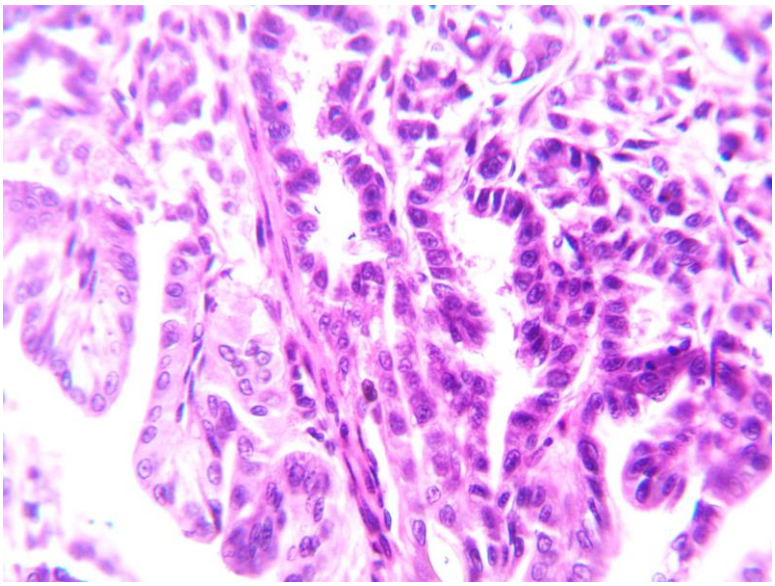

CORRELATION VS CAUSATION: THE PERILS OF AI

TGL 179, October 2020

ABSTRACT
This Report addresses the issue of Artificial Intelligence and its use in a variety of cancers. Our interest is in examining the advantages and disadvantages of these techniques. We focus on imaging and genomic inputs and examine how this has been addressed with various neural network approaches.

TERRENCE MCGARTY

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1 INTRODUCTION

There has been a significant proliferation of AI techniques as applied to a multiplicity of cancers. The approach is to take available data and using some AI technique deliver some diagnostic result. We examine some of these herein. For example, for colon cancer we examine a technique that use as an input the histology slides and determines certain classes of prostate cancer from them. The classes are based upon an amalgam of histological and genomic data. Thus in effect the AI process is inferring genomic data from cellular appearance. A second example is the use of miRNAs to ascertain the status of a putative prostate cancer.

In both cases, and the others we explore, using a neural net or other AI methodology provides a correlative result. This is in contrast to a causative result. A causative result is one where we know a fact and that fact can be measured and the results of that fact can be unambiguously stated. Newton's laws are such causative a set of results. Correlative is a data based supposition whereas causative is a scientifically provable fact. Correlation is often easy to assess, one needs just lots of data and a willingness to seek a relationship based solely on that data. Causation is fundamentally much more complex.

The danger of correlation is that a correlation may be ascertained between two factors wherein the fundamental relationship is some third hidden common factor.

For example, Linda et al recently provided an overview of AI techniques applied to cancer imaging. They summarize their efforts as follows:

Judgement, as one of the core tenets of medicine, relies upon the integration of multilayered data with nuanced decision making. Cancer offers a unique context for medical decisions given not only its variegated forms with evolution of disease but also the need to take into account the individual condition of patients, their ability to receive treatment, and their responses to treatment. Challenges remain in the accurate detection, characterization, and monitoring of cancers despite improved technologies.

Radiographic assessment of disease most commonly relies upon visual evaluations, the interpretations of which may be augmented by advanced computational analyses. In particular, artificial intelligence (AI) promises to make great strides in the qualitative interpretation of cancer imaging by expert clinicians, including volumetric delineation of tumors over time, extrapolation of the tumor genotype and biological course from its radiographic phenotype, prediction of clinical outcome, and assessment of the impact of disease and treatment on adjacent organs.

AI may automate processes in the initial interpretation of images and shift the clinical workflow of radiographic detection, management decisions on whether or not to administer an intervention, and subsequent observation to a yet to be envisioned paradigm. Here, the authors review the current state of AI as applied to medical imaging of cancer and describe advances in 4 tumor types (lung, brain, breast, and prostate) to illustrate how common clinical problems are being addressed. Although most studies evaluating AI applications in oncology to date have not

been vigorously validated for reproducibility and generalizability, the results do highlight increasingly concerted efforts in pushing AI technology to clinical use and to impact future directions in cancer care.

1.1 DEFINITIONS

The following is a list of characteristics for Causative and Correlative approaches and a comparison.

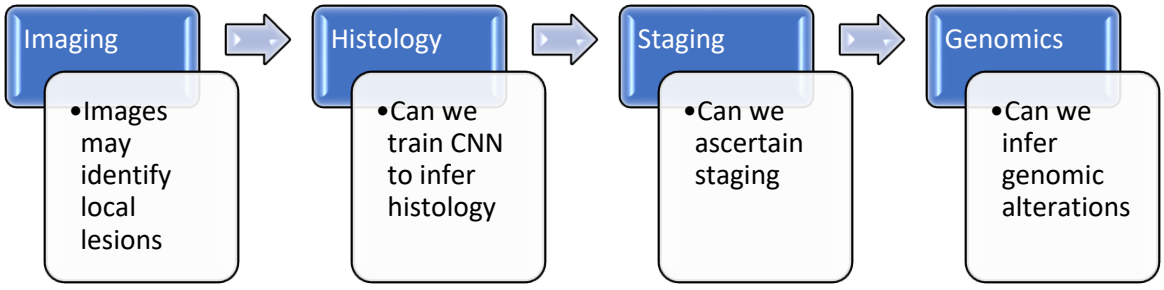
Characteristic	**Causative**	**Correlative**
Basis	Scientific validation and verification	Collective guess
Cause vs Effect	Direction of action is determinate	Uncertain
Usefulness	Can be therapeutic target	Problematic
Diagnostic	Dispositive	Suggestive
Prognostic	Suggestive	Uncertain
Repeatability	Open results allowing replication	Often proprietary methodologies

As we noted, causative is the basis of science. The repeatable demonstration of cause and effect. Correlative is all too often in the eye of the beholder. The underlying methodology to assert the relationship may all too often be hidden or worse unreproducible. Thus we should be wary in my opinion of AI qua proof.

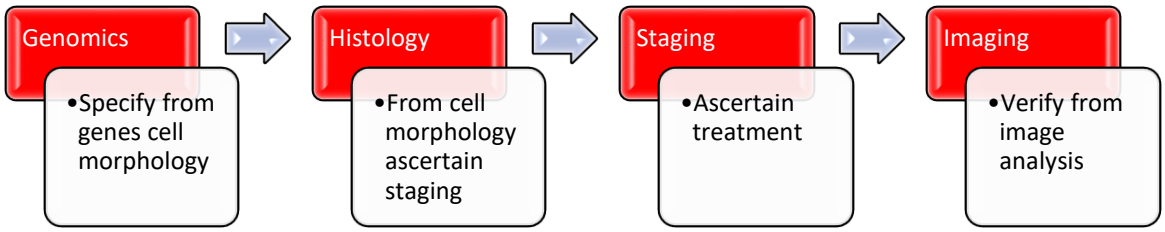
1.2 PARADIGMS

The core paradigm we will be focusing on will be of the following form:

The desirable outcome in a cancer is one where we can infer histology from say an imaging results and in turn infer malignant versus benign and localized vs invasive. Namely the goal is as below:



In contrast the paradigm in causative development is as follows:



This is more than a "chicken and egg" issue. We clearly know that the genomic issues control everything else, perhaps excluding certain extracellular environments. Thus when we try to reverse this natural paradigm one wonders if we have enough information to do so without error/

1.3 WHAT IS AI?

When one looks at much of the diagnostic and prognostic literature there is an ever growing reference to AI techniques. Having spent more than a half century using some of the methodologies one wonders if there is a bit too much hype. Namely can one just take an off the shelf algorithm, then take a set of training data, train the AI technique and then use it reliably and ethically in a medical application?

At the core belief of most if not all AI algorithms one has the belief that some form of data convergence can be obtained with an algorithm and enough well defined data. One can view most AI methodologies as follows:

1. Collect a data set of some measurements where we know a priori the outcome. For example blood test measurements and the presence or absence of some disease.

2. Use some AI type network where we enter the measurements and adjust weights on multiple paths across the network so that a selection of specific weights yields the a priori results with high probability.

3. Enter new unknown clinical data and measure the networks outcome.

4. That is the diagnosis.

There are many issues that result from this approach. First among then is the question of whether the new data entered is reflected in the training set. Thus, can we rely on the answer for new data? There are many others.

Now we are proposing taking this approach and applying it to images and working backward to genes. This dramatically compounds the complexity as we shall see.

Our objective herein is to examine a collection of AI applications and consider their benefits and concerns. There is an overall consideration that AI is viewed as a panacea for ascertaining results. We do not even have to select what to measure, what we are looking at, we just have to throw everything at a neural net, or better a convolutional neural net, make sure it is deep enough, turn the switch and out comes the answer. In a sense I call this a syndrome comparable to the Matlab generation. Before Matlab when one worked out complex problems, closed form solutions were often not at hand. But one could check boundary conditions, see how the system worked, then make linear assumptions, modify and expand them. The researcher lived in the bowels of the problem and from that came insight. This Matlab came along and we just write the equation and out come the answer. Easy but massive insight is lost. Taking a picture of the Mona Lisa does not compare to trying to paint it yourself.

2 NEURAL NETWORKS

Neural networks ("NN") have a long and storied history. They start with McCullough and Pitts and their work on neurons, then the development of the Perceptron, then an explosion of work in the 1980s, and finally as computer processing has expanded the neural network as we see it today. The term "deep learning" perhaps is just a euphemism for the massive amount of processing performed.

In essence, an NN is simply a large network with weights which uses a massive amount of input to determine a small amount of output, and it does so by having been trained with a massive amount of known inputs. The network uses the training in what is called a backward manner to establish weights across the network so that with many layers and many weights per layer an unknown input can be classified or identified.

Basically, there are two classes of interest. First is the neural network, NN, which takes in a measurement vector and output some classification. The NN uses several layers of processors and weights that are adjusted in an iterative manner using training sequences. The second is a convolutional neural network, where the input is an image and the output a vector which is then entered into a NN.

2.1 PATTERN RECOGNITION

Pattern recognition has been available in one form or another for decades. It has been used in a wide set of areas. Pattern recognition techniques allow for the "extraction" of key patterns to be used in discrimination. Thus using edge extraction in modifying say a US image we can then look for a pattern representative of a papilla like growth. We can look for pedunculated lesions, look for cyst like surfaces or look for clear nucleus or Orphan Annie eyes. Pattern recognition tools allow for the extraction of know or unknown patterns. If for example we know that certain patterns are pathognomonic then we can design a pattern recognition system to look for those specific patterns.

We believe that the development of pattern recognition and extraction methods will be at the heart of any successful classification scheme. The patterns will produce metrics which we can then use in the classification.

2.2 CLASSIFICATIONS; SUPERVISED AND UNSUPERVISED

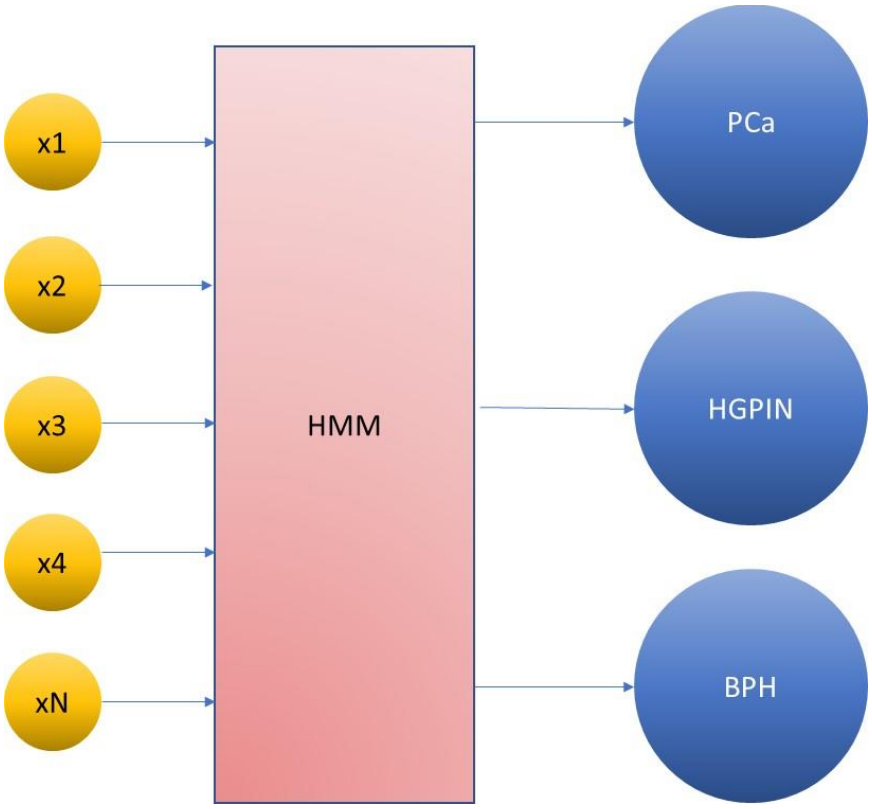
Classifiers take multidimensional data sets and establish lines of demarcation separating one class from another. The example of using PSA and %Free and seeking the dividing line between benign and malignant allows for a reasonable test. Multidimensional classifiers are much more highly structured.

We can now measure various miRNAs in body fluids and this gives rise to the liquid biopsy concept. However, the key question is how does one take a collection of miRNA measurements

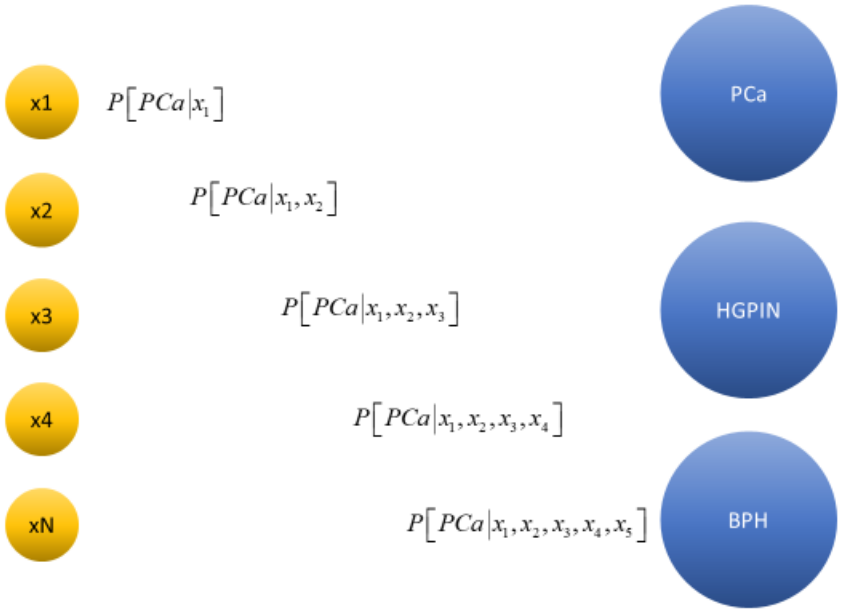
and ascertain, for example, that there is a prostate malignancy. For example we may from the previous presentation generate a vector of measurement of miRNA densities given by:

$$m_k = \begin{bmatrix} x_1 \\ \dots \\ x_n \end{bmatrix}$$

where this is for patient k and measures n miRNA densities. We want a discriminant function which takes these values and determines whether the patient has cancer of not. We could have a linear weighted discriminant or a more complex non-linear version.



We can look at a Markov model as below. However these transition probabilities are often difficult to determine.



$$P[x_1|PCa]$$

....

$$P[x_1,...,x_N|PCa]$$

or

$$P[PCa|x_1]$$

....

$$P[PCa|x_1,...,x_N]$$

where we have the two probabilistic ways to ascertain a condition based upon a data set.

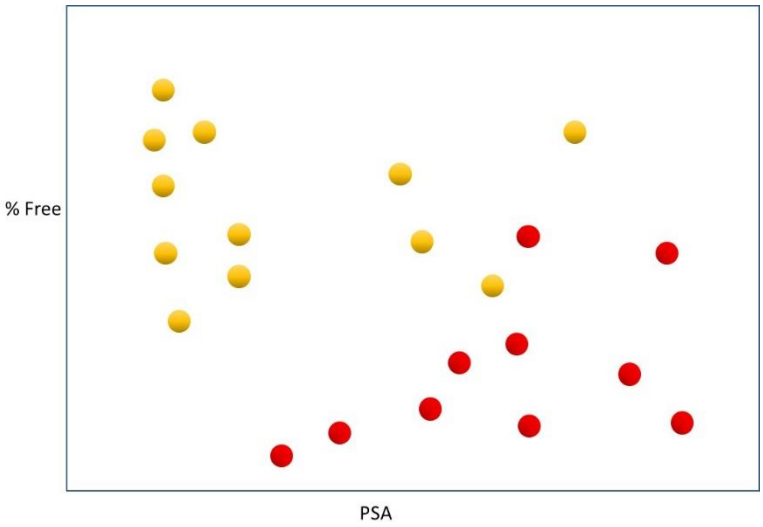
Let us consider a simple example. Assume we have to determine if a patient has prostate cancer or not. We are given three variables; PSA, % Free PSA, and PSA velocity[1]. Namely:

PSA=PSA
PF=% Free PSA

[1] See: Carter et al, Detection of Life-Threatening Prostate Cancer With Prostate-Specific Antigen Velocity During a Window of Curability, Journal NCI Vol 98 Nov 2006 pp 1521-1527, https://academic.oup.com/jnci/article/98/21/1521/2521858

V=PSA Velocity

Thus we have three measurements and they are somewhat related. Let us start with two of them; PSA and PF. The data may appear as shown below:



The red are PCa cells and the orange are benign. The higher the PSA the greater the chance for PCa. However, the higher the PF the greater the chance for benign, namely BPH. This is a simple case where we would have some discriminant where both variables count.

Now consider all three variables. We have PSA, PF and V. We need a discriminant so as to separate malignant from benign. We have data ex post facto so this is a supervised learning algorithm. We need to obtain some covering surface that maximizes the sensitivity and specificity. The algorithm must maximize the AUC. The more data the better the algorithm, yet we will always have aberrant cases.

The challenge in this case is that the discriminant is not a simple plane of some sort. It can be a complex surface winding its way around the 3-space. Namely the 2-space example shown in the above diagram may change for every V measure. For any V value we can obtain a 2-space profile. But that profile is different for every V and each has a different AUC. We can design a simple process where we enter all the data and calculate that surface on a cut by cut basis. Then any user can enter the three variable and get a result; benign/malignant, specificity, sensitivity.

Now let us consider a simple linear discriminant for PSA/PF and for a fixed V. Our goal is to select a curve:

$$PF = aPSA + PF_0$$

The goal is to obtain "a" and PF_0 so that we maximize both sensitivity and specificity. This can be readily accomplished by a variety of simple algorithms.

The next question would be; how many data points do we need and how frequently must they be updated? The answer can really only be obtained in an iterative manner with real data. We know that PSA alone has at best an AUC of 70%. Obtaining the AUC in this three element case is more complex. We may also want to add such elements as age, family history, prior biopsy results and the like. Each element adds another layer of complexity.

A simple and direct approach would be a linear classifier. Our metric is sensitivity and specificity. Namely:

$$Sensitivity = P\left[H_1 \middle| H_1 \right]$$

and

$$Specficity = P\left[H_0 \middle| H_0 \right]$$

If the discriminant plane is:

$$g(x) = ax + b$$

where

$$x = \begin{bmatrix} x_1 \\ \dots \\ x_N \end{bmatrix}$$

$$a = \left[a_1 \dots\dots\dots a_N \right]$$

The goal is given the data set, find the **a** vector and b to separate the data so as to maximize sensitivity and specificity[2].

There are a multiple set of classifiers and our selection of a linear classifier in a supervised environment is just one of many. We do not know the underlying statistics of the miRNA and also each miRNA itself may or may not be as strong an element in classification. Some miRNA that we choose may be a weak element and should be eliminated. That can only be ascertained after extensive data analysis.

Another way one could examine this partition problem is to assume that the two variables we discussed earlier, say PSA and PF, are independent Gaussian variable with mean and standard deviations:

[2] We refer to Theodoridis and Koutroumbas and their work on classification. We note that there are a multiplicity of algorithms to define this linear classifier. Also, there is a great deal on PCa learning algorithms in Hastie et al.

H_0 :

$m = m_0, \sigma = \sigma_0$

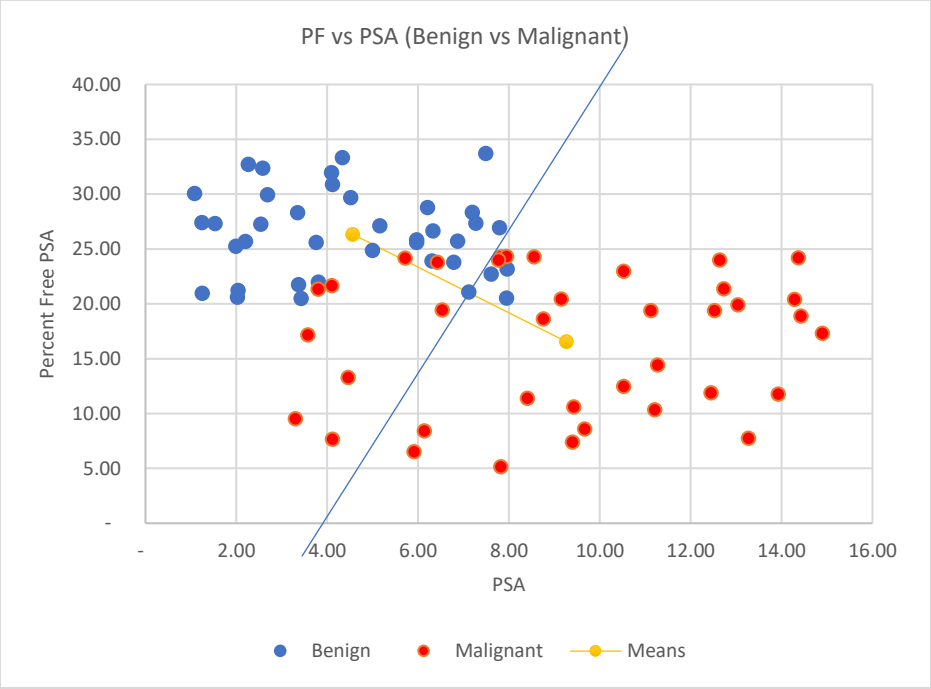
H_1 :

$m = m_1, \sigma = \sigma_1$

Then we could use classic decision analytical methods to determine optimal selection criteria. We could estimate the mean and variance from the given data and even ascertain a probability density function to see if it varies from Gaussian. It is not clear that such an approach yields better discrimination.

Finally, one could seek to use a Principal Component Analysis to determine optimal orthogonal axes[3]. However, again in my experience, this would not gain a great deal.

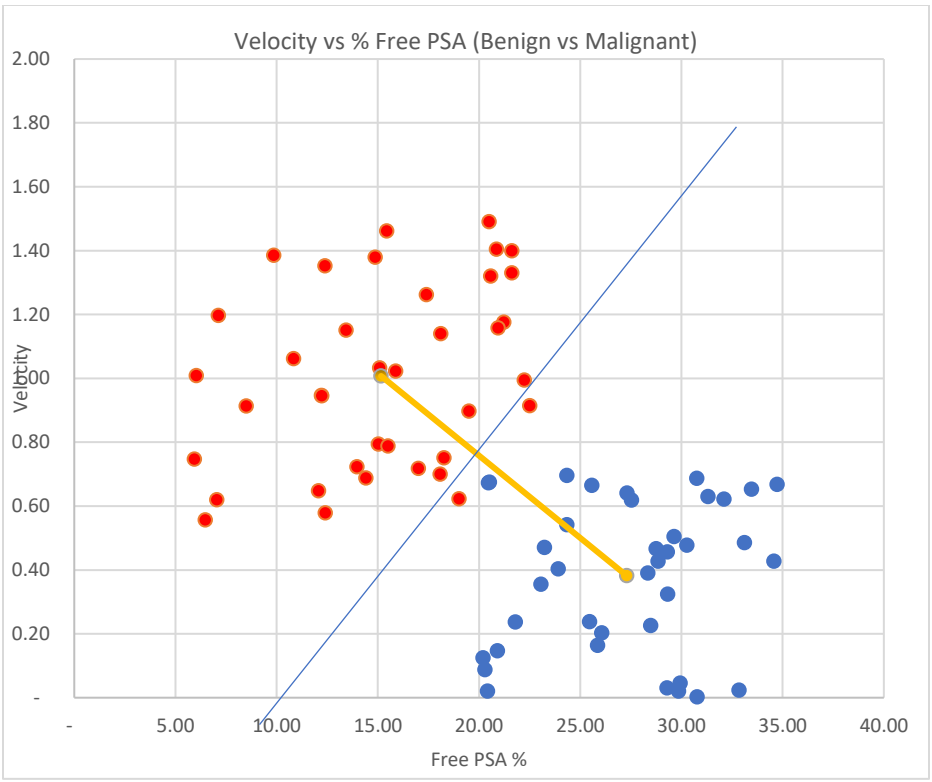
A linear classifier using the large data set may be more than adequate. We show below several examples of a linear classifier for PSA vs FP[4].



and for a second dimension we depict this below:

[3] See Dunteman, Principal Component Analysis, Sage University Paper, 1989.

[4] We use the reference of Duda and Hart, Pattern Classification, 1st Ed, Wiley, 1973.



Note all have different data yet all have same means on the two data sets. Thus the slope of the classifier is the same and intercept changes a bit. This same approach carries over to the miRNA context for multiple dimensions.

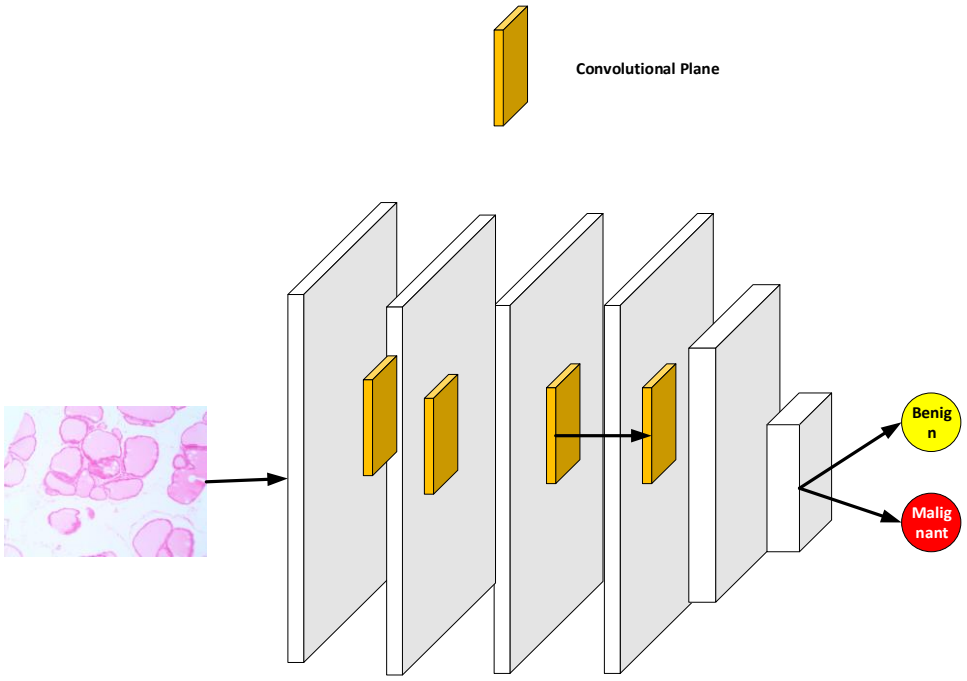
Now classifiers can often be nonlinear. The above simple example assumed a linear deign. However better performance may be obtained with a nonlinear classification scheme. The simplicity of the above classifier is based upon two facts. First, we know from the data who is benign and who is malignant. Second, we have selected elements, three in this case, upon which we can segment and classify.

In our study of images we do not necessarily have elements to be used to establish a classification. We start with a set of digital images. Perhaps from the images we can obtain a finite set of metrics, perhaps not. The next discussion is on neural networks, a more sophisticated from of classifier.

2.3 NEURAL NETS

Imaging analysis using neural networks often uses a convolutional neural network. The input may be an N X M matrix of k bit pixels. Namely a digital picture. We then collect are large set of such pictures and using knowledge of the disease state a priori we pass them through a network whose weights of connections are adaptively changed so as to maximize the probability that if we put in an unknown we get a very good guess as to whether it is benign or malignant.

In such a design we often use a convolution processor which passes on to the next layer a new meta-pixel which can be some integration or enhancement of the basic input. We show this construct below.



It functions as follows:

1. A two dimensional image, properly sized, is sampled into a two dimensional input plane in the network. It is identified as benign or malignant, and that is all.

2. The network has n planes and each plane has a convolutional filter as noted. Thus the entry on the following plane is comprised of (k x k) convolved samples of a segment of the prior plane.

3. A backward propagation algorithm is used as the network is trained. Namely M samples, each identified as malignant of benign, is passed through the network and the weights between layers are modified to optimize the output based on the known sample. Classically this may have been a least squares algorithm as was done in the classic Widrow Hoff optimizer for phased array antenna beam forming[5].

4. After the M samples are used to set the weights, the unknown is entered and the sample unknown identified.

Needless to say there are several key differences:

[5] See Monzingo and Miller, Introduction to Adaptive Arrays, Wiley, 1980, Chpts 3-4, VanTrees, Optimum Array Processing, Wiley, 2000.

1. No a priori patterns are selected. In fact, the network does not even assume that there are cells there. It has been trained on a large number of patterns.

2. The user may have no idea what the patterns emphasis may have been. Thus after some training period, the user is relying on the network to select what is a discriminant and what weight it may have.

The algorithm for neural networks with training is generally simple to grasp but it has many variations. Namely, data sets x consisting of say n x n arrays of 8 bit grey scale samples are used to put into a single hidden neural net, where we have say an m x m array of weights a which we want to train to discriminate between a disease state. The algorithms generally look like:

$$a = \begin{bmatrix} a_1 \\ ... \\ a_m \end{bmatrix}$$

and

$$x = \begin{bmatrix} x_1 \\ ... \\ x_N \end{bmatrix}$$

where

$$\hat{a}(k+1) = \hat{a}(k) - K(\hat{a}^T(k)x(k) - s)$$

where K is some convergence matrix. Namely we train the neural net with a massive number of samples whose sate "s", the disease, we know, and generate a(k), to reach some stable state, hopefully. Then with an unknown we send it into the net and hopefully get the correct disease state.

Now a brief overview of the Least Square Estimate procedures. Let us assume we are trying to estimate the slop and intercept of a straight line:

$$y = ax + b$$

we have N sets of x and y values, all somewhat noisy. That is:

$$x = \begin{bmatrix} x_1 \\ \dots \\ x_N \end{bmatrix}$$

and

$$y = \begin{bmatrix} y_1 \\ \dots \\ y_N \end{bmatrix}$$

Now we want a recursive estimator of the form (as we note to be a least square steepest descent model):

$$\hat{a}(k+1) = \hat{a}(k) + \Delta_a (y(k) - \hat{a}(k)x(k) - \hat{b}(k))$$

and

$$\hat{b}(k+1) = \hat{b}(k) + \Delta_b (y(k) - \hat{a}(k)x(k) - \hat{b}(k))$$

This is based on steepest descent algorithms and the choice of the function for the descent is based upon a least square performance function[6]. Namely we want to minimize:

$$\varepsilon^2 = \left[y(k) - \hat{a}(k)x(k) - \hat{b}(k) \right]^2$$

Classic least square has the descent function be that which minimizes the error for each element being minimized. Namely:

$$\frac{\partial \varepsilon^2}{\partial \hat{a}(k)} = \frac{\partial}{\partial \hat{a}(k)} \left[\left[y(k) - \hat{a}(k)x(k) - \hat{b}(k) \right]^2 \right]$$
$$= 2 \left[y(k) - \hat{a}(k)x(k) - \hat{b}(k) \right]$$

and the same for b. Thus, the steepest descent for a least square estimator is as we have shown above. The constants are chosen for convergence purposes and they are negative.

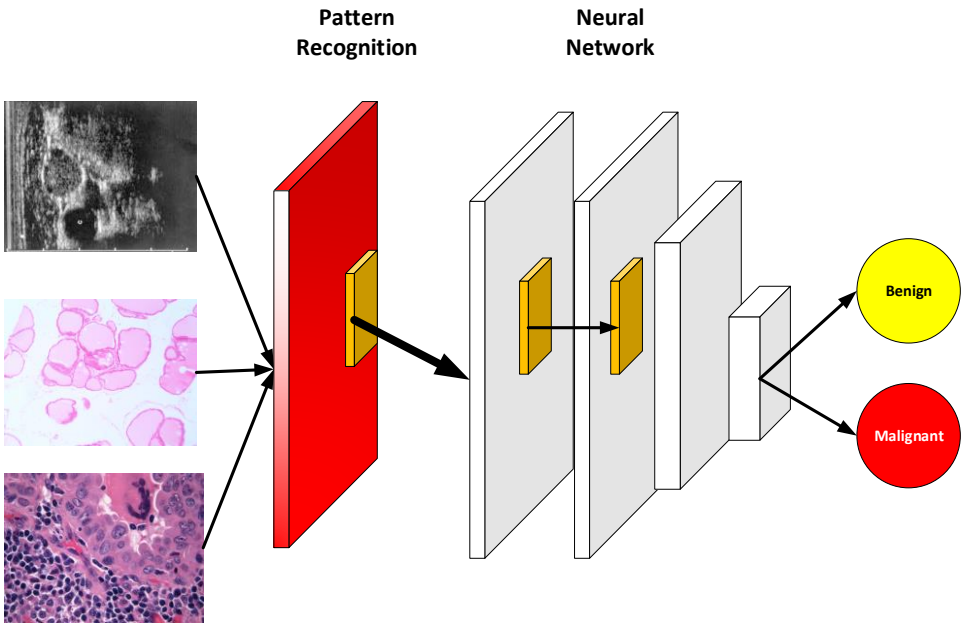
Now in a complex neural network we take the image which may be two dimensional and use each pixel as an input. Then we may convolve the image in some manner with a small m x m filter and pass it along. The weights at each step are adaptively changed if say a supervised test is performed. The neural net weights change in a manner similar to the linear estimator discussed above. We incrementally change them as we send identified image after image into the system[7].

[6] See Athans et al, Systems, Networks, and Computation, McGraw Hill, 1974

[7] See Hakim

There are now a massive number of algorithms to be used and with multiple layers as shown below we have deeper and deeper nets. Again the issue is that we are relying on the net to identify the diagnostic issues and we may never know what the net sees as important.

Now an added approach is to establish a pattern recognition front end where we can identify such things as edges, MDI artifacts, cell size, cell counts and the like. Then we feed those parameters to the Neural Net. We show this below.

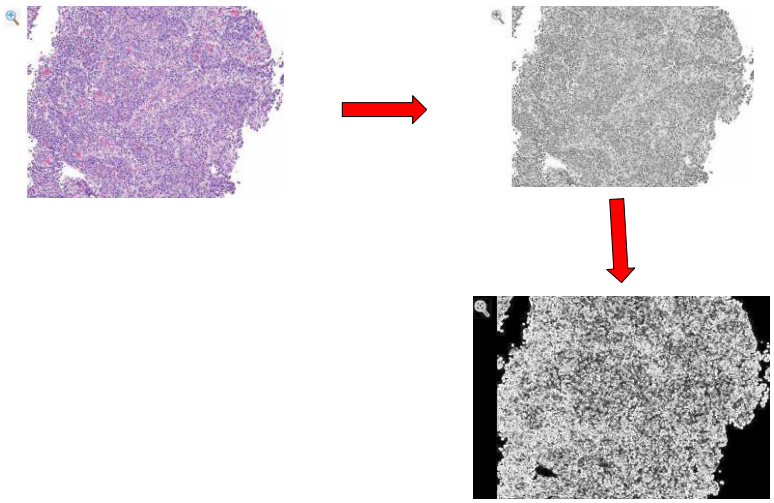


The above is a clear example of a pattern recognition system followed by a classifier. The classifier we have here is a neural network one but frankly we can use a variety of other classifier algorithms. It is critical to note that pure NN AI systems would just admit the images qua pixels. Here we add a priori knowledge of structures.

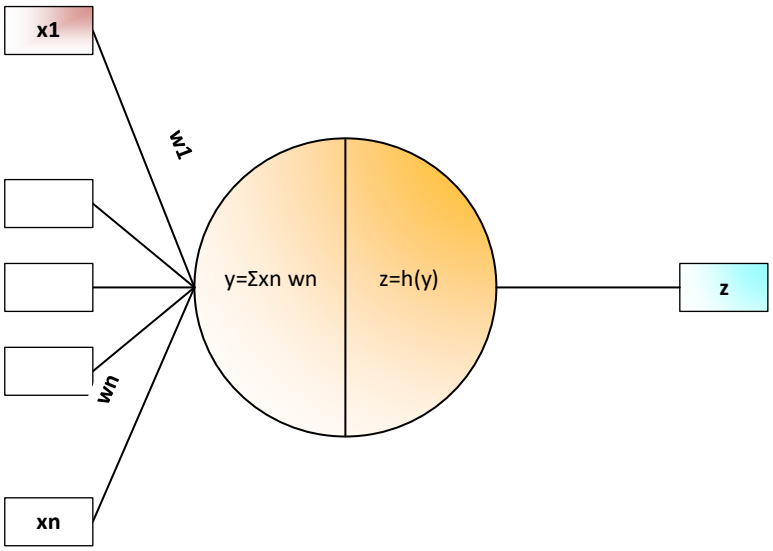
2.4 Some Details

Fundamentally many of the neural net applications deal with images. Unlike the prostate example where the input may be data measurements the image measurement is considerably more complex. We must first process the image and then use a NN as an adjunct. The concatenation of processing and image and a NN is called a Convolutional Neural Network, CNN. In reality we process the image in a manner similar to that of data, namely we ultimately reduce the image to a data vector but in so doing we apply the same techniques in the backward process in the previously least squared approach not only in the NN side but also in the image processing side.

Consider the image below of MSI colon cancer. We can process it by making it grey scale and then enhance it by say edge detection[8]. One may ask if this is what should be done first, namely a priori manual adjustments, and if so why or why not.

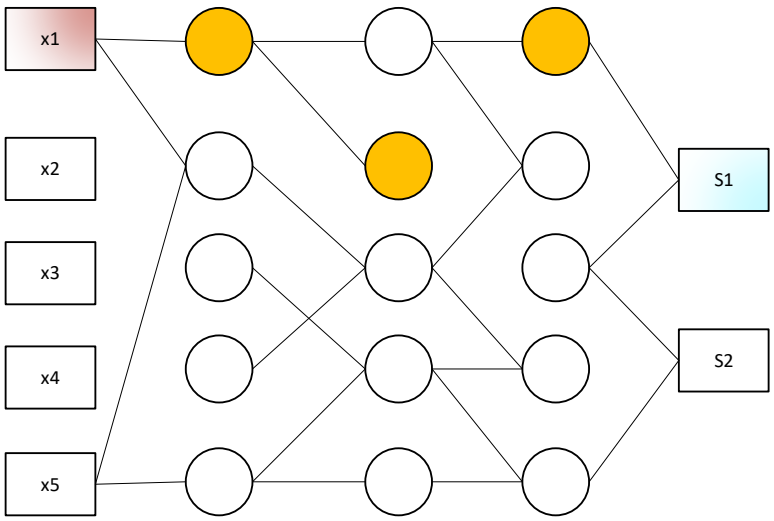


In reality we do not do this pre-processing. We use the same perceptron like elements on the pixels unprocessed. Recall that the perceptron processor is as below. We take incoming measurements, weight them by some algorithm, sum then up, then we apply some weighting function to smooth out the result. A typical function could be some exponential like function and there are a selection of many.



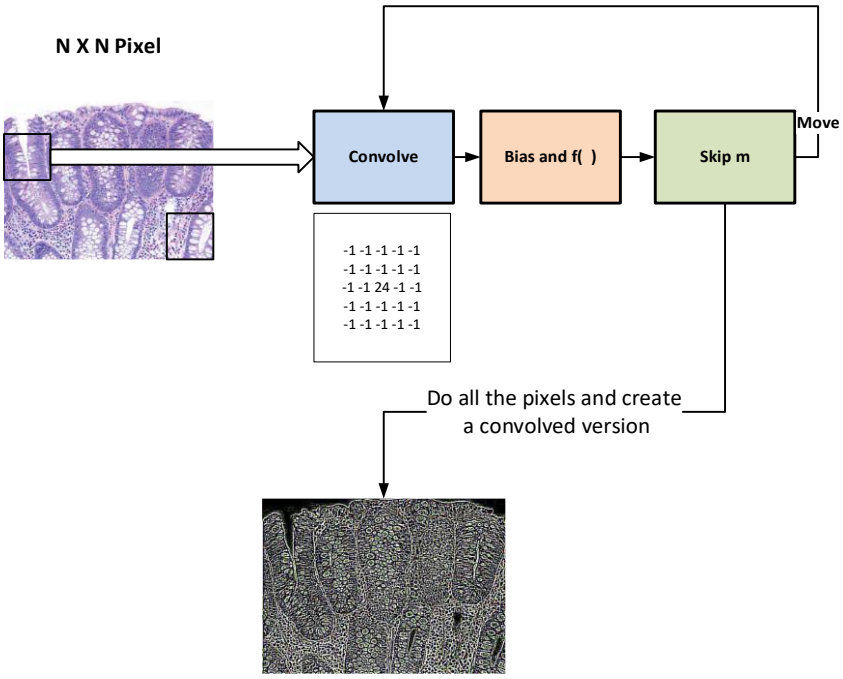
Note the output is just one from each such unit and this is made the input to the succeeding unit. A simple concatenation is shown below:

[8] Note, the processed images are done using Fiji and add-ons. The author finds it useful to use this tool to observe certain methodologies.



In the above case we take the 5 measurements, and look for one of two possible outputs; benign or malignant. We determine the w values by a backward training using a multiplicity of training data where we know the outcome. We use a least square backward training algorithm to adjust the w values at all layers of the NN and at some point their value reach some quasi steady state. One should not that there can be issue of unstable raining where it never converges and convergence on quasi optimum levels which may result in true biases on the determined output. The size of the training set can be quite large depending on the complexity of the net.

Now how do we deal with images? This we show below. We start with an image and we first convolve it. Here we show an image of a colon adenoma and we use a 5 by 5 convolving set. We may have converted the image to gray scale and use the amplitude of the gray scale and then in the convolution place the result in a cell. We seek to get some compression however. Namely instead of just doing each cell and mapping to another cell in some 1:1 mapping we may choose a cell, skip the next and do a 2:1 mapping on horizontal and 2:1 on vertical. This will yield a 4:1 compression. We show an example of this below



Now we can do this again and again but now, and this is critical, we do as follows:

1. Take the raw image

2. Process by some convolution compressing it as shown in a 4:1 manner.

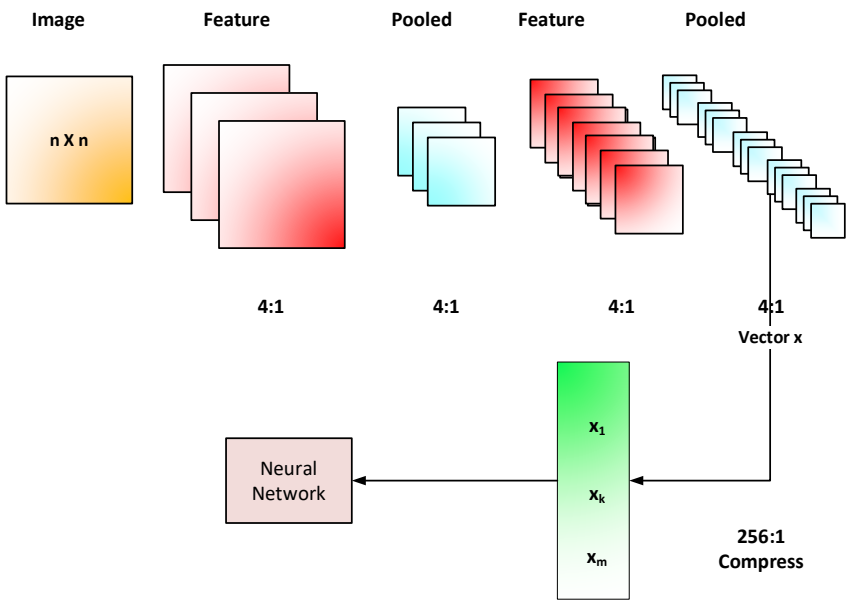
3. Now use weights as we do in an NN to generate a pooled quasi image and again doing a 4:1 compression

4. These yield a set of new images which we do the 4:1 again on

5. A pooled set using weights is used again

6. Finally we generate an input vector x which goes into an NN.

7. We then do backward training on data sets not only training the NN but also the we have used it in the pooled set.



The processing is highly complex and data intensive. This becomes a very deep NN complex and in some cases a special purpose process is best used. It will be this added architecture of the CNN we use when performing image analysis.

It is also worth summarizing the terms used in CNN. We follow Gonzalez and Woods, a superb reference book on this topic.

Term	***Definition***
Input Image	This is the raw image. It may be H&E or gray scale or any other form of a 2D image
Convolution	The weighted sum of a set of adjacent pixels
Bias	This is a selected bias
Activation	This is the function which processes the weighted value into a single result.
Feature Map	The 2D image resulting from the processing of an Image. The processing is the combination of the convolution, the stride used for compressing, and the activation.
Subsampling	This is convolution and activation
Pooled Feature Map	This is the image resulting from the Pooling process
Vectorization	The vectorization process is the process that take the final Pooled Image and converts it to an n dimensional vector which is to be placed into the following NN
Receptive Fields	These are small neighborhoods on the Image
Kernel	A set of weights in a Receptive field mapping
Stride	Number of spatial increments between sampling. For example, one may skip every other pixel or more.
Subsampling or Pooling	Pooling is a process whereby the Feature map is compressed again. The pooling process examines a small matrix of Feature Map pixels and produces a single resultant pixel according to some algorithm. For example, it may take a 2 X 2 selection and compute the average value.

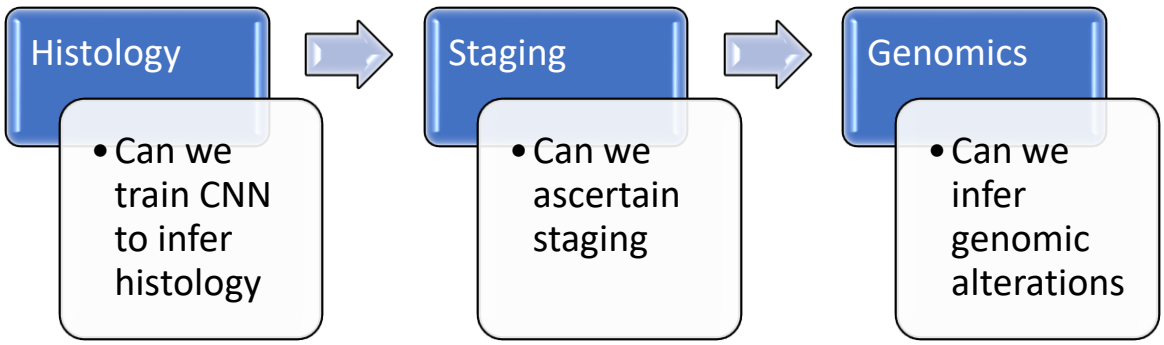
We now examine several cancers and the use of AI techniques as discussed.

3 COLON CANCER

Colon cancer has significant morbidity and mortality. Colonoscopies can provide an almost preventive procedure if performed frequently enough and by competent endoscopists. The process allows for the removal of polyps which may be pre malignant and thus any full invasive cancers can be avoided.

In diagnosing and treating CRC, colorectal cancer, understanding the histological character of the lesion and its genomic alterations is often essential in establishing a protocol. We examine here first the issue of lesion classification and then look at a recent effort to infer genomics from histologic data alone. From that inference, using a neural network, one then finds the class that the lesion falls in and from that an appropriate treatment.

The desirable outcome in colon cancer, CRC, is one where we can infer from histology results and in turn infer malignant versus benign and localized vs invasive. Namely the goal is as below:



Histological analysis is the classic method for identifying colon lesions. However even with histological diagnosis a genomic profile is also useful in treatment as well. Thus in today's environment it is incumbent upon the physician to seek as much information as would be possible.

3.1 CMS TYPES

There has been developed what is termed the "consensus molecular subtypes", CMS, of colorectal cancer as noted by Guinney et al. They summarize their work as follows:

Colorectal cancer (CRC) is a frequently lethal disease with heterogeneous outcomes and drug responses. To resolve inconsistencies among the reported gene expression–based CRC classifications and facilitate clinical translation, we formed an international consortium dedicated to large-scale data sharing and analytics across expert groups. We show marked interconnectivity between six independent classification systems coalescing into four consensus molecular subtypes (CMSs) with distinguishing features:

*CMS1 (microsatellite instability immune, 14%), hypermutated, **microsatellite** unstable[9] and strong immune activation;*

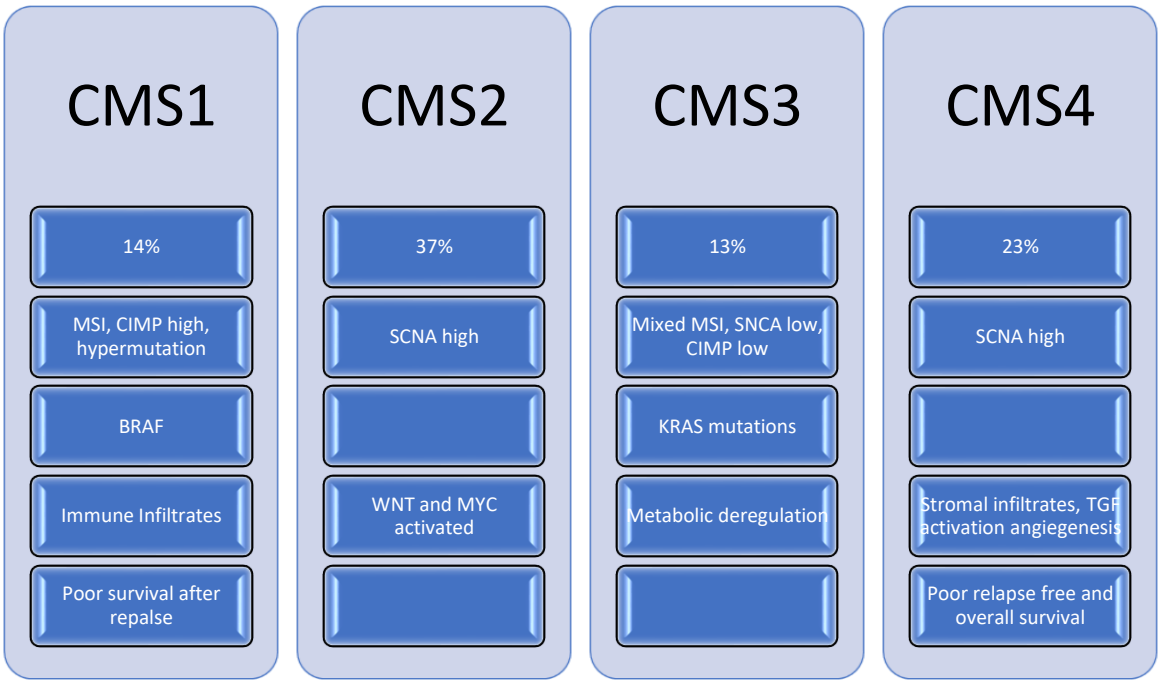
*CMS2 (canonical, 37%), epithelial, marked **WNT and MYC** signaling activation;*

CMS3 (metabolic, 13%), epithelial and evident metabolic dysregulation; and

*CMS4 (mesenchymal, 23%), prominent **transforming growth factor–β** activation, stromal invasion and angiogenesis.*

Samples with mixed features (13%) possibly represent a transition phenotype or intratumoral heterogeneity. We consider the CMS groups the most robust classification system currently available for CRC—with clear biological interpretability—and the basis for future clinical stratification and subtype-based targeted interventions.

Now this is a histological and genomic profiling of the tumor[10].



The CMS typing are useful not only for classification and prognosis but more importantly for treatment. The classification process is a complex integration of histological assessments and genomic profiling. Fontana et al have noted the use of automated classifiers in the assessment of CMS class as noted below:

[9] As we shall note the MSI is genomically controlled.

[10] From Guinney et al, Figure 5.

Using a network-based approach to match six distinct classifiers, we, along with other members of the CRC Subtyping Consortium (CRCSC), identified four robust consensus molecular subtypes (CMS):

CMS1, enriched for inflammatory/ immune genes;

CMS2, canonical;

CMS3, metabolic; and

CMS4, mesenchymal.

Stage-independent prognostic value and significant associations with multiple clinical and biological features were demonstrated. These data were subsequently validated in multiple retrospective analyses of prospectively collected clinical trial samples. Our group also demonstrated the potential predictive value of molecular subtypes with respect to the FOLFIRI (a combination of 5-flurouracil, leucovorin and irinotecan) chemotherapy regimen and the anti-epidermal growth factor receptor (EGFR)-targeted agent cetuximab using our previously published subtype classifier that is now reconciled into the CMS subtypes. Similar findings have been described in cell lines and retrospective clinical cases by others.

Recently, the CMS subtypes were evaluated as independent prognostic factors of survival demonstrating consistent results in correlative studies of phase III clinical trials; however, conflicting results were shown when tested as predictive factors of benefit from standard treatments in the metastatic setting.

Therefore, although the CMS subtypes show real promise for patient stratification to guide new biomarker-enriched clinical trials, a number of potential flaws and challenges must be accounted for when retrospectively analyzing studies or designing new ones.

The reason for such classifications, as noted, is that the treatment can be markedly different. For example, with MSI a recently discovered control gene, WRN, and be a useful target for therapeutics. We shall look at MSI in some detail. Yet one could surmise that it may be useful in the case of MSI to sequence for a WRN gene expression.

3.2 GENETIC FACTORS

It appears that all cancers have some set of underlying genomic aberrations. Colon cancer is no different and it was one of the first to understand the multiple gene hit paradigm. Vogelstein et al had done some of the early work in the area but as understanding has expanded the genes have also. From DeVita et al we have the following:

About 80% of CRCs show widespread chromosomal instability (CIN): gains, losses, and translocations that produce various gene amplifications, deletions, and rearrangements. Chromosomal segregation defects, mediated by segregation factors such as BUB1, may underlie CIN, but few genes are implicated directly. CRCs with CIN show a genome-wide bias toward

C:G to T:A transitions at 5′-CpG-3′, and fewer mutations in 5′-TpC-3′, dinucleotides than breast cancer, for example.3 On average, 17 genes are deleted or amplified to 12 or more copies per CRC,4 with deletions resulting in loss of heterozygosity (LOH).

In aggregate, the oncogenes ERBB2, MYC, KRAS, MYB, IGF2, CCND1, and CDK8 are amplified or overexpressed in most cases, usually along with neighboring genes, but nearly half the copy number alterations also occur in other cancers. CRCs thus reflect perturbation of selected pathways of replicative and tissue homeostasis, some common to many cancers and others restricted to CRC. Specific cytogenetic anomalies correlate poorly with clinical outcomes or disease features. Recent evidence suggests that beyond driving widespread gene alterations, CIN causes excessive spillage of DNA into the cytosol, hence activating noncanonical nuclear factor kappa B (NF-κB) signaling, which promotes metastasis.

Genetic pathways to colorectal carcinoma. All colorectal cancers (CRCs) arise within benign adenomatous precursors, fueled by mutations that serially enhance malignant behavior. Mutations that activate the Wnt signaling pathway seem to be necessary initiating events, after which two possible courses contribute to the accumulation of additional mutations. A: Chromosomal instability is a feature of up to 80% of CRCs and is commonly associated with activating KRAS point mutations and loss of regions that encompass P53 and other tumor suppressors on 18q and 17p, often but not necessarily in that order. B:

About 20% of CRCs are euploid but defective in DNA mismatch repair (MMR), resulting in high microsatellite instability (MSI-hi). MMR defects may develop sporadically, associated with CpG island methylation (CIMP), or as a result of familial predisposition in hereditary nonpolyposis colorectal cancer (HNPCC). Mutations accumulate in the KRAS or BRAF oncogenes, in p53 tumor suppressor, and in microsatellite-containing genes vulnerable to MMR defects, such as TGFβIIR.

They continue and include reference to multiple epigenetic factors as follows:

Epigenetic inactivation of the MMR gene MLH1 and activating BRAF point mutations are especially common in serrated adenomas, which progress, in part, through the silencing of tumor suppressor genes by promoter hypermethylation. Progression from adenoma to CRC takes years to decades, a process that accelerates in the presence of MMR defects. CIN, chromosomal instability. Although the remaining approximately 15% of CRCs are euploid, they carry thousands of small insertions and deletions (indels) or point mutations near nucleotide repeat tracts—collectively designated as MSI-hi—as a result of defective DNA mismatch repair.

Up to one-third of these cases occur in the setting of the familial Lynch syndrome, discussed at length in the following text. MSI-hi tumors usually arise in the ascending colon, have a high frequency of BRAFV600E mutation, resist adjuvant 5-fluorouracil treatment, and respond better to immunotherapy with programmed cell death protein 1 (PD-1) blockade than other cancers.

Stage for stage, aneuploidy in CIN confers a worse prognosis than MSI-hi disease.8 Some authors consider CIMP-positive serrated CRCs separately, but because their molecular, clinical, and pathologic features overlap with MSI-hi tumors, The Cancer Genome Atlas (TCGA) and

other groups pool the two CRC types into a single "hypermutated" category. Among the approximately 3% of sporadic CRCs cases that are hypermutated but lack MSI, the majority carry somatic mutations in the exonuclease domain of POLE. Moreover, rare kindreds with a dominantly inherited high risk of CRC carry specific germline defects in POLE or POLD1, the proofreading exonuclease genes for the leading- and lagging-strand DNA polymerases ε and δ. Although the tumors carry thousands of mutations, microsatellite tracts are stable; polymerase proofreading-associated polyposis (PPAP) thus represents a third "mutator" mechanism in CRC.

From DeVita et al (as modified) we have the following summary Table:

Gene	*Frequency*	*CRC Class*	*Known Cellular Function*
Oncogenes			
KRAS	35%–40%	CIN RTK	signaling
PIK3CA	18%–20%	Mostly CIN	RTK signaling
BRAF	7%–15%	MSI-hi, CIMP	RTK signaling
NRAS	9%	CIN	RTK signaling
ERBB3	<8%	CIN	RTK signaling
CTNNB1	<5%	All	Wnt pathway activation
Tumor Suppressor Genes			
APC	85%	All	Wnt pathway regulation
RNF43	18%	Mainly MSI-hi	Wnt pathway regulation
TP53	50%	Mainly CIN	Stress, hypoxia response
SMAD4	10%	CIN	TGF-β signaling
FBXW7	10%	CIN	Ubiquitin ligase
SOX9	5%–10%	CIN	Wnt-dependent ISC function
TGFBR2		MSI-hi	TGF-β signaling
MSH2, etc		MSI-hi	DNA mismatch repair
POLE		Hypermutated (non-MSI)	DNA polymerase ε
Epigenetic Modifier Genes (Roles Are Emerging)			
ARID1A		MSI-hi	Chromatin remodeling
SIN3A			Transcriptional repression
SMARCA5			Chromatin remodeling
NCOR1			Transcriptional repression
JARID2			Histone modification
TET1, 2, 3			DNA demethylation

The above lists the three classes: Oncogene, Suppressor Genes, and Epigenetic Modifiers. We can note that such a listing is quite common across the board. Recent work by Piskol et al to CMS deserves merit.

Now NN studies have also been applied to colon cancer genomics (see Liu et al 2017). In the referenced article they conclude:

We applied a systems biology approach, namely, WGCNA (Weighted gene co-expression network analysis), to analyse one mRNA expression dataset comprising 461 CC patients to identify the networks and genes associated with clinical variables and prognosis indicators. We then confirmed our findings by using an independent validation dataset. WGCNA can be applied to determine complex biological mechanisms responsible for the target phenotypes; this method is effective because the algorithm aims to clarify the relationships between genes above noise and maintain consistency among all of the samples.

The unsupervised hierarchical clustering method selected by WGCNA avoids potential biases and subjective decisions attributed to the selection of the candidate genes previously reported as associated with CC or to the early distinction of control samples for supervised methods.

This is an interesting alternative of not having a supervised or training set. It basically takes the raw data and then allows the NN to process.

In our study, 11 distinct gene modules from 3600 genes that satisfied our pre-filtering standard for the co-expression analysis were identified. The increased expression of the green module containing 170 genes mostly related to the cell cycle was associated with low tumour grade and correlated with positive RFS outcome. The association relationship reached statistical significance in the discovery dataset ($p = 1.37 \times 10{-}3$, FDR = $7.52 \times 10{-}3$), and marginal significance ($p = 6.67 \times 10{-}2$) in the validation dataset. This marginal significance may account for the small sample size (n = 111) and lack of statistical power. Since we can make conclusions depend on the effect size and its precision rather than just the p-value.

The result of HR and 95% CI of module green in the validation dataset suggested that the association relationship between module green and RFS of CC patients was clinically significance. After conducting the single gene survival analysis of each member of the green module, we found that approximately 65% of the genes were significantly related to RFS ($p < 0.05$), and all of the genes yielded HR < 1.

Furthermore, the hub gene CENPA was identified as a potential novel marker. CENPA, a protein-coding gene, is the histone-H3-like variant essential for centromere functioning and structure. This gene is implicated in cell cycle and mitotic pathways; the GO annotations of this gene include protein heterodimerisation activity and chromatin binding. CENPA is also a potential prognostic biomarker of breast cancer, and the increased expression of this gene is associated with the poor survival of breast cancer patients.

Among various core markers in neoplasic intratubullar germ cells, such as CD9, CENPA and PODXL, CENPA is overexpressed, and this finding suggests that this gene may be a potential biological marker of human diseases. … CENPA is overexpressed at a transcriptional level in all 11 primary human CC tissues. Furthermore, the immunostaining with anti-CENPA antibodies revealed that the CENPA signals in tumour cells increase; therefore, the overexpression of CENPA may be critical in aneuploidy in colorectal cancers. However, the role of CENPA in CC should be further validated. The yellow module containing 179 genes involved in the cell cycle was correlated with RFS in the type 3 subtypes, including MSI-low, CIMP-negative, negative for BRAF mutation and positive for KRAS mutation.

We also identified CDK1 as a marker. The specific activity of CDK1 is a promising biomarker of the metastasis risk in stage II CC. The type 3 subgroup is the only subgroup with KRAS mutation. KRAS, a proto-oncogene, encodes a small 21 kD guanosine triphosphate/guanosine diphosphate binding protein that modulates cellular proliferation and differentiation. Approximately 97% of KRAS mutations are caused by seven different DNA base-pair substitutions in codons 12 and 13 of exon 2; as a result, an amino acid substitution in the protein occurs.

Therefore, KRAS may affect cell cycle processes. In a recent study, CDK1 is reported as a synthetic lethality target for KRAS mutation in colon cancer. Our study highlights RAD51AP1 as a prognostic marker and therapeutic target. It has been reported that Overexpression of RAD51 is a negative prognostic marker for colorectal adenocarcinoma. However, the roles of the hub gene KIF11 in module yellow and OTUD6B in module tan in CC have yet to be determined.

One can now compare this to the prior list from DeVita et al.

3.3 MSI

Microsatellites are the result of improper gene reproduction resulting in dysmorphic gene structures and the resultant gene instability. The appear in several of the CMS classes. From NCI:

A change that occurs in certain cells (such as cancer cells) in which the number of repeated DNA bases in a microsatellite (a short, repeated sequence of DNA) is different from what it was when the microsatellite was inherited. Microsatellite instability may be caused by mistakes that don't get corrected when DNA is copied in a cell. It is found most often in colorectal cancer, gastric cancer, and endometrial cancer, but it may also be found in many other types of cancer. Knowing whether a cancer has microsatellite instability may help plan the best treatment. Also called MSI.

A recent NCI study on MSI has noted[11]:

Last year, scientists discovered that cancer cells with a genetic feature called microsatellite instability-high (MSI-high) need WRN to survive. Getting rid of WRN killed MSI-high cancer cells but not healthy cells—sparking excitement about a potential new treatment approach. Around 1 in 3 endometrial cancers, and 1 in 7 colorectal, stomach, and ovarian cancers, are MSI-high. It's a hallmark of tumors that develop in people who have Lynch syndrome, a hereditary disorder that increases the risk of cancer.

Specific tests can determine whether a person's cancer is MSI-high. The new study addresses the big question of why MSI-high cancer cells depend so heavily on WRN…WRN unwinds unusual DNA structures that occur more frequently in MSI-high cancer cells than in other

[11] https://www.cancer.gov/news-events/cancer-currents-blog/2020/microsatellite-instability-cancer-wrn?cid=eb_govdel

cancer cells, the NCI researchers found. In MSI-high cancer cells that lacked WRN, these unusual structures eventually cause the DNA to shatter, killing the cells. … DNA Breaks in MSI-High Cancer Broken DNA. … "In 40% of the cells, the chromosomes look like dust…It's like a bomb went off." …

The breaks occurred in the same areas in the genome, even in different kinds of lab-grown colorectal cancer cells. Nearly all of the breaks were near a long series of the same two DNA letters, or bases. The letters, T and A, were repeated back-to-back, as if there was a stutter in the DNA. Repeats of back-to-back Ts and As are found in healthy cells and cancer cells without MSI, too. But in MSI-high cancer cells, many TA repeats were much longer than normal. Some were expanded several hundred times. … the standard way to read, or sequence, DNA skips over sections with long, repetitive sequences, he explained. But with new technologies, scientists can finally read these elusive sections of DNA. "It's becoming clear that [we] are missing too many important [DNA] elements" with the standard way of sequencing…" …As researchers eventually shift to using the newer technologies, more features of DNA are likely to be discovered, he said. But at the moment, the new technologies are only available at a handful of large research institutions.

Thus it seems important to gain information on the operations of such genes as WRN[12]. Namely WRN is required to the aberrant DNA to be sustained and thus produce the cells one sees in colon cancer. In effect, one can assert from the above, that MSI is driven by WRN. Here we see a classification of genomics first and histology second.

As Koncina et al note:

Microsatellites are short tandem repeats of DNA sequences located throughout the genome. MSI status results from a deficient DNA mismatch repair (MMR) system, commonly caused by the inactivation of the four MMR genes (MSH2, MLH1, MSH6 and PMS2). A deficient MMR system leads to a failure in the correction of the insertion or deletion of repeating units during DNA replication, leading to a hypermutable phenotype (MSI-high is characterized by instability at two or more loci). MSI status can be determined by two distinct methods—immunohistochemistry analysis (IHC) or PCR..

Reduced expression of the MLH1, MSH2, MSH6, and PMS2 genes, determined by immunohistochemistry analysis, identifies tumors as MSI (microsatellite instable, also referred to as deficient MMR, dMMR) in contrast to MSS (microsatellite stable, also referred to as proficient MMR, pMMR). Alternatively, standard PCR can be used to compare microsatellite length in tumors versus normal tissue in order to determine aberrant microsatellite lengths detected in the tumor

[12] WRN: This gene encodes a member of the RecQ subfamily of DNA helicase proteins. The encoded nuclear protein is important in the maintenance of genome stability and plays a role in DNA repair, replication, transcription and telomere maintenance. This protein contains a N-terminal 3' to 5' exonuclease domain, an ATP-dependent helicase domain and RQC (RecQ helicase conserved region) domain in its central region, and a C-terminal HRDC (helicase RNase D C-terminal) domain and nuclear localization signal. Defects in this gene are the cause of Werner syndrome, an autosomal recessive disorder characterized by accelerated aging and an elevated risk for certain cancers. https://www.ncbi.nlm.nih.gov/gene/7486

As Montgomery notes:

MSI evaluation typically involves microdissection (usually manual) of tumor and normal tissue from submitted formalin-fixed paraffin-embedded tissue sections followed by DNA isolation, a polymerase chain reaction (PCR) using primers directed at a number of microsatellite markers and analysis of the PCR products by capillary electrophoresis for patterns of MSI. As such, when colorectal cancers are biopsied, we encourage our clinical colleagues to also sample benign flat mucosa to facilitate this testing, but of course, the testing can always be performed later on resected material in which there is abundant normal tissue to use as a control.

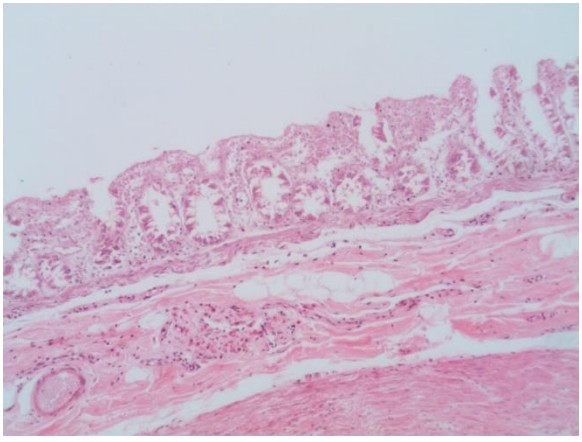
Electropherograms from both tumor and normal are usually compared when conducting this analysis. The two most commonly used panels are a Promega Microsatellite Analysis System and a reference panel recommended by an NCI-sponsored consensus committee. The Promega system consists of five mononucleotide markers, whereas the NCI-sponsored reference panel consists of three dinucleotides and two mononucleotides.

However, mononucleotide markers show the greatest sensitivity for MSI. MSI is diagnosed when microsatellite lengths are shifted from the germline pattern. Three possible data interpretations exist: MSI-high (MSI-H), MSI-low (MSI-L), and microsatellite stable (MSS). When five markers are used, a tumor that shows MSI in greater than or equal to two loci is considered MSI-H, one that shows MSI in one locus is termed MSI-L, and one that shows no MSI at any locus is diagnosed MSS.

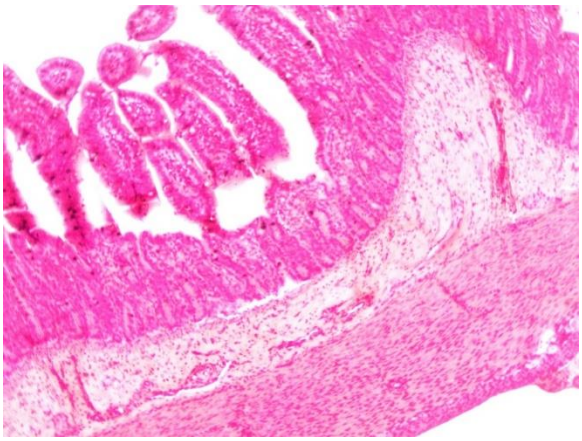
This is important in that the recommendation is the unambiguous identification of genomic markers.

3.4 Histological Interpretations

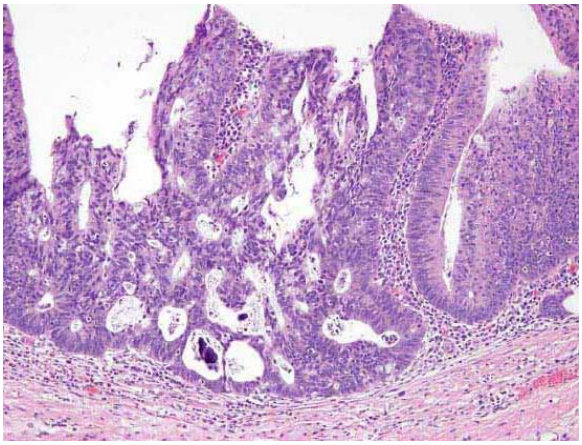
We can now examine some typical histological results. It will then be from these that we allegedly can ascertain the genomic aberrations. A normal colon section is shown below. As we shall note, the structure is somewhat normal in appearance with regular cell conformation.



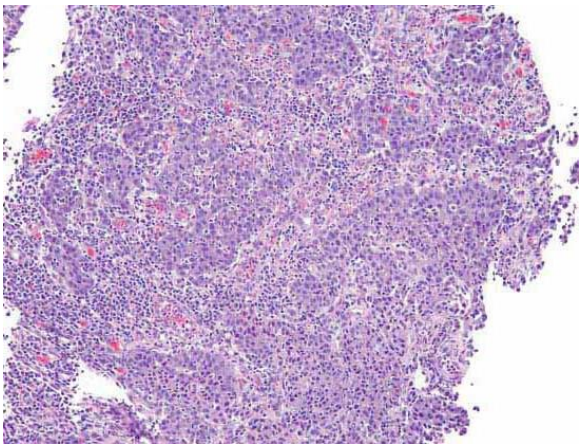
The figure below is a similar view but at higher resolution.



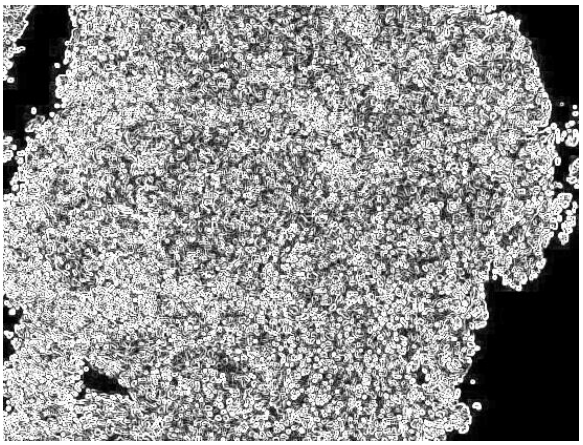
The image below is a high-grade dysplasia (Fig 4.34). The structure is highly non-uniform



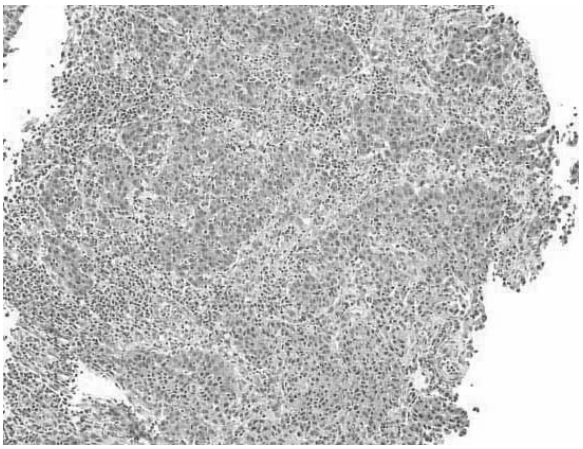
The image below is a microsatellite unstable mismatch repair (Fig 4.46). It is highly dysplastic.



We can now process this in gray scale as shown below. Generally we may not lose a great deal of information.



Then we may further process using edge detection as shown below. The question is; are we losing diagnostic information? What is best as an input to the set of images we use to train?



The above are just a few examples of the CRC potentials that must be considered. The demonstration of image enhancement is just an expression of what could possibly be done with pre-processing. We shall discuss this in the following section.

3.5 AI APPLIED TO CMS

There is a recent paper by Kather et al regarding the use of AI in the identification of CMS in colorectal cancer. This paper addresses the paradigm of histology to genomics. The authors allege:

Molecular alterations in cancer can cause phenotypic changes in tumor cells and their microenvironment. Routine histopathology tissue slides, which are ubiquitously available, can reflect such morphological changes. Here, we show that deep learning can consistently infer a wide range of genetic mutations, molecular tumor subtypes, gene expression signatures and standard pathology biomarkers directly from routine histology. We developed, optimized, validated and publicly released a one-stop-shop workflow and applied it to tissue slides of more than 5,000 patients across multiple solid tumors.

Our findings show that a single deep learning algorithm can be trained to predict a wide range of molecular alterations from routine, paraffin-embedded histology slides stained with hematoxylin and eosin.

This is the significant assertion made by the authors. Namely they assert the ability using an NN one can determine genomic variants independent of specific sequencing.

These predictions generalize to other populations and are spatially resolved. Our method can be implemented on mobile hardware, potentially enabling point-of-care diagnostics for personalized cancer treatment. More generally, this approach could elucidate and quantify genotype–phenotype links in cancer.

Simply stated this means that by examining the histology we can identify the genomic changes adequately to assert a CMS class. The assertion states that using a neural net approach the underlying genomic structure of the lesion can be asserted. We have examined the opposite approach, namely seeking what genes lead to what structure, and why. Here they make assertions on genomics from structure via the NN.

Two steps are worth note. First the image processing:

Scanned WSIs of diagnostic tissue slides (FFPE tissue) stained with hematoxylin and eosin were acquired in SVS format. All images were downsampled to 20× magnification, corresponding to 0.5µm px−1. Each WSI was manually reviewed and the tumor area was annotated under direct supervision of a specialty pathologist. During annotation, all observers were blinded with regard to any molecular or clinical feature. Only those images containing at least 1 mm2 contiguous tumor tissue were used for downstream analysis.

In total, 6% of WSIs, corresponding to 5% of patients, were excluded due to technical artefacts or a lack of tumor (Supplementary Table 2). Tumor tissue on all other slides was tessellated into square tiles of 512 px×512 px edge length, corresponding to 256µm×256µm at a resolution of 0.5µm px−1. Tiles with more than 50% background were discarded. Background pixels were defined by a brightness of >0.86 (220/255). For the benchmark task (identification of an optimum neural network model), these images were resized to 224 px×224 px

and second the neural net approach:

Deep neural networks were trained on image tiles with the aim of predicting molecular labels. All neural networks were pre-trained on the ImageNet database, as described previously9, and were specifically modified for the classification task at hand by replacing the three top layers with a 1,000-neuron fully connected layer, a softmax layer and a classification layer. For training, we used on-the-fly data augmentation (random horizontal and vertical reflection) to achieve rotational invariance of the classifiers. Hyperparameter selection was performed for five commonly used deep neural networks: ResNet-18, AlexNet, Inception-V3, DenseNet-201 and ShuffleNet.

In a similar paper by Sirinukunwattana et al the authors note:

This study shows that a prediction of RNA expression classifiers can be made from H&E images, opening the door to simple, cheap and reliable biological stratification within routine workflows….

The aim of this study was to develop an image analysis framework to associate features of tissue organisation on standard histology slides with molecular classification and outcome data in patients with CRC. Training and test cohorts were selected to represent relevant clinical scenarios in the management of patients with CRC including postoperative resection specimens (FOCUS and TCGA) and endoscopic biopsy material (GRAMPIAN). A total of 1540 slides from three independent datasets were utilised in this study including 666 slides of resection specimens from 362 patients in the FOCUS cohort, 468 slides of resection specimens from 463 patients in the TCGA cohort and 406 slides from preoperative biopsies of 223 patients in the GRAMPIAN cohort.

Tumour areas on each slide were annotated by a pathologist, and the molecular analysis was performed on material obtained from strict serial sections to derive the CMS calls. Clinical and molecular data are summarised in online supplementary table S1. The imCMS classifier was trained against CMS calls on the transcriptionally classified samples of the FOCUS cohort (510 slides, 278 patients) and tested on the TCGA (431 slides, 430 patients) and GRAMPIAN (265 slides, 144 patients) cohorts (online supplementary materials and methods).

With the assumption that each CMS class is associated with unique histological patterns localised in different regions of the tumours,14 inception V319 deep neural networks were trained for prediction of CMS calls for small overlapped image regions (tiles) of 512×512 pixels within the annotated regions (figure 1C). The size distribution of annotated areas per slide and the number of tiles per slide is …

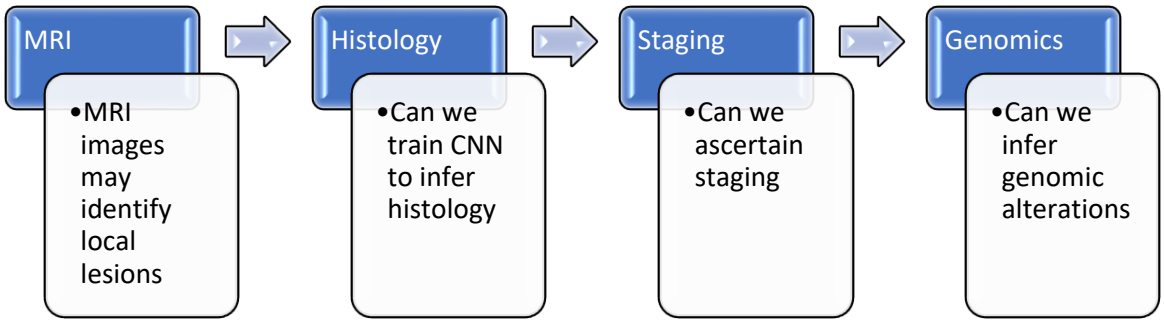
The imCMS class, prediction score and spatial location for each tile were recorded. …

An overall imCMS call for each slide was assigned based on the majority classification of tiles. image-based cMs (imcMs) accurately classified slides in unseen datasets from Tcga (n=431 slides, AUC)=0.84) and rectal cancer biopsies (n=265 slides, AUC=0.85). imcMs spatially resolved intratumoural heterogeneity and provided secondary calls correlating with bioinformatic prediction from molecular data. imcMs classified samples previously unclassifiable by RNA expression profiling, reproduced the expected correlations with genomic and epigenetic alterations and showed similar prognostic associations as transcriptomic cMs

Thus there has been and continues to be a significant amount of effort to go from image to gene and RNA.

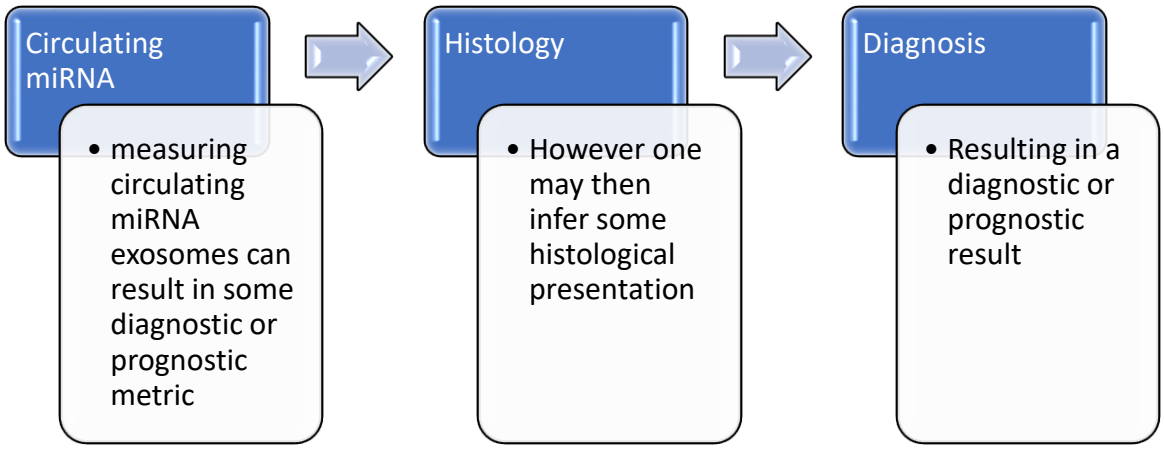
4 PROSTATE CANCER

We have done considerable amounts in the area of prostate cancer, PCa. This is a highly complex cancer and one which we still remain to fully understand. The current standard of care is PSA, then mpMRI, followed by targeted biopsy, then whatever may result from that. The desirable outcome in prostate cancer, PCa, is one where we can infer histology from MRI results and in turn infer malignant versus benign and localized vs invasive. Namely the goal is as below:



The issue at hand is can we diagnose with MRI alone? Does the MRI, such as PIRad numbers really mean anything. We have noted previously that PIRad numbers on MRI on a prostate which has had multiple previous biopsies may very well show poor diffusion on scar tissues generated from previous biopsies.

Another paradigm might be:



4.1 MRI IMAGING

There has been a recent increase in mpMRI, multiparameter MRI, as applied to PCa. mpMRI allows for the examination of the prostate before a biopsy and it has been alleged that it can reduce the need for biopsies in benign appearing prostates. As Khosravi et al note:

Magnetic Resonance Imaging (MRI) is routinely used to visualize the prostate gland and manage prostate cancer. The Prostate Imaging Reporting and Data System (PI-RADS) is used to evaluate the clinical risk associated with a potential tumor. However the PI-RADS score is ***subjective and its assessment varies between physicians. As a result, a definite diagnosis of*** *prostate cancer requires a biopsy to obtain tissue for pathologic analysis. A prostate biopsy is an invasive procedure and is associated with complications, including hematospermia, hematuria, and rectal bleeding.*

We have noted previously that the PIRAD score can be compromised due top scarring effects of previous biopsies. Namely diffusion is compromised through scar tissue and it may appear as a lesion. There does not appear to be any significant analysis of this typeof phenomenon. The authors continue:

We hypothesized that an Artificial Intelligence (AI) can be trained on prostate cases where both imaging and biopsy are available to distinguish aggressive prostate cancer from non-aggressive lesions using MRI imaging only, that is, without the need for a biopsy. Our computational method, named AI-biopsy, can distinguish aggressive prostate cancer from non-aggressive disease with an AUC of 0.855 and a 79.02% accuracy.

We used Class Activation Maps (CAM) to highlight which regions of MRI images are being used by our algorithm for classification, and found that AI-biopsy generally focuses on the same regions that trained uro-radiolosts focus on, with a few exceptions. In conclusion, AI-biopsy provides a data-driven and reproducible way to assess cancer aggressiveness from MRI images and a personalized strategy to reduce the number of unnecessary biopsies.

The question then is: does AI improve the assessment of the prostate by mpMRI? As Harmon et al note:

Pathologic grading plays a key role in prostate cancer risk stratification and treatment selection, traditionally assessed from systemic core needle biopsies sampled throughout the prostate gland. Multiparametric magnetic resonance imaging (mpMRI) has become a well-established clinical tool for detecting and localizing prostate cancer. ***However, both pathologic and radiologic assessment suffer from poor reproducibility among readers.*** *Artificial intelligence (AI) methods show promise in aiding the detection and assessment of imaging-based tasks, dependent on the curation of high-quality training sets. This review provides an overview of recent advances in AI applied to mpMRI and digital pathology in prostate cancer which enable advanced characterization of disease through combined radiology-pathology assessment.*

Unfortunately, AI may not resolve the putative ambiguities. As Ishioka et al have noted:

Our present results suggest that a CAD system can be applied to the interpretation of prostate MRI. Automated detection of prostate cancer regions in pre-biopsy prostate MRI offers several advantages.

First, it is self-evident that machines make consistent diagnoses because an algorithm makes the same diagnosis on a specific image every time.

Second, CAD improves cost-effectiveness and efficiency, because a machine can diagnose almost instantly and diligently without tiring. Moreover, such intelligent systems are designed to learn from their mistakes and have the potential to improve over time.

Third, physicians can objectively establish flexible usage depending on the situation.

Sensitivity and specificity can be fine-tuned depending on the screening environment. Although few attempts have been made to apply such a CAD system to prostate assessment before the emergence of deep learning, future studies will likely employ CAD for prostate cancer diagnosis with increased accuracy. If the diagnostic precision of the CAD system exceeds that achieved by humans, and the pathological diagnosis can be highly accurately predicted, it may become realistic to treat or to survey prostate cancer based on image diagnosis without biopsy.

The diagnostic measures of the two algorithms we developed showed AUC values of 0.645 and 0.636 for estimating the labelled area in which targeted biopsy confirmed the presence of cancer. These values are by no means very good results, because the ground truth label we used was an annotation based on the interpretation of the radiologists, which may not have exactly matched the true cancer area. Such results might be inevitable as long as biopsy results are used as ground truth labels. However, this is not a serious problem in clinical application.

We think that the marginal areas of the annotation do not need to be reproduced in detail by the algorithm, because targeted biopsy is typically directed on the centre of the annotation area. Assuming that the centre of the area specified by an algorithm is targeted by prostate biopsy, seven and 16 false detections and two and zero oversights were found in data set 1 and data set 2, respectively. We think that these results are satisfactory because it is essential to first reduce oversights in cancer diagnosis, particularly in primary screening

The above may not really resolve the issues of artifacts and prior examinations, not matter how well trained the CNN is. Now Linda et al have noted:

Prostate cancer is the most frequently diagnosed, noncutaneous male malignancy and the second leading cause of cancer-related mortality among men in the United States. Statistics of prostate cancer frequency, morbidity, and mortality can be examined in many different ways. It is a very common cancer, as it is a "tumor of aging," but it has a very low disease-specific mortality, all of which reinforce its characterization as a complex public health concern that impacts a large population. Although prostate cancer is a serious disease, most men diagnosed with prostate cancer do not die of it.

The 5-year survival rate for patients with prostate cancer ranges from approximately 30% in patients with metastatic disease to 100% in patients with localized disease.

The key clinical problems in prostate cancer diagnosis today include:

1) overdiagnosis and overtreatment resulting from an inability to predict the aggressiveness and risk of a given cancer; and

2) inadequate targeted biopsy sampling, leading to misdiagnosis and to disease progression in men with seemingly low-risk prostate cancer.

In a meta-analysis,176 the reported rate of overdiagnosis of nonclinically significant prostate cancer was as high as 67%, leading to unnecessary treatment and associated morbidity. Because of this range of clinical behavior, it is necessary to differentiate men who have clinically significant tumors (those with a biopsy Gleason score 7 and/or pathologic volume 0.5 mL)177 as candidates for therapy from those who have clinically insignificant tumors and can safely undergo active surveillance.

It has been noted that potential survival benefits from aggressively treating early-stage prostate cancer are undermined by harm from the unnecessary treatment of indolent disease. The biological heterogeneity of prostate cancer leads to different clinical outcomes, ranging from indolent to highly aggressive tumors with high morbidity and mortality, and differences in therapy planning, therapy response, and prognosis of patients. This is reflected by the incorporation of genomic profiling in the National Comprehensive Cancer Network guidelines, …

In parallel with molecular characterization, AI also has the potential to empower clinicians in the detection, localization, characterization, staging, and monitoring of prostate cancer. There are no widespread multicenter trials as yet, and therefore much of the initial work is limited to single-center, single-algorithm analyses and on small data sets. However, some groups, such as the National Institutes of Health and MICCAI, are developing infrastructure to allow larger, well annotated data sets to become available for AI development.

They continue:

In the past years, deep learning networks, and particularly CNNs, have been revolutionizing investigative research into prostate cancer detection and diagnosis. These methods use different modality types, CNN architectures, and learning procedures to train deep networks for prostate cancer classification and have achieved state-of-the-art performance.

Some investigators use CNNs to classify MRI findings with an auto-windowing mechanism to overcome the high dynamic range of MR images and normalization, whereas others use different combinations of mpMRI images by stacking each modality as a 2D channel of RGB images and use them as training examples. Furthermore, 3D CNNs can be designed that use specific MRI-based parameters such as apparent diffusion coefficient, high b-value, and volume transfer constant (Ktrans) modalities.

Deep learning systems have been applied to localize and classify prostate lesions at the same time. Both de novo training194,196,197 and transfer learning of pretrained models195 have been successful for training CNNs for prostate cancer diagnosis in MRI. The explicit addition of anatomically aware features to the last layers of CNNs has been used successfully to boost their performance. In addition to MRI, AI techniques have achieved promising results by incorporating ultrasound data, specifically radiofrequency, for prostate cancer classification. Here again, both classic machine learning approaches and deep learning have been used to train classifiers to grade prostate cancer in temporal ultrasound data. The results of the ongoing research into the use of AI for the detection and characterization of prostate cancer are promising and demonstrate ongoing improvement.

The recent body of research in prostate cancer image analysis reveals a transition from feature engineering and classic machine learning methods toward deep learning and the use of large training sets. Unlike lung and breast cancers, clinical routines in prostate cancer have not yet adopted regulated CAD systems. However, the recently achieved results of deep learning techniques on midsize data sets, …, are promising. It is now evident that there has been a rapid growth in prostate MR examination volumes worldwide and increasing demand for accurate interpretations.

Accurate CAD systems will improve the diagnostic accuracy of prostate MRI readings, which will result in better care for individual patients, because fewer patients with benign and indolent tumors (false-positives) will need to undergo invasive biopsy and/or radical prostatectomy procedures, which can lower their quality of life.

Conversely, early detection of prostate cancer improves the prognosis of patients who have clinically significant prostate cancer (Gleason pattern 4). ***Computer-assisted detection and diagnosis systems for prostate cancer help clinicians by potentially reducing the chances of either missing or overdiagnosing suspicious targets on diagnostic MRIs, although this merits additional validation in trials before routine clinical incorporation.***

This conclusion may still be a bit optimistic. Each lesion is often unique. Also there is the problem of biopsies having limited sampling, albeit directed by the mpMRI. Biopsies, albeit costly and with some risk, are often just the first step in a diagnostic process.

4.2 MIRNA

miRNAs are small RNA sequences about 22 nucleotides in length. They have a strong impact on gene expression in multiple dimensions and as more has been learned they are often associated with various cancers. As Guo et al have recently noted for PCa:

MicroRNAs (miRNAs/miRs) are a group of non-coding RNAs of 17-27 nucleotides in length that regulate gene expression by binding to the 3'untranslated regions of messenger RNAs (mRNAs). miRNAs have been demonstrated to serve important roles in a number of cellular processes as post-transcriptional regulators, in addition to roles in cancer development and progression.

Dysregulation of miRNAs has been demonstrated to contribute to tumorigenesis by stimulating proliferation, angiogenesis and invasion.

Previous studies have investigated miRNA expression profiles in primary prostate cancer (PPC) or MPC and several miRNAs have been suggested as diagnostic markers for PC (9,10). However, the molecular mechanisms underlying the roles of miRNAs and their target differentially expressed genes (DEGs) in PC metastasis remain unclear…

In conclusion, the significantly upregulated miR-144, miR-494, miR-30d, miR-181a, miR-196a, miR-708 and miR-486-5p screened in the present study may participate in the metastasis of PC cells via the downregulation of their corresponding target DEGs, particularly those with large |log2 FC| values, including MYH11, SLC22A3, DPP4, SORBS1, PDE5, MYLK and ACTG2. The effects on these target DEGs require further experimental verification. A number of these DEMs or DEGs have been associated with the occurrence of PC; however, the molecular mechanisms underlying their roles in the occurrence of MPC remain unclear and require further investigation

Thus, it is clear that miRNAs are worth examining. In a recent paper by Wei-Lin et al:

We describe the development of a novel biostatistical based approach for the diagnosis and classification of prostate cancer based on the interrogation of miRNAs and snoRNAs isolated from urinary exosomes. The performance characteristics of this interrogation methodology demonstrate that the diagnostic tests have the potential to significantly reduce unnecessary core needle biopsies and incorrect disease classification, reducing morbidities and cost associated with the diagnosis and prognosis of prostate cancer….

The Sentinel PCa, Sentinel CS and Sentinel HG Tests are based on a classification algorithm that takes as input the sncRNA expression signature for a participant with unknown disease status (emulated by the validation set in this paper) and produces a Sentinel Score. The participant is classified by comparing this score to the predetermined cutoff value, referred to as the classification boundary (obtained from cross-validation in the training data set) that controls sensitivity for classifying a future patient with unknown disease status (but known expression signature), at a user-defined level (typically 95% or greater).

As with many proprietary classifiers one is left wondering just what is happening.

For each test the classification boundary is shown as a vertical dashed line. The Sentinel PCa, CS and HG Tests operate analogously, using unique sncRNA expression signatures, however the classification rules and boundaries are different among the 3 tests. The tests are stand-alone and do not incorporate any information about other clinical markers such as prostate specific antigen, % core involvement, CAPRA (Cancer of the Prostate Risk Assessment) Score or Prostate Cancer Prevention Trial criteria….

In this comprehensive evaluation of the urinary exosome based miR Sentinel PCa, CS and HG Tests, high accuracy for identifying the presence of cancer and the presence of high grade cancer was demonstrated. These data demonstrate that the evaluation of a panel of urinary

exosomal sncRNA offers the ability to accurately and noninvasively screen, diagnose, characterize and monitor prostate cancer.

In the paper by Bryzgunova et al:

Urine of prostate cancer (PCa) carries miRNAs originated from prostate cancer cells as a part of both nucleoprotein complexes and cell-secreted extracellular vesicles. The analysis of such miRNA-markers in urine can be a convenient option for PCa screening. The aims of this study were to reveal miRNA–markers of PCa in urine and design a robust and precise diagnostic test, based on miRNA expression analysis. The expression analysis of the 84 miRNAs in paired urine extracellular vesicles (EVs) and cell free urine supernatant samples from healthy donors, patients with benign and malignant prostate tumours was done using miRCURY LNA miRNA qPCR Panels (Exiqon, Denmark).

Sets of miRNAs differentially expressed between the donor groups were found in urine EVs and urine supernatant. Diagnostically significant miRNAs were selected and algorithm of data analysis, based on expression data on 24-miRNA in urine and obtained using 17 analytical systems, was designed. The developed algorithm of data analysis describes a series of steps necessary to define cut-off values and sequentially analyze miRNA expression data according to the cut-offs to facilitate classification of subjects in case/control groups and allows to detect PCa patients with 97.5% accuracy

4.3 GRADING

PCa grading is generally done via a histological test. Gleason score from a best and worst areas are determined and the score may get as high as a 10, 5 plus 5, which implies an highly aggressive infiltration of malignant cells. As Abraham and Nair have noted:

Prostate Cancer (PCa) is one of the most prominent cancer among men. Early diagnosis and treatment planning are significant in reducing the mortality rate due to PCa. Accurate prediction of grade is required to ensure prompt treatment for cancer. Grading of prostate cancer can be considered as an ordinal class classification problem. This paper presents a novel method for the grading of prostate cancer from multiparametric magnetic resonance images using VGG-16 Convolutional Neural Network and Ordinal Class Classifier with J48 as the base classifier. Multiparametric magnetic resonance images of the PROSTATEx-2 2017 grand challenge dataset are employed for this work. The method achieved a moderate quadratic weighted kappa score of 0.4727 in the grading of PCa into 5 grade groups, which is higher than state-of-the-art methods. The method also achieved a positive predictive value of 0.9079 in predicting clinically significant prostate cancer.

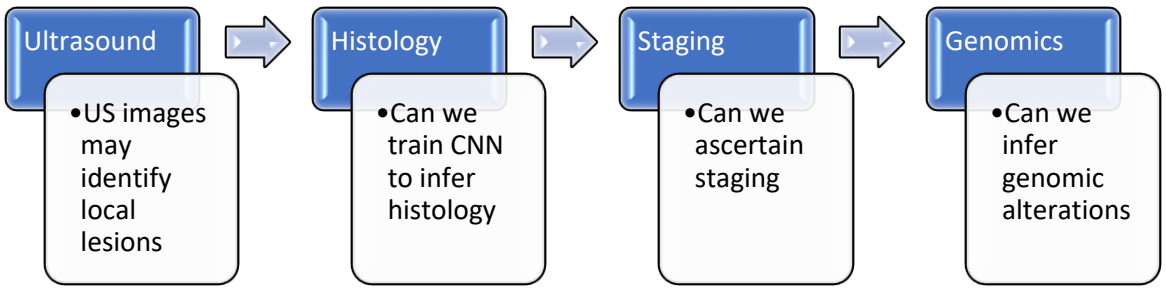
Grading is often a difficult task with biopsy samples. Oftentimes the grade in materially increased after a prostatectomy when a much more significant sample of the lesion is obtained. Thus the grading of biopsies is not fully dispositive of the extent of the malignancy and it is not clear what machine learning can add.

4.4 AI APPLICATIONS

A recent paper by Kwak and Hewitt present a summary of various CNN in assessing prostate H&E slides for machine interpretation. In the previous discussions we have seen that CNN are used in mpMRI and H&E histological studies as well as miRNA compilations.

5 THYROID CANCER

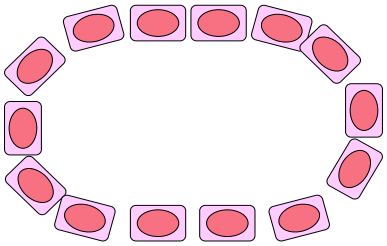
The desirable outcome in Thyroid cancer, TCa, is one where we can infer histology from ultrasound, US, results and in turn infer malignant versus benign and localized vs invasive. Namely the goal is as below:



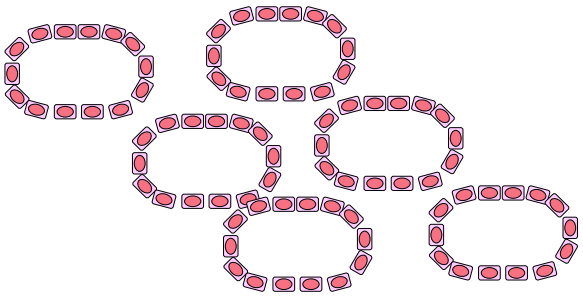
5.1 CELLULAR PRESENTATION

The third form of diagnostic analysis is cytological, both worth fine needle aspirations, FNA, and that of the excised tumor mass. The papillary form of TCa is interesting in that its diagnosis is a cellular diagnosis of specific forms and presentations. Unlike say PCa, where there is a proliferation of basal and or luminal cells, TCa has proliferation in the follicular form, and a papilla like growth in the papillary form, but the dispositive elements are those of how the cell appears, as we will show below.

Basically the thyroid cells is the outer side of a thyroid follicle. It is the boundary. This is shown below for a simple thyroid boundary. The cells on the boundary are well behaved and connected. Cell interfaces such as E-cadherin stabilize these cells. Internally to this glandular structure is a collagen internal fill. From this colloid under the pituitary control the T3 and T4 hormones are released. From this is the basis of the thyroid control path.

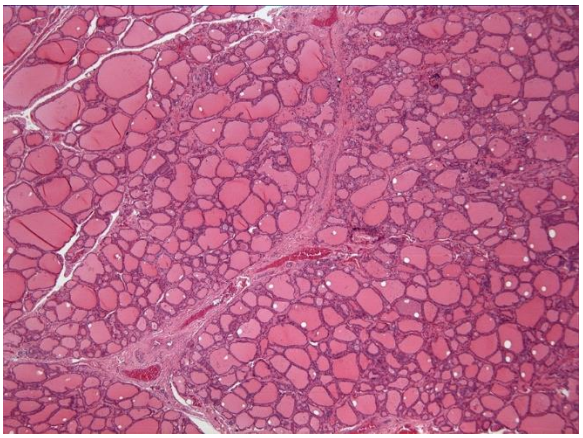


Now the thyroid gland is a compilation of these follicles as shown below. There are blood organs between the follicles and also C cells, cells separate from those that form the effective gland. The highly simplistic view is seen below.

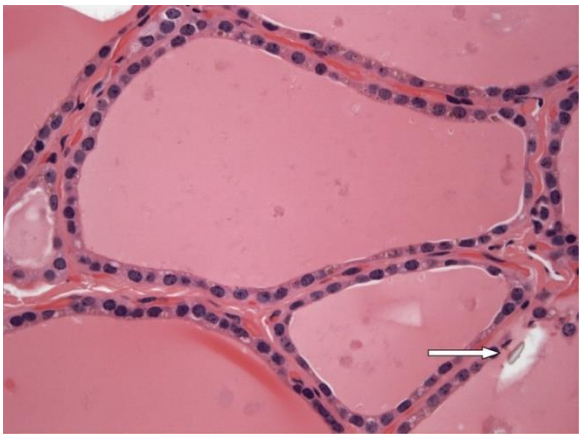


Inside is the collagen material used by the cells to produce T3 and T4.

The normal thyroid cells are shown below (From *Epstein, Biopsy Interpretation of the Thyroid*). The separate cells for enclosures which contain colloid and then it is processed and released by the cell. Surrounding the cell is and there are blood networks throughout the thyroid providing the cells with their requirements and transporting the cell products.



Further specific detail of a follicle is shown below (again from Epstein). Note the clarity and simplicity as well as structure of the cells in each small gland portion:

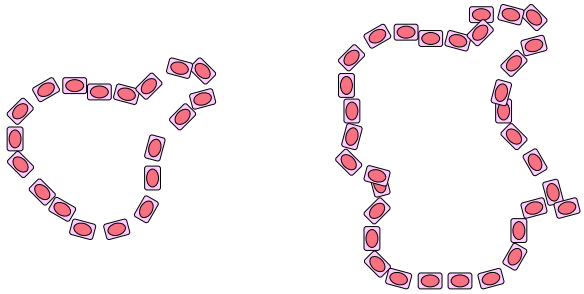


Note that the cells are well demarcated and organized. Now as we shall see, several variations occur as the cell becomes malignant. Growth results in a proliferation of cells, everywhere, loss of adhesion via EMT results in cell dislocation, and the morphology of individual cells change as well.

5.1.1.1 Papillary Growth

Now papilla are the small bumps or perturbances of the normal cells which generally are somewhat uniform as we have depicted.

The papillary like cells are shown as below:



The above show the papilla, the bumps or offshoots. It can be argued that this papilla formation is a result of a quasi-EMT process where the E-cadherin bond structure is starting to deteriorate. Namely the genetic control of this is breaking down because of the suppression of the pathways that control epithelial like structure[13]. Now as Nucera and Pontecorvi have noted:

Most human thyroid cancers are differentiated papillary carcinomas (PTC). Papillary thyroid microcarcinomas (PTMC) are tumors that measure 1 cm or less. This class of small tumors has proven to be a very common clinical entity in endocrine diseases. PTMC may be present in 30-40% of human autopsies and is often identified incidentally in a thyroid removed for benign clinical nodules.

Although PTMC usually has an excellent long-term prognosis, it can metastasize to neck lymph nodes; however deaths related to this type of thyroid tumor are very rare. Few data exist on molecular pathways that play a role in PTMC development; however, two molecules have been shown to be associated with aggressive PTMC.

S100A4 (calcium-binding protein), which plays a role in angiogenesis, extracellular matrix remodeling, and tumor microenvironment, is over-expressed in metastatic PTMC. In addition, the BRAFV600E mutation, the most common genetic alteration in PTC, is present in many PTMC with extra thyroidal extension and lymph node metastasis.

[13] See McGarty, EMT and Cancers, January 2019, https://www.researchgate.net/publication/330222973_EMT_and_Cancers

The above observation is interesting. Namely that almost 40% of people will be harboring small PTCs which unless sampled by a good ultrasound examiner would never be found. Furthermore they would never grow. They also note regarding the papillary growth above:

BRAFV600E triggers a cascade that leads to human papillary thyroid microcarcinoma (PTMC) proliferation. The constitutive kinase activity of BRAFV600E phosphorylates and activates MEK1/2. Phospho-MEK1/2 induces hyperphosphorylation of ERK1/2 which translocates into the nucleus, triggering cell cycle progression, and abnormal cell proliferation by up-regulating cyclins (e.g., Cyclin D1) crucial for the checkpoint machinery in G1-S phases and inhibiting anti-cell cycle cyclins (e.g., p27). Up-regulation of cyclins (e.g., Cyclin D1) leads to hyper-proliferation of papillary thyroid microcarcinoma cells and increase in papillae size.

Now there are several additional and specific histological characteristics. For example, as Das notes[14]:

Psammoma bodies (PBs) are concentric lamellated calcified structures, observed most commonly in papillary thyroid carcinoma (PTC), meningioma, and papillary serous cystadenocarcinoma of ovary but have rarely been reported in other neoplasms and nonneoplastic lesions. PBs are said to represent a process of dystrophic calcification.

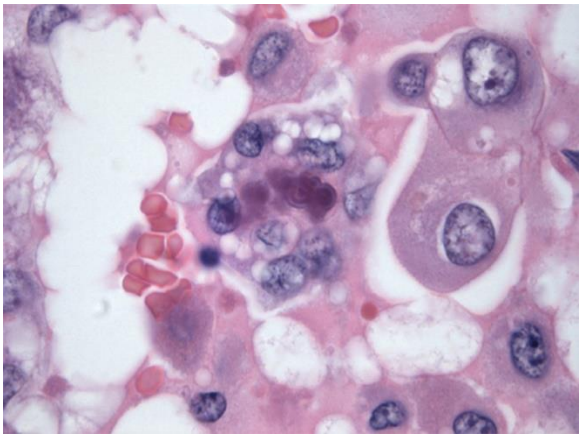
Despite numerous ancillary studies over a span of three and half decades, formation of PBs remains a poorly understood mechanism. Ultrastructural study of PTC has shown that thickening of the base lamina in vascular stalk of neoplastic papillae followed by thrombosis, calcification, and tumor cell necrosis leads to formation of PBs. Studies on serous cystadenocarcinoma of ovary and meningioma, however, revealed that collagen production by neoplastic cells and subsequent calcification was responsible for the formation of PBs.

The existence of some precursor forms of PBs was reported in meningiomas and more recently in PTC, which were mostly in the form of extracellular hyaline globules surrounded by well-preserved neoplastic cells or in a smaller number of cases intracytoplasmic bodies liberated from intact tumor cells.

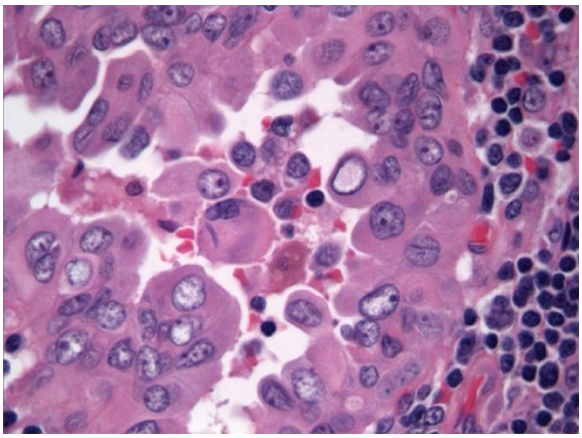
Cellular degeneration and necrosis, leading to the disappearance of neoplastic cells, were noticed by us only around PBs but not around the precursor forms. Based on the above findings, it is suggested that rather than being the outcome of dystrophic calcification of dead or dying tissue, PBs may indeed represent an active biologic process ultimately leading to degeneration/death of tumor cells and retardation of growth of the neoplasm. It may also serve as a barrier against the spread of neoplasm.

We show psammoma bodies below.

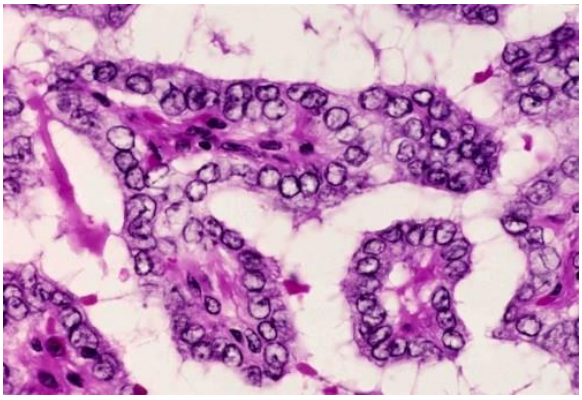
[14] Note: A psammoma body is a round collection of calcium, seen microscopically. The term is derived from the Greek word ψάμμος (psámmos), meaning "sand".



An additional example of specific histological characteristics is one with clear nuclei as shown below:

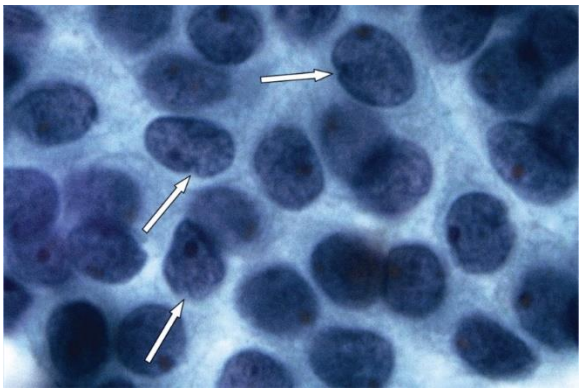


Another example of one type of papillary carcinoma is one with "Orphan Annie" eyes, the wide open white eyes of the nucleus in the cells below.



Note also the well demarcated papillary form with outstretches of the otherwise well-structured cell.

Finally an added one is cells with nuclear grooves is a characteristic that is part of this diagnosis as shown below:



These are small notches seen in the side of the nucleus.

Now these are what a trained histopathologist would be looking for. However, an underlying question is; why are they present and what causes these specific characteristics. We frequently see in medicine the answers to what but not why. Some of these answers are yet to be determined.

5.1.1.2 Follicular Growth

In contrast to follicular carcinoma, where the boundary patency gets deformed, follicular carcinoma is where there is a proliferation of the follicular cells.

Baloch and LiVolsi have noted:

Follicular carcinoma comprises about 5% of thyroid cancers; however, in iodide-deficient areas, this tumor is more prevalent making up 25-40% of thyroid cancers. The true incidence of follicular carcinoma is difficult to determine since the follicular variant of papillary carcinoma may still be placed into this category. Risk factors include iodine deficiency, older age, female gender, and radiation exposure (although the relationship of radiation to follicular carcinoma is far less strong than with papillary cancer).

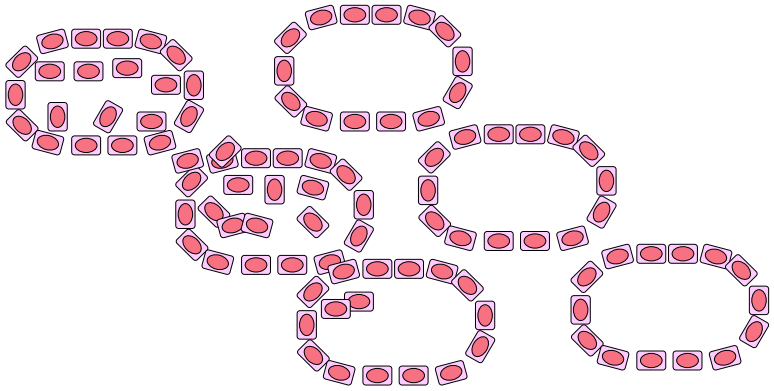
Clinically, follicular carcinoma usually presents as a solitary mass in the thyroid. Follicular carcinoma has a marked propensity for vascular invasion and avoids lymphatics; hence, true embolic lymph node metastases are exceedingly rare. Follicular carcinoma disseminates hematogenously and metastasizes to bone, lungs, brain, and liver …

What are the minimum criteria for making this diagnosis? Invasion of the capsule, invasion through the capsule, and invasion into veins in or beyond the capsule represent the diagnostic criteria for carcinoma in a follicular thyroid neoplasm. The criterion for vascular invasion applies solely and strictly to veins in or beyond the capsule, whereas, the definition of capsular invasion is controversial. Some authors require penetration of the capsule to diagnose a

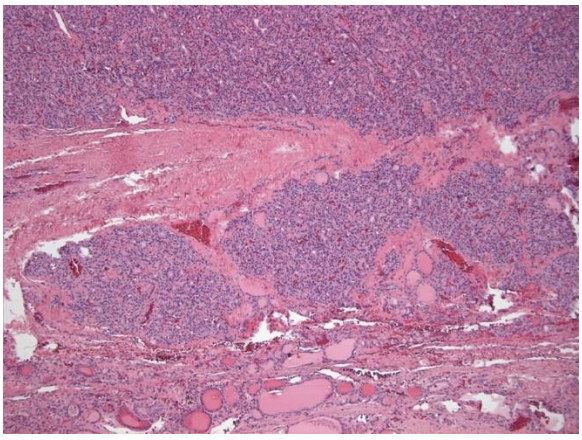
follicular tumor as carcinoma, while others need tumor invasion through the capsule into the surrounding normal thyroid.

Is capsular invasion insufficient for the diagnosis of follicular cancer? Distant metastases have been reported in follicular carcinoma diagnosed only on the basis of capsular and not vascular invasion, however, in some cases, metastases were already present at initial diagnosis. The presence of vascular invasion is also indicative of malignancy in a follicular tumor. Invasion of vessels within or beyond the lesional capsule is necessary for a definitive diagnosis of vascular invasion. The lesions with vascular invasion should be separated from the minimally invasive follicular carcinomas that show capsular invasion only, because angio-invasive lesions have a greater probability of recurrence and metastasis.

Thus a simplistic view of a follicular cancer is shown below.



We depict a follicular cancer below:



This shows the multiplicity of cells in what was initially a well ordered cell structure filled with collagen.

Note the extensive infiltration. Again in simplistic terms, papillary is a form where we lose shape, namely a putative EMT transition and follicular is where we see extensive proliferation. Clearly both forms may occur.

5.1.1.3 Neuroendocrine Growth and Medullary Thyroid Cancer

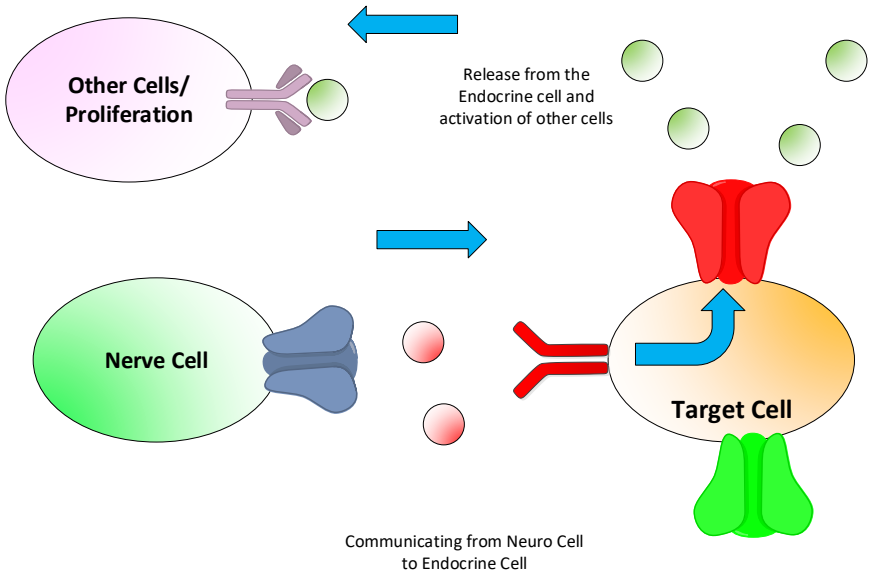
Medullary thyroid cancer is basically a neuroendocrine cancer. Neuroendocrine cancers are an interesting subset of many cancers and it worth reviewing the overall paradigm of their growth.

Namely we look at neuroendocrine type effects and thus it requires a slightly more detailed understanding of the prostate As NCI notes[15]:

Neuroendocrine: Having to do with the interactions between the nervous system and the endocrine system. Neuroendocrine describes certain cells that release hormones into the blood in response to stimulation of the nervous system.

We then, in a rationalistic manner, can try and connect the other empirical facts and see if the initial observation can also be logically correct and from that logic ascertain a new therapeutic approach.

A simplistic view of a neuroendocrine system is shown below. Basically the neuro cell activates the endocrine cell which in turn sends out signals to other collections of cells to do whatever they are supposed to do.



The above is simplistic but based upon a substantial base of validated cellular signalling factors. Namely these results are empirical in a broad sense. Now when examining various cancers we

[15] https://www.cancer.gov/publications/dictionaries/cancer-terms/def/neuroendocrine

often look at the cancer cell as being the driving factor. However in a neuroendocrine environment, the cancer cell may be getting its signalling from a cancer initiating cell which in turn is being signaled by a neuro cell. The cancer initiating cell may be blocked by blocking the signalling between it and the causative neuro cell. That is the logical or rationalistic part of this exercise.

The questions now are;

(i) If the malignancy occurs in the neuroendocrine cell, then does it create an environment for proliferation of other cells?

(ii) If the malignancy occurs in the neuroendocrine cell does it send out signals that either block other homeostatic processes or does it accelerate angiogenesis in the new malignancy?

(iii) If the malignancy starts in a non-neuroendocrine cell, are there processes that effectively "turn on" the neuroendocrine cell to facilitate such effects as proliferation, angiogenesis, gene suppression or activation in other cells?

These are but a few of the questions which may be posed. Again we indicate that this is a bit simplistic but it does present the key issues related hereto.

We have examined neuroendocrine driven cancers when examining the prostate. They are simply cancers where a local neuroendocrine cell starts controlling the proliferation process.

As Franz notes:

Medullary thyroid cancer (MTC) is a tumor of the parafollicular C cells that accounts for approximately 10% of all thyroid malignancies. An estimated 75% of MTC cases are sporadic, and the remaining 25% are familial. Embryologically, these cells originate within the neural crest and function similarly to other neuroendocrine cells within the amine precursor uptake and decarboxylation system.

C cells are distributed throughout the entire thyroid gland, although they tend to predominate in the upper poles. Calcitonin, a hormone active in calcium metabolism, is synthesized and secreted by C cells and therefore serves as a useful serum marker for the presence of MTC. Calcitonin levels are most useful in screening individuals who are genetically predisposed to the disease and in following patients who already have been treated. The recent identification of the gene responsible for heritable forms of MTC has allowed earlier identification of individuals at risk for the disease

Kim and Kuo have noted:

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor derived from the thyroid C cells producing calcitonin. MTC accounts for 0.6% of all thyroid cancers and incidence of MTC increased steadily between 1997 and 2011 in Korea. It occurs either sporadically or in a

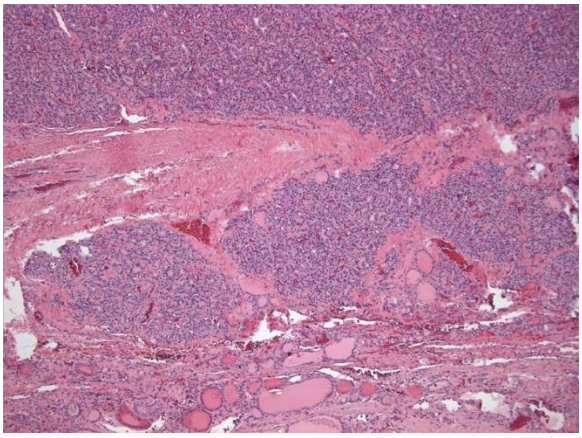
hereditary form based on germline rearranged during transfection (RET) mutations. MTC can be cured only by complete resection of the thyroid tumor and any loco-regional metastases.

The most appropriate treatment is still less clear in patients with residual or recurrent disease after initial surgery or those with distant metastases because most patients even with metastatic disease have indolent courses with slow progression for several years and MTC is not responsive to either radioactive iodine therapy or thyroid-stimulating hormone suppression. Recently, two tyrosine kinase inhibitors (TKIs), vandetanib and cabozantinib, are approved for use in patients with advanced, metastatic or progressive MTC.

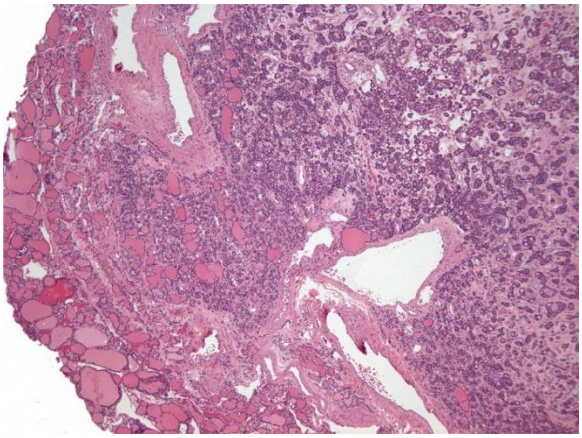
Baloch and LiVolsi note:

Medullary thyroid carcinoma comprises less than 10% of all thyroid malignancies. This tumor is of great diagnostic importance because of its aggressiveness, its close association with multiple endocrine neoplasia syndromes (MEN2A and 2B), and a relationship to a C cell hyperplasia, a probable pre cursor lesion.

While the majority of medullary carcinomas are sporadic, about 10-20% are familial. Since these familial cases have been identified, a gene associated with medullary carcinoma has been identified on chromosome 10 and involves mutations in the RET oncogene.



and below:



5.1.1.4 Anaplastic

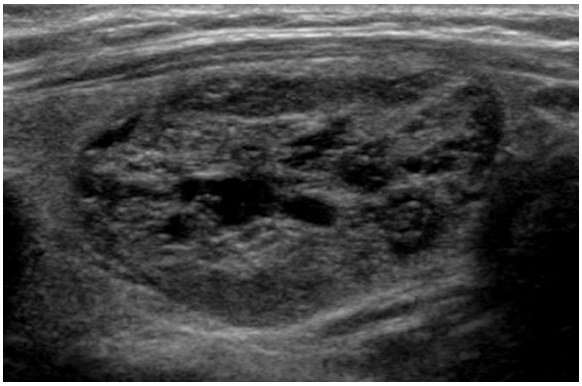
This is a highly aggressive cancer with nearly 100% mortality in 6 to 12 months. It is also quite rare but seems to be a sequella to a Graves diseased thyroid. Given its rarity and complexity it is worth just a mention.

5.2 ULTRASOUND

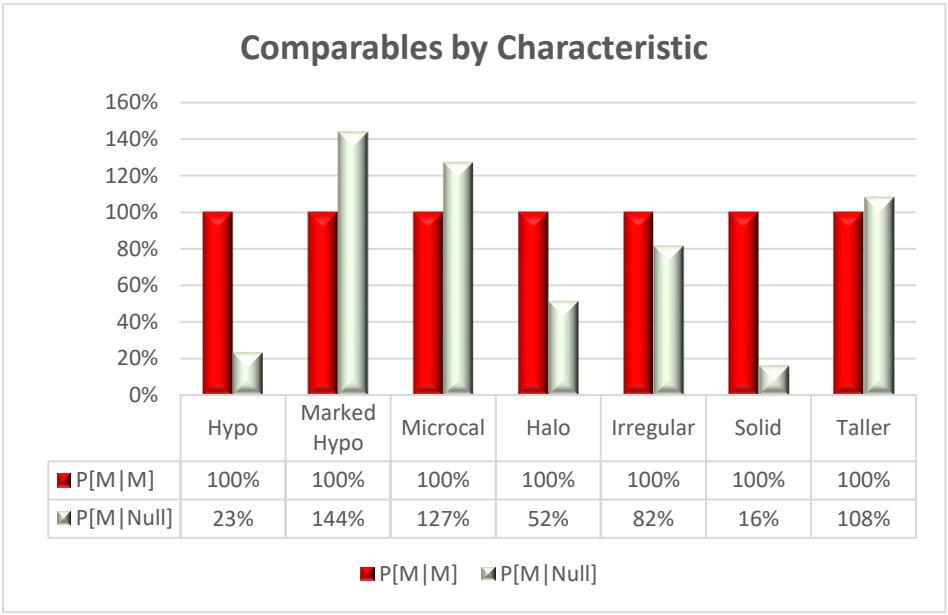
The thyroid is near the surface of the body and is highly amenable to ultrasound examination. In fact US examination is the most cost effective diagnostic tool available. It is not dispositive of a malignancy but is highly suggestive. Below is a table of typical landmarks found in US scans.

Characteristic	*Sensitivity*	*Specificity*
Hypoechoic c/w surrounding thyroid	81% (48-90%)	53% (36-92%)
Marked hypoechogenicity c/w strap muscle	41% (27-59%)	94% (92-94%)
Microcalcifications	44% (26-73%)	89% (69-98%)
Macrocalcifications	10% (2-17%)	94% (84-98%)
Absence of halo	66% (33-100%)	43% (30-77%)
Irregular, microlobulated margins	55% (17-84%)	80% (62-85%)
Solid consistency	86% (78-91%)	48% (30-58%)
Taller-than-wide shape on transverse view	48% (33-84%)	92% (82-93%)

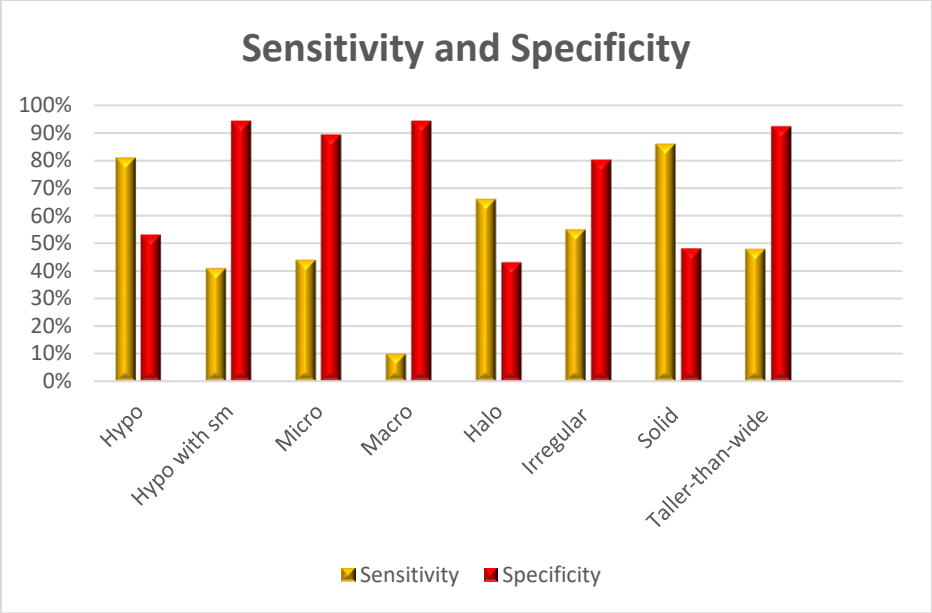
A typical US of a thyroid nodule is shown below evidencing many of the features consistent with a suspicious node.



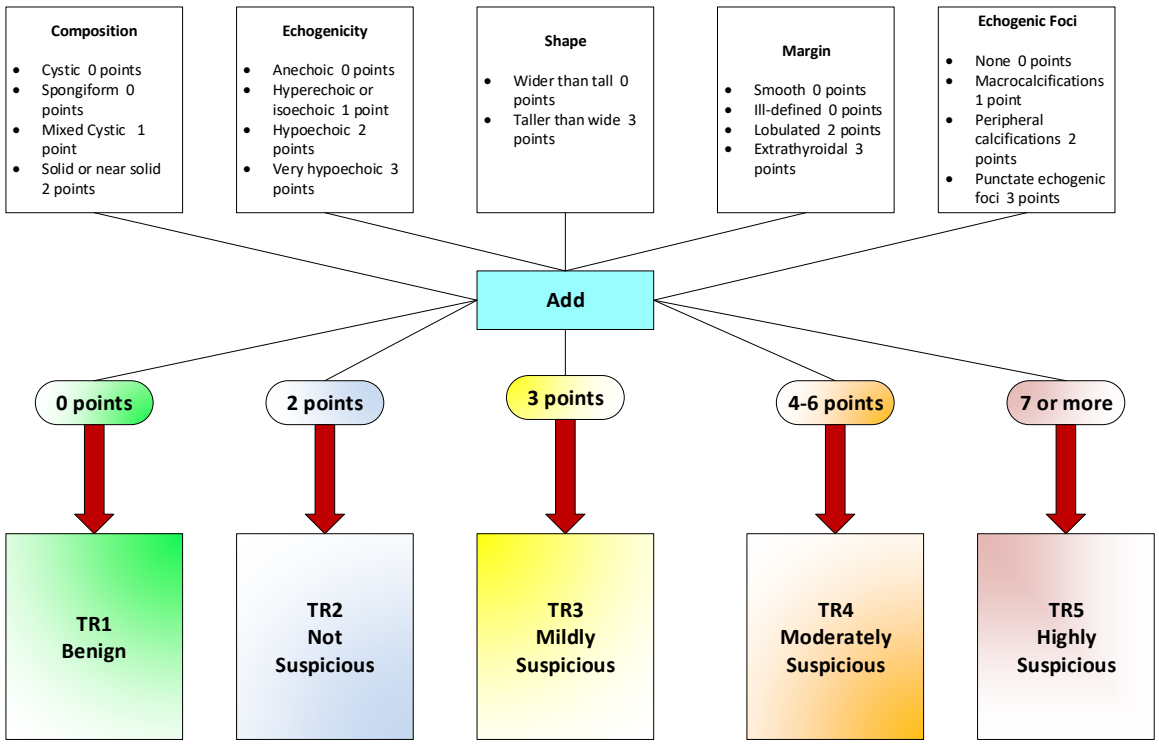
Statistically we can summarize these characteristics in the following chart.



Note that we have normalized the ones where it is malignant to 100% to give the relative frequency to the same measure when there is no malignancy. We then plot the sensitivity and specificity below:



TI-RADS is the set of metrics which are used with thyroid US to assess if the lesion is suspicious. It is similar to the PI-RADS used in PCa. From Tessler et al, we have the following summary for TI-RADS:



The following is a sample US analysis of a patient with multiple nodules. It yields a TR3 level. However it is also the case that when there are multiple nodules even at this level the risk of a malignancy is reduced.

Semantic feature	***Variations***	***Value***	***Nodule 1***	***Nodule 2***	***Nodule 3***
Size			***1.7 by 0.7 by 1.3***	***1.5 by 1.0 by 1.0***	***0.8 by 0.8 by 1.1***
			Right Upper	***Right Middle***	***Right Lower***
Composition					
	Solid	2			
	Predominantly Solid	1	1	1	1
	Predominantly Cystic	0			
	Cystic	0			
	Spongiform	0			
Echogenicity					
	Anechoic	0			
	Hyperechoic	1		1	1
	Isoechoic	1			
	Hypoechoic	2	2		
	Very hypoechoic	3			
Shape					
	Taller than wide	3			
	Wider than tall	0	0	0	0
Border					
	Smooth	0	0	0	0
	Irregular	2			
	Lobulated	2			
	Ill defined	0			
Halo					
	Present				
	Absent				
Extrathyroidal extension					
	Present	3			
	Absent	0	0	0	0
Punctate echogenic foci					

Semantic feature	*Variations*	*Value*	*Nodule 1*	*Nodule 2*	*Nodule 3*
	Present	3			
	Absent	0	0	0	
Macrocalcifications					
	Present	1			
	Absent	0			
Peripheral calcifications					
	Present	2			
	Absent	0			
Comet-tail artifacts					
	Present	0	0		
	Absent	0			
Score			**3**	**2**	**2**

Thus if we were to consider ways to automate these measurements we have multiple means and methods. For example let us consider two:

1. Pattern Recognition: In this approach we start with the identifying patterns as given. Then we must process the image to extract the patterns.

2. Neural Network: Let us assume a supervised neural network. Namely we assume we know the diagnosis and we have for each image a well-defined diagnosis. Then we may use a multilayer NN and have it trained so that when presented with a new US it can classify it as a score of say 1 to 7. The image is scanned via some form of convolutional NN, CNN, and based upon extensive training we can assess any unknown.

As Chamrara and Ying have note for CADS systems:

This review suggests that CAD of thyroid ultrasound features has a good diagnostic performance which is comparable to that of radiologists' qualitative assessment with the potential for improved overall diagnostic accuracy when qualitative and quantitative approaches are combined. The nodule size, the experience of the operator and the choice of TIRADS system are potential influencers of CAD diagnostic performance. Future multi-center studies that compare similar CAD software based on standardized approaches and assess the diagnostic performance of combined Doppler ultrasound CAD and grey scale ultrasound CAD of the same thyroid nodules are recommended to further evaluate the clinical role of CAD in thyroid nodule characterization.

5.3 GENOMICS

The common gene mutations examined for in thyroid cancer are listed below. We examine some in detail.

Gene	***Function***
GNAS[16] {7}	This locus has a highly complex imprinted expression pattern. It gives rise to maternally, paternally, and biallelically expressed transcripts that are derived from four alternative promoters and 5' exons. Some transcripts contain a differentially methylated region (DMR) at their 5' exons, and this DMR is commonly found in imprinted genes and correlates with transcript expression. An antisense transcript is produced from an overlapping locus on the opposite strand.
RET[17](10;11;13;15;16)	This gene encodes a transmembrane receptor and member of the tyrosine protein kinase family of proteins. Binding of ligands such as GDNF (glial cell-line derived neurotrophic factor) and other related proteins to the encoded receptor stimulates receptor dimerization and activation of downstream signaling pathways that play a role in cell differentiation, growth, migration and survival.
AKT1[18] (3)	he serine-threonine protein kinase encoded by the AKT1 gene is catalytically inactive in serum-starved primary and immortalized fibroblasts. AKT1 and the related AKT2 are activated by platelet-derived growth factor. The activation is rapid and specific, and it is abrogated by mutations in the pleckstrin homology domain of AKT1. It was shown that the activation occurs through phosphatidylinositol 3-kinase.
HRAS[19] {2;3)	This gene belongs to the Ras oncogene family, whose members are related to the transforming genes of mammalian sarcoma retroviruses. The products encoded by these genes function in signal transduction pathways. These proteins can bind GTP and GDP, and they have intrinsic GTPase activity. This protein undergoes a continuous cycle of de- and re-palmitoylation, which regulates its rapid exchange between the plasma membrane and the Golgi apparatus. Defects in this gene are implicated in a variety of cancers, including bladder cancer, follicular thyroid cancer, and oral squamous cell carcinoma.
NRAS[20] {2;3)	This is an N-ras oncogene encoding a membrane protein that shuttles between the Golgi apparatus and the plasma membrane. This shuttling is regulated through palmitoylation and depalmitoylation by the ZDHHC9-GOLGA7 complex. The encoded protein, which has intrinsic GTPase activity, is activated by a guanine nucleotide-exchange factor and inactivated by a GTPase activating protein. Mutations in this gene have been associated with somatic rectal cancer, follicular thyroid cancer, autoimmune lymphoproliferative syndrome, Noonan syndrome, and juvenile myelomonocytic leukemia

[16] https://www.ncbi.nlm.nih.gov/gene/2778
[17] https://www.ncbi.nlm.nih.gov/gene/5979
[18] https://www.ncbi.nlm.nih.gov/gene/207
[19] https://www.ncbi.nlm.nih.gov/gene/3265
[20] https://www.ncbi.nlm.nih.gov/gene/4893

Gene	*Function*
BRAF[21] (11;15)	This gene encodes a protein belonging to the RAF family of serine/threonine protein kinases. This protein plays a role in regulating the MAP kinase/ERK signaling pathway, which affects cell division, differentiation, and secretion. Mutations in this gene, most commonly the V600E mutation, are the most frequently identified cancer-causing mutations in melanoma, and have been identified in various other cancers as well, including non-Hodgkin lymphoma, colorectal cancer, thyroid carcinoma, non-small cell lung carcinoma, hairy cell leukemia and adenocarcinoma of lung. Mutations in this gene are also associated with cardiofaciocutaneous, Noonan, and Costello syndromes, which exhibit overlapping phenotypes. A pseudogene of this gene has been identified on the X chromosome.
PIK3CA[22] {2;5;8;10;14;21)	Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. This gene has been found to be oncogenic and has been implicated in cervical cancers.
TP53[23] {2;4;5;6;7;8;10}	This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers.
KRAS[24] {2;3;4)	This gene, a Kirsten ras oncogene homolog from the mammalian ras gene family, encodes a protein that is a member of the small GTPase superfamily. A single amino acid substitution is responsible for an activating mutation. The transforming protein that results is implicated in various malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma.
CTNNB1[25] (3)	The protein encoded by this gene is part of a complex of proteins that constitute adherens junctions (AJs). AJs are necessary for the creation and maintenance of epithelial cell layers by regulating cell growth and adhesion between cells. The encoded protein also anchors the actin cytoskeleton and may be responsible for transmitting the contact inhibition signal that causes cells to stop dividing once the epithelial sheet is complete. Finally, this protein binds to the product of the APC gene, which is mutated in adenomatous polyposis of the colon. Mutations in this gene are a cause of colorectal cancer (CRC), pilomatrixoma (PTR), medulloblastoma (MDB), and ovarian cancer
PTEN[26] {1;3;6;7;8)	This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. It negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells and functions as a tumor suppressor by negatively regulating AKT/PKB signaling pathway. The use of a non-canonical (CUG) upstream initiation site produces a longer isoform that initiates translation with a leucine, and is thought to be preferentially associated with the mitochondrial inner membrane.

We now provide a brief summary of some of the key genes involved.

[21] https://www.ncbi.nlm.nih.gov/gene/673
[22] https://www.ncbi.nlm.nih.gov/gene/5290
[23] https://www.ncbi.nlm.nih.gov/gene/7157
[24] https://www.ncbi.nlm.nih.gov/gene/3845
[25] https://www.ncbi.nlm.nih.gov/gene/1499
[26] https://www.ncbi.nlm.nih.gov/gene/5728

5.3.1 BRAF V600

BRAF is a RAF gene (protein) which plays a role in many pathways and has been a key player in many malignancies. Mutations in somatic cells of BRAF have resulted in multiple malignancies. These mutations have been found in many malignancies such as melanoma, prostate and breast.

As noted in Nucera and Pontecorvi:

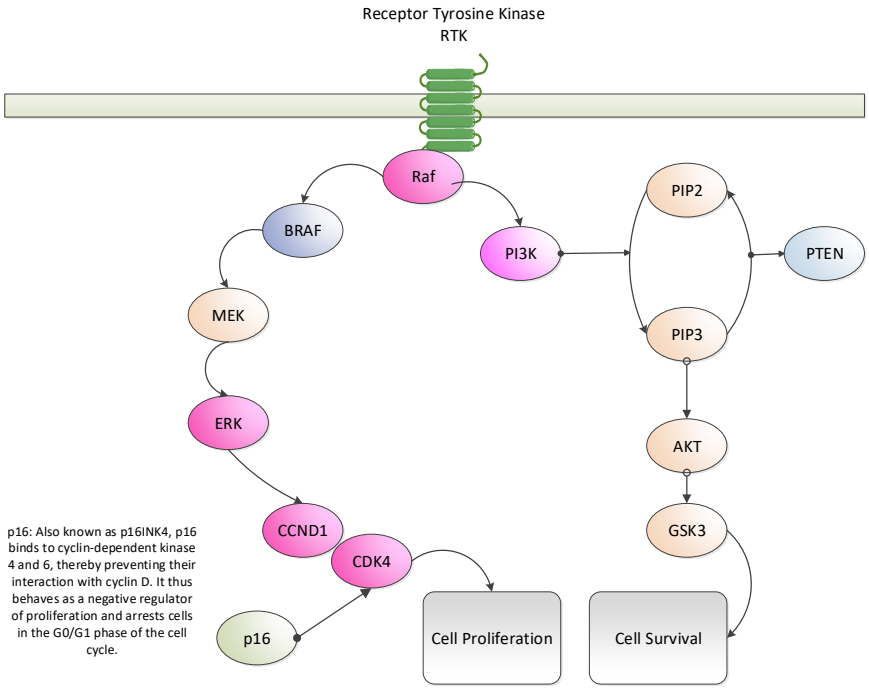
The management and treatment of malignant thyroid micro nodules (i.e., PTMC) can be a challenge for physicians. Most PTMC are indolent and have an excellent prognosis; however, a subgroup shows an aggressive biological and clinical behavior similar to PTC. While additional robust prospective studies are required, there is now a body of evidence suggesting that BRAF V600E-positive PTMCs show aggressive behavior, whereas BRAFV600E-negative PTMCs have a good prognosis. This suggests that it will be valuable to consider the BRAF V600E mutation as a prognostic marker of PTMC aggressiveness and to undertake prospective studies with systematic screening for the BRAF V600E mutation and long-term follow-up to validate this marker of tumor aggressiveness.

As Ascierto et al have noted:

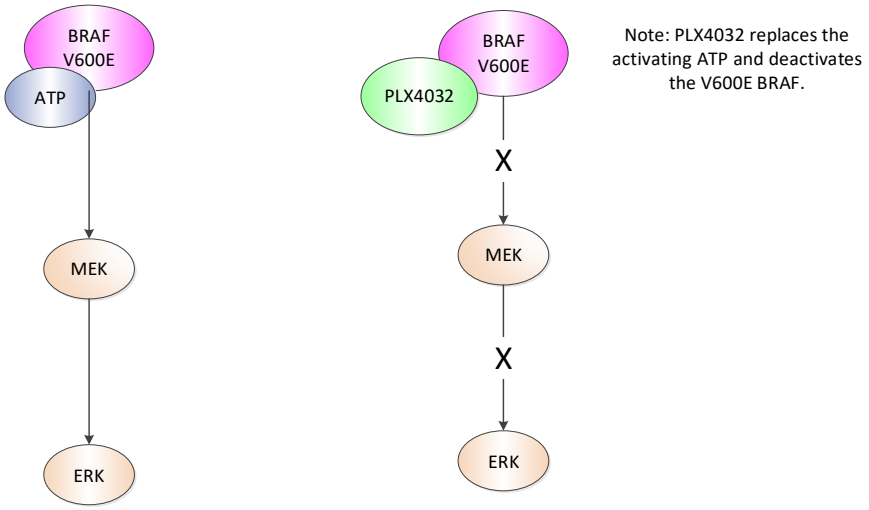
BRAF is a serine/threonine protein kinase, encoded on chromosome 7q34, that activates the MAP kinase/ERKsignaling pathway. BRAF is the family member most easily activated by Ras. In addition, the basal kinase activity of BRAF is higher than that of other family members. This provides a potential rationale for the frequent mutational activation of BRAF observed in human tumors. In fact, approximately 50 % of melanomas harbor activating BRAF mutations.

Among the BRAF mutations observed in melanoma, over 90 % are at codon 600, and among these, over 90 % are a single nucleotide mutation resulting in substitution of ***glutamic acid for valine*** *(BRAFV600E: nucleotide 1799 T>A; codon GTG>GAG). The second most common mutation is BRAFV600K substituting* ***lysine for valine****, that represents 5-6 % (GTG>AAG), followed by BRAFV600R (GTG>AGG), an infrequent two-nucleotide variation of the predominant mutation, BRAF V600 'E2' (GTG>GAA), and BRAF V600D (GTG>GAT) [6]. The prevalence of BRAFV600K has been reported as higher in some populations*

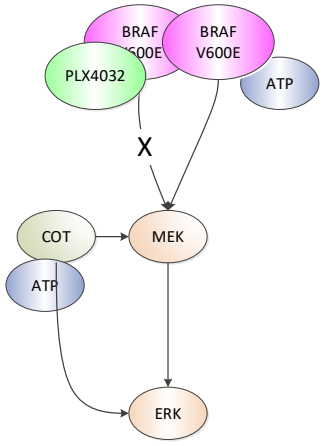
Note in the Figure below the BRAF functions:



BRAF V600 blocking can be achieve as shown below:



with the following details:



From Solit and Rosen Fig 1: *"the overexpression of RAF1 or the activation of RAS as a result of RAS mutation or upstream activation of a receptor tyrosine kinase promotes:*

(i) the formation of RAF dimers. In cells expressing RAF dimers, binding of RAF inhibitors to one member of the dimer transactivates the other, nonbound member.

(ii) In such cells, PLX4032 does not inhibit MAP kinase signaling, which leads to drug resistance.

(iii) Alternatively, the overexpression of mitogen-activated protein kinase kinase kinase 8 (MAP3K8, or COT) results in RAF-independent activation of MEK and ERK and thus resistance to PLX4032.

(iv) The activation of upstream receptor tyrosine kinases may also cause resistance to PLX4032 by activating RAS, as well as by activating parallel signaling pathways, which results in diminished dependence of the cell on RAF signaling. PDGFRβ denotes platelet-derived growth factor receptor β, and RAS-GTP RAS in its active, GTP-bound state."

5.3.2 RAS

From Mendelsohn et al

Point mutations in RAS genes are among the most common oncogenic abnormalities in all cancers, and DTC is no different. Mutations in the RAS protein lead to constitutive activation through alterations in the binding affinity of the kinase for GTP or through inactivation of its intrinsic GTPase activity. Thus, mutant RAS can signal downstream through both the MAPK and PI3K/Akt pathways without upstream activation derived from ligand-bound RTK. All three RAS genes (H-RAS, K-RAS, and N-RAS) are implicated in thyroid tumor formation from follicular cells, including 20% to 40% of benign follicular adenomas, 40% to 50% of FTC (including 15% to 20% of oxyphilic variants), 10% to 20% of PTC (almost exclusively follicular variants of PTC), and 25% of PDTC.

The presence of a RAS mutation may portend more aggressive disease with worse outcomes, but this has not been extensively examined.19,23 Each of these histologies has also been observed in transgenic mice expressing RAS mutations, although the presence of mutant RAS proteins alone is likely insufficient to cause tumor formation.24,25
PI3K/Akt Pathway

Inactivating germline mutations of the tumor suppressor gene PTEN cause Cowden syndrome, which carries a 50- to 70-fold increased risk for the development of DTC, especially FTC.26,27 Loss of this tumor suppressor function leads to activation of PI3K, Akt, and mTOR, thus contributing to enhanced cell cycle progression, decreased apoptosis, and increased tumor proliferation. However, mutations in individual genes in this pathway are otherwise uncommonly reported as early oncogenic events.

Instead, somatic mutations and/or overexpression of PIK3CA (which encodes the class I p110α catalytic subunit of PI3K), AKT, and PTEN are observed as frequent later events, especially in FTC, PDTC, and ATC.20,28,29 Gene amplification as well as activating point mutations are

observed in 10% to 20% of PDTC and 40% of ATC and can be found in tumors also bearing either BRAF or RAS mutations. AKT activation is also characteristic of the invasive fronts of aggressive DTC and has been reported to trigger increased cellular motility.30
PAX8/PPARγ

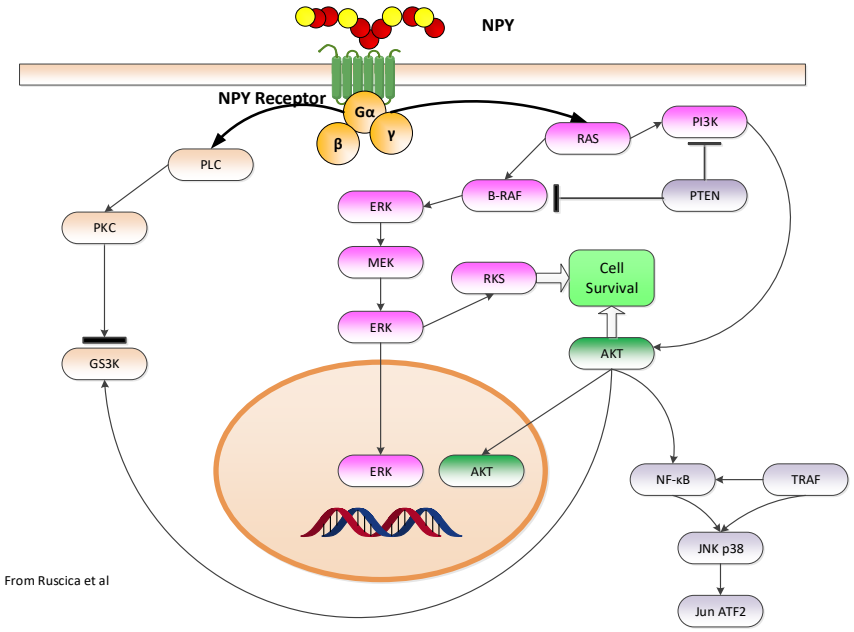
A chromosomal translocation, t(2:3) (q13;p25), results in the PAX8/PPARγ mutation, which couples the DNA binding domains of the thyroid transcription factor PAX8 with the entire coding sequence of the nuclear peroxisome proliferator-activated receptor subtype γ1.31 The actual mechanisms by which the encoded fusion protein contributes to thyroid tumorigenesis remain unclear. However, several critical pathways may be affected, including reduced expression of PTEN leading to increased activation of Akt, and a dominant-negative effect on the normal PPARγ transcription factor permitting enhanced cellular proliferation and reduction of apoptosis. This mutation may be preferentially seen in younger patients with smaller tumors, which are generally better prognostic signs, but conversely are also seen in tumors with solid or nested histologies as well as with vascular invasion.

From Ruscica et al we have:

The neuropeptide Y (NPY) family of peptides, in addition to its many physiological actions, has also been involved in the modulation of tumor progression, with specific reference to endocrine-related cancers such as neuroendocrine tumors, breast and prostate cancers. These have been found either to express NPY receptors, or to secrete NPY-related peptides, or both.

The study of the role of the NPY family of peptides in the biology of endocrinerelated tumors, specifically concerning cell proliferation, angiogenesis, invasion and metastatization, may help to clarify some aspects of tumor pathophysiology, as well as to indicate novel diagnostic markers and therapeutical approaches…..

Proposed mechanisms of ERK1/2 activation by NPY in human prostate cancer cells. In PC3 cells, NPY activates ERK1/2 via PKC and, possibly, via RAS/RAF, whereas in DU145 cells, PKC activation is not required for NPY-induced ERK1/2 phosphorylation

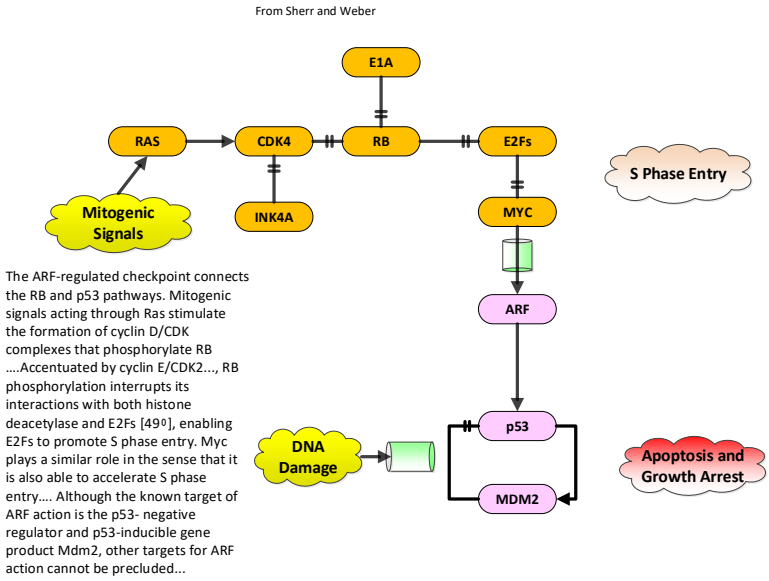


Sherr and Weber discuss the function of RAS as below. The first state regarding the Figure:

The ARF-regulated checkpoint connects the RB and p53 pathways. Mitogenic signals acting through Ras stimulate the formation of cyclin D/CDK complexes that phosphorylate RB in mid to late G1 phase. Accentuated by cyclin E/CDK2 (not shown), RB phosphorylation interrupts its interactions with both histone deacetylase and E2Fs, enabling E2Fs to promote S phase entry. Myc plays a similar role in the sense that it is also able to accelerate S phase entry.

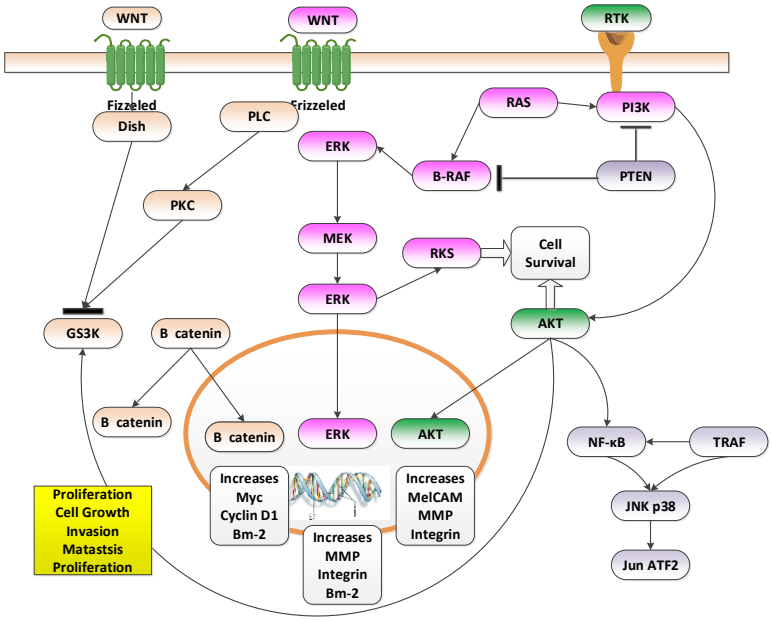
By dampening cyclin D-dependent kinase activity, p16INK4a acts as a potent tumor suppressor. One of the oncogenic effects of adenovirus E1A is to interfere with RB function. Inappropriately increased E2F or Myc signals, stemming from oncogene activation, trigger ARF expression (the vertical barrel designates the checkpoint) and activate p53 to induce either cell-cycle arrest or apoptosis, depending on the biologic context. Although the known target of ARF action is the p53- negative regulator and p53-inducible gene product Mdm2, other targets for ARF action cannot be precluded.

Similarly, Mdm2 probably interacts with proteins other than p53. DNA damage (checkpoints collectively indicated by the horizontal barrel) is shown to access the Mdm2–p53 machinery independently of ARF. However, ARF loss enables Mdm2 to work more efficiently in countering p53 function in response to DNA damage. All proteins illustrated can act as oncoproteins (light shading, black letters) or tumor suppressors (



5.3.3 PTEN

PTEN is a major controlling gene. We show the overall pathway elements below.



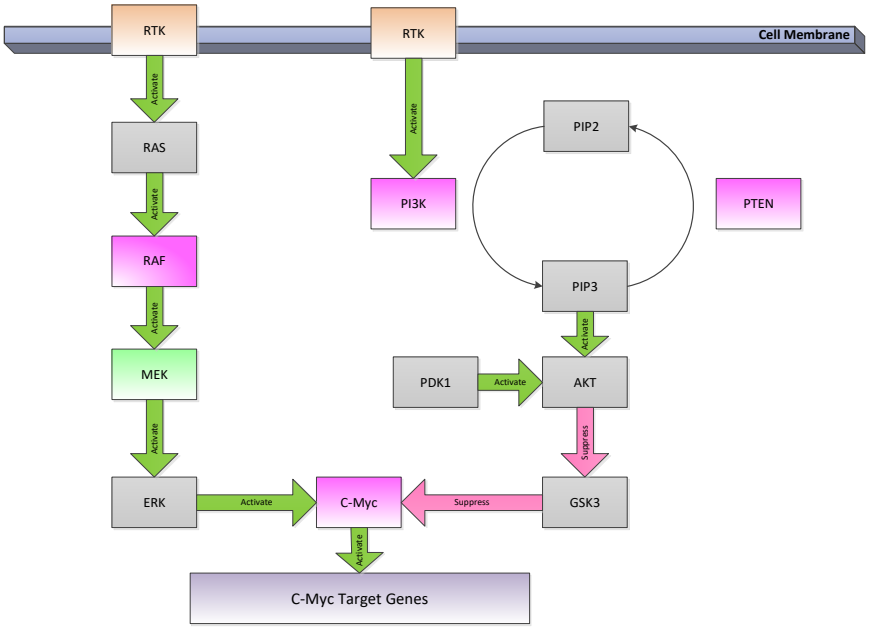
PTEN is a significant gene which controls the Akt pathway which in turn controls the replication of cells. Loss of PTEN is often seen in metastatic prostate cancer. In many ways it is the hallmark of this change. As stated in NCBI[27]:

This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. The protein encoded this gene is a phosphatidylinositol-3,4,5-trisphosphate 3-

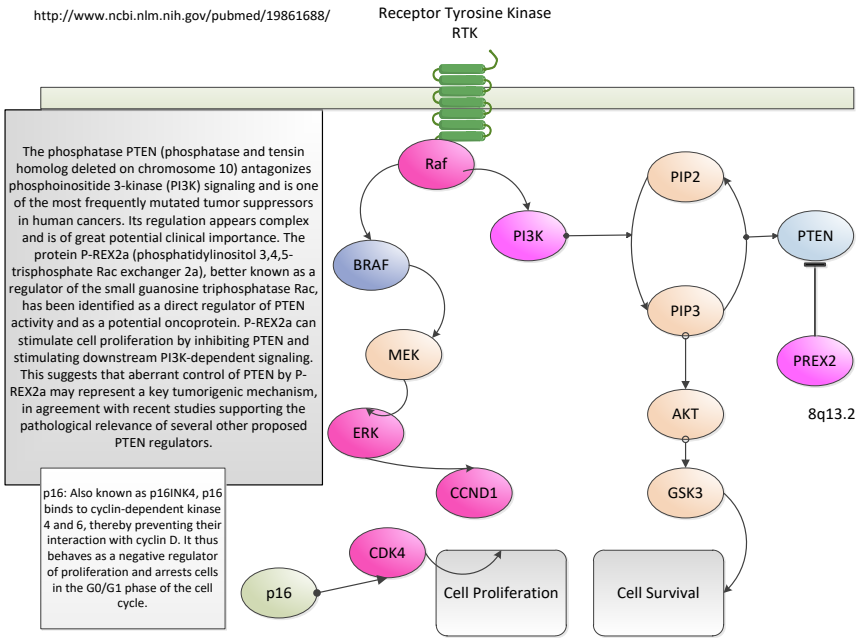
[27] http://www.ncbi.nlm.nih.gov/gene/5728

phosphatase. It contains a tensin like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. Unlike most of the protein tyrosine phosphatases, this protein preferentially dephosphorylates phosphoinositide substrates. It negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells and functions as a tumor suppressor by negatively regulating AKT/PKB signaling pathway.

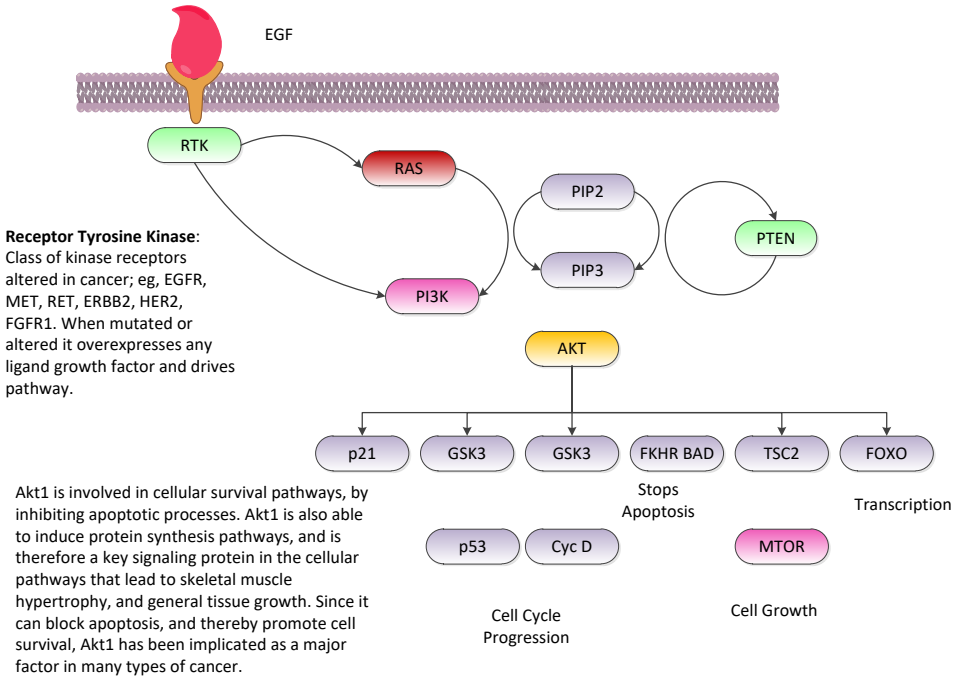
First the PTEN pathway as shown below:



Note PTEN modulates the production of Akt which in turn modulates c-Myc which in turn controls cell reproduction. Any effect which causes PTEN to not be expressed will in turn result in unfettered cell growth.



PTEN has become a key gene in the development of prostate cancer. It controls a pathway leading up to c-myc control and once PTEN is lost the PCa can be considered as very aggressive. Its loss results in an activation of Akt and then c-myc causing uncontrolled cell growth. The pathway is shown below:



As Jelovac and Park state[28]:

[28] http://jama.ama-assn.org/content/304/24/2744.full

The phosphatase and tensin homolog gene (PTEN) is a tumor suppressor located on the human chromosome 10q arm and is an important mediator of carcinogenesis in a variety of human malignancies. By the strictest definition, a tumor suppressor is a gene whose loss confers an increased lifetime risk of developing tumors. The most illustrative examples of genes that fulfill this criterion are those associated with familial cancer syndromes whereby heritable inactivation of 1 allele and subsequent increased tumor risk is passed along to each generation in an autosomal-dominant fashion.

Using this as a framework, PTEN is a bona fide tumor suppressor gene in that heritable germline mutations have been described in Cowden syndrome (CS), giving rise to a number of human tumors and cancers, most notably thyroid and breast cancers. As is the paradigm of tumor suppressor genes, affected patients with CS inherit 1 mutant inactive copy of PTEN from either parent, and the ensuing loss of the second allele results in tumor formation with subsequent genetic events that eventually lead to cancer. Although there are notable exceptions to this model, most heritable cancer syndromes are believed to adhere to this pattern.

From the work of McMenamin et al we have the slides below. Here is a case where PIN is still expressing PTEN but as we increase the grade of PCa we see the elimination of PTEN expression. Thus we can say that PIN is a state prior to PTEN suppression and a corollary may be that PCa aggressiveness is reflective of loss of PTEN and activation of Akt pathway.

5.3.4 p53

p53 is one of the earliest gene products that has been correlated with cancers. First known as an oncogene it was found that its function actually inhibited cancerous growth. As Malaguarnera et al have noted:

At variance with other human malignancies, p53 mutations are not frequent in thyroid cancer and are believed to be responsible mainly for cancer progression to poorly differentiated and aggressive phenotype. p63 and p73, two proteins with a high degree of homology with p53, are overexpressed in thyroid cancer, but their role in cancer initiation or progression is controversial. Regulation of p53 family protein function depends on:

(1) the balance between the expression of transcriptionally active (p53, TAp63, and TAp73) and inactive isoforms (DNp63 and DNp73);

(2) their interaction and competition at DNA-responsive elements;

(3) their interaction with regulatory proteins, either inhibitory or activating.

In thyroid cancer, therefore, although mutations of the p53 oncosuppressor protein family are rare, other mechanisms are present, including aberrant expression of p53 family dominant negative isoforms, up-regulation of inhibitory proteins, and functional inhibition of activating proteins. The overall result is a defective oncosuppressor activity.

These inactivating mechanisms may be present in the early stages of thyroid cancer and in different cancer histotypes. A better understanding of this complex network may not only ameliorate our comprehension of cancer biology, but also open the possibility of innovative diagnostic procedures and the development of targeted therapies.

Thus the p53 mutation may not be common its presence can be highly suggestive of a severe malignancy.

5.3.5 GNAS

GNAS appears to be a protein common to hormone glands. From the NLM database:[29]

The GNAS gene provides instructions for making one component, the stimulatory alpha subunit, of a protein complex called a guanine nucleotide-binding protein (G protein).Each G protein is composed of three proteins called the alpha, beta, and gamma subunits. In a process called signal transduction, G proteins trigger a complex network of signaling pathways that ultimately influence many cell functions by regulating the activity of hormones.

The G protein made with the subunit produced from the GNAS gene helps stimulate the activity of an enzyme called adenylate cyclase. This enzyme is involved in controlling the production of several hormones that help regulate the activity of endocrine glands such as the thyroid, pituitary gland, ovaries and testes (gonads),and adrenal glands. Adenylate cyclase is also believed to play a key role in signaling pathways that help regulate the development of bone (osteogenesis). In this way, the enzyme helps prevent the body from producing bone tissue in the wrong place (ectopicbone).

5.3.6 RET

RET appears to be an influential gene involved in thyroid malignancies. From NLM database:[30]

The RET gene provides instructions for producing a protein that is involved in signaling within cells. This protein appears to be essential for the normal development of several kinds of nerve cells, including nerves in the intestine (enteric neurons) and the portion of the nervous system that controls involuntary body functions such as heart rate (the autonomic nervous system). The RET protein is also necessary for normal kidney development and the production of sperm (spermatogenesis).

The RET protein spans the cell membrane, so that one end of the protein remains inside the cell and the other end projects from the outer surface of the cell. This positioning of the protein allows it to interact with specific factors outside the cell and to receive signals that help the cell respond to its environment.

[29] https://ghr.nlm.nih.gov/gene/GNAS

[30] https://ghr.nlm.nih.gov/gene/RET

When molecules that stimulate growth and development (growth factors) attach to the RET protein, a complex cascade of chemical reactions inside the cell is triggered. These reactions instruct the cell to undergo certain changes, such as dividing or maturing to take on specialized functions.

From Mendelsohn we have:

About 20% of MTC occurs in one of several familial syndromes: multiple endocrine neoplasia (MEN) 2A (which also includes parathyroid tumors and pheochromocytomas); MEN 2B (which also includes pheochromocytomas, intestinal ganglioneuromatosis, neuromas of the tongue and subconjunctiva, and Marfanoid habitus); and familial MTC (FMTC, which lacks the other clinical features of MEN 2A).

Additional variants of MEN 2A have been reported that include cutaneous lichen amyloidosis and with Hirschsprung disease. Germline mutations in RET were identified as causative of these hereditary forms of MTC in two landmark 1993 studies.34,35 Today, more than 99% of all cases of hereditary MTC can be attributed to one of numerous point mutations in RET that cause activation of the tyrosine kinase function of the RTK (Table 43-1). Given the ubiquitous nature of the mutation, it is not surprising that the disease begins with diffuse hyperplasia of all of the C cells, with eventual development of one or more malignant foci.

The most common germline mutation, a cysteine-to-arginine substitution at codon 634 (denoted C634R), accounts for at least half of all cases of MEN 2A and has also been extensively studied in vitro in the well-characterized TT cell line.36 This mutation is found in the cysteine-rich extracellular domain of RET, a region responsible for ligand-dependent dimerization. However, in the setting of the C634R mutation, RET is capable of ligand-independent dimerization, leading to autophosphorylation of the intracellular tyrosine residues that are responsible for interaction with downstream signaling pathways.

In contrast, a methionine-to-threonine substitution at codon 918 (denoted M918T) is associated with the more aggressive phenotype of MEN 2B. The M918T mutation occurs in the intracellular domain of RET, changing the conformation of the tyrosine kinase domain and allowing marked enhancement of autophosphorylation in the absence of dimerization. In addition, allelic imbalance, due to either increased copy number of the mutant RET allele or deletion of part or all of the wild-type allele, has been reported in several cases of MEN 2A as well as the TT cell line itself.

Sporadic MTC, on the other hand, is not associated with germline changes in RET, but nonetheless, somatic RET mutations have been commonly reported in 25% to 50% of sporadic MTC cases. In this instance, the most frequent somatic mutation is the M918T alteration, but numerous other codon changes have also been observed, including selected deletions as well as point mutations. Of note, about 6% to 7% of patients with clinically sporadic MTC are found to carry germline mutations diagnostic of hereditary forms of the disease despite the absence of a positive family history, thus leading to the consensus recommendation to recommend RET germline testing for all newly diagnosed cases of apparently sporadic MTC.37,38

Extensive genotype:phenotype correlations have been established in the two decades since RET was identified as causing MTC. In addition to identifying specific clinical syndromes associated with each mutation, these analyses have also demonstrated that disease penetrance, typical age of development of C-cell hyperplasia and malignancy, and the aggressiveness of the malignancy vary in a manner that is based to a large degree on the individual mutation. Thus, the intracellular domain mutations, which tend to be associated with the aggressive MTC characteristic of MEN 2B, are also found to cause aggressive sporadic MTC when they occur as somatic mutations. Patients who present with sporadic MTC associated with a somatic M918T mutation of RET have worse outcomes, including overall survival.39 These genotype:phenotype correlations are also useful in determining the role and outcomes of genetic screening in hereditary disease.

Recently published guidelines from the American Thyroid Association divide known RET germline mutations into four risk categories that guide earliest age for RET testing of potential familial carriers, earliest age for recommended first thyroid ultrasound and serum calcitonin testing to detect early presymptomatic evidence of disease, and role for potentially curative prophylactic thyroidectomy.38 Using this type of approach, most young patients identified by prospective genetic screening as carriers for FMTC or MEN 2A can be cured with prophylactic thyroidectomy, although a small percentage remain with biochemical evidence of residual disease.40
RAS

Mutations of RAS have recently been recognized as common in sporadic MTC in the absence of documented RET mutations.41,42 A wide range of frequency has been reported, however, between 10% and 80% of all RET–wild-type sporadic cases, using differing techniques for identifying RAS mutations. In the largest study, tumor samples from 108 sporadic disease patients without somatic RET mutations were subjected to RAS sequencing, yielding a frequency of 17% in that setting.42 Of the three potential genotype combinations, patients who were (mutant)RAS (wt)RET were more likely to be disease free after a median follow-up of 5 years than those who were (wt)RAS (wt)RET or (wt)RAS (mutant)RET.

As Subbiah et al note:

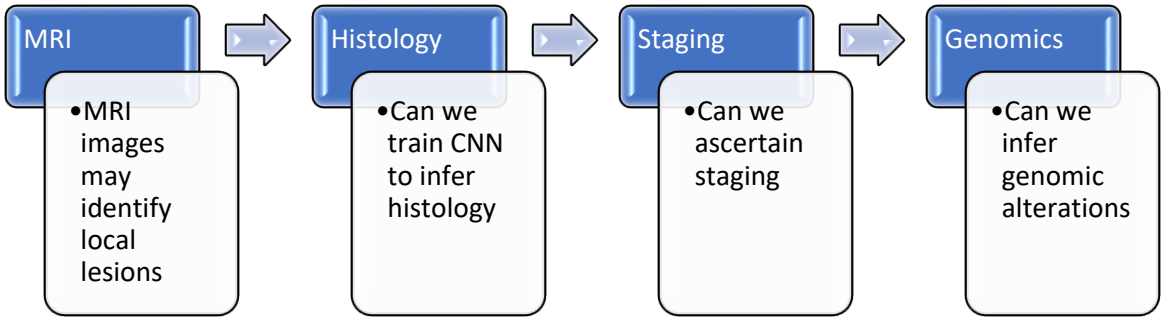
The receptor tyrosine kinase RET can be oncogenically activated by gene fusions or point mutations. RET fusions occur in a variety of malignancies, including 1%-2% of lung cancers, up to 10%-20% of papillary thyroid cancers, and rarely in many other solid tumors. RET mutations affect most medullary thyroid cancers (MTCs) , and next generation sequencing (NGS) analysis of large numbers of patient tumors has uncovered RET alterations at low frequency in other tumor types. Such alterations possess the hallmarks of cancer drivers: constitutive kinase and signaling activity, transformation of primary cells, and mutual exclusivity from other drivers. Until recently, only multikinase inhibitors (MKIs) with nonselective RET inhibitory activity have been available for patients with RET-altered cancers. Clinical experience with these nonselective RET inhibitors has been disappointing, with only modest activity in RET-mutant MTCs and RET fusion-positive lung cancers.

Other MKIs approved for other indications (e.g. sorafenib) possess similar, nonselective anti-RET activity preclinically. In part, this may be due to substantial 'off-target' side-effects that limit the degree of RET-specific inhibition and lead to frequent dose reductions. Together with weak anti- RET potency and poor pharmacokinetic (PK) properties, these limitations prevent potent RET pathway inhibition in patients.

6 BLADDER

Bladder cancer can often be a chronic disease if it has penetrated into the muscle[31]. However once in the muscle it can become very aggressive. Managing a non-muscle invasive bladder cancer can be problematic and quite costly. Thus having a better diagnostic and prognostic and non-invasive methodology is of primary import. We discuss some of the options herein.

The desirable outcome in Bladder cancer, BCa, is one where we can infer histology from MRI results and in turn infer malignant versus benign and localized vs invasive. Namely the goal is as below:



6.1 MPMRI

Imaging has significant potential for bladder cancer. As Hoshi et al have recently noted:

For high-grade non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC), MRI may detect and stage tumors with high sensitivity and specificity. mpMRI has demonstrated high diagnostic accuracy in differentiating NMIBC from MIBC and organ-confined disease from non-organ confined disease; exceeding that of T2 weighted imaging (T2W) or diffusion weighted imaging (DWI)-MRI used alone. Compared with CT, MRI offers improved soft-tissue resolution, making it easy to distinguish between NMIBC and MIBC.

It has even been proven to be superior to CT in identifying bladder-wall invasion. There are a number of reports on the usefulness of mpMRI for the detection of tumor recurrence and differentiation of non-muscle invasive UC from muscle-invasive UC. Afifi et al reported on the usefulness of mpMRI in detecting metastatic lymph nodes. The largest size of the metastatic lymph nodes detected was 42 mm, and lymph nodes with low apparent diffusion coefficient values were considered positive. However, in general, data on lymph node staging from mpMRI remains limited.

Moreover, the capabilities of mpMRI add dimensions not seen before. Salmanoglu et al. has noted:

31 https://www.researchgate.net/publication/336460663_Bladder_Cancer_An_Interesting_Set_of_Diagnostic_Options

Multiparametric MRI (mpMRI) is composed of T1W-MRI, T2W-MRI and functional MRI methods, including DCE-MRI and DW-MRI. mpMRI combines anatomic and functional MRI sequences and plays a role for detection, staging, and local recurrence of BCa. In a recent study, efficacy of mpMRI for staging of BCa after TURB in 45 patients was examined. mpMRI was found both sensitive (92%) and specific (84%) method for MIBC detection. The investigators concluded that, mpMRI may be helpful for local staging of BCa after TURB. However, to assess its efficacy carefully, clinical studies in large patient groups are required.

mpMRI protocols allow for the potential of complex identification. As Bagheri et al has noted:

Routine MRI protocol includes mpMRI sequences, i.e., T1, T2, diffusion-weighted precontrast; T1-weighted gradient echo pre- and contrast-enhanced MRI sequences targeted to the urinary bladder/pelvis; and, when appropriate, a single non-contrast, T1-weighted sequence of the lumbar spine in the axial plane for further evaluation of possible bone metastasis.

The mpMRI bladder/pelvis and lumbosacral sequences can be accomplished in a single session without moving the patient. For MIBC and high-grade NMIBC, MRI can detect and stage tumors with relatively high sensitivity and specificity, with the advantage of not having the ionizing radiation associated with CT. Though not commonly used, MRI of the pelvis can stage the bladder tumor after cystoscopic diagnosis. Although TURBT is the preferred, and most accurate, technique for staging NMIBC and MIBC tumors, retrospective analyses have found that TURBT has understaged cases by 42%.

MRI, on the other hand, provides extensive soft-tissue resolution. It has even been shown to detect T3 and T4 disease and has proven superior to CT in distinguishing between stages T2a and T2b by identifying bladder-wall invasion. Traditional T1-weighted imaging can identify extravesical fat infiltration, pelvic lymphadenopathy, and suspected bone metastases, but is of limited use in local staging of urothelial carcinoma because these tumors appear similar to the normal detrusor muscle of the bladder wall due to similar signal intensities.

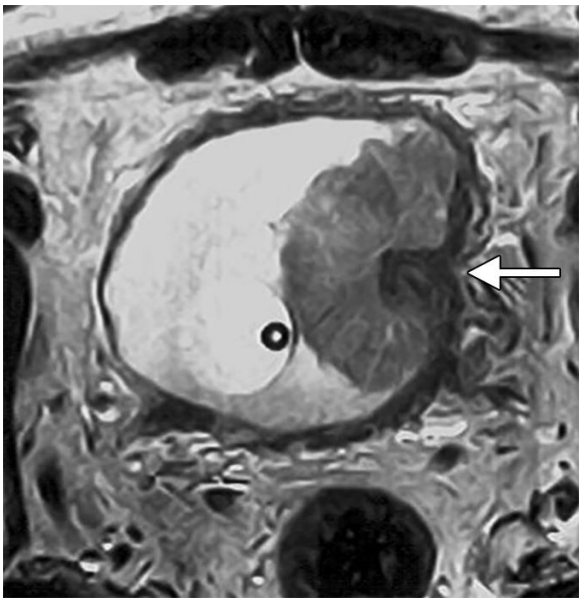
On the other hand, T2-weighted imaging can differentiate between tumor edges and the bladder wall because urothelial carcinomas have increased T2-weighted signaling compared to normal detrusor muscle. Triplanar anatomical T1- and T2-weighted sequences are used for local tumor staging of urothelial carcinoma, and have been used to make 3D virtual MR cystoscopies which have detection rates of > 90%. In cases of conventional visualization obscured by significant hematuria, urothelial stricture disease, or lesions within the diverticula, virtual MR cystoscopy can provide an alternative to, and perhaps better visualization than, conventional modalities.

Other forms of MRI, including DWI-MRI and DCE-MRI, are commonly used in RCC and provide functional sequences that can differentiate between tumor and normal detrusor muscle. DWI-MRI can characterize tissue by measuring the Brownian motion of water molecules. In DWI-MRI, malignant tissue has lower apparent diffusion coefficient values than normal tissue. Sensitivity and specificity have been shown to be 80% and 79%, respectively, but when used in combination with anatomic T2-weighted MRI, sensitivity and positive predictive value were

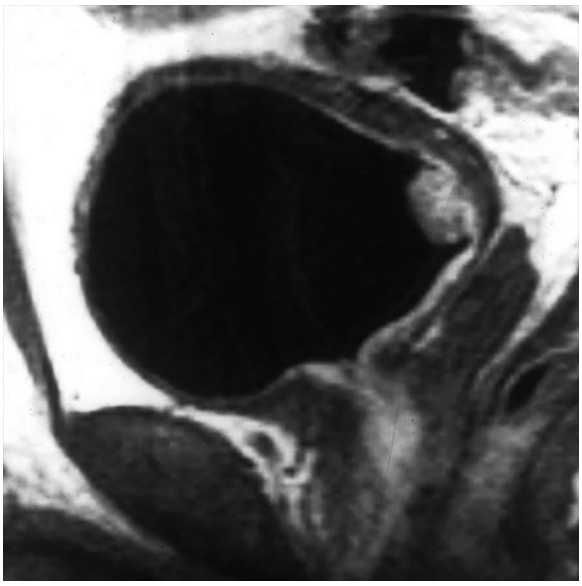
100% in a small cohort (n = 15) of patients. This combination has been shown to provide more accurate staging in patients in clinical stage ≤ T2.

On the other hand, DCE-MRI has been found to be superior to traditional unenhanced T1- and T2-weighted anatomic imaging owing to its ability to distinguish urothelial carcinoma from normal bladder wall. The hypervascularity of tumor tissue leads to earlier and better contrast enhancement compared to normal bladder wall. One study revealed 84% accuracy for DCE-MRI in tumor staging compared to 67% accuracy for unenhanced T1- and T2- weighted imaging. A prospective study investigating the role of DCE-MRI in 122 patients demonstrated an 87.5% sensitivity for differentiation of lymph node-negative organ confined UC from non-organ-confined urothelial carcinoma and accuracy of 74%. However, the specificity of MRI findings were mediocre at 47.6%.

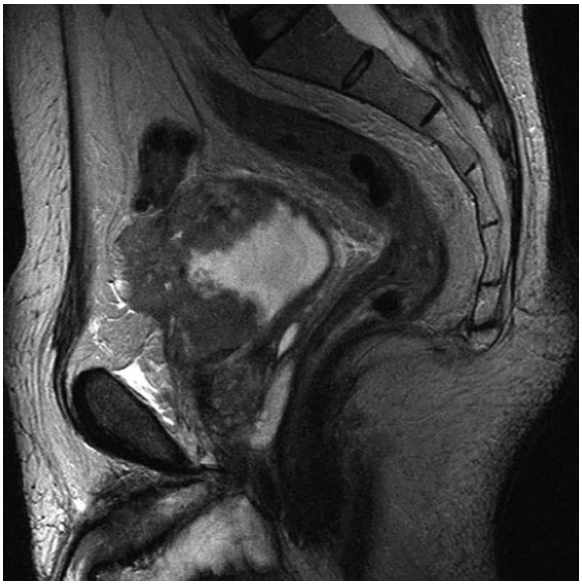
We now consider several MRI scans. The one below demonstrates a clear lesion of substantial size.



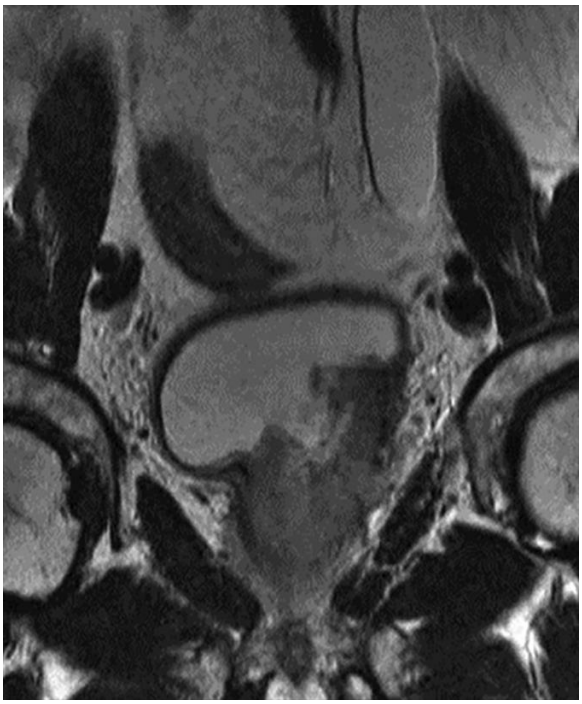
The following MRI demonstrates what appears to be a papillary lesion with a stem. The clarity is quite reasonable and most likely would be seen visually upon cystoscopy.



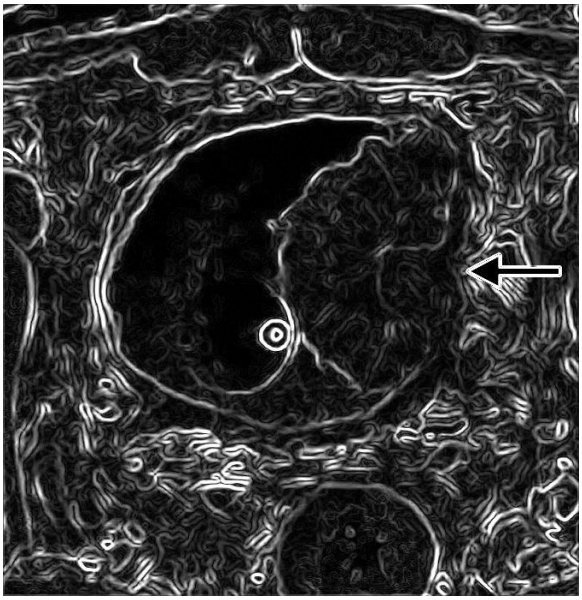
Again we see in this sagittal view the same lesion very prominently.



This is a transverse view which we will then image process.



The following is an edged processed version of the above. Edge processing is a preprocessing which may then feed into some identifier[32].



6.2 CYSTOSCOPY

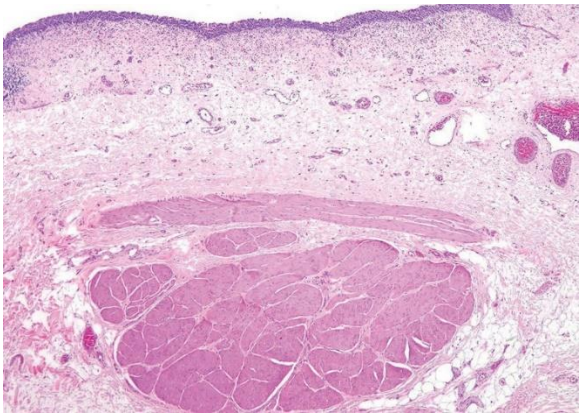
Cystoscopy is a visual examination of the bladder via an optical scope. Salmanoglu et al. has noted:

[32] Note we have used Fiji to process this and other images.

White light cystoscopy (WLC), a widely available technique, that allows visualization of the mucosa within the bladder, is considered a gold standard method for detecting BCa [8]. There are two forms of WLC, rigid and flexible cystoscopy (FC). Rigid cystoscopy provides better image quality, enables working with a large lumen, and provides improved flow. FC on the other hand allows alternative patient positioning, easy passage, and enables examination of all parts of the bladder. Therefore, FC is usually applied for initial assessment of patients. However, FC may miss up to 10% of papillary tumors. Furthermore its small working channel lumen does not allow resection of BCa. Although technology has improved the WLC image quality significantly, WLC cannot reliably determine flat and carcinoma in situ (CIS) lesions, and cannot distinguish benign lesions from malignant masses.

Such a distinction is particularly important when Transurethral Resection of Bladder (TURB) is to be performed. However, cystoscopy is recommended by national comprehensive cancer network (NCNN) and American urological association (AUA) guidelines for imaging patients with macroscopic hematuria

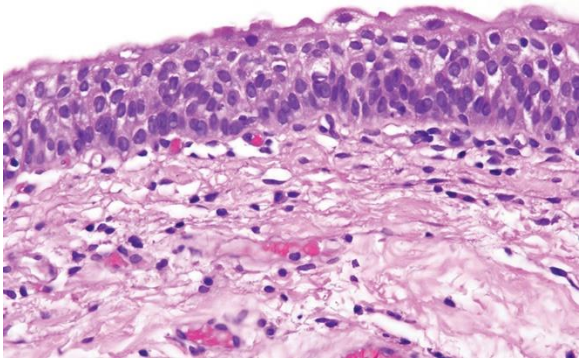
The normal anatomy of the bladder cells is shown below. It includes the urothelium, the lamina propria and submucosa with the thick muscle bundles[33].



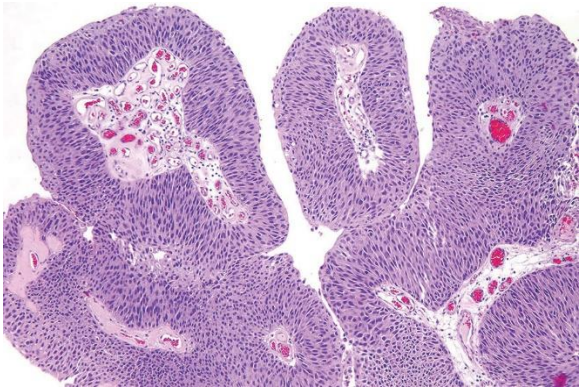
The normal urothelium is depicted below. It is a well-organized surface[34].

[33] See Epsetin, Bladder, Fig E 1.1

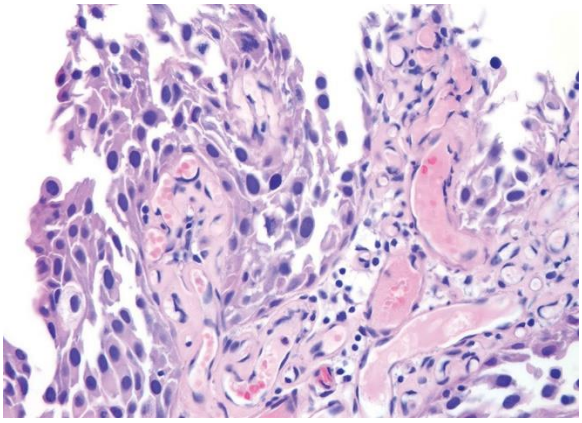
[34] See Epstein, Bladder, Figure 1.1



The figure below is a low grade non-invasive papillary cancer[35]. Note the papilla like structures and the organization.



In contrast a high-grade version is depicted below[36]:

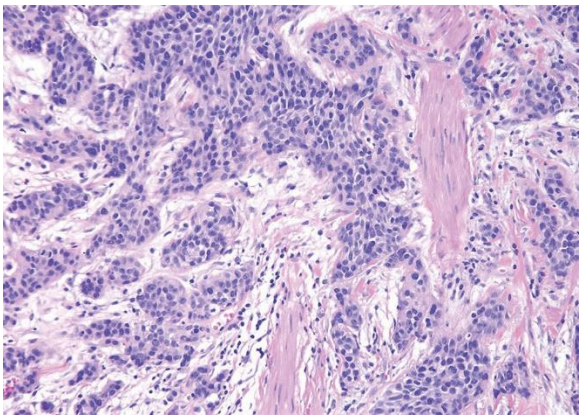


Here we have increased disorganization and remnants of the papilla.

Now with muscle invasive bladder cancer we have an example shown below:

[35] See Epstein, Bladder, Fig 3.23

[36] See Epstein, Bladder, 3.128



Here we have a clear invasion of the muscle layer.

6.3 GENOMICS

As we noted, genomics is the study of the genetic variations in the tumor cells. We are reaching the point where by cell flow cytometry we can examine the genetic issues on a cell by cell basis. Unlike some cancers, such as thyroid, we do not have cell specific morphological markers such as notched nuclei and clear cytoplasm[37]. From Meeks et al we have the following table listing the most significant genetic alterations:

Gene Altered	Non progressor	Progressor Baseline	Progressor Muscle Invasive	Metastatic
TERT	10/15 (66%)	7/10 (70%)	5/8 (62%)	9/11 (81%)
TP53	9/15 (60%)	6/10 (60%)	6/8 (75%)	4/11 (36%)
RB1	5/15 (33%)	1/10 (10%)	0/8 (0%)	0/11 (0%)
PIK3CA	6/15 (40%)	3/10 (30%)	2/8 (25%)	4/11 (36%)
PTEN	1/15 (6%)	1/10 (10%)	1/8 (12%)	0/11 (0%)
2D (MLL2)				4/11 (36%)
ARID1A	6/15 (40%)	2/10 (20%)	2/8 (25%)	1/11 (9%)
CDKN2A/B	1/15 (6%)	2/10 (20%)	3/8 (37%)	7/11 (63%)
CCND1 amp	2/15 (12%)	2/10 (20%)	3/8 (37%)	3/11 (27%)
FGFR/FGF	9/15 (60%)	6/10 (60%)	5/8 (62%)	6/11 (54%)

[37] See, https://www.researchgate.net/publication/335404502_Thyroid_Cancer_and_Genetic_Differentiation , https://www.researchgate.net/publication/334429457_miRNAs_Genes_and_Cancer_Cytology , https://www.researchgate.net/publication/331935614_Thyroid_Cancer_Seek_and_Ye_Shall_Find

Nothing in the above are surprising given the profile of most somatic cancers. As Vandekerkhove et al note:

*The analysis of **circulating tumor DNA (ctDNA)** in plasma has shed light on the somatic landscape of metastatic disease in several solid malignancies. Prognostic and predictive ctDNAbased biomarkers are beginning to emerge in prostate, colon and pancreatic cancer. In non-small cell lung cancer, a 'companion diagnostic' **ctDNA test for the EGFR T790M mutation** recently received FDA approval.*

However, the abundance and utility of ctDNA in advanced BCa remains largely unexplored, with prior studies limited by small cohort sizes and dependency on single gene sequencing and/or digital droplet PCR (ddPCR) assays to detect specific missense mutations. While ddPCR provides the sensitivity required to detect extremely low levels of ctDNA (e.g. for predicting disease relapse after curative therapy, only broad nextgeneration sequencing assays can deliver a comprehensive analysis of clinically-relevant alterations such as PI3K/mTOR pathway deregulation or somatic hypermutation. In this study we applied a custom sequencing approach to plasma cell-free DNA (cfDNA) collected from a cohort of patients with aggressive BCa. We reveal the landscape of somatic alterations detected across 50 clinically-relevant driver genes in metastatic BCa, demonstrating the promise of liquid biopsies as both a discovery tool and a biomarker for therapy selection. …

Across the 26 patients with quantifiable ctDNA in at least one cfDNA sample (or in two cases, metastatic tissue), we detected 281 somatic mutations including 121 protein altering mutations. We identified 22 missense or truncating mutations in TP53 in 17/26 patients...

The large number of mutations make any identification problematic.

Incorporating copy number results, 24/26 patients carried either a TP53 inactivating change (n=19), RB1 inactivating change (n=8), MDM2 gain (n=2), or CDKN2A loss (n=6), likely resulting in disrupted cell cycle regulation (Figure 3C). For the two patients without alterations in these four genes, E-006 had non-metastatic disease, and BC-008 had a low ctDNA fraction not amenable to copy number analysis.

Four patients exhibited definitive evidence for biallelic inactivation of RB1 or CDKN2A. Over half the cohort (19/26 patients) had mutations or disrupting rearrangements in chromatin modifier genes (Figure 3C), including eight truncating mutations within ARID1A that remove the DNA binding domain and/or glucocorticoid receptor binding domain, and eight truncating mutations in KMT2D (MLL2). TERT promoter mutations were identified in 12/26 patients: ten patients harboured the chr5:1295113:G>A mutation (reported in 65% of BCa), while two carried the chr5:1295135:G>A mutation (reported in 10% of BCa).

The majority of patients had alterations to the PI3K/mTOR pathway, including six with hotspot missense mutations in PIK3CA (K111E, E542K, E545K (n=2), E674Q or E726K), four with truncating mutations in TSC1 (mutually exclusive with PIK3CA hotspot mutations), one with PIK3R1 stopgain, one with PTEN stopgain, and one with a TSC2 truncating rearrangement (Figure 3C). Six patients showed evidence for PTEN or PIK3R1 deletion.

Nine patients (35%) carried ERBB2 activating somatic changes, including amplification in five patients (Supplementary Figure S8), and hotspot mutations (S310F, L755S, I767M, V777L) in four patients. Four patients carried ERBB3 activating alterations, including two amplifications and two hotspot mutations (M91I, V104L) (Figure 3C). Remarkably, one patient had an average of 71 copies ERBB2 in his ctDNA. Three more patients carried activating RAS mutations (KRAS G12D (n=2), HRAS Q61R), and one patient carried a KRAS amplification. In total, 15/26 patients carried activating somatic alterations in the MAPK pathway

We summarize these genes in the following table using reference to NCBI data bases. Also details are in Cantley et al regarding pathways.

Gene Altered	Normal Function
TERT[38]	Telomerase is a ribonucleoprotein polymerase that maintains telomere ends by addition of the telomere repeat TTAGGG. The enzyme consists of a protein component with reverse transcriptase activity, encoded by this gene, and an RNA component which serves as a template for the telomere repeat. Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells resulting in progressive shortening of telomeres. Deregulation of telomerase expression in somatic cells may be involved in oncogenesis
TP53[39]	This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome.
RB1[40]	The protein encoded by this gene is a negative regulator of the cell cycle and was the first tumor suppressor gene found. The encoded protein also stabilizes constitutive heterochromatin to maintain the overall chromatin structure. The active, hypophosphorylated form of the protein binds transcription factor E2F1. Defects in this gene are a cause of childhood cancer retinoblastoma (RB), bladder cancer, and osteogenic sarcoma
PIK3CA[41]	Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. This gene has been found to be oncogenic and has been implicated in cervical cancers
PTEN[42]	This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. The protein encoded by this gene is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase. It contains a tensin like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. Unlike most of the protein tyrosine phosphatases, this protein preferentially dephosphorylates phosphoinositide substrates. It negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells and functions as a tumor suppressor by negatively regulating AKT/PKB signaling pathway. The use of a non-canonical (CUG) upstream initiation site produces a longer isoform that initiates translation with a leucine, and is thought to be preferentially associated with the mitochondrial inner membrane. This longer isoform may help regulate energy metabolism in the mitochondria.
2D (MLL2)[43]	The protein encoded by this gene is a histone methyltransferase that methylates the Lys-4 position of histone H3. The encoded protein is part of a large protein complex called ASCOM, which has been shown to be a transcriptional regulator of the beta-globin and estrogen receptor genes. Mutations in this gene have been shown to be a cause of Kabuki syndrome.

[38] https://www.ncbi.nlm.nih.gov/gene/7015

[39] https://www.ncbi.nlm.nih.gov/gene/7157

[40] https://www.ncbi.nlm.nih.gov/gene/5925

[41] https://www.ncbi.nlm.nih.gov/gene/5290

[42] https://www.ncbi.nlm.nih.gov/gene/5728

[43] https://www.ncbi.nlm.nih.gov/gene/8085

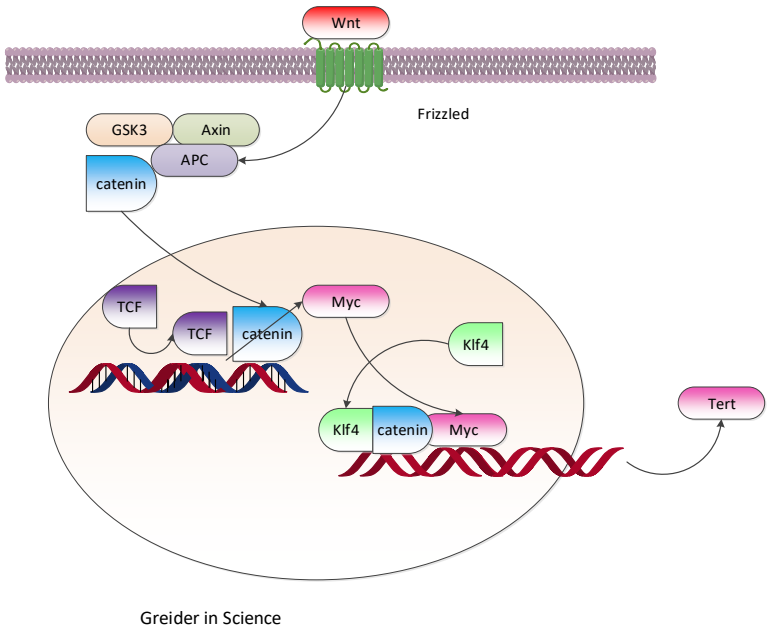
Gene Altered	Normal Function
ARID1A[44]	This gene encodes a member of the SWI/SNF family, whose members have helicase and ATPase activities and are thought to regulate transcription of certain genes by altering the chromatin structure around those genes. The encoded protein is part of the large ATP-dependent chromatin remodeling complex SNF/SWI, which is required for transcriptional activation of genes normally repressed by chromatin. It possesses at least two conserved domains that could be important for its function. First, it has a DNA-binding domain that can specifically bind an AT-rich DNA sequence known to be recognized by a SNF/SWI complex at the beta-globin locus. Second, the C-terminus of the protein can stimulate glucocorticoid receptor-dependent transcriptional activation. It is thought that the protein encoded by this gene confers specificity to the SNF/SWI complex and may recruit the complex to its targets through either protein-DNA or protein-protein interactions.
CDKN2A/B[45]	This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase. The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the products of the other variants. This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, the E3 ubiquitin-protein ligase MDM2, a protein responsible for the degradation of p53. In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control. This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene.
CCND1[46] **amp**	The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance throughout the cell cycle. Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. This cyclin forms a complex with and functions as a regulatory subunit of CDK4 or CDK6, whose activity is required for cell cycle G1/S transition. This protein has been shown to interact with tumor suppressor protein Rb and the expression of this gene is regulated positively by Rb. Mutations, amplification and overexpression of this gene, which alters cell cycle progression, are observed frequently in a variety of tumors and may contribute to tumorigenesis
FGFR/FGF[47]	This gene encodes a member of the fibroblast growth factor receptor (FGFR) family, with its amino acid sequence being highly conserved between members and among divergent species. FGFR family members differ from one another in their ligand affinities and tissue distribution. A full-length representative protein would consist of an extracellular region, composed of three immunoglobulin-like domains, a single hydrophobic membrane-spanning segment and a cytoplasmic tyrosine kinase domain. The extracellular portion of the protein interacts with fibroblast growth factors, setting in motion a cascade of downstream signals, ultimately influencing mitogenesis and differentiation. This particular family member binds acidic and basic fibroblast growth hormone and plays a role in bone development and maintenance.

We depict some of the key pathways below:

[44] https://www.ncbi.nlm.nih.gov/gene/8289

6.3.1 TERT

TERT is associated with the hedgehog pathway as shown below. It is a controller of the telomerase.



Greider in Science

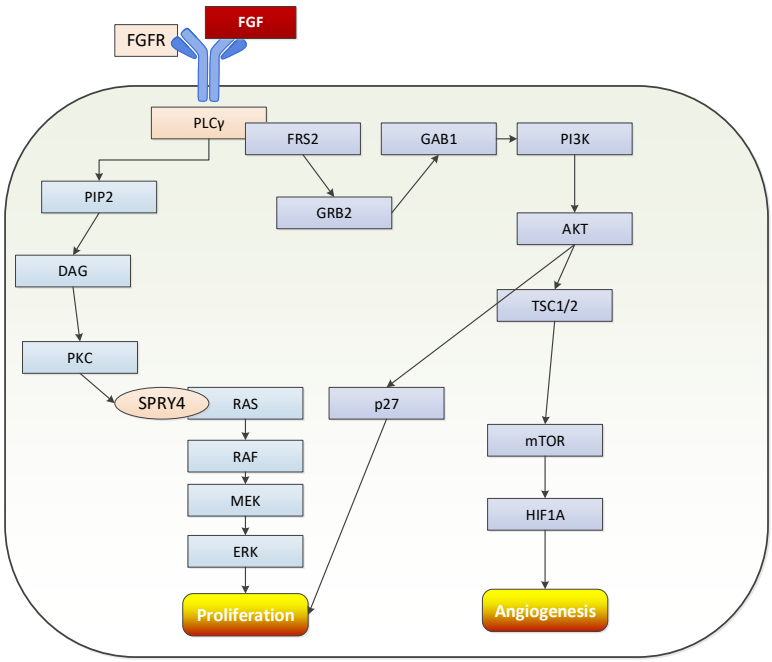
6.3.2 FGR

Growth factors and their receptors play a significant role in almost all malignancies[48]. We depict below the FGF complex and the resulting proliferation and angiogenesis that results. FGF and FGFR can be attractive targets across many cancers.

[45] https://www.ncbi.nlm.nih.gov/gene/1029

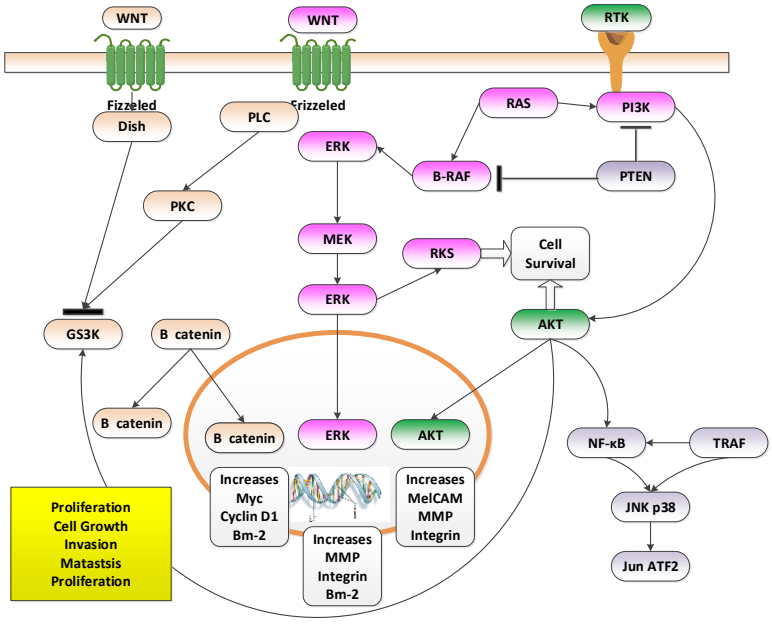
[46] https://www.ncbi.nlm.nih.gov/gene/595

[47] https://www.ncbi.nlm.nih.gov/gene/2261 and
[48] https://www.researchgate.net/publication/329702571_Growth_Factors_Pathways_and_Cancers



6.3.3 PTEN

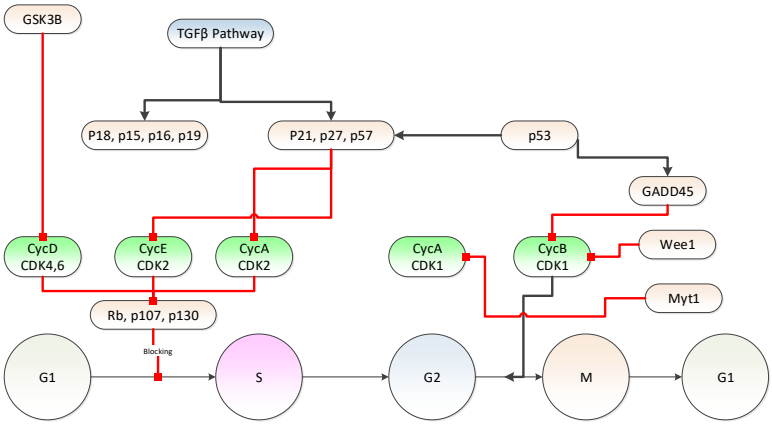
PTEN is a classic gene and protein involved in regulating cells. Loss of PTEN is well known as a cancer inducer in prostate cancer[49].



6.3.4 CDKs

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

Cyclin dependent kinases play a significant role in the * related to proliferation. We depict them below:



From Solimini et al: "*Known pathways with multiple shRNAs in the validation screen. Genes that validated in the secondary screen by a factor of ≥4 with multiple shRNAs are denoted with circles corresponding to three to five shRNAs (orange) and six or more shRNAs (red).*"

7 OBSERVATIONS

The issue of correlation vs causation goes to the heart of deep learning algorithms. Fundamentally all of these algorithms are correlative. They take massive amounts of data and either with learning or without seek to ascertain some outcome, diagnostic or otherwise. One can imagine in some malpractice trial having to explain to twelve people that a physician relied on this massive computer which fundamentally just kept making guess after guess, albeit under an algorithm, and the treatment and outcome was based upon a set of such compounded guesses.

7.1 AI APPROACHES ARE OFTEN NOT DISPOSITIVE

As we have repeatedly noted, the NN/AI approach is fundamentally based upon placing a massive amount of data into a "guessing machine" which in turn tries to process it so as to minimize some error metric. It is not an experiment whose steps are revealed and which can be repeated with the same results over and over again.

7.2 NN AND CNN MAY OFTEN HAVE STABILITY ISSUES

Stability of NNs can be an issue. Local optima can allow the NN to bounce around and thus depending on where one is in the cycle the output does have a potential to reflect the existence of these multiple optima. Namely malignant today may be benign tomorrow. Now clearly this effect is often the case with humans. Pathologists and radiologists may differ on certain results. We see in the literature many discussions of the human error. This is all too often a reason for using some AI artifact whose outcome may very suffer the same condition.

7.3 NN AND CNN MAY OFTEN HAVE SINGULAR CONVERGENCE ISSUES

Convergence is an essential necessity for any such decision system. Unlike stability, which may have multiple convergent points, fundamental convergence means that we never get to a reliable answer.

7.4 AI RESULTS OFTEN LACK SUGGESTIVE EXPERIMENTAL VERIFICATION

All AI approaches are deficient in any lab verification. It is merely input in and verdict out. What data should we put in and does any of that extraneous data bias the result, suppress the results and so forth. In an experimental world we have protocols to address these issue. Also in the experimental world we make observation, often unexpected, that lead to new insights. All of this at best is hidden in the bowels of the NN.

7.5 CAN THE AI APPROACH RESULT IN RELIANCE WITHOUT UNDERSTANDING?

Trust can be a double edged sword. If founded in a tested and time honored system, then perhaps it works most of the time. We have trust in the airline when we go from point A t point B but planes still crash. But we have a century of seeing this system function and with a multiplicity of

checks and balances we do board airlines. In contrast can one trust a NN without fully understanding it?

7.6 Cell Identification En Masse vs Cancer Cell of Origin

Identifying a cancer cell of origin, stem cell, or the like is critical. Not all the cancer cells in a tumor are alike genetically and there is often a cell or class of cells with certain genomic profiles that is pathognomonic for a certain level of malignancy. One must be careful in placing all the cells in a blender and then sequencing. Single cell sequencing is becoming de regur in the field of cancer genomics.

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