metastasis. …

Patients with mismatch repair deficient (MMRd) tumors historically have poor prognoses, although this is now changing with the advent of immune checkpoint inhibitors which may lead to durable responses in this population. These patients are known to have higher PD-L1 expression, with 40% of MMRd mutated tumors having ≥1% PD-L1 positive cells (versus only 10% of MMR normal tumors). These patients have increased T-cell lymphocyte infiltration, which has made them a target for immune checkpoint inhibitors. …

B7-H3 was found to be expressed on tumor cells, but not the stroma. There were interspersed B7-H3 expressing and non-expressing cells, often within close proximity (20-39 µm), which has

[11] https://www.urotoday.com/conference-highlights/esmo-2024/esmo-2024-prostate-cancer/154827-esmo-2024-b7-h3-as-therapeutic-target-for-prostate-cancer.html

important treatment implications, given that these negative cells may be subject to a 'bystander' tumor-killing effect targeting the B7-H3 positive cells. … Next, from a clinical standpoint, there are numerous ways of targeting B7-H3 in clinical practice. These include:

- *'Naked' antibodies that block the receptor*
- *Antibody-drug conjugates*
- *Targeted radioligand therapy*
- *Bi-specific antibodies that simultaneously target B7-H3 and T cells*
- *Bi-specific killer engagers (BIKEs) and tri-specific killer engagers (TriKEs)*
- *CAR-T and CAR-NK cells*

Carmichael highlighted the trial of vobramitamab duocarmazine (NCT05551117) in mCRPC patients. Vobramitamab duocarmazine is a humanized anti-B7-H3 IgG1 monoclonal antibody with a topoisomerase 1 inhibitor payload. Part 1 (dose escalation) demonstrated good tolerability with early signs of anti-tumor activity. 12.0 mg/kg was selected as the dose for Part 2 (dose expansion).

Carmichael concluded as follows:

- *Membranous B7-H3 is frequently overexpressed in CRPC. It is frequently already present at initial diagnosis and associated with DNA repair defects*
- ***B7-H3 expression is relatively homogenous in CRPC, with positive and negative cells in close proximity***
- *The exact function of B7-H3 in prostate cancer and whether it is a key driver of tumour growth remains unknown*
- ***B7-H3 is minimally expressed in normal tissue and is, therefore, an attractive therapeutic target***
- *B7-H3 antibody-drug conjugates have potent and selective anti-tumor activity in B7-H3 positive human CRPC models, with early signs of anti-tumor activity in trials*
- ***B7-H3 targeting has huge promise against mCRPC***

This is a prototypical example of targeting specific proteins on cancer cells. The therapeutic approaches are consistent in what we shall discuss herein. We shall now examine this protein in more detail.

From Getu et al:

The biology of B7-H3 B7 family proteins

The B7 family proteins are a type of integral membrane proteins found on activated antigen-presenting cells and consists of structurally related cell-surface protein ligands that bind to receptors on lymphocytes. B7.1 (CD80) and B7.2 (CD86) are the two major types of B7 proteins, but currently, there are other proteins grouped in the B7 family, including inducible co-stimulator ligand (ICOS-L), and co-inhibitory programmed death-1 ligand (PD-L1), programmed death-2 ligand (PD-L2), B7-H3, and B7-H4 (Table 1).

The B7 family produces a costimulatory or a coinhibitory signal to enhance or decrease the activity of the MHC-TCR signal between the antigen presenting cells (APC) and the T cells. Interaction of B7-family members with costimulatory receptors augments immune responses while interaction with coinhibitory receptors attenuates immune responses [8, 9]. B7-H3 shares 20–27% amino acid identity with other B7 family members [10]. It is a type-I transmembrane protein that primarily functions as a negative immunoregulatory protein, and is overexpressed in various human tumor tissues [4–6, 11].

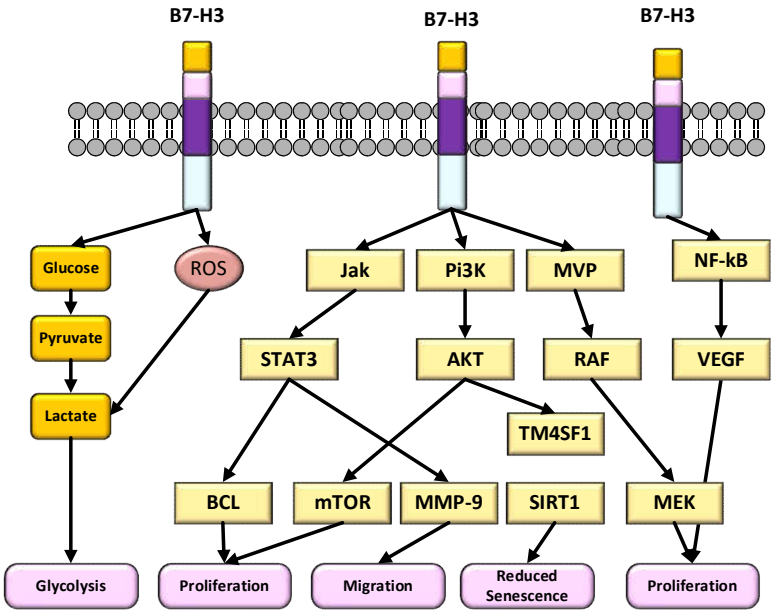
Structure of B7-H3

The basic structure (2Ig form) of B7-H3 contains a single pair of IgV-like and IgC-like immunoglobulin domains, a transmembrane region, and a short highly diverse cytoplasmic tail . The dominantly expressed form of human 4IgB7-H3 contains tandemly duplicated VC domains with four Ig-like domains. Although human B7-H3 has two isoforms (2IgB7-H3 and 4IgB7- H3), the mouse B7-H3 has only one isoform (2IgB7-H3). Serine and arginine-rich splicing factor 3 (SRSF3) involves the splicing of B7-H3 by directly binding to its exon 4 and/or 6. B7-H3 crystallized as an unusual dimer arising from the exchange of the G strands in the IgV domains of partner molecules, which indicates the dynamic nature and plasticity of the immunoglobulin fold

The Cellular Localization of B7-H3

B7-H3 has been observed to be expressed in different cellular compartments and different cancer types may have different B7-H3 localization profiles. Several immunostaining results show B7-H3 was expressed on the cell membrane and in cytoplasm of tumor tissues

The pathway controls of B7-H3 are as shown below:



3.4 STEAP1

Danila et al have reported on a transmembrane protein STEAP1:

Six-transmembrane epithelial antigen of the prostate 1 (STEAP1) is highly expressed in prostate cancers.

DSTP3086S is a humanized immunoglobulin G1 anti-STEAP1 monoclonal antibody linked to the potent antimitotic agent monomethyl auristatin E*. This study evaluated the safety and activity of DSTP3086S in patients with metastatic castration-resistant prostate cancer…. Six-transmembrane epithelial antigen of the prostate 1 (STEAP1) is a multitransmembrane protein believed to act as an ion channel or transporter protein.*

As a cell surface protein frequently expressed in prostate cancer, with limited expression in nonprostate tissues, STEAP1 is an ideal candidate for antibody-derived therapies in patients with mCRPC.

DSTP3086S is an antibody-drug conjugate (ADC) that contains the humanized immunoglobulin G1 antiSTEAP1 monoclonal antibody MSTP2109A linked through a protease labile linker, maleimidocaproylvaline-citrulline p-aminobenzyloxycarbonyl, to a potent antimitotic agent, monomethyl auristatin E (MMAE). …

In summary, DSTP3086S demonstrated an acceptable safety profile, with evidence of antitumor activity confirming that the targeting of STEAP1-expressing mCRPC tumors with an ADC is feasible. Although DSTP3086S would require optimization for further clinical development, these data may inform development of novel ADCs, chimeric antigen receptor T cells, and immune cell–recruiting bispecific antibodies that target STEAP1.

This appears to be another attractive target. If we were to generate a polyAb with targets of STEAP1 and PSMA then one suspects the specificity would be exceptionally high. It is through mechanisms such as these that we believe both therapeutic delivery and immunotherpeutics can be successfully achieved. However we still face the TME issues which we believe can dominate mets in PCa.

3.5 STEAP2

As NCBI notes[12]:

This gene is a member of the STEAP family and encodes a multi-pass membrane protein that localizes to the Golgi complex, the plasma membrane, and the vesicular tubular structures in the cytosol. A highly similar protein in mouse has both ferrireductase and cupric reductase activity, and stimulates the cellular uptake of both iron and copper in vitro. Increased transcriptional expression of the human gene is associated with prostate cancer progression.

[12] https://www.ncbi.nlm.nih.gov/gene/261729

As Wang et al note:

*One of the prostate-specific genes upregulated in prostate cancer is **STAMP1 (also known as STEAP2**). STAMP1 expression is **androgen independent but mainly occurs in androgen receptor (AR)–positive cells, suggesting that AR signaling may have a role in its expression[13].***

Furthermore, STAMP1 expression is significantly increased in prostate cancer compared with normal prostate. STAMP1 was found to localize to the Golgi, trans-Golgi network, and the plasma membrane and may have a role in endocytic/secretory trafficking pathways (6). STAMP1 belongs to a recently discovered six-transmembrane protein family. STAMP2 (also known as STEAP4 and TIARP) is another member of this family whose expression is increased in prostate cancer compared with matched normal prostate epithelial cells (6), which may also have a role in metabolic disease.

Other members of the STAMP family include pHyde, a rat protein that has been implicated in apoptosis of prostate cancer cells, and its human homologue TSAP6 (also known as STEAP3), a p53-inducible gene involved in apoptosis and the cell cycle in prostate cancer and HeLa cells. Recent reports indicate that STAMP family members have ferrireductase and cupric reductase activities in HEK-293T cells …

In addition to extending this to larger numbers of specimens, immunohistochemical analysis indicated that STAMP1 was localized in the cytosol and the cell membrane of human prostate cancer cells in situ. Furthermore, STAMP1 expression was increased, especially the fraction localized to the plasma membrane, in cancer cells compared with normal prostate. These data are consistent with the in vitro and in vivo studies and indicate that STAMP1 is involved in prostate cancer growth.

They summarize as follows:

1. ***STAMP1 increases cell proliferation***
2. ***STAMP1 affects cell cycle–related gene expression***
3. ***Downregulation of STAMP1 significantly increases apoptosis***
4. *STAMP1 knockdown inhibits growth of human prostate cancer xenografts*
5. *STAMP1-regulated ERK activation in human prostate cancer cells*
6. *Downregulation of STAMP1 significantly increases apoptosis induced by TRAIL or combination of TRAIL and AKT inhibitor*
7. *STAMP1 expression is upregulated in human prostate cancer specimens*

3.6 TROP2

[13] https://www.researchgate.net/publication/370125480_Androgen_Receptor_Whither_Goest_Thou

TROP2 is a cell surface receptor that transduces calcium signals[14]. As Shvartsur and Bonavida note:

Trop2 has been implicated in numerous intracellular signaling pathways. Trop2 transduces an intracellular calcium signal.

Trop2-induced signal transduction can occur without extracellular Ca2+, suggesting a mobilization of Ca2+ from internal stores. Specific antibodies are used for cross-linking Trop2. This cross-linking leads to a significant rise in cytoplasmic Ca2+ . Trop2 provides crucial signals for cells with requirements for proliferation, survival, self-renewal, and invasion.

Trop2 has several ligands, inlcluding claudin-1, claudin-7, cyclin D1, and potentially IGF-1.

Trop2 has stem cell-like qualities and regulates cell growth, transformation, regeneration, and proliferation, which explains why its overexpression can lead to tumor progression. It is expressed on the surface of many stem/progenitor cells and has a role in maintaining tight junction integrity.

Trop2 might be a modulator and/or an enhancer of EpCAM-induced cell signaling.

Trop2 modulation of EpCAM can cause EpCAM to proliferate and migrate into liver parenchyma.

Trop2 can foster cell migration without the presence of growth factors. Induced foci formation represents a loss of the ability to maintain cell growth and movement.

Regulated Intramembrane Proteolysis (RIP) is required for Trop2 activity; it is necessary for Trop2's enhanced cell growth and self-renewal activity in prostate cancer. RIP cleaves Trop2 through the TNF-α converting enzyme (TACE) followed by γ-secretase cleavage within the transmembrane domain. Cleavage is mediated by presenilin 1 (PS-1), which is the dominant enzyme, and presenilin 2 (PS-2). This cleavage makes two products, namely the extracellular domain (ECD) and the intracellular domain (ICD). The ECD is shed and found only on the plasma membrane and in the cytoplasm.

Secreted ECD causes an increase in sphere size but not in sphere number, which suggests that the ECD increases the proliferation of progenitor cells, specifically of prostate stem cells. Treating prostate cells with secreted ECD leads to the appearance of small 6 kD fragments, suggesting Trop2 cleavage. It is uncertain whether the ECD induces Trop2 cleavage via distinct binding partner interactions or through direct hydrophilic interactions. The ICD is released from the membrane, for the most part, and accumulates in the nucleus. Nuclear ICD is only detected in cancer specimens.

[14] https://www.ncbi.nlm.nih.gov/gene/4070

Cleavage and activation is required for its transformation activity and it has been associated with human prostate cancer, but it could also be associated with other cancers. The ICD is the functionally dominant part of Trop2.

It promotes self- renewal, initiates prostatic intraepithelial neoplasia (PIN) and is involved in a β-catenin-dependent signaling cascade[15].

As Foersch et al note:

TROP2 is a trans-membranous protein expressed in a variety of normal tissues (especially trophoblast cells and squamous epithelia) and physiologically acts as a calcium signalling transducer that regulates cell growth, migration, and proliferation. TROP2-positive epithelia have been linked with stem cell properties in normal tissues of several organs and TROP2 expression has been linked with an adverse prognosis in a variety of cancers.

For CRC, a recent study investigated TROP2 expression in metastatic CRC and demonstrated prognostic relevance in this subgroup. However, the association and the prognostic value of TROP2 in comparison to conventional histopathological parameters (tumour budding, tumour grade, histopathological subtypes) is still poorly understood and has not yet been comprehensively studied in large CRC collectives

As Ajkunic notes:

Therapeutic approaches targeting proteins on the surface of cancer cells have emerged as an important strategy for precision oncology. To capitalize on the potential impact of drugs targeting surface proteins, detailed knowledge about the expression patterns of the target proteins in tumor tissues is required. In castration-resistant prostate cancer (CRPC), agents targeting prostate-specific membrane antigen (PSMA) have demonstrated clinical activity. However, PSMA expression is lost in a significant number of CRPC tumors.

]The identification of additional cell surface targets is necessary to develop new therapeutic approaches. Here, we performed a comprehensive analysis of the expression heterogeneity and co-expression patterns of trophoblast cell-surface antigen 2 (TROP2), delta-like ligand 3 (DLL3), and carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) in CRPC samples from a rapid autopsy cohort.

We show that DLL3 and CEACAM5 exhibit the highest expression in neuroendocrine prostate cancer (NEPC), while TROP2 is expressed across different CRPC molecular subtypes, except for NEPC.

We further demonstrated that AR alterations were associated with higher expression of PSMA and TROP2. Conversely, PSMA and TROP2 expression was lower in RB1-altered tumors. In

15 https://www.researchgate.net/publication/325047485_PROSTATIC_INTRAEPITHELIAL_NEOPLASIA_PROGRESSION_REGRESSION_A_MODEL_FOR_PROSTATE_CANCER

addition to genomic alterations, we show a tight correlation between epigenetic states, particularly histone H3 lysine 27 methylation (H3K27me3) at the transcriptional start site and gene body of TACSTD2 (encoding TROP2), DLL3, and CEACAM5, and their respective protein expression in CRPC patient-derived xenografts. Collectively, these findings provide insights into patterns and determinants of expression of TROP2, DLL3, and CEACAM5 with implications for the clinical development of cell surface targeting agents in CRPC.

3.7 CEACAM5

CEACAM5 is described by NCBI as follows[16]:

This gene encodes a cell surface glycoprotein that represents the founding member of the carcinoembryonic antigen (CEA) family of proteins. The encoded protein is used as a clinical biomarker for gastrointestinal cancers and may promote tumor development through its role as a cell adhesion molecule. Additionally, the encoded protein may regulate differentiation, apoptosis, and cell polarity. This gene is present in a CEA family gene cluster on chromosome.

As Ajkunic et al note:

Of the constantly expanding spectrum of cell-surface targets in oncology, delta-like ligand 3 (DLL3), carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), and trophoblast cell-surface antigen 2 (TROP2) have been a focus for pre-clinical and clinical drug development efforts for advanced PC23–28.

DLL3 is a ligand that inhibits the Notch signaling pathway and is expressed in the spinal cord and nervous system during embryonic development24. Importantly, DLL3 is expressed at high levels in the majority of tumors that exhibit high-grade neuroendocrine/small cell carcinoma features, making it a potentially valuable target for NEPC23–25,29.

Similarly, CEACAM5, a member of the carcinoembryonic antigen family, is overexpressed in a larger fraction of solid tumors, with high expression observed in NEPC.

Notably, several antibody-drug conjugates (ADCs) targeting CEACAM5 have been developed and explored in the context of different solid tumors.

TROP2 is a transmembrane protein that is expressed in multiple malignancies. Clinical trials using TROP2-targeting agents have shown efficacy and a TROP2 ADC sacituzumab govitecan has been approved for triple-negative breast cancer and urothelial carcinoma, and phase 2 studies in CRPC are currently ongoing

3.8 KLK2

[16] https://www.ncbi.nlm.nih.gov/gene/1048

As NCBI notes[17]:

This gene encodes a member of the grandular kallikrein protein family. Kallikreins are a subgroup of serine proteases that are clustered on chromosome 19. Members of this family are involved in a diverse array of biological functions. The protein encoded by this gene is a highly active trypsin-like serine protease that selectively cleaves at arginine residues. This protein is primarily expressed in prostatic tissue and is responsible for cleaving pro-prostate-specific antigen into its enzymatically active form. This gene is highly expressed in prostate tumor cells and may be a prognostic maker for prostate cancer risk.

As Paniagua-Herranz et al note:

The identification of surfaceome proteins is a main goal in cancer research to design antibody-based therapeutic strategies. T cell engagers based on KLK2, a kallikrein specifically expressed in ***prostate cancer (PRAD)****, are currently in early clinical development. Using genomic information from different sources, we evaluated the immune microenvironment and genomic profile of prostate tumors with high expression of KLK2. KLK2 was specifically expressed in PRAD but it was not significant associated with Gleason score.*

Additionally, KLK2 expression did not associate with the presence of any immune cell population and T cell activating markers. A mild correlation between the high expression of KLK2 and the deletion of TMPRSS2 was identified. KLK2 expression associated with high levels of surface proteins linked with a detrimental response to immune checkpoint inhibitors (ICIs) including CHRNA2, FAM174B, OR51E2, TSPAN1, PTPRN2, and the non-surface protein TRPM4. However, no association of these genes with an outcome in PRAD was observed.

Finally, the expression of these genes in PRAD did not associate with an outcome in PRAD and any immune populations. We describe the immunologic microenvironment on PRAD tumors with a high expression of KLK2, including a gene signature linked with an inert immune microenvironment, that predicts the response to ICIs in other tumor types. Strategies targeting KLK2 with T cell engagers or antibody–drug conjugates will define whether T cell mobilization or antigen release and stimulation of immune cell death are sufficient effects to induce clinical activity.

3.8.1 Kallikreins

As Lawrence et al note:

The 15 members of the kallikrein-related serine peptidase (KLK) family have diverse tissue-specific expression profiles and putative proteolytic functions.

The kallikrein family is also emerging as a rich source of disease biomarkers with KLK3, commonly known as prostate-specific antigen, being the current serum biomarker for prostate cancer.

[17] https://www.ncbi.nlm.nih.gov/gene/3817

The kallikrein locus is also notable because it is extraordinarily responsive to steroids and other hormones. Indeed, at least 14 functional hormone response elements have been identified in the kallikrein locus. A more comprehensive understanding of the transcriptional regulation of kallikreins may help the field make more informed hypotheses about the physiological functions of kallikreins and their effectiveness as biomarkers. In this review, we describe the organization of the kallikrein locus and the structure of kallikrein genes and proteins.

We also focus on the transcriptional regulation of kallikreins by androgens, progestins, glucocorticoids, mineralocorticoids, estrogens, and other hormones in animal models and human prostate, breast, and reproductive tract tissues. The interaction of the androgen receptor with androgen response elements in the promoter and enhancer of KLK2 and KLK3 is also summarized in detail. There is evidence that all kallikreins are regulated by multiple nuclear receptors.

Yet, apart from KLK2 and KLK3, it is not clear whether all kallikreins are direct transcriptional targets. Therefore, we argue that gaining more detailed information about the mechanisms that regulate kallikrein expression should be a priority of future studies and that the kallikrein locus will continue to be an important model in the era of genome-wide analyses.

3.8.2 PSA Functions

We have discussed the androgen receptor and PSA elsewhere[18]. However in the context of markers we can include the following. As Lawrence et al note:

Androgens regulate the prostatic expression of several human kallikreins, in particular KLK2 and KLK3.

The earliest evidence for androgen-regulated KLK3 expression came from immunohistochemistry experiments showing that prostatic KLK3 levels mirror serum testosterone concentrations: low in prenatal development and childhood, greater in puberty, and highest in adulthood. Soon after the KLK2 and KLK3 genes were cloned, their androgen responsiveness was confirmed at the mRNA level using Northern blots of androgen-treated LNCaP prostate cancer cells. These observations were verified with a range of in vitro and in vivo experiments.

Numerous studies have since used KLK2 and KLK3 as prototypical AR target genes to investigate different aspects of androgen signaling in prostate cells. KLK3 levels are also monitored in patients undergoing androgen ablation therapy for prostate cancer because KLK3 is re-expressed when AR signaling is reactivated in castrate-resistant tumors. KLK3 levels, however, are highly heterogeneous in castrate-resistant prostate cancer and do not directly correlate with tumor growth. This variability may be due to the different ways that tumors adapt to castrate androgen levels including overexpression and mutation of the AR, up-regulation of transcriptional coactivators, and intratumoral steroidogenesis. …

[18] https://www.researchgate.net/publication/370125480_Androgen_Receptor_Whither_Goest_Thou

AREs were identified within the promoter of KLK3 soon after its androgen-dependent expression was established.

… the KLK3 promoter is bound by nuclear proteins in LNCaP cells.

They then identified the first KLK3 ARE, AREI (AGAACAgcaAGTGCT), at 170 to 156 bp from the TSS using a series of promoter deletion and mutation constructs.

Other groups confirmed this finding using similar reporter experiments and EMSAs. The results from reporter assays suggested that another ARE might be present between 320 and 539 bp from the KLK3 TSS.

Subsequently, AREII (GGATCAgggAGTCTC) was identified at 400 bp from the TSS and found to be a low-affinity AR binding site that cooperates with AREI. This was confirmed by other studies that also suggested that Fos-related complexes, distinct from AP-1, might be important in mediating AR transactivation of the KLK3 promoter …

Kallikreins can be used as markers of particular cell types, especially when their patterns of tissue-specific expression and hormonal regulation converge. KLK3 is a good example because it is one of the most highly expressed genes in the prostate. This means that KLK3 may have several clinical applications in prostate cancer. In addition to its use as the serum biomarker, KLK3 has been tested as a marker of circulating tumor cells, as an antigen to prime dendritic cells for targeted immunotherapy, and as an enzyme to activate cytotoxic prodrugs. Furthermore, the KLK3 promoter and enhancer have been used to design prostate-specific expression vectors for gene therapy. KLK3 is more precisely a marker of terminally differentiated luminal epithelial cells of the prostate.

It is not produced by stem, transit amplifying, or intermediate cells, which make up the basal layer of the epithelium and express little or no AR.

Although the prostate stroma is androgen-responsive, it does not express KLK3.

This suggests that KLK3 expression in luminal epithelial cells depends on more than just androgens and AR.

Recent genome-wide ChIP studies have shown that epigenetic marks, such as histone 3 lysine 4 methylation and pioneer coactivators guide hormone receptors to enhancers of tissue-specific target genes. This holds true for AR-mediated expression of KLK3.

Prostate cancer cell lines that express endogenous KLK3 have high levels of di- and trimethylated histone 3 lysine 4 at the promoter and enhancer of KLK3.

Furthermore, pioneer factors like GATA2 bind to the KLK3 enhancer in prostate cells and are re quired for maximum androgen-regulated gene expression. Within the prostate, GATA2 and KLK3 are both produced by luminal epithelial cells, but not the stroma. As previously noted,

KLK3 is expressed in some other tissues, but at much lower levels. Presumably, these tissues lack the precise combination of methylation, coactivator expression, and AR activity that stimulates such high levels of KLK3 in the prostate.

As Kalinska et al note:

The majority of studies on kallikreins and their physiological functions have focused on KLK3, also known as Prostate Specific Antigen (PSA). Since its identification and characterization in the 1970s, KLK3 has been investigated extensively with respect to its biochemical and cellular functions as an enzyme and prostate cancer biomarker.

Investigations into KLK3 as a prostate cancer marker, performed mostly by pharmaceutical companies, were fueled by its tissue specificity and incredibly high expression in prostate cancer.

These studies eventually led to development of diagnostic kits for the detection of the prostate cancer. However, KLK3 elevation needs to be considered in a broader biological context since it leads to frequent false-positive diagnoses followed by unnecessary treatments that have resulted in some instances in treatment-associated health problems.

Originally, the physiological activity of KLK3 was associated with its ability to perform semen liquefaction and enhance sperm motility, which it achieves by cleaving fibronectin and seminal-gel-forming proteins semenogelin 1 and semenogelin .

Recent reports, however, highlight the expression and mechanisms of action of other kallikreins in semen liquefaction, including proKLK3 (pro-PSA) activation by KLK-4, -5, -14, and -15, as well as the direct proteolytic activity of KLK-14 and -5 .

Following its activation, KLK3 degrades numerous proteins [extracellular matrix proteins, insulin-like growth factor (IGF)- binding proteins 3 and 5, and parathyroid-hormone-related protein (PTHRP)] facilitating metastasis of prostate cancer cells.

KLK2 is the second-best-characterized kallikrein biomarker used in prostate cancer diagnosis.

Despite its relatively low expression compared with PSA, utilization of KLK2 as a secondary biomarker increases the specificity and sensitivity of cancer detection . To date, the only known protein substrate of KLK2 is the ARA70 - the androgen receptor coregulator , suggesting that KLK2 has potential function in maintaining tissue balance in the testis. Another kallikrein highly expressed in prostate cancer is KLK4, an androgen regulated enzyme . Along with PSA, KLK4 facilitates metastasis of prostate cancer to the bone because it facilitates the degradation of extracellular matrix proteins.

3.9 PSCA

As NCBI notes[19]:

[19] https://www.ncbi.nlm.nih.gov/gene/8000

*This gene encodes a glycosylphosphatidylinositol-anchored **cell membrane glycoprotein**. In addition to being highly expressed in the **prostate** it is also expressed in the bladder, placenta, colon, kidney, and stomach.*

*This gene is up-regulated in a large proportion of **prostate cancers** and is also detected in cancers of the bladder and pancreas. This gene includes a polymorphism that results in an upstream start codon in some individuals; this polymorphism is thought to be associated with a risk for certain gastric and bladder cancers.*

As Frieling et al note:

Critically for CAR-T efficacy, a reliable and highly expressed tumor antigen needs to be identified. In this regard, more than 90% of prostate cancers express prostate stem cell antigen (PSCA), with even higher positivity (>99%) noted in bone metastatic disease (20, 21). PSCA expression is also significantly lower in normal prostate tissue, minimizing the potential for "on target, off tumor" effects (20). In the present study, we demonstrate the efficient expression of an anti-PSCA CAR expressed in human γδ T cells expanded from peripheral blood and their potent cytotoxicity against bone mCRPC cells in vivo.

Furthermore, we show that this effect is augmented by the nBP, ZOL. We observed no overt toxicities in tumor-bearing mice and that the anti-PSCA γδ CART treatment significantly reduced cancer-associated bone disease. Together, the data reveal that γδ T cell–based CAR therapies effectively mitigate bone metastatic prostate tumors and that the infusion of anti-PSCA γδ CAR-T cells in bone metastatic prostate cancer patients has the potential to be highly effective, due to the preexisting therapeutic application of ZOL in this patient population. …

Our work demonstrates that anti-PSCA γδ CAR-T cells potently promote the cytotoxicity of CRPC cell lines (C4-2B and 22Rv1) and that this effect could be enhanced via the addition of ZOL. Similar results were found in vivo, where the anti-PSCA γδ CAR-T induced regression and, in some mice, eradication of established tumors, especially in the ZOL treatment arm (60% with no evidence of disease at study endpoint). This effect was associated with increased degranulation and cytokine secretion, but not with an increase in PD-1 expression induced by ZOL. These tumor regressions are remarkable given the robustness and aggressiveness of the C4-2B model of CRPC in bone. However, some tumors did recur subsequent to anti-PSCA γδ CAR-T, raising the question of whether these recurrent prostate cancer cells retain PSCA expression and to what degree, or if a subpopulation of PSCA-negative cancer cells evolved. PSCA is expressed strongly in >99% of human bone metastatic tumor cells and minimally in other tissues, suggesting high potential efficacy of the approach in the clinical setting (20). In future studies, we will dissect this mechanism with experiments that include a second infusion of anti-PSCA γδ CAR-T cells to test rechallenging.

3.10 P2X4

NCBI notes regarding P2X4 as follows[20]:

The product of this gene belongs to the family of purinoceptors for ATP. This receptor functions as a ligand-gated ion channel with high calcium permeability. The main pharmacological distinction between the members of the purinoceptor family is the relative sensitivity to the antagonists suramin and PPADS. The product of this gene has the lowest sensitivity for these antagonists. Multiple alternatively spliced transcript variants, some protein-coding and some not protein-coding, have been found for this gene.

Maynard et al have reported on a target as follows:

P2X4 belongs to the P2 purinergic receptor family that is commonly upregulated in cancer and is associated with poorer outcomes.

Herein, we report that the P2X4 purinergic receptor is overexpressed in PCa, associated with PCa metastasis, and a driver of tumor development in vivo. We observed P2X4 protein expression primarily in epithelial cells of the prostate, a subset of CD66+ neutrophils, and most CD68+ macrophages. Our analysis of tissue microarrays representing 491 PCa cases demonstrated significantly elevated P2X4 expression in cancer compared to benign tissue spots, in prostatic intraepithelial neoplasia, in cancer from White compared to Black men, and in PCa with ERG positivity or with PTEN loss.

High P2X4 expression in benign tissues was likewise associated with the development of metastasis after radical prostatectomy. Treatment with P2X4-specific agonist CTP increased transwell migration and invasion of PC3, DU145, and CWR22Rv1 PCa cells.

P2X4 antagonist 5-BDBD treatment resulted in a dose-dependent decrease in viability of PC3, DU145, LNCaP, CWR22Rv1, TRAMP-C2, Myc-CaP, BMPC1, and BMPC2 cells and decreased DU145 cell migration and invasion. Knockdown of P2X4 attenuated growth, migration, and invasion of PCa cells. Finally, knockdown of P2X4 in Myc-CaP cells resulted in significantly attenuated subcutaneous allograft growth in FVB/NJ mice. Collectively, these data strongly support a role for the P2X4 purinergic receptor in PCa aggressiveness and identifies P2X4 as a candidate for therapeutic targeting.

3.11 DLL3

As NCBI notes[21]:

This gene encodes a member of the delta protein ligand family. This family functions as Notch ligands that are characterized by a DSL domain, EGF repeats, and a transmembrane domain. Mutations in this gene cause autosomal recessive spondylocostal dysostosis 1. Two transcript variants encoding distinct isoforms have been identified for this gene

[20] https://www.ncbi.nlm.nih.gov/gene/5025

[21] https://www.ncbi.nlm.nih.gov/gene/10683

As Matsuo et al note:

Delta-like canonical Notch ligand 3 is a member of the DSL Notch receptor ligands, which include five ligands in mammals: DLL1, DLL3, DLL4, JAG1, and JAG2*.*

Delta-like canonical ligand 3 plays a crucial role in Notch signaling, which influences various cellular processes, including ***differentiation, proliferation, survival, and apoptosis****. DLL3 is expressed throughout the presomitic mesoderm and is localized to the rostral somatic compartments; mutations in DLL3 are known to induce skeletal abnormalities such as spondylocostal dysostosis. …*

Delta-like canonical Notch ligand 3 is a structurally divergent DSL family member. Unlike other DSL ligands, DLL3 localizes in the Golgi apparatus and emerges on the cell surface when overexpressed.10 Delta-like canonical Notch ligand 3 does not bind to Notch receptors, and inactivates Notch signaling in cis. Delta-like canonical Notch ligand 3 also prevents the localization of Notch and/or DLL1 on the cell surface through intracellular retention.12 Thus, DLL3 is regarded as a cell-autonomous inhibitor of Notch signaling. It is also one of several notch ligands that is a direct downstream target of ASCL1, a transcription factor associated with pulmonary neuroendocrine cell development. These findings suggest that DLL3 is related to neuroendocrine tumorigenesis, especially in lung cancer[22]*. …*

A subset of patients with advanced prostate cancer show histologic transformation to small-cell neuroendocrine prostate cancer. Castration-resistant small cell neuroendocrine prostate cancer is typically associated with poor outcomes, and patients are treated with platinum-based chemotherapy regimens. Because the clinical behavior of CRPC-NE shares similarities with SCLC, the association of DLL3 expression with the CRPC-NE phenotype in prostate cancer was investigated and the antitumor activity of SC16LD6.5 (humanized Ab against DLL3) was evaluated in DLL3- expressing prostate cancer models.

Delta-like canonical Notch ligand 3 was found to be expressed in most CRPC-NE and some castration-resistant prostate adenocarcinoma cases, but not in the localized benign prostate cancer. Moreover, a single dose of SC16LD6.5 induced a complete and durable response in DLL3- expressing prostate cancer xenografts.38

Overall, these findings indicate that DLL3 is a potential therapeutic target in neuroendocrine prostate cancer …

3.12 OTHERS

3.12.1 LLT1

As NCBI notes[23]:

[22] https://www.researchgate.net/publication/325497685_Neuroendocrine_PCa_Galen_Logic_and_Rationalism

[23] https://www.ncbi.nlm.nih.gov/gene/29121

This gene encodes a member of the natural killer cell receptor C-type lectin family. The encoded protein inhibits osteoclast formation and contains a transmembrane domain near the N-terminus as well as the C-type lectin-like extracellular domain.

3.12.2 GRP-R

As NCBI notes[24]:

Gastrin-releasing peptide (GRP) regulates numerous functions of the gastrointestinal and central nervous systems, including release of gastrointestinal hormones, smooth muscle cell contraction, and epithelial cell proliferation and is a potent mitogen for neoplastic tissues. The effects of GRP are mediated through the gastrin-releasing peptide receptor. This receptor is a glycosylated, 7-transmembrane G-protein coupled receptor that activates the phospholipase C signaling pathway. The receptor is aberrantly expressed in numerous cancers such as those of the lung, colon, and prostate. An individual with autism and multiple exostoses was found to have a balanced translocation between chromosome 8 and a chromosome X breakpoint located within the gastrin-releasing peptide receptor gene

3.12.3 CELSR3

As NCBI notes[25]:

Predicted to enable G protein-coupled receptor activity and calcium ion binding activity. Involved in dopaminergic neuron axon guidance; planar cell polarity pathway involved in axon guidance; and serotonergic neuron axon guidance. Acts upstream of or within several processes, including cilium assembly; generation of neurons; and regulation of protein phosphorylation. Predicted to be located in plasma membrane. Predicted to be integral component of membrane. Is expressed in several structures, including central nervous system; embryo ectoderm; epiblast; peripheral nervous system; and retina

[24] https://www.ncbi.nlm.nih.gov/gene/2925

[25] https://www.ncbi.nlm.nih.gov/gene/107934

4 THERAPEUTIC APPROACHES

There are a large selection of therapeutic approaches that entail surface markers. The now classic approaches include targeting PD-1, CART cells on CD19, and CTLA4 targeting. We briefly summarize the other putative approaches.

From Kono we have the following Table which presents some putative targets:

Target	*Biological function*	*Antibody (fusion protein)*	*Phase*	*Cancer type*
CTLA4	Inhibitory receptor	Ipilimumab	FDA approved Phase II and III	melanoma, multiple cancers
PD1	Inhibitory receptor	MDX-1106 MK3475 CT-011 AMP-224	Phase I/II Phase I Phase I Phase I	melanoma, renal, lung multiple cancers multiple cancers multiple cancers
PDL1	Ligand for PD1	MDX-1105	Phase I	multiple cancers
LAG3	Inhibitory receptor	IMP321	Phase II	breast cancer
B7-H3	Inhibitory ligand	MGA271	Phase I	multiple cancers
B7-H4	Inhibitory ligand			Preclinical
TIM3	Inhibitory receptor			Preclinical

Paul et al have recently noted:

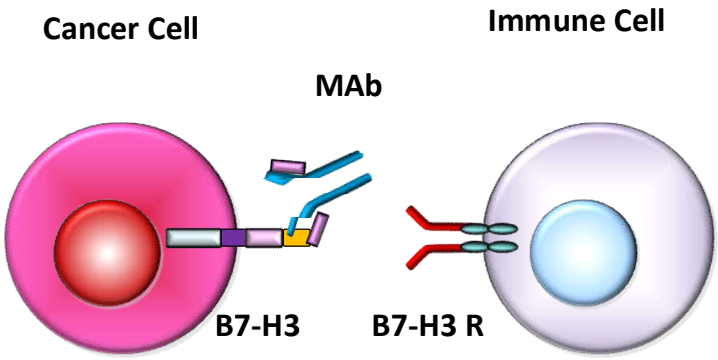
The greatest challenge in cancer therapy is to eradicate cancer cells with minimal damage to normal cells.

Targeted therapy has been developed to meet that challenge, showing a substantially increased therapeutic index compared with conventional cancer therapies. Antibodies are important members of the family of targeted therapeutic agents because of their extraordinarily high specificity to the target antigens. Therapeutic antibodies use a range of mechanisms that directly or indirectly kill the cancer cells. Early antibodies were developed to directly antagonize targets on cancer cells. This was followed by advancements in linker technologies that allowed the production of antibody–drug conjugates (ADCs) that guide cytotoxic payloads to the cancer cells.

Improvement in our understanding of the biology of T cells led to the production of immune checkpoint-inhibiting antibodies that indirectly kill the cancer cells through activation of the T cells. Even more recently, bispecific antibodies were synthetically designed to redirect the T cells of a patient to kill the cancer cells. In this Review, we summarize the different approaches used by therapeutic antibodies to target cancer cells. We discuss their mechanisms of action, the structural basis for target specificity, clinical applications and the ongoing research to improve efficacy and reduce toxicity

4.1 ANTIBODIES (AB)

Monoclonal antibodies can be designed to attach to B7-H3. Usually the B7-H3 binds to its conjugate receptor and sends a "do not kill" signal allowing the cancer cell to be left un-attacked. However by blocking this with a Mab allows for the attack to proceed. This is akin to the PD-1, PDL-1 blockage in other malignancies.



B7-H3 and B7-H3R are blocked by Mab. Thus Immune cell can attack the cancer cell since the "don't kill" signal has been eliminated

4.2 ANTIBODY DRUG CONJUGATES (ADC)

As Paul et al have noted:

ADCs are constructed by linking a tumour-targeting antibody to a

. The binding of ADC molecules to the cell-surface antigen leads to their internalization followed by the release of the cytotoxic drug inside the cell. This allows selective delivery of the cytotoxic drug to cancer cells while sparing most of the healthy tissues.

Key components of an ADC include a tumour-targeting antibody, a cytotoxic drug and a linker connecting the antibody to the cytotoxic drug. The success of ADCs depends on the optimal selection of these key components, along with the conjugation method used to attach the linker to the antibody which often determines the drug–antibody ratio (DAR).

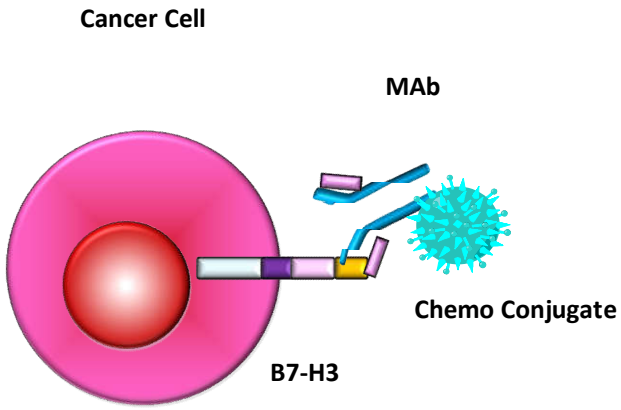
Most ADCs use a humanized or human IgG1 as the tumour targeting antibody (except for brentuximab, which uses a chimeric IgG1, and gemtuzumab and inotuzumab, which use a humanized IgG4)

As alluded to above, the popularity of using IgG1 is owing to its long plasma half-life of ~21 days (for example, compared with the half-life of IgG3, which is ~7 days)114, and its ability

to bind Fc receptors leading to enhanced target cell killing by ADCC and ADCP (for example, compared with IgG2 and IgG4, which are less efficient at ADCC and ADCP)115. Two ADCs, gemtuzumab and inotuzumab, use IgG4, which has a lower affinity for FcγRII and FcγRIII, thus limiting ADCP, along with a possible reduction in toxicity owing to diminished nonspecific uptake of the ADC into immune cells through the Fc receptor.

The majority of the linkers connect the cytotoxic drug to the antibody at random lysine or cysteine residues on the IgG1 antibody backbone. An effective linker minimizes the early release of the cytotoxic drug in the bloodstream while facilitating the controlled release of the active drug at preferred targeted locations.

Linkers are broadly classified as cleavable and non-cleavable. Ten out of the twelve approved ADCs use a cleavable linker such as a peptide linker, hydrazone linker, disulfide linker or the CL2A linker. One of the first linkers developed for drug attachment was a cleavable linker using hydrazone bonds. This linker was used to attach the antitumour antibiotic calicheamicin to the ADCs gemtuzumab and inotuzumab.



ADCs are Mab with an attached or conjugated chemotherapeutic element. Once attached to the target cell it enterr as an exosome and kills the targeted cell.

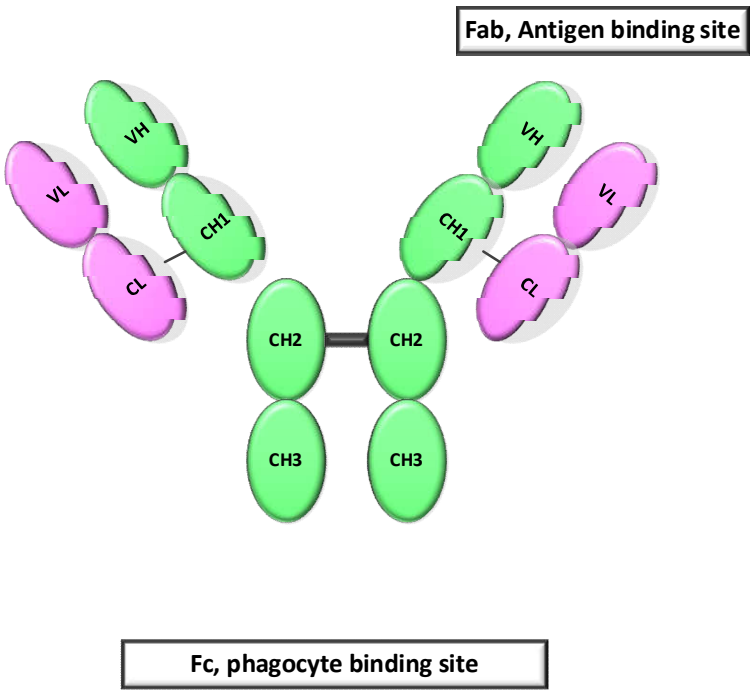
A recent presentation by Tarantino stated[26]:

The success obtained with HER2-targeted ADCs has ignited research for additional targets for the development of ADCs Among the most promising targets is Trop2, given its expression in >90% of breast cancers, surface localization and rapid internalization upon binding by monoclonal antibodies

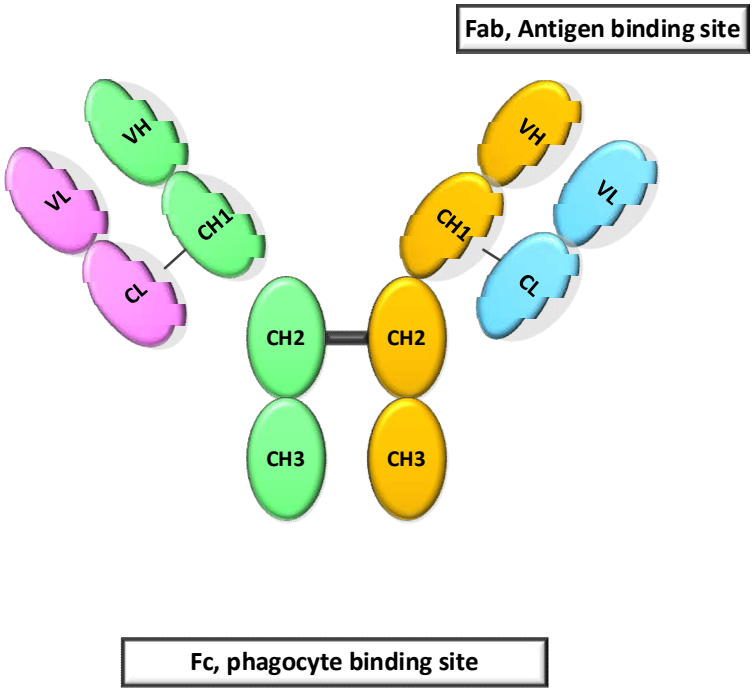
[26] July 14, 2024 Harvard Med School Breast Cancer Symposium

4.3 BITES

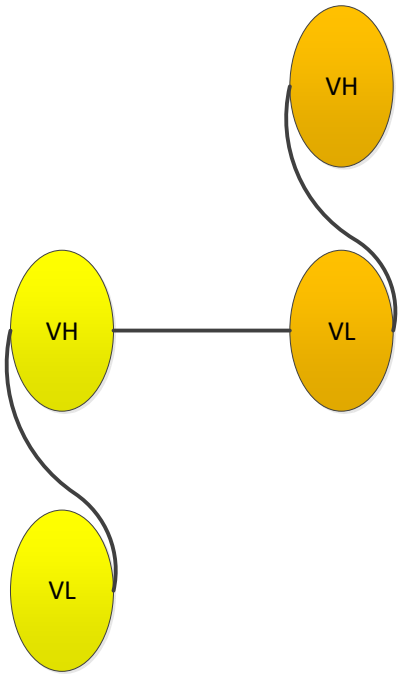
BITES are one of many polyspecific antibodies[27].



[27] https://www.researchgate.net/publication/346245151_Poly-specific_Antibodies



BiTE is a more mature bispecific. It contains the two motifs that we see below and no Fc element. Namely it does not contain the lower segment as shown above. It has two segments attachable to targeted cells.



The Bispecific T cell approach has seen limited use. As Huehls et al note:

Bispecific T cell engagers are a new class of immunotherapeutic molecules intended for the treatment of cancer. These molecules, termed BiTEs, enhance the patient's immune response to tumors by retargeting T cells to tumor cells. BiTEs are constructed of two single chain variable fragments (scFv) connected in tandem by a flexible linker. One scFv binds to a T cell-specific molecule, usually CD3, while the second scFv binds to a tumor-associated antigen. This structure and specificity allows a BiTE to physically link a T cell to a tumor cell, ultimately stimulating T cell activation, tumor killing and cytokine production. BiTEs have been developed that target several tumor-associated antigens for a variety of both hematological and solid tumors.

Several BiTEs are currently in clinical trials for their therapeutic efficacy and safety. This review examines the salient structural and functional features of BiTEs as well as the current state of their clinical and preclinical development….

The concept of using T cell retargeting for cancer therapy stretches back to the 1970s. Unlike macrophages, dendritic cells, and other accessory cells, T cells are present in copious numbers, expand rapidly upon activation, give robust and durable cytotoxic responses, and have the potential to generate immunologic memory. Furthermore, T cells have been found to attack tumors from the outside as well as infiltrating into the tumor. These features make T cells optimal therapeutic effectors for cancer. T cell redirection does suffer one significant challenge, which is the requirement of a second stimulatory signal to achieve full T cell activation and prevent anergy. Multiple bispecific formats have been developed to meet or circumvent this requirement.

Then Abbas et al also have noted:

Bispecific T cell engagers (BiTEs) facilitate the targeting of host T cells of any specificity to attack tumor cells. These reagents are recombinant antibodies engineered to express two different antigen binding sites, one specific for a tumor antigen and the second specific for a T cell surface molecule, usually CD3. In many of these antibodies, each antigen binding site is composed of a single chain variable fragment containing Ig heavy and light chain variable domains, similar to the CARs described earlier.

The presumed mechanism of action of BiTEs, based on in vitro studies, is the formation of immune synapses between the tumor cells and the T cells and the activation of the T cells by CD3 crosslinking. A CD19-specific BiTE is approved for treatment of acute lymphocytic leukemia. BiTEs specific for many other tumor antigens have been developed, including CD20, EpCAM, Her2/neu, EGFR, CEA, folate receptor, and CD33, and are at various stages of preclinical and clinical trials.
As Ross et al note:

For targets that are homogenously expressed, such as CD19 on cells of the B lymphocyte lineage, immunotherapies can be highly effective. Targeting CD19 with blinatumomab, a CD19/CD3 bispecific antibody construct (BiTE®), or with chimeric antigen receptor T cells

(CAR-T) has shown great promise for treating certain CD19-positive hematological malignancies.

In contrast, solid tumors with heterogeneous expression of the tumor-associated antigen (TAA) may present a challenge for targeted therapies. To prevent escape of TAA negative cancer cells, immunotherapies with a local bystander effect would be beneficial. As a model to investigate BiTE®-mediated bystander killing in the solid tumor setting, we used epidermal growth factor receptor (EGFR) as a target. We measured lysis of EGFR-negative populations in vitro and in vivo when co-cultured with EGFR-positive cells, human T cells and an EGFR/CD3 BiTE® antibody construct. Bystander EGFR-negative cells were efficiently lysed by BiTE®-activated T cells only when proximal to EGFR-positive cells.

Our mechanistic analysis suggests that cytokines released by BiTE®-activated T-cells induced upregulation of ICAM-1 and FAS on EGFR-negative bystander cells, contributing to T cell induced bystander cell lysis.

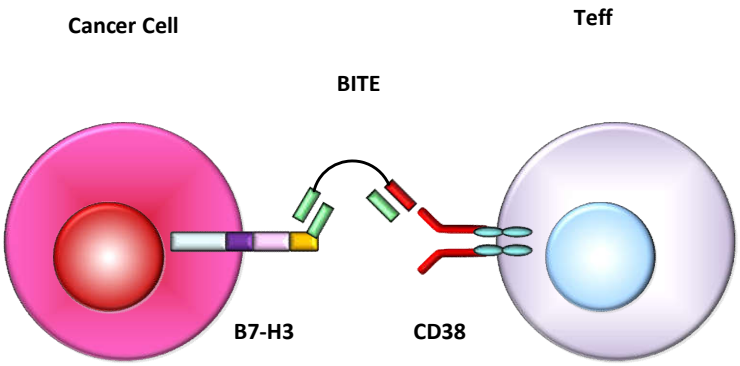
Namely the BITE approach is to create using an Ab a molecule which is CD3 on one end and say CD19 on the other and use this to cover a target and then to attract a T cell. In some ways this is akin to CAR-T where we place the receptor to the target on a T cell, here we use a T cell and attach the target to a known receptor on a T cell.

Furthermore, Zahavi and Weiner have recently noted:

Recently, the most successful mAb-based strategies have moved away from targeting tumor antigens and instead focused on targeting immune cells in order to enhance their anti-tumor capabilities. One of the first mAb approaches to stimulate T cell anti-tumor immunity was the development of bispecific T Cell Engager (BiTE) antibodies that both target a tumor antigen such as CD19 and the activating receptor, CD3, on T cells. BiTEs combine direct targeting of tumor cells with recruitment of cytotoxic T cells into the tumor microenvironment and led to tumor regressions even when administered at doses three orders of magnitude less than the parent mAb alone. The CD19-CD3 BiTE blinatumomab conferred significant clinical benefit to acute lymphoblastic leukemia patients and was FDA approved in 2017 .

Clinical trials are currently underway using BiTEs generated from the widely used anti-HER2 and anti-EGFR mAbs trastuzumab and cetuximab. Other mAb approaches seek to enhance T cell specific immunity against tumor cells by stimulating activating receptors such as 4-1BB, OX40, CD27, CD40, and ICOS. Agonist antibodies towards CD40 stimulate antigen presentation by dendritic cells and mAbs to OX40 and 4-1BB activate T cells while simultaneously dampening the activity of inhibitory T regulatory cells (Tregs) . mAbs designed to stimulate these activating receptors are in various stages of clinical trials both alone and in combination with other immunotherapy approaches. Additional mAbs that deplete inhibitory Tregs directly, such as daclizumab, which targets CD25 on Tregs, are also undergoing clinical trials

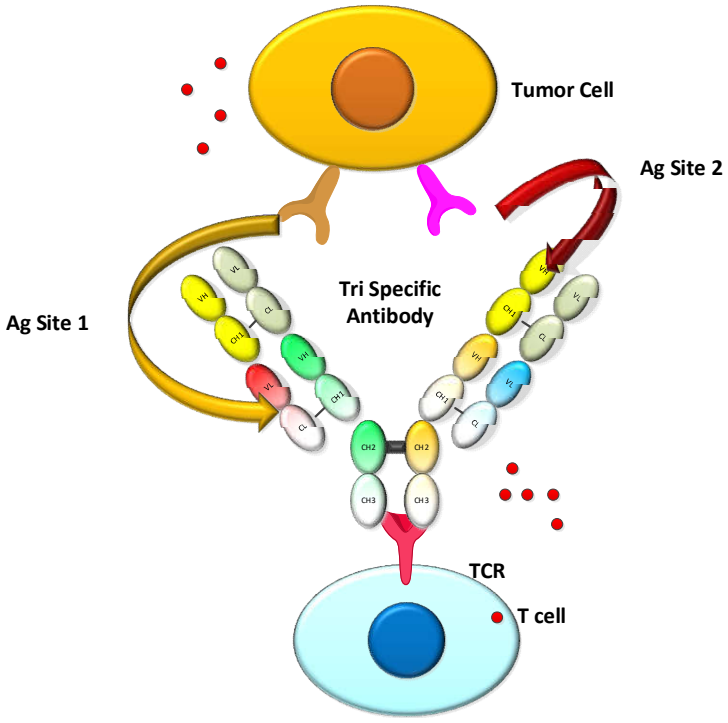
We demonstrate a BITE below.



BITE acts by attaching to cancer cell and a Teff cell via CD38 thus activating the Teff to kill cancer cell

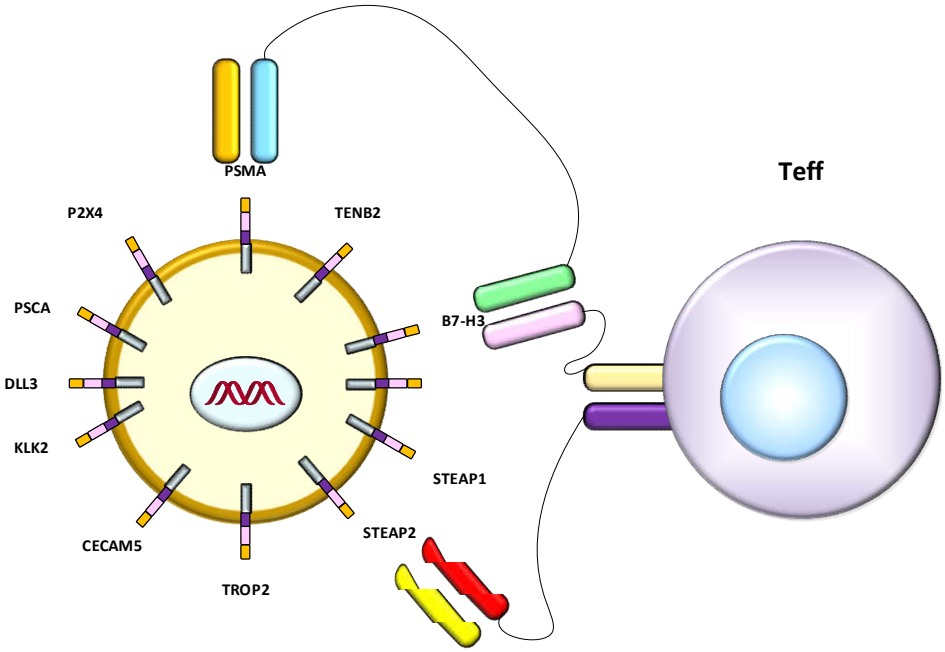
4.4 TRIKES

TRIKES are merely three element as compared to the two just discussed.



4.5 POLYSPECIFIC AB

Poly specific are extensions of the previous elements. They can be crafted to cover multi targeted attacks as we have discussed in our previous NOTE. The example below show targeting three such targets and the related poly.

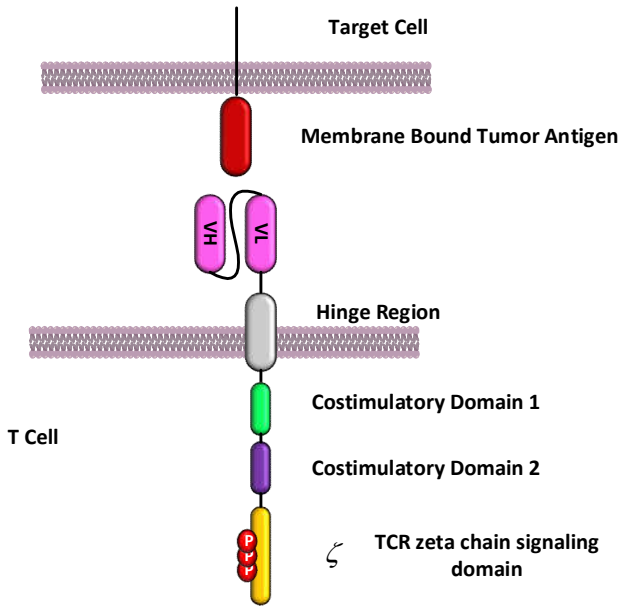


4.6 CART(NK)

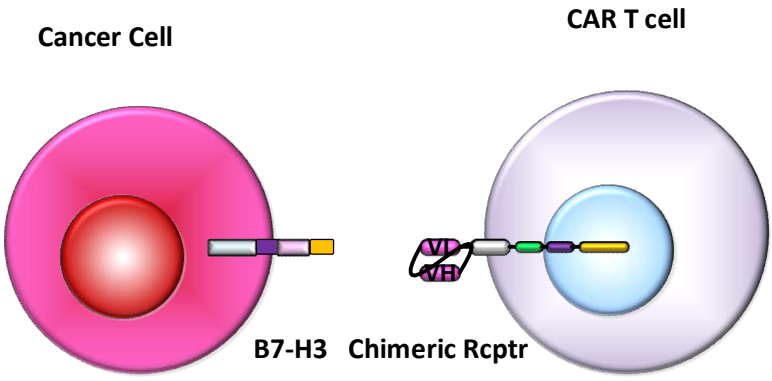
We have examined CAR cells for the past decade[28]. Simply, they are the patients T cells changed with receptors targeting the protein fragments on the surface of the tumor cells. This created the chimera and then uses T cells to attack it. NK cells can also be used creating CARNK cells therapeutics. NK cells are often better since they do not rely on the specific patient.

The following is an example of a typical CAR.

[28] https://www.researchgate.net/publication/309419224_CAR_T_Cells_and_Cancer



The figure below shows the CAR on a T cell and it attack on a cancer cell.



The T cell modified by the CAR receptor recognizes the tumor B7-H3 and then attacks that cell

4.7 Current Clinical Examples

We summarize some current clinical examples. Clearly multiple approaches are warranted. The utilization of some of these techniques are both neoadjuvant and adjuvant. Surgical resection is often still performed. The issue is avoiding or combatting metastatic spread.

As Pulido et al have recently noted:

B7-H3 (encoded by CD276), ***an immune checkpoint protein, is a highly glycosylated Type 1 transmembrane protein that is abundantly expressed on the surface of cancer cells****, including PCa cells. B7-H3 plays a double role in oncogenesis, acting as an inhibitory immune checkpoint and as a protumorigenic protein.*

The interaction of B7-H3 with the tumor microenvironment is manifested by B7-H3- mediated promotion of M2 polarization of tumor-associated macrophages and a decrease in tumor-infiltrated cytotoxic T (Tc) and natural killer (NK) cells, among other immune-evasive effects. In bladder cancer, the expression of B7-H3 on M2 macrophages cooperates to decrease Tc cells' tumor infiltration.

Whether this interaction occurs in PCa deserves investigation. B7-H3/CD276 is one of the most expressed immunomodulators in PCa, mainly in the tumor cells but also in endothelial and other tumor microenvironment cells, and its expression is positively associated with the level of androgen receptor (AR), AR signaling proteins, and major vault protein (MVP) involved in multidrug resistance. Androgen acts as a negative regulator of the transcription of B7-H3/CD276, suggesting that androgen deprivation therapy could obtain additional benefit in combination with targeting B7- H3 in patients under hormone-naïve PCa treatment.

Some studies reported a negative correlation between the expression of B7-H3 and both phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and TP53, making targeting B7-H3 a relevant therapeutic option in PCa harboring PTEN or TP53 deficiencies. The expression of B7-H3/CD276 in metastatic castration-resistant PCa (mCRPC) is associated with defective DNA repair (DDR), which is likely to make these tumors more sensitive to DDR-related inhibitors. Therefore, it would be interesting to test the efficacy of targeting B7-H3 in combination with poly (ADP-ribose) polymerase (PARP) inhibitors in PCa.

Targeting B7-H3 in PCa B7-H3 is emerging as a versatile actionable target in PCa therapy. Several strategies to target B7-H3 in PCa are summarized …

These include approaches directly targeting and killing the tumor cells, such as monoclonal antibody (mAb)-mediated cytotoxicity (ADCC), the use of mAb drug conjugates (ADC), radio-conjugated mAb, bispecific mAb, or chimeric antigen receptor (CAR)-T cells.

In addition, strategies actively blocking immune evasion or the tumorigenicity facilitated by B7-H3, using inhibitory or ligand-receptor-blocking mAb, are also under scrutiny. …

The Phase 1 clinical trial NCT01391143 tested the fragment crystallizable (Fc)-optimized ***humanized antiB7-H3 mAb enoblituzumab*** *in patients with B7-H3-positive solid tumors, including PCa, and who had previously been treated with chemotherapy or immunotherapy. This was followed by a* ***Phase 2 trial, NCT02923180****, testing* ***neoadjuvant enoblituzumab****, followed by* ***prostatectomy****, in 32 patients with localized PCa.*

The treatment appeared relatively safe, with favorable declines in prostate-specific antigen (PSA) when compared with historical outcomes from high-risk postprostatectomy patients.

Importantly, significant activation of the immune system in the tumor microenvironment, which involved both T cells and myeloid cells, was observed.

A larger randomized trial is necessary to corroborate these clinical findings. ADC studies targeting B7- H3 include the trials NCT03729596 (Phase 1) and NCT05555117 (Phase 2), using the humanized mAb ***vobramitamab loaded with duocarmazine****, a DNA-alkylating agent that binds to the minor groove of DNA. The NCT03729596 trial is completed and showed interim results of a tolerable safety profile as well as evidence of clinical activity by PSA decrease and tumor lesion reduction in patients with mCRPC (n = 26), although an expanded cohort is required to confirm these results.*

Combined use of ***vobramitamab and lorigerlimab****, a* ***bispecific dual-affinity retargeting mAb (DART) that recognizes PD-1 and CTLA-4, is under study in the trial NCT05293496, opening the clinical perspective of anti-B7-H3 combinatorial therapies in the treatment of advanced PCa****.*

The ADC clinical trial NCT05914116 is testing DB-1311, a humanized anti-B7- H3 mAb linked to a cleavable DNA topoisomerase I inhibitor, P1021. More recently, the clinical trial NCT04145622 with anti-B7-H3 ***ifinatamab deruxtecan*** *(ADC DS-7300a) has been initiated, showing a good safety profile and promising antitumor activity. In this regard, positive results have been obtained in preclinical PCa models using DS-7300a, alone or in combination with decitabine, a panDNA methyltransferase inhibito.*

Decitabine treatment increased the expression of B7-H3 in B7-H3-low PCa cells and increased the cytotoxicity of DS-7300a, which could be exploited to increase the sensitivity of PCa cells with low B7-H3 levels to anti-B7-H3-based therapies. Additional trials are warranted using other inhibitors of DNA repair in combination with targeting B7-H3. The Phase 1 studies NCT02628535 and NCT03406949 have tested (alone or in combination with anti-PD-1 mAb) obrindatamab (formerly orlotamab), a humanized DART that recognizes both B7-H3 and CD3. Obrindatamab redirects T cells, via CD3, to kill B7-H3-expressing cells, a strategy showing antitumor activity in preclinical models, including PCa cell lines.

Thus, combined use of ***obrindatamab*** *with other targeted therapies provides a great avenue to combat PCa. Several B7-H3-based clinical trials for therapies adopting CAR-T are ongoing. The Phase 1 study NCT04691713 is testing autologous CAR-T in advanced PCa and other solid tumors. A more recent clinical trial, NCT04432649, is evaluating the side effects and effective doses of a B7-H3 CAR-T fused to an inducible apoptotic caspase 9 domain.*

Preclinical studies addressing B7-H3-based CART immunotherapy approaches in PCa have shown efficient antitumor activity. A study using anti-B7-H3 376.96 mAbbased CAR-T alone or in combination with fractionated irradiation revealed high cytotoxic efficacy in PCa cell lines, especially with the combined treatment. Since irradiation upregulates the expression of B7-H3 in PCa cells, this combinatorial CAR-T strategy is promising for treating radioresistant PCa. Potent cytotoxicity was also obtained in a study using anti-B7-H3 8H9 mAb-based CART approach in PCa cell lines.

In summary, a variety of therapeutic options targeting B7-H3 are under clinical scrutiny for advanced PCa. Clinical outcomes from the ongoing clinical trials, as well as an analysis of expanded cohorts, will inform us of the (dis)advantages of these treatment options.

Likewise Mortezaee noted:

Combination of anti-B7-H3 with common immune checkpoint inhibitor therapy

Anti-PD-1 therapy recruits CD8+ T cells into tumor area, and IFN-γ released from the recruited cells induces B7-H3 expression, which defines a mechanism of resistance to ICI therapy.

A pilot study showed promising objective responses to the PD-L1 inhibitor durvalumab and the CTLA-4 inhibitor tremelimumab in TNBC patients, but luminal cancer cases had no response. mRNA expression profile of cancer cell lines showed high expression of PD-L1, PD-L2 and CTLA-4 in TNBC cells, whereas B7-H3 was overexpressed considerably in luminal cells, which is indicative of dynamic expression of immunoregulatory molecules in breast cancer subtypes, thereby representing diverse responses to anti-checkpoint therapy. Anti-B7-H3 therapy promotes vascular normalization in TNBC mice. Normalization in tumor ecosystem is a breakthrough in cancer therapy and it can be a mechanism for boosting anti-PD-1 efficacy.

Blockade of B7-H3, but not PD-1, increased survival of ovarian cancer mice models, and deficiency of B7-H3 on ovarian cancer cells enhanced the efficacy of anti-PD-L1 therapy. Outcomes of animal tumor models showed promising impact of anti-B7-H3 combination with anti-PD-1 in powering the immune system particularly against late-stage cancers. Combination of the PD-1 inhibitor pembrolizumab with the B7-H3 inhibitor enoblituzumab is evaluated in patients with advanced cancers.

The combination regimen was safe and effective in ICI naïve HNSCC and NSCLC patients with the respective objective response rate (ORR) of 33% and 36% (NCT02475213).

B7-H3 chimeric antigen receptor-modified T cells

CARs can be developed from conversion of established anti-B7-H3 antibodies into single-chain variable format (scFv) and cloning B7-H3 scFvs into CAR-T cells, which is called B7-H3 CAR-T. The binder (namely scFvs) preferentially attaches to tumor tissues [80]. This indicates the specific attraction of B7-H3 CAR-T toward tumor area but not toward normal tissues where low level of B7-H3 is expressed. TAA06 is a humanized B7-H3 CAR-T that its efficacy is evaluated in solid tumor models. TAA06 shows limited impact on xenograft models with HCT-15 CRC cells, but pre-treatment with irradiation considerably increases infiltration of CAR-T cells into tumor tissue and boosts their tumor-killing abilities. This is due to the increased expression of B7-H3 on HCT-15 cells after irradiation. B7-H3 CAR-T provides potential therapeutic opportunities in solid cancer patients due to displaying extensive expression of B7-H3. Considerable control of cancer growth without evident adverse effects is reported in a syngeneic tumor model.

The efficacy of B7-H3 CAR-T cell therapy can be increased after co-stimulation with related molecules. CD137 (also called 4–1BB) is an example of such molecules. B7-H3 stimulation

hampers activation of the co-stimulatory receptor CD137, whereas treatment with enoblituzumab augmented the proportion of CD137 expressing NK, CD4+ and CD8+ T cells. In line with the positive impact of enoblituzumab on NK and T cells, the CD137 agonist urelumab further boosted tumor-killing activity of enoblituzumab. CD137 co-stimulation induces lower PD-1 representation on B7-H3 CAR-T cells, promotes their resistance to PD-L1, and increases the efficacy of therapy upon targeting PD-L1 expressing tumor cells

5 OBSERVATIONS

We now make some observation regarding extensions and open issues.

5.1 WHAT ARE THE SPATIAL DYNAMICS OF PCA CELLS?

We have examined various cell surface elements. These are however but a small number of them. The question is; how many of each are on a cell, how are they distributed, is there temporal ebb and flow of these elements and do some mutually support or interfere with others.

Consider the fact that a cancer cell may be 20 microns in diameter. That means an area of about 1200 sq micro m. Now cell proteins diameters may be on the order of 1-10 nm. Thus a single protein could occupy 25 nm sq. Thus for tight packing we may have 48 million surface proteins! We know that not to be the case but it may very well be in the hundreds of thousands!

Thus the question is; as we look at targets, how many of each are there and how accessible would they be with say polyspecific Ab?

5.2 WHAT IMPACT DOES THE TME HAVE IN SURFACE TARGET THERAPEUTICS?

We have discussed the TME extensively in the past[29]. The TME can be both a protective and supportive environment. One suspects that it is essential to attack the TME first and then the malignant cells. Fibroblasts and M2 macrophages protect and support the tumor and despite effective immunotherapy this shell may make it impervious.

We recently examine macrophages especially M2 macrophages which dominate the TME[30]. In that examination we presented various therapeutics being considered to reduce M2s. We believe that such a reduction is essential.

Similarly we examined fibroblasts, especially CAF, cancer associate fibroblasts[31]. We suggest referring to that document. Not as supportive of tumor cells as M2s it is highly protective.

5.3 WHAT IS THE CELL BY CELL VARIANCE OF SURFACE TARGETS?

[29] https://www.researchgate.net/publication/383547930_Macrophages_REDUX and https://www.researchgate.net/publication/336116071_Tumor_Associated_Immune_Cells_On_the_one_hand_and_on_the_other_hand

[30] https://www.researchgate.net/publication/383547930_Macrophages_REDUX

[31] https://www.researchgate.net/publication/341788660_Fibroblasts_and_Cancer_The_Wound_That_Would_Not_Heal

This is a critical factor. All too often cell variants are examined as a gross examination. However we need cell by cell analysis. The Protocol that we suggested in the first section details how this may be accomplished and then tied in with therapeutics.

5.4 Is there a stem cell target that should be found?

We examined stem cells in PCa more than a decade ago[32]. The argument is that there is some cell that facilitates and drives the other cells and if you can get that cell then all others collapse. Yet as we know PCa is highly heterogeneous. What is on the left may not be on the right etc. Thus the stem cell argument may be limited at best. If we take a melanoma, a thyroid Ca or many others, perhaps the paradigm may hold. For PCa it is challenging. Does PCa start with one aberrant cell? What are the temporal and spatial characteristics of PCa.

5.5 PCa is highly heterogeneous. This most likely means that lesions in one part of the prostate may have different signatures than those in other parts. Thus identifying targets may be complex. Does this demand multiple signature identification?

PCa lesions are genetically heterogenous. This is especially true as to surface targets. Again the suggested protocol details ways to address this issue.

5.6 In metastatic PCa, are the signature of the metastatic cells varied, and if so is this a temporally changing process as well as spatially?

Is there temporal changes in targets. In human targets we have great difficulty ascertaining this. In mice it may be possible but the genetic characteristics may significantly distort this. In vivo analysis may be attempted via patients in watchful waiting but proper experimental procedures would likely be lacking.

5.7 Is there some optimal set of surface targets that maximizes the reduction of PCa cells?

How many targets are necessary. The balance is getting the PCa cells while avoiding other cell damage. Polyspecific may be optimized by extensive clinical examination.

5.8 If there exists an optimal set then is there a change in that set temporally?

The temporal characteristics may distort optimal targeting. If the initial targeting eliminates the PCa cell first pass then this is a non-issue. However if TME protected cells allow temporal drift then subsequent targeting may vary.

[32] https://www.researchgate.net/publication/301542243_Cancer_Stem_Cells_and_Cancer_of_Origin_Redux(2016) and https://www.researchgate.net/publication/301222986_Prostate_Cancer_Stem_Cells (2012)

5.9 Is a Neo-adjuvant therapy appropriate as in other cancers?

We have noted that the TME may be a hinderance. Further we noted that in other cancers the use of Ab conjugates reduce tumor loads. Perhaps addressing the two before surgery may be efficacious. For example:

The TME must deal with both M2 macrophages as well as blocking fibroblasts[33].

5.10 Can Staging using PSMA PET assist in Targeting?

We have discussed PSMA at length herein and elsewhere[34]. As Udovicich et al have recently noted:

Prostate-specific membrane antigen (PSMA) positron emission tomography or computed tomography (PET/CT) has emerged as a superior imaging option to conventional imaging for prostate cancer. The majority of early evidence and prospective trials evaluated PSMA PET/CT in the biochemical recurrence or metastatic setting. However, there has been an increasing number of prospective trials in the primary setting. The purpose of this narrative review was to describe the role of PSMA PET/CT in localized primary prostate cancer. This narrative review focuses on the prospective evidence available in this setting. We detail the current practice and future potential for PSMA PET/CT to be used in multiple stages of localized primary . The most common practice currently for PSMA PET/CT is in the primary nodal and metastatic staging of high-risk prostate cancer.

We describe other roles of PSMA PET/CT, including in intermediate-risk prostate cancer as well as local staging and the impact on radiation therapy and surgical management. ***We also discuss the potential future roles of PSMA PET/CT in prediagnosis such as risk stratification for biopsy, prognosis, and specific surgical roles. Potential pitfalls of PSMA PET/CT are also***

[33] https://www.researchgate.net/publication/383547930_Macrophages_REDUX and https://www.researchgate.net/publication/341788660_Fibroblasts_and_Cancer_The_Wound_That_Would_Not_Heal

[34] https://www.researchgate.net/publication/352554812_PSMA_A_Prostate_Cancer_Target

addressed. PSMA PET/CT has already had a significant influence on prostate cancer, and there will continue to be a greater role for this imaging modality in localized primary prostate cancer.

Thus using the prestaging one may believe that it is also useful for assessing the application of neoadjuvant therapy.

5.11 NK vs T cell Attacks

There is a discussion regarding the use of NK cells vs T cells as immunotherapeutic effectors. NK cells have several advantages as compared to T cells[35]. As Vivier et al have noted:

The ten hallmarks of tumour immunity of NK cells compared with T cells

	NK cells	**T cells**
Natural recognition of cancer cells		
Detection of stressed cells	Yes	Yes
Multiple ligands: tumour-antigen-agnostic activity against a vast array of tumour cells	Yes	No (TCR mediated)
Combat tumour cells with low mutation load	Yes	No
No antigen-specific priming required	Yes	No
No need for MHC-I expression activity increased in absence of expression	Yes	No
Elimination of cancer cells		
Direct killing of tumour cells	Yes**	Yes
Production of cytokines and chemokines that shape T cell responses	Yes	Yes
Activity against primary tumours and metastasis	Yes	Yes
Clinical studies have demonstrated Efficacy in haematological malignancies	Yes	Yes
Excellent safety profile of cell infusions	Yes	No (graft-versus-host disease)

And as the authors note:

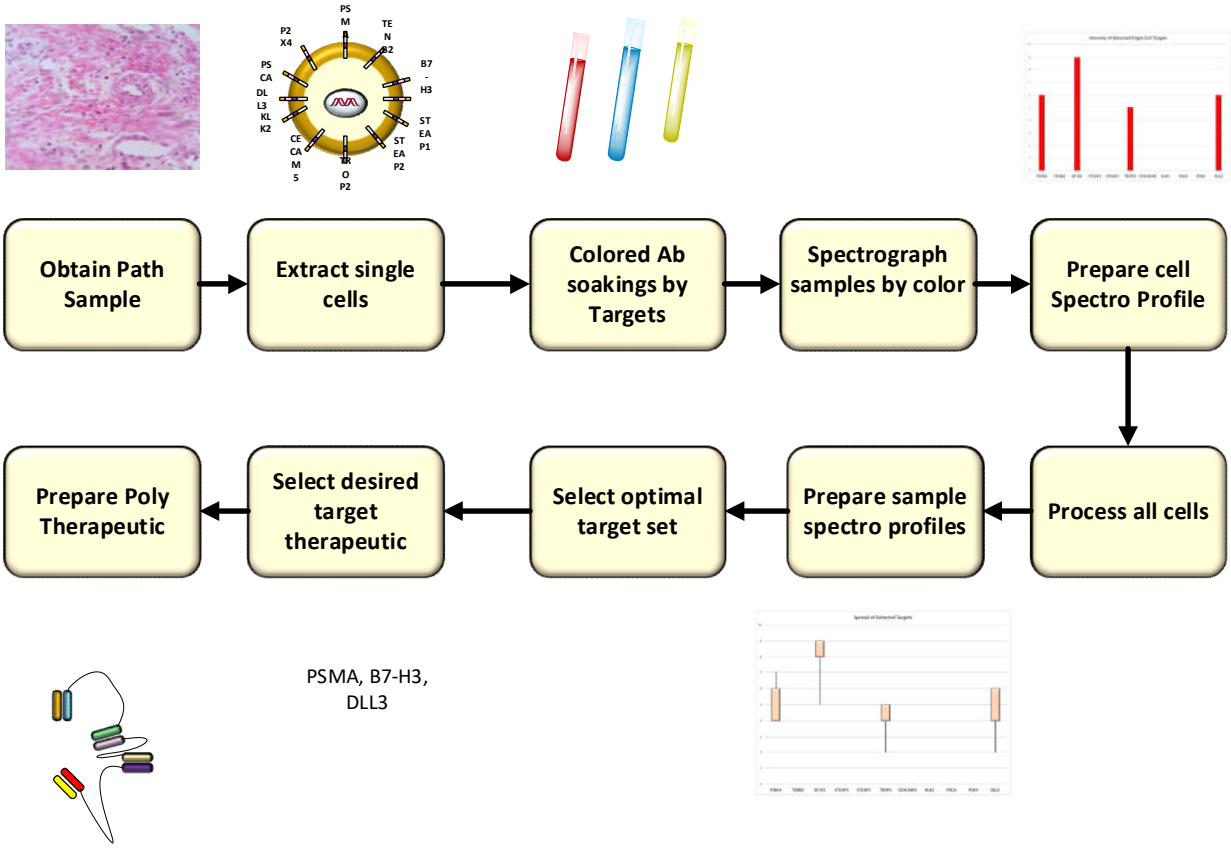
[35] https://www.researchgate.net/publication/367997547_Prostate_Cancer_CAR-NK_or_Ab

Two primary therapeutic strategies are being explored to enhance the antitumour efficacy of NK cells: monoclonal-antibody-based therapies and cell-based therapies. The monoclonal-antibody based NK therapies encompass the activation of NK cell antitumour immunity using immune checkpoint inhibitors (red antibodies) such as anti-LAG3, antiNKG2A, anti-TIM-3 and anti-TIGIT monoclonal antibodies, and the augmentation of NK cell antitumour response through monoclonal-antibody-derived tools that stimulate their activating receptors, such as NK cell engagers. The cell-based NK therapies use various sources of NK cell products that are injected into the patients, such as ex vivo conditioned NK cells, genetically manipulated NK cells and CAR NK cells. Activating and inhibitory NK cell receptors and their cognate ligands expressed on tumour cells are shown.

We believe that there are compelling reasons to use NK cells as noted above.

5.12 A Proposal for a Polyspecific Protocol

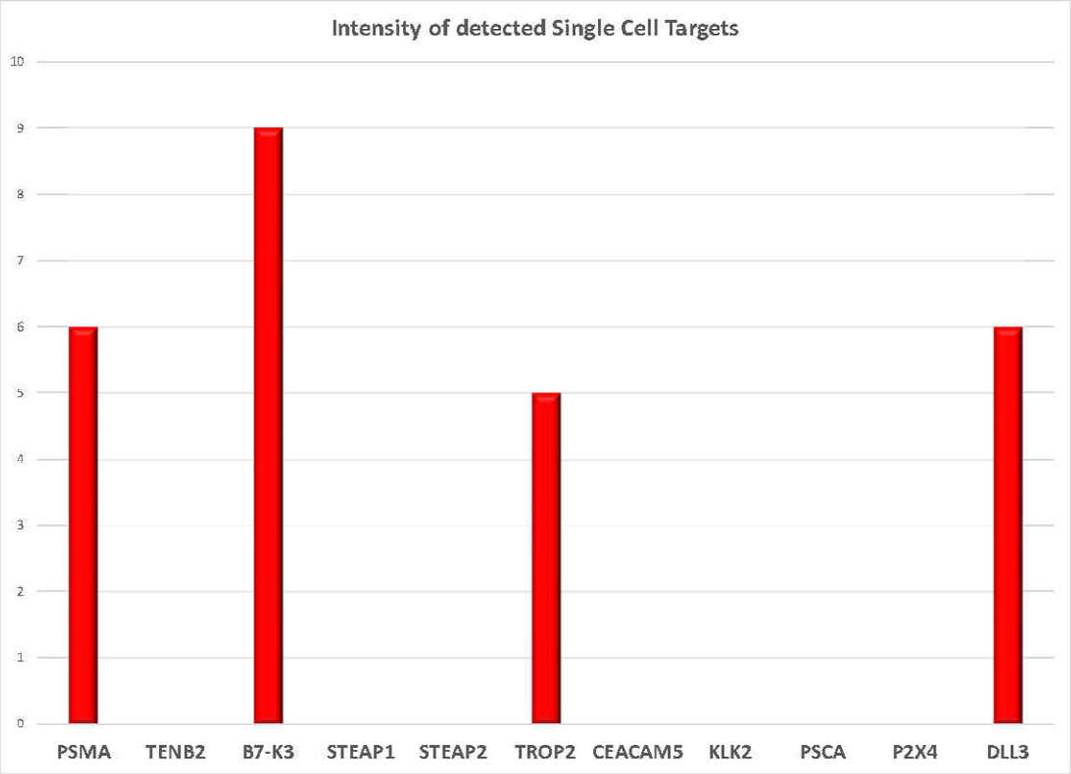
We repeat the proposal of a protocol as how to select targets and prepare therapeutics. The following is the process proposed:



We now follow through the steps:

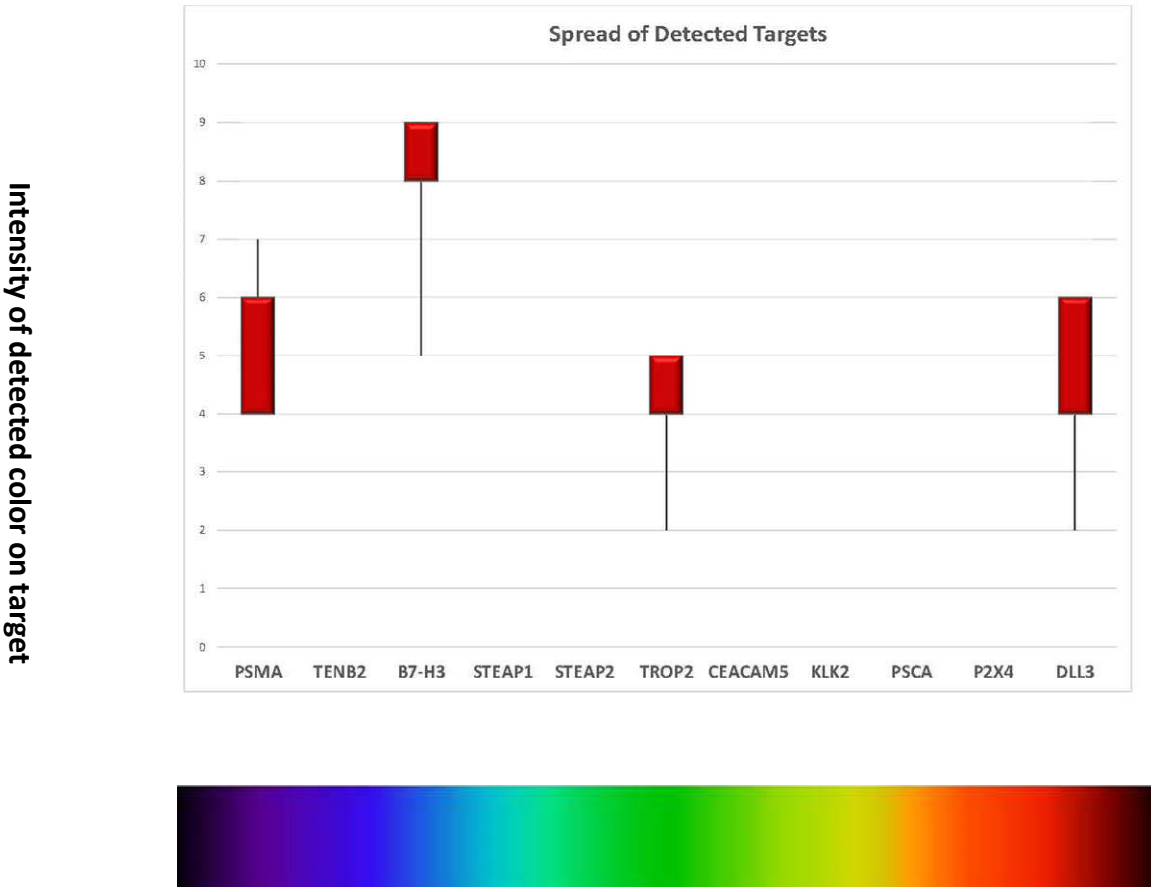
11. First obtain a path sample

12. The select a cell by cell from the sample. This allows a detection of the targets
13. Then using colorable Abs for each target select a specific color which can be determined by spectrographic means
14. Scan the cell to obtain spectrographic intensity
15. Prepare the cell spectrographic intensity as follows: Note that we see only 4 targets.



16. Then continue for all cells examining the targets spectrographically.
17. Process the cells

18. Prepare combined spectrographic data by spread analysis as shown below:



19. Select the optimal set of targets and then cull to a desired set. Here we show three selected targets
20. Prepare a polyspecific therapeutic based on procedures outline later.

This proposal allows individualized targeting for a specific malignancy. In fact, based upon collected clinical data these therapeutic polys can have been pre-prepared and used in a timely and cost effective manner.

The design and implementation complexity of this appears reasonable. Logically is should have broad usage for many cancers.

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NOTE: These references are inclusive of those directly used herein as well as those supporting the material herein.

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