Prostate Cancer: Removing the Uncertainty

Prostate cancer (PCa), excluding skin cancer, is the most commonly diagnosed malignancy in men over the age of 50 living in the developed world. In the simplest terms, one in six men will be diagnosed with PCa in their lifetime. Given that it is a relatively slow-developing cancer affecting so many, PCa is an ideal candidate for early detection. Accurate identification of the beginning stages of PCa pathology confers to clinicians the greatest opportunity to successfully treat the disease.

Conventional management of PCa relies primarily on digital rectal exams (DREs), blood tests for elevated and rising levels of prostate-specific antigen (PSA), and prostate gland needle biopsies (cores). Each of these tests has limitations that may be unrelated to PCa, or that falsely indicate the presence of malignancy. For example, elevated levels of PSA can be caused by other disorders such as a prostate infection, and needle biopsies that rely on histology (visual examination under a microscope) will only detect PCa if the needle collects prostate core samples from actual tumor tissue, which can easily be missed.

The uncertainty of conventional testing often leads to patient anxiety that PCa may be present; conversely, the apparent absence of disease results in a false sense of security, when, in fact, the needle biopsies may have missed the cancerous tissue. The Prostate Core Mitomic Test™ (PCMT) closes this gap in conventional management of PCa. The ability to detect large-scale mitochondrial DNA (mtDNA) deletions in prostate biopsy samples enables clinicians to accurately discriminate between benign and malignant prostate tissue. Using the same initial biopsy samples that are collected for histology, PCMT utilizes the tumor field effect to identify the molecular signature of elevated levels of mtDNA genomic mutations in histologically benign cells adjacent to tumor tissue. This significantly reduces the risk of needle biopsy sampling error that might otherwise return a false-negative result to the patient.

Ideal biomarkers predict the onset of disease at the earliest possible point and reduce the need for additional invasive and often painful testing. In addition to meeting this expectation, PCMT also has a sensitivity of 85% and a negative predictive value of 92% at first biopsy.

The following diagram depicts the field effect surrounding malignant prostate tissue:
The Suitability of the Mitochondrial Genome as a Biomarker

Within a cell, each mitochondrion will contain multiple copies of the mitochondrial genome, ranging from 10 to even dozens of copies. Each cell can contain thousands of mitochondria. Hence small tissue samples or biofluids such as blood and urine can provide enough copies of the mitochondrial genome to detect deletions that may indicate the presence or onset of cancer. In the instance of PCa, the same tissue samples that are collected for histology can also be used to detect an mtDNA deletion.

Mutations in the mitochondrial genome have been identified as playing a role in the etiology of nearly every type of cancer and numerous other diseases. Literature surrounding this area of research also suggests that the tumor field effect may enable the onset of tumorigenesis rather than simply indicating its presence in nearby cells. Unlike nuclear DNA, mitochondrial DNA is not protected by histones. Histones are structural proteins that nuclear chromatid DNA is wound around; they help to maintain the integrity of the DNA sequence. The absence of this safeguard makes mtDNA particularly susceptible to damage from oxidative stress. The mitochondrial respiratory chain is the primary means of converting NADH into ATP for the production of cellular energy, making this process the main source of reactive oxygen species (ROS) production within a cell. As a result, unprotected mtDNA is constantly subjected to oxidative stress. The outcome of mtDNA damage associated with oxidative stress causes somatic mutations, or genetic mutations that occur after conception.

Since each cell contains such a high copy number of the mitochondrial genome, approximately 80% of the copies can be damaged before the cell experiences a decline in function.
energy production. Detecting mtDNA signatures of disease at their onset gives the clinician the greatest opportunity to successfully treat the illness.

Mitochondrial DNA (mtDNA) Analysis

Real-time polymerase chain reaction (RT-PCR) has been shown to be a valid, reliable, and rapid means of detecting mtDNA deletions. The relative simplicity of RT-PCR, its ability to accurately detect mtDNA deletions using minute amounts of sample collected by a biopsy needle core section, and its economical implementation make it an ideal technology platform upon which to base a PCa biomarker. Since the discovery of the 3.4kb mtDNA deletion, Mitomics™ has taken steps to optimize the assay, significantly improving the efficiency and sensitivity of the assay, and enhancing the specificity of the test by achieving a very high negative predictive value (NPV) for PCMT. This lends to PCMT’s exceptional strength in terms of preventing unnecessary re-biopsies, and the ability to more closely monitor elevated risk groups such as those whose histology displays atypical small acinar proliferation (ASAP).

The refinement of this test can be demonstrated through Mitomics’ research concerning the tumor field effect5, 6, and the three-stage progression of PCMT development.

Detecting the Tumor Field Effect

Some of Mitomics, early research made it possible to successfully identify the tumor field effect, which vastly enhances the power of PCMT. The concept of the tumor field was first devised by Slaughter in the mid-1940s7, 8 and formalized in a ground-breaking publication in 19539. The tumor field effect can be described as the presence of molecular changes in histologically benign cells adjacent to tumor tissue. These molecular changes can be correlated with the presence of a tumor. This effect does not just occur within a single cell, but rather, it occurs within a cellular region surrounding the malignancy. PCMT utilizes the expansiveness of the field effect to detect PCa. The molecular subtleties of the field are essentially a molecular signal, which is not visible at the microscopic level, and consequently they remain unrecognized by conventional histology. For this reason PCMT is an immensely powerful tool for the elimination of false negatives in PCa screening.

Devising a means of detecting the field was a subsequent result of work that had two primary goals: 1) to differentiate between true somatic mutations and wildtype variations in mitochondrial genomic material and 2) to distinguish between nuclear germplasm and somatic mitochondrial genomic material.

The first undertaking was to distinguish between somatic and wildtype genomic variations10. Forty-six overlapping DNA segments were amplified using 36 primer sets and then
sequenced, and this was representative of the entire mitochondrial genome. This work was carried out on samples collected from prostatectomies and needle biopsies, and involved 24 malignant patients and 12 benign. Laser dissection was used to capture cells from three different areas of a cross-section slice from each prostatectomy. For each patient, cell collections were taken from the tumor, the region proximal to malignancy (PTM), and normal tissue. DNA mutation rates were found to be the same over all three regions, with most mutations found in the “D-loop” of the sequence, which is a non-coding region. This is indicative of the non-selective nature of cancerous mutations once they begin.

The second part of this work involved comparing mitochondrial genomic material from human tissue to rho-zero cells (controls), which had been depleted of mitochondrial DNA but still retained nuclear genomic material. This made it possible to make a clear distinction between nuclear pseudogenes and somatic mitochondrial genomic material. Blood samples were also taken from each patient to be used as a source of germlasm that was sequenced and used to score somatic mutations and ensure that the mutations under study were the result of cumulative mitochondrial damage associated with PCa.

**PCMT mtDNA Deletion: Stages of Discovery and Validation**

The entire process of discovery and validation involved 396 patients and close to 1,700 prostate core samples. This included 143 patients with benign histology and 253 patients with malignant histology. The extensive development process included three primary stages.

Stage 1 encompassed the actual discovery of the deletion and two pilot studies to confirm the discovery and integrate the use of the tumor field effect. Stage 2 involved an internal validation study carried out by Mitomics and a second external validation study carried out by the National Institute of Standards and Technology (NIST). Stage 3 was conducted within the framework of a clinical trial. The diagram below outlines the flow of work and the study population used in each stage of assay development:

<table>
<thead>
<tr>
<th>Stage 1: Discovery</th>
<th>Stage 2: Validation</th>
<th>Stage 3: Clinical Trial</th>
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<tbody>
<tr>
<td><strong>Discovery</strong></td>
<td><strong>Internal validation</strong></td>
<td><strong>Initial biopsy</strong></td>
</tr>
<tr>
<td>30/33 (91%) of prostatectomy samples exhibit the 3.4 kb mtDNA deletion</td>
<td>183 patients: 296 cores</td>
<td>94 patients with benign histology results</td>
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<tr>
<td><strong>Pilot Studies (2)</strong></td>
<td><strong>External validation (NIST)</strong></td>
<td><strong>Repeat biopsy within one year</strong></td>
</tr>
<tr>
<td>86 patients: 132 cores</td>
<td>22 benign patients: 98 benign cores</td>
<td>74 patients with benign histology results</td>
</tr>
<tr>
<td>- 27 benign patients: 43 benign cores</td>
<td>161 malignant patients: 75 malignant cores</td>
<td>20 patients with malignant results</td>
</tr>
<tr>
<td>- 59 malignant patients: 54 malignant cores, 35 PTM cores</td>
<td>123 PTM cores</td>
<td>553 cores (6 cores per patient; 11 patients with only 5 cores)</td>
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The PCMT predicted the outcome of the repeat biopsy with a SEN of 85% and a NPV of 92%.
**Stage 1:** The discovery of the 3.4 kb mtDNA deletion was made in a pilot study that utilized frozen prostatectomy samples, and the deletion was used to accurately identify PCa in 30 out of 33 or 91% of the samples. The following illustration depicts the amplified DNA using gel electrophoresis associated with the study:

Confirmation of the discovery was established through two studies in which 86 patients participated and provided 132 prostate biopsy samples. These studies involved 59 PCa positive patients who were able to provide 54 malignant cores and 35 PTM cores; and 27 benign patients who provided 43 benign cores. Significantly, it was shown that the 3.4 kb mtDNA deletion was only present in PCa positive samples and was not associated with non-malignant conditions such as benign prostatic hypertrophy, prostatic atrophy, and inflammation of the prostate gland. The deletion was also present in PTM samples that were histologically normal, providing additional confirmation of the field effect. Together, these studies comprised stage 1 in the development of PCMT.

**Stage 2:** The next phase of PCMT development encompassed an internal study carried out at Mitomics and an external validation study conducted by the Biochemical Science Division of the NIST. The internal validation study utilized a total of 296 cores contributed by 183 patients. The sample size for this blinded confirmation study was statistically determined using a power calculation based on the mean residual mitochondrial DNA scores. This ensured that the internal study would accurately assess the robustness and reproducibility of PCMT. Sixty-five malignant patients provided 75 malignant cores, 96 malignant patients provided 123 PTM cores, and 22 benign patients provided 98 cores. At this point in development, the RT-PCR amplification utilized a C(t) cutoff of 37. A significant observation of the internal study was that the rate of detection of a prostate tumor could be increased by evaluating two or more histologically normal samples from the initial biopsies performed on each patient.
The external study was carried out on DNA extracts from a subset of the samples from the internal validation study, and the results affirmed the internal study conclusions. Researchers at NIST evaluated 46 benign, 25 malignant, and 41 PTM core extracts. While the internal study showed sensitivity and specificity of the test to be 80% and 71% respectively, these rates increased in the external study to 83% sensitivity and 79% specificity.

**Stage 3:** A retrospective blinded clinical study embodied stage 3 development for PCMT. This study involved 94 patients that underwent initial biopsies, each providing six needle cores, which were all classified as histologically benign. Within 14 months, all 94 patients underwent a second round of biopsies. In this second set of samples, 20 of the patients were found to have malignant cores while 74 patients’ results remained benign.

In evaluating the initial results of stage 3, PCMT identified 17 of the 20 men who were found to have malignant results on the second biopsy, giving the test a sensitivity of 85%. In this study, PCMT also displayed a negative predictive value of 92%, and this is of great clinical importance. In terms of managing prostate cancer, these results mean that PCMT is extremely successful in identifying men who do not require a follow-up biopsy procedure in the near term.

Additionally, the results suggest that the majority of men who display ASAP require closer monitoring for the onset of PCa. Of the 20 patients who had malignant results on the second biopsy, 46% of these patients had a classification of ASAP on the initial procedure. This call rate is consistent with the frequency of men who have malignancy on a secondary biopsy after having initial benign results that indicate the presence of ASAP.

A further result of this study was the significant optimization of the procedure, as a Ct cutoff of 31 was established while still maintaining a sensitivity of 85%. This was accomplished by redesigning the RT-PCR primers for the mtDNA deletion, and by using PCR reagents that had been specifically optimized for efficiency and sensitivity. Most importantly, this leg of development was able to show that the clinical performance of PCMT is greatly enhanced when all of the available biopsies (up to six) are included in the assay, as shown in by the following graph:
Since PCMT requires only 20ng of mtDNA, molecular detection of the field effect can be carried out on the same samples used for histology. Not only does this ensure continuity in the patient’s results, it greatly reduces the risk of missing a tumor since the results are not reliant solely on histology of the needle biopsy. The ability to use existing (or previously collected) histology samples for PCMT evaluation also reduces patient stress and risk of infection since no additional biopsies are required.

**The Economic Reality of Prostate Cancer**

Due to the subtle nature of PCa and the extremely small tumor cross-section that is collected with a needle biopsy, the histological identification of prostate cancer is one of the most difficult diagnoses in pathology\(^\text{12}\). PCMT effectively expands the detection perimeter of the biopsy needle by utilizing the tumor field effect. This not only makes the prostate core biopsy a more effective screening method for the patient, it also adds value to the process by increasing its accuracy.

The North American population demographic over the age of 65 has been steadily growing in recent decades. This, coupled with the fact that over 60% of prostate cancer diagnoses occur in men 65 years and older, makes it readily apparent that the economic impact of prostate cancer is steadily growing. Screening programs for PCa have been developing since the mid-1970s, resulting in earlier detection of the disease. This has contributed significantly to an increased incidence of prostate cancer (due to better detection) and long-term survivability of those diagnosed with PCa. The most immediate impact of a diagnosis of PCa is the impact on a man’s quality of life, and this is directly related to the overall long-term survivability of the disease. The longer it takes to diagnose PCa, the less chance there is to arrest or cure the
disease, resulting in the need for much more aggressive and extreme treatment for a longer period of time. Ultimately this means that more cost-effective strategies are needed to detect the onset of PCa to ensure the greatest number of men have the best access to treatment and a potential cure for the disease.

The economic burden of managing and treating prostate cancer has been reviewed by two prominent studies: Wilson, et al\textsuperscript{13}, and Snyder, et al\textsuperscript{14}. Both studies show that the cost patterns associated with PCa are high, but that they vary greatly depending upon the initial diagnosis and treatment strategy selected. Current financial data indicates that active surveillance, which includes closely monitoring serum PSA levels and repeat prostate biopsies, is associated with the lowest initial-year average cost of $4,270. Other strategies such as hormone therapy, radiation therapy, surgery, and combined hormone and radiation therapy have much higher initial annual costs, ranging from $11,034 to $17,474. Since PCMT increases the accuracy of detection for PCa, a much earlier determination can be made regarding the need for more aggressive treatments. This could eliminate active surveillance periods, which may more accurately be regarded as incubation periods for aggressive PCa. The ability to advance the patient to the most effective treatment strategy at the earliest point reduces the overall cost of treatment and lessens the potential for future complications such as the risk of sepsis due to antibiotic-resistant infections resulting from repeat biopsies, or metastatic cancer attributable to unnecessary delays in the diagnosis of PCa.

PCMT provides the opportunity to more accurately stratify the PCa-suspicious patient population, and in some instances, safely remove patients from monitoring altogether. Substantially reducing uncertainty in instances where histology alone is inconclusive would eliminate or delay the need for re-biopsies in many cases, as well as directly reduce the risk of sepsis associated with prostate biopsies. Additionally, by lowering the rate of false negatives, PCMT would hasten treatment for potentially tens of thousands of men, arresting the progress of their disease at an earlier state. The overall potential for decreasing the economic burden of PCa management as a direct result of utilizing PCMT is substantial as it will increase access to more cost-effective treatment due to early detection, reduce the rate and cost of complications such as infections and sepsis, and improve the quality of life for those living with the disease.

**Specific Health Economic Benefits of PCMT**

When patients undergo a prostate needle biopsy and the outcome is deemed to have a negative pathology, these same biopsy samples should be assayed with PCMT. The 92%
negative predictive value, coupled with 85% sensitivity for PCa, adds an essential component of assuredness to the negative pathology.

Reconfirmation of a negative pathology report places the patient in a position to proceed with an annual follow-up to monitor the status of his prostate, rather than enduring multiple visits within a one-year time period that are normally associated with active surveillance. The average cost of a prostate biopsy procedure and standard ancillary care administered within a clinical setting is about $4,200 US$\textsuperscript{14}. Taking into account a negative PCMT result along with other clinical signs may delay or even circumvent the need for an additional visit to a urologist to undergo a second biopsy procedure within that year, resulting in an annual savings of at least $4,200.

When negative pathologies are correlated with positive PCMT results, this indicates that the original pathology may have been a false negative, which occurs in 15 to 30% of biopsy results. The high PCMT sensitivity of 85% would serve to minimize the number of false negatives that occur, by initiating a re-biopsy much sooner than it would have occurred in instances where only conventional histology has been utilized to diagnose PCa. Expediting a correct diagnosis via PCMT means that therapeutic treatment of PCa would be initiated at an earlier date, usually resulting in better outcomes for the patient at a lower treatment cost. Identifying the disease at an earlier time point generally means it will be possible to treat PCa with less aggressive regimens such as radiation therapy alone instead of the more aggressive combined treatment of hormonal and radiation therapy, or prostatectomy. The monetary savings within the first year alone runs into several thousands of dollars, and over time, may ultimately mean a savings into the tens of thousands\textsuperscript{14}. Clearly, PCMT represents a substantial and practical economical savings to the patient.

**Optimizing Patient Outcome, Minimizing the Impact of Prostate Cancer**

From the clinician’s perspective, ensuring the best possible outcome for the patient is the primary goal. Though a biopsy may come back negative, the clinician is well aware that current screening methodologies can miss up to 30% of actual incidents of prostate cancer. PCMT offers the clinician an invaluable additional means of being sure of the diagnosis and helps determine the best way forward in managing patient outcome through increased clinical insight.

Increased clinical insight also benefits the patient by affording greater confidence in the decisions that have to be made regarding their treatment. For men who have just received news that they must undergo screening for prostate cancer, some of the most worrisome issues they must face include whether or not their diagnosis will be accurate; and, if their
Each year, nearly 1,400,000 North American men are screened for prostate cancer. Out of that 1,400,000 men:

- 242,330 men will be diagnosed with prostate cancer (~69% of annual incidents of prostate cancer)
- 1,050,000 men will receive negative or benign pathologies attributable to non-cancerous prostate conditions

Of the 1,050,000 negative or benign diagnoses:

- Between 36,350 and 72,700 incidents of prostate cancer will be missed
- 52,500 men will receive a diagnosis that is suspicious of cancer but not confirmed
- 15,750 of these men will receive the same inconclusive diagnosis upon second opinion
- More than 3,600 men will receive false-positive results and undergo unnecessary treatments for prostate cancer

PCMT works to correctly identify PCa, reduce the number of false-negative diagnoses, and remove uncertainty for the patient.

diagnosis is positive, what strategy for managing the disease will best suit their needs.

Accuracy of diagnosis does not only pertain to whether or not the cancer will be detected, but also to the possibly that the prostate cancer will be incorrectly diagnosed as positive. For worried patients awaiting the outcomes of their tests, knowing that every measure has been taken to ensure their results are correct provides the greatest comfort. This is the peace of mind they receive when their biopsies undergo PCMT in addition to conventional histological examination.

In 2006 there were approximately 1,400,000 prostate biopsies performed\(^\text{15}\), with the number of biopsies expected to increase 6 to 9% by 2011. In Canada and the U.S., the estimated number of new prostate cancer diagnoses for 2010 was 242,330\(^\text{16, 17}\). Nearly 75% of the time histological examinations of prostate core biopsies do not identify cancer. In these instances, enlarged prostate and elevated PSA levels are attributed to other prostate conditions such as benign prostate hypertrophy or prostatitis\(^\text{12}\). This translates into nearly 1,050,000 benign diagnoses in North American men who were screened for prostate cancer in 2010.

Tragically, a significant portion of the negative or benign pathologies are actually false-negative results. Studies show that the combined use of PSA and DRE screening only detects up to a maximum of 69% of all tumors\(^\text{18, 19}\). Approximately 15 to 30% of true-positive cancers are missed, totaling between 36,350 and 72,700 incidents of prostate cancer that elude detection every year. The high PCMT sensitivity of 85% would substantially lower this rate of false negatives.

A diagnosis of “suspicious of cancer” generally means that there are abnormal indications such as ASAP present in the tissue sample. Since this diagnosis is pathologist-dependent, the rate of tissues being deemed as suspicious of cancer can range from between 1 to 23%, though the average rate is 5%\(^\text{12, 20, 21}\). Such a diagnosis brings with it a much greater risk of detection of cancer on a subsequent procedure, and typically results in a re-biopsy within three to six months. Within the annual North American screening population, the initial diagnosis of suspicious of cancer corresponds with about 52,500 diagnoses, and when the re-biopsy is conducted, 15,750 or 30% of these will remain as suspicious of cancer. The PCMT negative predictive value of 92% could eliminate the vast majority of these suspicious diagnoses and the subsequent need to undergo re-biopsy.

About 1.3 to 1.5% of the time, pathologists will deem a prostate biopsy cancerous when it is in fact benign\(^\text{12}\). These erroneous results are called false positives, and though when stated as a percentage of error this sounds like an excellent result, it means that more than 3,600 men a year will go through unnecessary prostate cancer interventions such as radical
prostatectomies. Considering that even two years after radical prostatectomy surgery, 44% of men report impotence and 9% report incontinence\textsuperscript{22}, undergoing the procedure only to find out it was unnecessary has devastating implications for quality of life. Again, the enhanced detection capacity and accuracy of PCMT would eliminate a tremendous portion of this unnecessary suffering.

Staying within conventional bounds, one possibility for capturing a larger number of prostate cancer-positive cases using PSA as a screening tool would be to lower the PSA cutoff value. Currently, for men ages 50 to 70, DRE results indicative of prostate enlargement as well as PSA values greater than 4.0 ng/mL are generally considered to be the optimal cutoff for deeming a biopsy necessary\textsuperscript{23}. However, lowering the PSA cutoff would mean that more men would be undergoing biopsies, and since a lower PSA would likely correspond to a smaller tumor size, this strategy would require taking larger numbers of biopsies to select cancer-positive samples. This is particularly the case for younger men. For obvious reasons, lowering the PSA cutoff is a less-than-optimal strategy, as from the patient perspective it means undergoing a larger number of painful biopsies, and facing much higher risks for problems such as bleeding, post-procedure pain, and infection. From an economic standpoint, it would greatly increase the number of men requiring biopsies. The simple solution remains implementing a test that utilizes extant biopsy samples, optimizes the outcome for the patient, and does not increase the number of men who have to undergo prostate screening. This is precisely the solution provided by the Prostate Core Mitomic Test\textsuperscript{™}. The overall rate of false-negative diagnoses should be reduced, and patients will enter the most effective treatment path more quickly. This will have the overall outcome of improving survival rates and decreasing the costs associated with the care and management of PCa.

**The Mitomic Technology\textsuperscript{™} Advantage: Empowering Clinical Insight\textsuperscript{™}**

In addition to completing design and development of the assay, Mitomics has developed a high complexity CLIA-compliant laboratory in the U.S. With this CLIA laboratory capability, Mitomics is firmly established for commercial availability of PCMT in the North American marketplace.

The commercial launch of PCMT represents a culmination of an extensive body of research that stands to revolutionize the diagnosis and management of prostate cancer, from both the clinician and patient perspectives. The fact that PCMT can be done on the same tissues collected for conventional histological screening – and that it uses well-established and economical high-throughput RT-PCR methodology – makes this test highly accessible and
easy to implement. The efficient use of extant biopsy tissue also minimizes both risk and stress for the patient.
The Prostate Core Mitomic Test™ was developed using Mitomic Technology™, a highly advanced proprietary technology for detecting and comparing novel discoveries of mtDNA deletions against a library of known mitochondrial genomic deletions. The assay detects PCa by identifying underlying molecular alterations that have occurred in normal-appearing tissue that is adjacent to a malignancy. These molecular alterations, known as the tumor field effect, enhance tumor detection in instances where biopsy cores appear histologically benign because they have failed to collect tumor tissue from patients with PCa. As a result, PCMT provides the clinician with insights far surpassing those of conventional screening. The concurrent high sensitivity of 85% and negative predictive value of 92% contribute unprecedented strength to this assay through its ability to identify PCa.

The economic reality of PCMT is that it provides a much more accurate stratification of the patient population. By increasing confidence in the identification of true-negative outcomes, a substantial number of men will avoid PCa treatment. Other men whose cancer would have previously gone undetected before reaching a more advanced stage will now receive earlier treatment. The number of men receiving results that are suspicious of cancer will also be minimized as the clinician will have an extra screening test available to identify PCa. For all of these groups, the end result of having PCMT will be quite optimal, as both their health management and outcomes will be delivered with the highest level of confidence available. From the clinical standpoint of saving lives, the decision to use the PCMT is a simple one.
References


