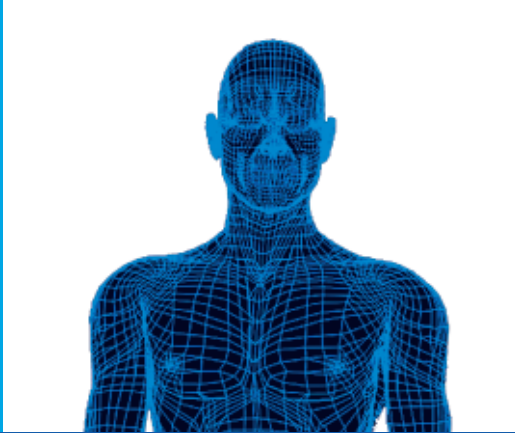
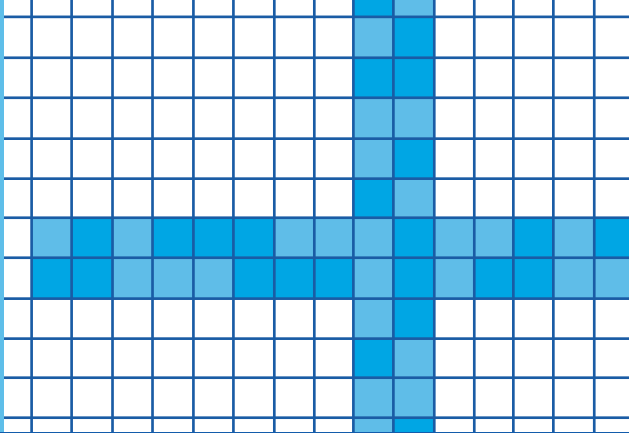


PROSTATE  
THE POWER TO PREDICT



# Comprehensive, Personalized Prostate Cancer Disease Assessment

Prostate cancer affects one in six men; more than 218,000 men in the United States were diagnosed with the disease in 2007, and >27,000 men died from it.<sup>1</sup> As baby boomers age, the incidence of all cancers is expected to rise significantly, with prostate cancer being the most prominent.

The widespread introduction of PSA screening in the late eighties was critical for identifying prostate cancer early in the disease course. Today PSA is widely used, with annual PSA tests recommended for Caucasian men beginning at age 50 and African-American men at age 45. However, an unintended consequence of PSA screening is the early identification of indolent prostate cancer: cancer which previously might not have been identified nor become a problem for the patient. Consequently, an important objective in the management of patients with prostate cancer is the ability to stratify individuals based on their likelihood of cancer recurrence. In other words, determine which men are at high risk for prostate cancer progression and which men are at low risk.<sup>2,3</sup> Answers to these questions can influence patient decision making and result in modified treatment plans.

## Risk Assessment Challenges

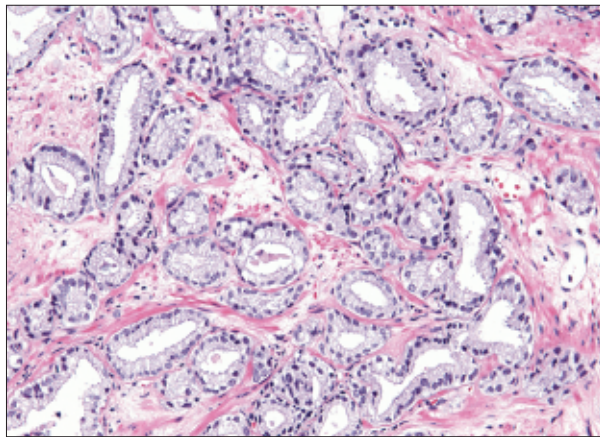
The current challenge to predicting individual patient risk is complicated by the multiple variables that must be considered as well as the applicability and accuracy of available predictive tools. Risk can be assessed either at the time of diagnosis or post definitive treatment. A fair amount of research has been published on prognostic features that can be utilized to assess disease severity and the likelihood of cancer recurrence. At diagnosis, a number of features are considered to have a degree of prognostic value and include biopsy Gleason grade and score, the number of positive tumor cores, PSA and clinical tumor stage. After prostatectomy, different features are used to assess the probability of cancer recurrence, including pathological tumor stage, surgical margin status, Gleason grade and score, as well as other features.<sup>4-7</sup>

Although individual indicators have a degree of prognostic utility, no one variable can accurately predict a patient's outcome post-treatment. Even Gleason score, widely considered one of the most important prognostic indicators of patient outcome, has deficiencies. The biopsy Gleason score, assessed at the time of diagnosis, can be significantly up- or down-graded when the prostatectomy tissue is evaluated after surgery.<sup>8</sup> The change in Gleason score is primarily due to the very narrow needle-biopsy view of patients' tumors compared with the broader tissue context observed after prostatectomy. In addition, Gleason grading is a descriptive morphology metric and therefore subjective. Different pathologists can assign different Gleason grades to the same tissue section – especially in difficult cases. Finally, Gleason grading has been shown to change (stage migration) over time; a tissue sample assigned a specific Gleason score a decade ago might receive a different score if re-graded today using contemporary guidelines.<sup>9</sup>

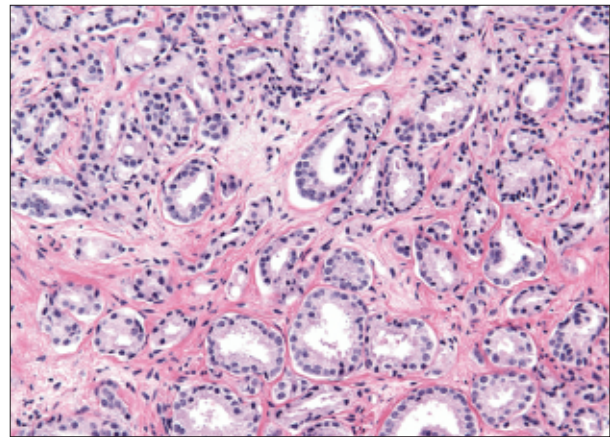
The art of histology (examining cells and tissues under a light microscope) has not substantially changed in the last 150 years. Gleason grading, introduced in 1966, is based on changes in tissue morphology. As normal prostate cells change, the Gleason grading system represents five tissue architectural and morphology patterns. These are commonly referred to as Gleason patterns 1 through 5, with pattern 1 being the most well differentiated and therefore the most favorable. Pathologists assign a Gleason grade to the most common pattern (dominant Gleason grade) and a second Gleason grade to the next most observed pattern. The two grades are added to provide the Gleason score (ranging from 4–10).

Light-based microscopic examination of a patient's tissue sample can not discriminate between indolent versus life-threatening disease (Figure 1). Because the foundation of Gleason grading is tissue morphology, the pathologist's visual assessment can significantly underestimate the underlying biological complexity of the disease process. Prostate cancer is a multi-focal disease and each prostate cancer case can contain multiple tumors with different morphologies.<sup>10</sup> In addition, two tumors with the same Gleason score can progress very differently: as an example, most physicians have had some Gleason 6 patients who progressed very quickly and other patients who appeared progression-free many years later.

Figure 1



Gleason 6, Indolent Disease



Gleason 6, Disease Progression

Multiple prognostic features can be discordant when reviewed as a group. For example, a patient can have a high PSA but a "low-risk" Gleason score (e.g., 6 or less). In addition, some features comprise a large subjective component as observed with surgical margin status. After surgery, the excised prostate tissue is examined for extent of residual cancer and to evaluate margin status. Pathological examination demonstrating cancerous cells at the excised border are considered positive surgical margins. Positive surgical margins as prognostic indicators of disease recurrence are controversial. Some research indicates that margin status may be prognostic while other research belies their utility.<sup>11</sup> Conflicting reports may be the result of the subjective definition and the degree of positive margins. Moreover, not all "positive" margins are representative of residual cancerous cells and may reflect surgical artifacts.<sup>12</sup>

## Existing Risk Assessment Methods

Prostate cancer risk stratification is currently assessed by a variety of pre-operative as well as post-operative models; this discussion will solely focus on pre-operative risk assessment. Practical implementation of these models ranges from an individual practitioner's knowledge/experience to paper-based tables, internet-based calculators and, recently, to Aureon's advanced technology. A majority of existing methodologies predict disease recurrence primarily as PSA recurrence (biochemical recurrence) after primary therapy. Other approaches, such as the Partin tables, pre-operatively provide the likelihood that, after surgery, the patient's cancer will be organ confined or will have spread beyond the prostate.<sup>13</sup>

Many patients are placed into clinically-localized recurrence risk categories following guidelines based upon clinical and pathological features (from biopsy) such as Gleason score, PSA and clinical stage. The four groups are low risk (PSA <10ng/ml, Gleason 2–6, stage T1–T2a), intermediate risk (PSA 10–20ng/ml,

Gleason 7, stage T2b–T2c), high risk (PSA >20ng/ml, Gleason 8–10, stage T3a) and locally advanced, very high risk (stage T3b–T4).<sup>14</sup> The challenge with this method of stratification is that individual outcomes are not possible and patients are lumped into broad risk groups based upon clinical and pathologic parameters.

Some physicians use their experience and knowledge to assign patients to risk groups but others use nomograms which are stratification tools: graphical methods used to represent numerical relationships. Nomograms can be paper tables, internet calculators or PDA-based programs. There are many nomograms in the literature designed to predict various prostate cancer-related endpoints both before and after various treatment options.<sup>15</sup> There are well-known published nomograms that predict PSA recurrence pre-operatively<sup>16</sup> and post-operatively<sup>17</sup> as well as attempt to discriminate indolent disease.<sup>18</sup> In addition, other research has resulted in nomograms that predict recurrence after radiotherapy.<sup>19,20</sup> Nomograms can be useful, but the same challenge exists as classifying patients based upon broad risk groups: personalized outcomes are not achievable. In addition, nomograms are powered by PSA and Gleason scores, which have become less informative in assessing individual risk.

## Prostate Px<sup>⊕</sup>

Prostate Px<sup>⊕</sup> uses biopsy tissue at diagnosis to predict disease progression. In addition, the test also may indicate indolent disease as assessed by favorable pathology. Prostate Px<sup>⊕</sup> helps physicians to properly assess disease severity for newly diagnosed men and make more-informed treatment decisions. Prostate Px<sup>⊕</sup> provides the following information:

- **Disease Progression**  
This describes the post-operative likelihood of developing disease progression, including metastasis, death of disease or androgen independent recurrence secondary to hormonal/salvage radiation
- **Indolent Disease as Assessed by Favorable Pathology**  
The indolent disease endpoint predicts the likelihood of a favorable outcome post-prostatectomy, i.e., prostatectomy Gleason score ≤6 (no pattern 4 or 5), ≤pT2c and PSA nadir post-prostatectomy
- **Px<sup>⊕</sup> SCORE™**  
Provides a number between 0 and 100 that directly correlates to the likelihood of prostate cancer disease progression within 8 years of a radical prostatectomy

## Clinical Benefits

At diagnosis, Prostate Px<sup>⊕</sup> provides physicians with objective, actionable information that significantly adds to the clinical information currently used to assess risk and that helps physicians guide and counsel patients as treatment options are considered.

At diagnosis, Prostate Px<sup>⊕</sup> can:

- Predict disease progression after definitive treatment (i.e., prostatectomy)
- Assist objective, informed treatment decisions
- Identify high-risk patients presenting as lower risk
- Re-classify intermediate-risk patients as either high or low risk
- May indicate indolent disease as assessed by favorable pathology
- Help alleviate some of the anxiety of the unknown for patients

## Study Design

A multi-institutional cohort of 1,487 patients and associated outcome data was assembled from multiple physicians practicing at The Mayo Clinic, Duke University Medical Center/Durham Veterans Administration, The University of Graz (Austria), University Hospital at Uppsala (Sweden) and The University of Connecticut. After review, there were 1,027 evaluable cases in the cohort. The median time to progression was eight years post radical-prostatectomy. The cohort was used to build two separate predictive models employing systems pathology: the prediction of disease progression and indolent disease as assessed by favorable pathology. Each model was designed using a training set derived from the cohort and independently validated on a blinded set of the cohort. Stratification of the cohort into balanced training (2/3 of patients) and validation (1/3) sets was independently executed by a third party at Rutgers University.

## Disease Progression

This prospective clinical study used retrospective data to predict disease progression within 8 years after diagnosis. The model was developed using clinical-pathological features retrospectively collected from de-identified patient information. Only patients with complete clinical-pathological, morphometric, and molecular data, as well as accurate/verified outcome information, were further studied. In the final analysis, 686 evaluable cases were used to train the model.

### Validation

The model was independently validated by investigators at the University of California San Francisco using 341 patients. The cut point in the  $Px\oplus$  SCORE between low-risk and high-risk identification was 30.2.

### Features Selected

Clinical features that were incorporated into the model included PSA, biopsy Gleason score and dominant biopsy Gleason grade. There were two histomorphometric features and a biomarker expression profile which included Androgen Receptor and Ki-67, as well as a number of cellular location and differentiation protein markers.

## Indolent Disease

Prostate  $Px\oplus$  may indicate indolent disease as assessed by favorable pathology. Patients who present at biopsy with a pathologic evaluation of Gleason score  $\leq$ six (6) and organ-confined disease may have a higher Gleason pattern and non-organ-confined disease upon prostatectomy. The indolent disease endpoint predicts patients who are likely to still have Gleason score  $\leq$ six (6) and organ-confined disease. Only patients with complete clinical-pathological, morphometric, and molecular data, as well as complete outcome information, were further studied. In the final analysis, 628 evaluable cases were used to train the model.

### Validation

The model was validated using 280 cases: 102 cases had indolent disease as assessed by favorable pathology. Patients with a probability  $>41\%$  of favorable stage were considered low risk.

## Features Selected

Similar to the disease progression model, clinical features that were incorporated included PSA, biopsy Gleason score and dominant biopsy Gleason grade. There were two different histomorphometric features and a biomarker expression profile which included Androgen Receptor and Ki-67, as well as a number of cellular location and differentiation protein markers.

## Results

The Hazard Ratio (HR) is a measure of the effectiveness of a factor/variable to stratify patients into different risk populations: low- and high-risk. A HR of 1 means there is no difference in risk between the two groups. A HR of >1 means patients in the predicted high-risk group are indeed at higher risk than the predicted low-risk group. As the HR increases, the more accurate the low/high risk stratification.

An analysis of the disease progression endpoint in the validation cohort (N=341) looked at the hazard ratios, concordance index and associated *p* values of the available clinical information as well as the Px⊕ SCORE.

Variable (Validation)	Concordance Index	Hazard Ratio	<i>p</i> -Value (HR)
Age	0.47	0.81	0.521
PSA	0.67	1.93	0.030
Clinical Stage	0.53	1.19	0.769
Dominant Gleason Grade	0.60	2.29	0.007
Px⊕ SCORE	0.73	3.47	<0.001

As Table 1 indicates, age and clinical stage were not statistically significant. In addition, the Px⊕ SCORE had the best hazard ratio, which was highly statistically significant, as well as the most favorable concordance index.

An analysis of the indolent disease endpoint in the validation cohort (N=280) examined the area under the curve. As demonstrated in Table 2, area under the curve (AUC) measurements were, at best, marginal for all clinical factors; the singular exception was the AUC for the Px⊕ SCORE, which was also highly statistically significant.

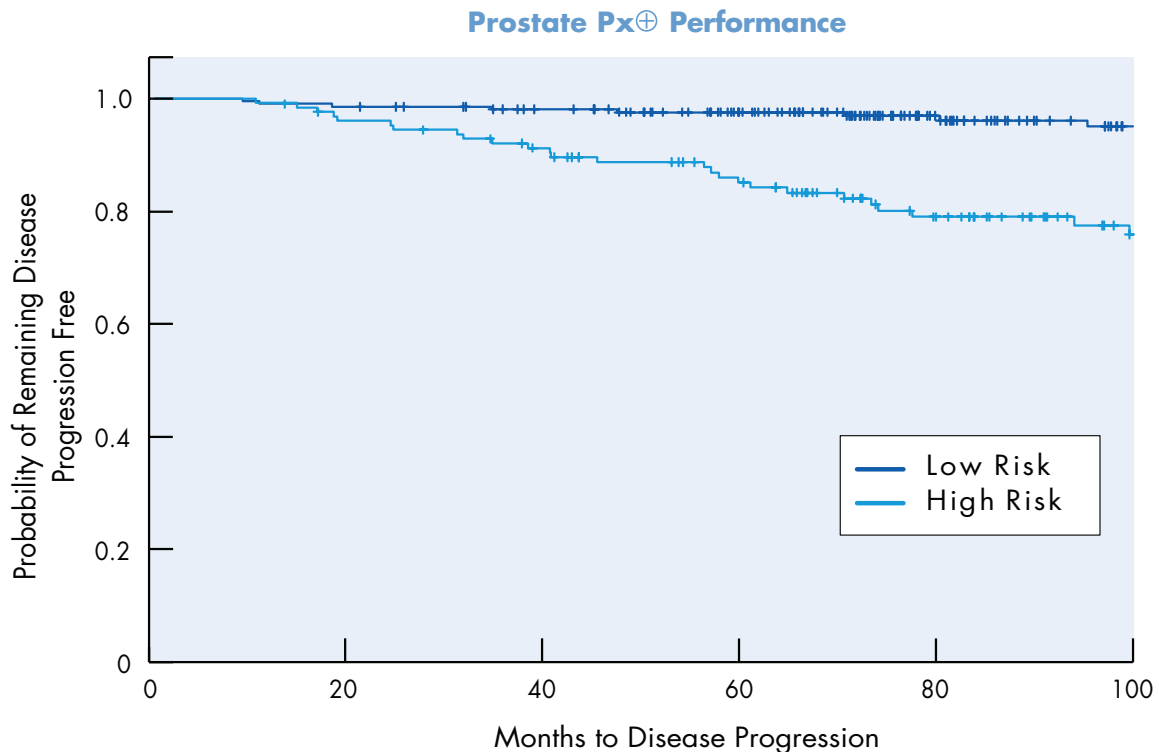
**Table 2**  
**Indolent Disease**

Variable (Validation)	AUC	p-Value (HR)
Age	0.60	0.003
PSA	0.69	<0.001
Clinical Stage	0.55	0.102
Dominant Gleason Grade	0.65	<0.001
Prostate Px⊕	0.74	<0.0001

A Kaplan Meier curve (Figure 2) visually illustrates the statistically significant ( $p < 0.001$ ) separation of low-risk and high-risk patients as defined by Prostate Px⊕.

The power of Aureon’s Systems Pathology approach (see Technology description) can best be observed by comparing existing methods of risk stratification within the same validation cohort of patients. This ensures an equal comparison. The primary purpose of risk stratification tools is to detect high-risk patients masquerading as lower (low and intermediate) risk patients. Accordingly, all validation patients identified as low-risk (PSA <10ng/ml, Gleason 2–6, stage T1–T2a) or intermediate-risk (PSA 10–20ng/ml, Gleason 7, stage T2b–T2c) patients by AUA classification (N=267) were analyzed.

**Figure 2**



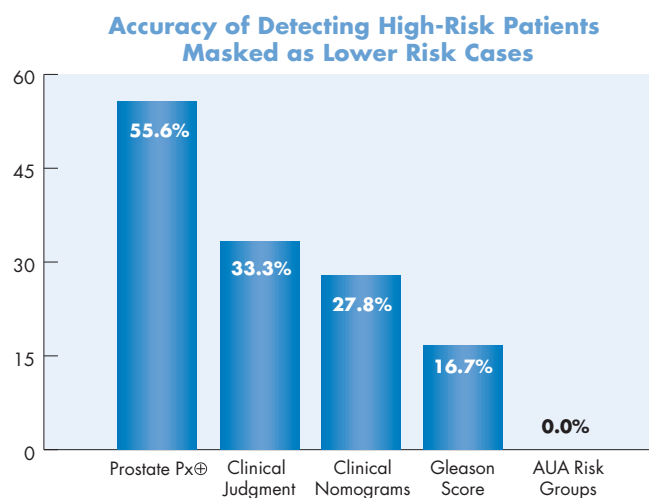
## Predicting Disease Progression for Low- and Intermediate-Risk Patients

The following methods were examined head-to-head with Prostate Px $\oplus$  for disease progression:

- **Clinical judgment:** Defined by using PSA and Gleason grade to define risk. Clinical judgment splits both Gleason score and PSA at the midpoints of these features for intermediate-risk patients. Therefore, Gleason 4+3, Gleason 8–10 are defined as high risk, Gleason 3+4, Gleason 5–6 are considered low risk. PSA  $\leq 15$ ng/ml is low and  $>15$ ng/ml is high risk.
- **Clinical nomograms:** A number of pre-operative and post-operative nomograms exist for predicting disease recurrence risk prior to prostatectomy, radiotherapy, etc. The most well known pre-operative nomograms use clinical and pathological information to make risk determinations and were used for these comparisons.<sup>16, 21</sup> Critically important to understand before making any comparison, nomograms provide an assessment of PSA recurrence, which is typically the first step in disease recurrence. Prostate Px $\oplus$  provides a disease progression endpoint utilizing serious symptoms of advanced disease (metastasis, progression through secondary therapy, etc). Consequently, comparisons between the tools should be considered carefully because assessed disease endpoints are different.
- **Gleason score:** Gleason grade and Gleason score are considered by many to be the most useful of the currently available clinical and pathological features.
- **AUA risk groups (D’Amico Risk Classification):** These risk groups are based on PSA, Gleason score and clinical stage.<sup>14</sup> Although straight-forward and widely used, they are arguably the least useful because they lump people into broad risk groupings based upon limited information. In addition, there is no way to discriminate hidden, aggressive disease in patients placed in the low-risk group. Patients that cannot be classified as high or low risk are grouped together as intermediate-risk or medium-risk patients; these patients are assigned the “gray” area of intermediate risk because a better risk assessment was previously impossible.

Using the same validation set, each of these methods was assessed for its ability to detect high-risk patients initially appearing as low or intermediate risk. By definition, the AUA classification scheme was unable to detect a high-risk case from these categories. The Systems Pathology approach exemplified by Prostate Px $\oplus$  was 67% better than clinical judgment, 100% better than clinical nomograms and 233% better than Gleason score (Figure 3).

Figure 3

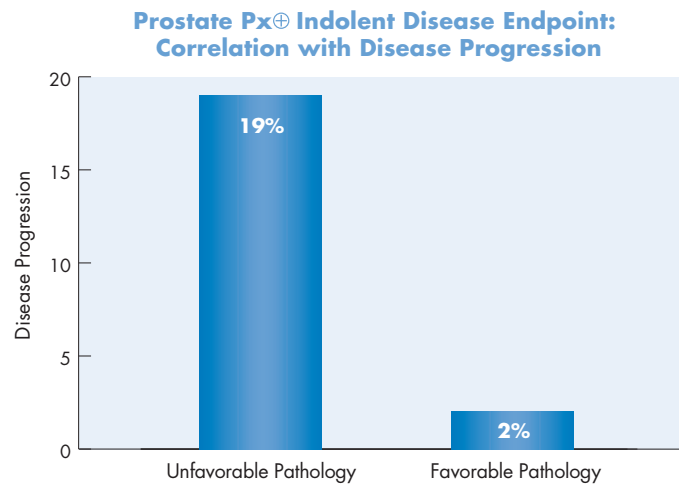


## Predicting Indolent Disease

In addition to disease progression, Prostate Px $\oplus$  predicts indolent disease as assessed by favorable pathology. The endpoint predicts the likelihood of a favorable outcome post-prostatectomy, i.e., prostatectomy Gleason score  $\leq 6$  (no pattern 4 or 5),  $\leq$ pT2c and PSA nadir post-prostatectomy.

The importance of assessing favorable pathology can not be underestimated. Patients with unfavorable pathology were much more likely to have disease progression within 8 years post-diagnosis (Figure 4).

Figure 4



Analyzing the validation cohort data (N=280) for indolent disease, Prostate Px $\oplus$  was able to identify patients likely to have indolent disease as assessed by favorable pathology with a sensitivity of 74% and a specificity of 65%. Analyzing the validation data, Prostate Px $\oplus$  is almost twice as accurate as AUA risk group designations (Figure 5a) for making this determination.

It is well known that biopsy-based risk assessment is impacted by biopsy Gleason patterns that are often upgraded or downgraded after prostatectomy.<sup>8</sup> An examination of the entire study cohort observed 453 cases diagnosed as  $\leq$ Gleason score six (6) at biopsy. At prostatectomy, 28% were reclassified as  $\geq 7$ . In addition, 35% of clinical stage  $\leq$ T2 were reclassified as  $\geq$ T3 upon prostatectomy (Figure 5b).

Figure 5a

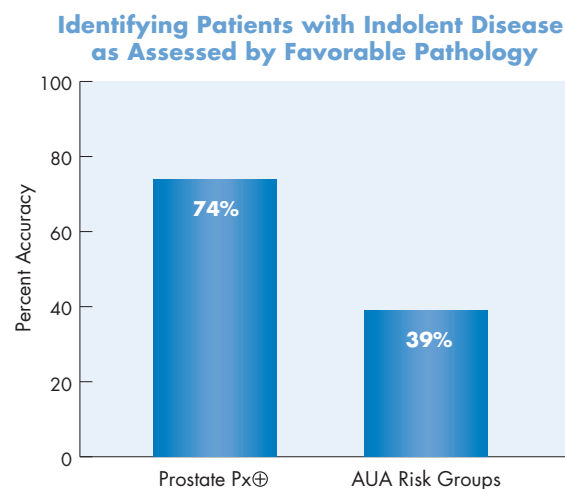
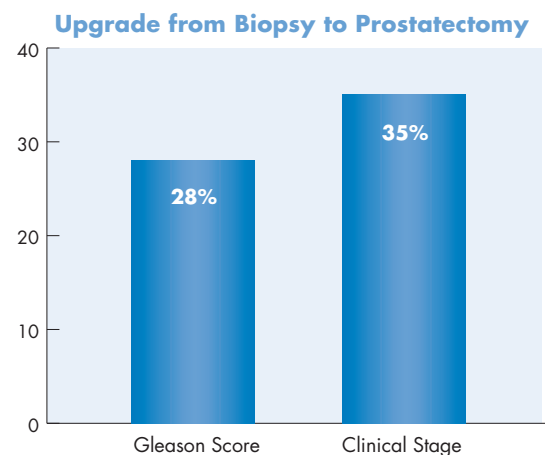


Figure 5b



## Systems Pathology Technology

Systems pathology is an integrated methodological approach for personalized patient risk assessment. Traditional pathology bridges both basic and clinical biomedical sciences. However, there is a degree of subjectivity to these analyses, and they lack the ability to provide quantitative results. Aureon's Systems Pathology is an approach that overcomes these deficiencies by integrating information from tissue architecture, clinical data and the cellular localization and quantification of molecular information.<sup>22</sup> Systems Pathology tools provide results that can be assessed by mathematical formulas or algorithms.

By using the patient's biopsy tissue, Prostate Px<sup>⊕</sup> can better assess disease progression [metastasis, death of disease, progression through primary adjuvant therapy (ADT or radiation)] post-prostatectomy. In addition, Prostate Px<sup>⊕</sup> has an indolent disease endpoint (assessed by favorable pathology) that provides better insight into the likelihood of a less aggressive tumor based upon Gleason pattern. Previously, the Systems Pathology approach was validated using Prostate Px, a prostatectomy-based model which assesses PSA recurrence and disease progression for up to five years post-surgery.<sup>23, 24</sup>

The Systems Pathology platform combines histological, molecular and clinical information to predict cancer recurrence by integrating three advanced technologies:

- **Image Analysis:** Digital images of cancer tissue are used to generate a variety of statistical measurements. This image platform is the result of the latest technologies in image processing and analysis aided by significantly enhanced computational power. Most cancer image-analysis systems have been developed for cytological specimens and do not utilize the architectural information available at the tissue level.
- **Biomarker Detection:** Multiplexed *in situ* protein detection utilizing fluorescently-tagged antibodies and analysis via spectral imaging. The automated, high-throughput system allows the separation of true signal from tissue auto-fluorescence, enabling the cellular detection and localization of specific signal as well as a more sensitive assessment of biomarkers.
- **Clinical Data:** Clinical information, such as the Gleason score, pathological stage or PSA values, can be integrated.

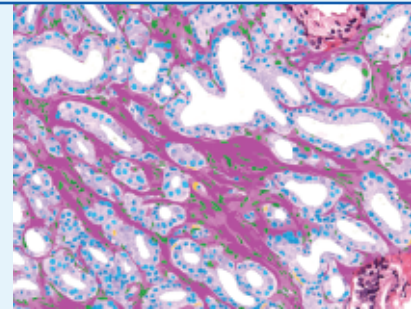
Features extracted from histologic, clinical, and molecular data are analyzed using advanced mathematics via novel "machine learning" methods. Machine learning approaches are becoming more widely used to analyze complex and varied sets of data, and the field is a subset of the artificial intelligence domain. Machine learning focuses on algorithms that enable computers to learn, recognize patterns and make decisions thereby improving their performance: it is related to data mining, pattern recognition and statistical analysis. There are many applications for machine learning technology including bioinformatics, internet search engines, stock market analysis, and predicting cancer recurrence. Aureon's machine learning methods can learn from prostate cancer data and improve upon the existing ability to provide an accurate prediction for patients.

## The Underlying Foundation of Aureon's Breakthrough Technology is Comprised of:

### Aureon PathoMetrix™

PathoMetrix is an automated machine vision tissue image analysis system that uses innovative image processing algorithms in order to segment, classify and measure properties of histopathological objects.<sup>25</sup>

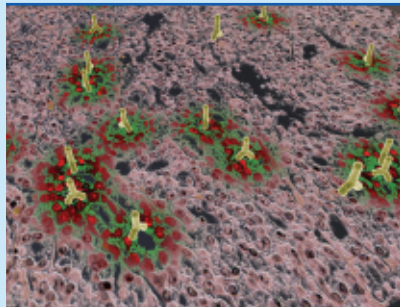
Currently, pathologists rely on the Gleason scoring system to provide an effective evaluation of how advanced and aggressive the cancer is. It is graded based on the architecture of the prostate tissue as seen under a light microscope. The more advanced the cancer is, the higher its Gleason score.



Although Gleason grading is widely considered by pathologists and physicians in general to be reliable, it is a subjective scoring system. The pathologist's experience, as well as other factors, can considerably affect the final Gleason scores. Moreover, the Gleason score is categorized into discrete levels to make it easier for pathologists to use. Meanwhile, new techniques in image processing and analysis have emerged, aided by significantly increased computational power. Many cancer image analysis systems have been developed for images taken from cytological specimens, which only capture cells and do not utilize the architectural information available at the tissue level. However, the structure of different pathological elements at the tissue level plays a more important role in diagnosis than the appearance of individual cells. For example, in a prostate tissue specimen, the shape and size of the gland are two of the most critical features pathologists use to determine the progression of the cancer.

Aureon PathoMetrix, one of the fundamental technologies of Prostate Px<sup>⊕</sup>, enables the analysis of prostate digital tissue images, detecting and measuring such significant architectural features.<sup>26</sup> The features extracted from the images are then integrated with clinical and molecular information to assess disease severity and predict disease progression at diagnosis.

### Aureon M-Plex™

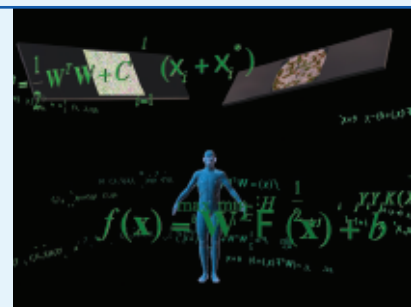


Multiplexed *in situ* protein detection consists of strategies developed at Aureon to fluorescently tag individual antibody:antigen complexes whose distribution and intensity is then selectively analyzed with spectral imaging. Our automated high-throughput imaging system allows the separation of multiple different real fluorescent signals (simultaneously identifying different biomarkers) from each other and from tissue autofluorescence, enabling a more accurate, sensitive and quantitative measurement of biomarkers. Furthermore, an elaborate quality control system has been implemented to guarantee day-to-day reproducibility.

The captured images are processed with the immunofluorescence image technologies developed within Aureon PathoMetrix to create area- and/or intensity-based features, representing cellular identification and quantitative biomarker data, which are then selected—through clinical outcome-based univariate and multivariate analyses—for inclusion in the predictive model.

### Aureon Discovery-Path™

Aureon has developed a novel methodology that incorporates the concepts of machine learning into the arena of clinical outcomes prediction. Machine learning is an area of artificial intelligence—akin to how the brain works—whose engineering can be explained in a statistical framework. This methodology enables a tool set that learns from patients' data to improve the accuracy of prediction in an individual patient. Discovery-Path is a supervised multivariate analytic toolbox capable of computing an optimized model to predict clinical outcomes.



# Appendix

## Supportive Data

Existing risk assessment relies exclusively on clinical and pathologic information gleaned from the biopsy sample. An analysis of the Px $\oplus$  SCORE indicates that integrating molecular and cellular data enables useful information that goes beyond what is available with existing clinical and pathologic features.

The following histograms demonstrate the distribution of Px $\oplus$  SCOREs across existing clinical variables:

- Px $\oplus$  SCOREs were evenly distributed across clinical stages (Figure 6)
- Px $\oplus$  SCOREs were similarly dispersed regardless of PSA level with only a slight narrowing of the distribution in the patient population with PSA >20ng/ml (Figure 7)
- Px $\oplus$  SCOREs were dispersed across age groups ranging from 50 to  $\geq 70$  (Figure 8)
- Px $\oplus$  SCOREs were also distributed across Gleason scores, although as expected, there was a tendency for Gleason  $\leq 6$  patients to trend towards lower Px $\oplus$  SCOREs and Gleason  $\geq 8$  to trend higher (Figure 9)

Figure 6

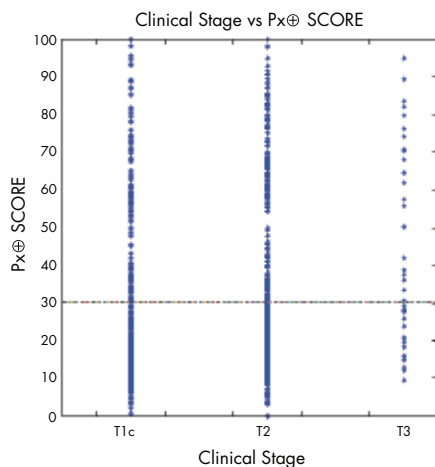


Figure 7

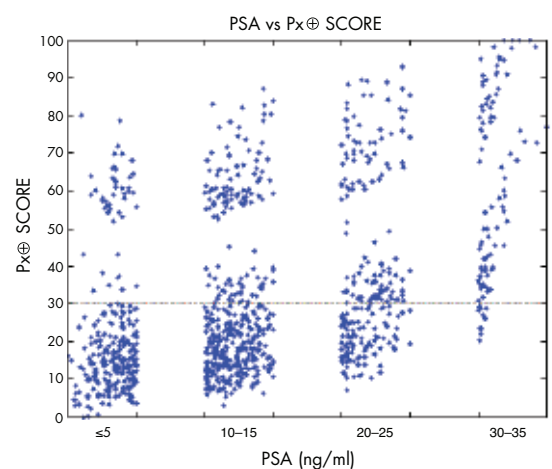


Figure 8

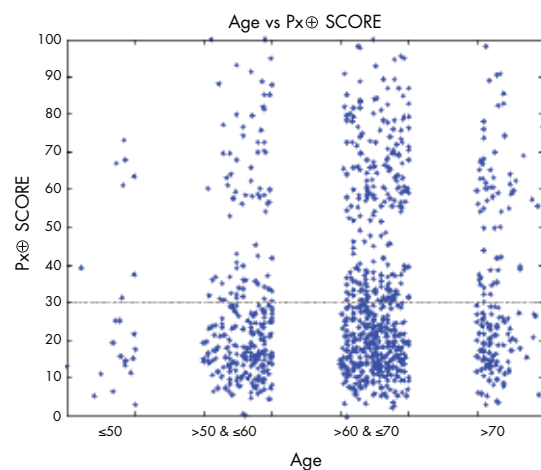
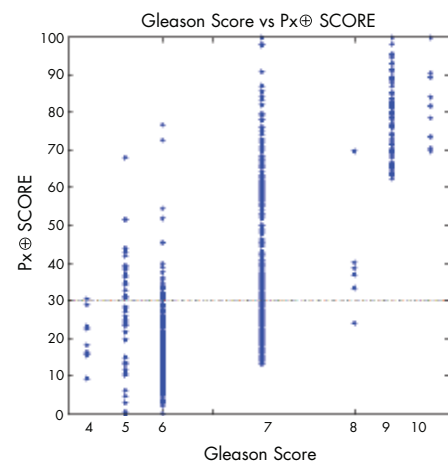


Figure 9



## Sample Case Study

A sample case study has been provided using patient data and the associated tissue image from the validation data.

### Challenge

Available clinical and pathologic data would suggest a low-risk case at diagnosis (Figure 10). The PSA level is below the widely used 4.0ng/ml standard but above 2.5ng/ml. The biopsy Gleason score of six (6) and the clinical stage indicate low-risk, organ-confined disease.

Existing methodologies that employ similar clinical and pathological data would indicate a low-risk patient.

- The AUA guidelines do not have the ability to discriminate high-risk patients with these clinical and pathological parameters
- A physician's clinical judgment based upon PSA and Gleason score would have identified this case as low risk
- Online nomograms would have suggested that this patient had an excellent likelihood of disease-free recurrence up to 10 years post diagnosis

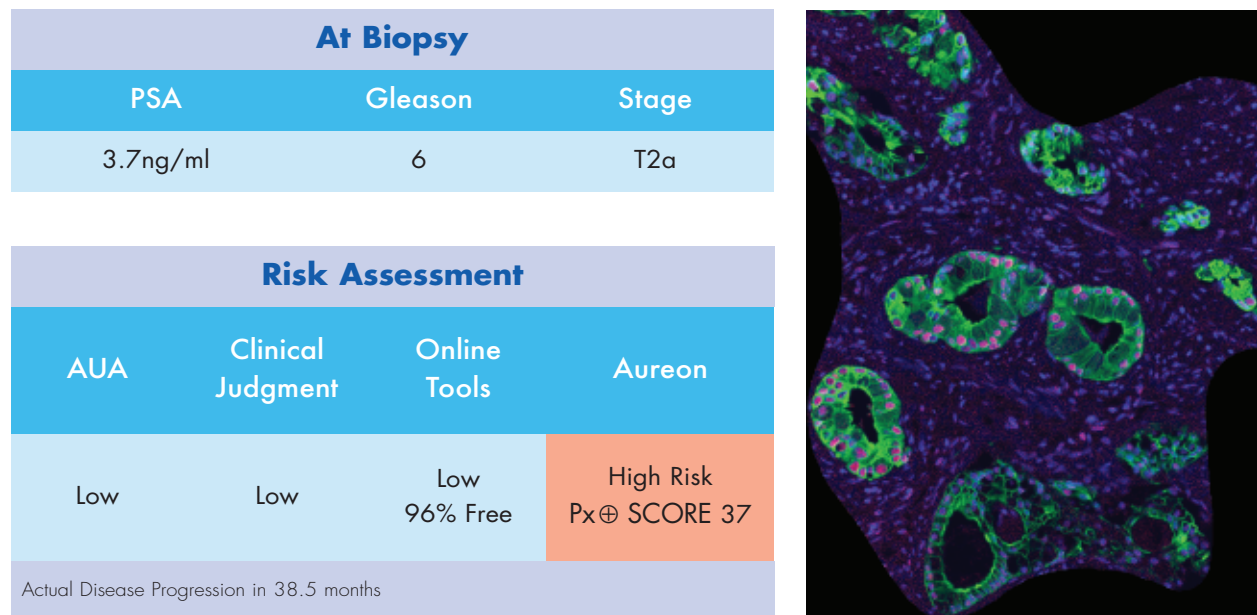
### Result

The Systems Pathology approach enables discrimination of high-risk disease in this "low-risk" patient by integrating cellular and molecular features. The Px $\oplus$  SCORE of 37 indicated a high-risk case (the cut point between high and low risk is 30.2).

### Outcome

This patient had actual disease progression within 38.5 months of diagnosis.

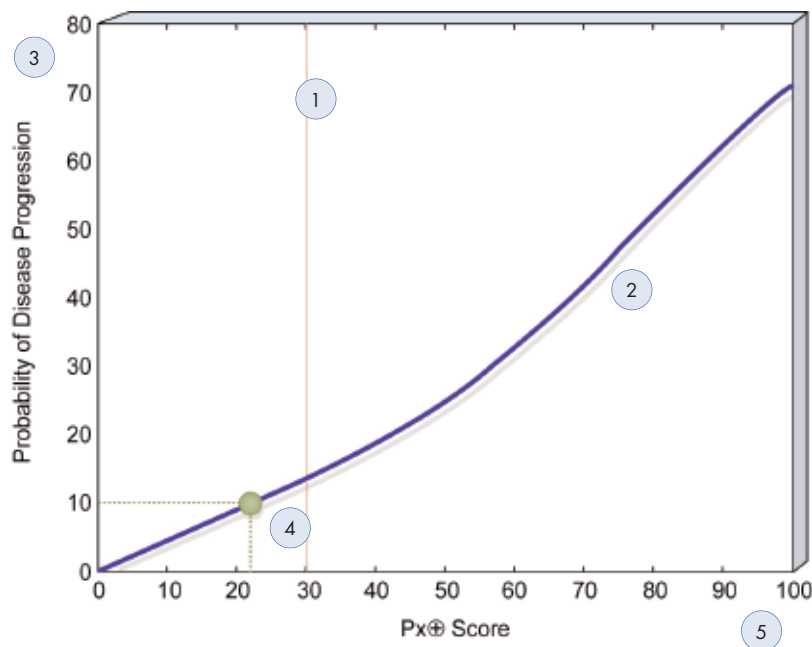
Figure 10



## Patient Report

A report will be sent to the physician within five business days of the biopsy specimen and associated clinical data arriving at Aureon. Test results are mailed and/or faxed. Each report focuses on the patient's individualized results including:

- **Disease Progression:** This describes the likelihood of developing metastasis, progression through androgen deprivation therapy and or death of disease
- **Indolent Disease:** This endpoint predicts the likelihood of a favorable outcome post prostatectomy, i.e., prostatectomy Gleason score  $\leq 6$  (no pattern 4 or 5),  $\leq pT2c$  and PSA nadir post-prostatectomy
- **Px $\oplus$  SCORE™:** Provides a number between 0 and 100 that directly correlates to the likelihood of prostate cancer disease progression within 8 years of radical prostatectomy
- **Quantitative Morphometric Features** which describe the objective quantification of a number of features from the digital image of the H&E and IF specimens
- **Photomicrographs** which show segmented and classified H&E and immunofluorescence images



1. Cut point: the “dividing” line between low-risk and high-risk determination. The cut point (30) is determined during model development to provide optimal risk discrimination
2. Px $\oplus$  SCORE curve: determined by the training cohort of patient data
3. Y axis: probability of remaining free of disease progression
4. Personalized patient Px $\oplus$  SCORE: Note that below the cut point the dot is green; above the cut point the dot would be red
5. X axis: Px $\oplus$  SCORE ranges from 0 to 100. A case with a higher score is more likely to recur before a case with a lower score

# References

1. Cancer Facts and Figures 2007. American Cancer Society, Inc. Available at [http://www.cancer.org/docroot/STT/content/STT\\_1x\\_Cancer\\_Facts\\_Figures\\_2007.asp](http://www.cancer.org/docroot/STT/content/STT_1x_Cancer_Facts_Figures_2007.asp)
2. Freedland SJ, Humphreys EB, Mangold LA, et al. Risk of prostate cancer-specific mortality following biochemical recurrence after radical prostatectomy. *JAMA*. 2005;294:433–439
3. D'Amico AV, Chen M-H, Roehl KA and Catalona WJ. Identifying patients at risk for significant versus clinically insignificant postoperative prostate-specific antigen failure. *J Clin Oncol*. 2005;23:4975–4979
4. Fitzsimons NJ, Presti Jr JC, Kane CJ, et al. Is biopsy Gleason score independently associated with biochemical progression following radical prostatectomy after adjusting for pathological Gleason score. *J Urol*. 2006;176:2453–2458
5. Vollmer RT, Keetch DW, Humphrey PA. Predicting the pathology results of radical prostatectomy from preoperative information. *Cancer*; 1998;83:1567–1580
6. Montironi R. Prognostic factors in prostate cancer. *BMJ*. 2001;322:378–379
7. Lieberfarb ME, Schultz D, Whittington R, et al. Using PSA, biopsy Gleason score, clinical stage, and the percentage of positive biopsies to identify optimal candidates for prostate-only radiation therapy. *Int J Rad Oncol Biol Phys*. 2002;53:898–903
8. Pinthus JH, Witkos M, Fleshner NE, et al. Prostate cancers scored as Gleason 6 on prostate biopsy are frequently Gleason 7 tumors at radical prostatectomy: Implication on outcome. *J Urol*. 2006;176:979–984
9. Albertsen PC, Hanley JA, Barrows GH, et al. Prostate cancer and the Will Rogers phenomenon. *J Natl Cancer Inst*. 2005;97:1248–1253
10. Ruijter E Th, Van De Kaa CA, Schalken JA, et al. Histological grade heterogeneity in multifocal prostate cancer. Biological and clinical implications. *J Pathol*. 1996;180:295–299
11. Swindle P, Eastham JA, Ohori M, et al. Do margins matter? The prognostic significance of positive surgical margins in radical prostatectomy specimens. *J Urol*. 2005;174:903–907
12. Epstein JI, Pizov G, and Walsh PC. Correlation of pathologic findings with progression after radical retropubic prostatectomy. *Cancer*. 1993;71:3582–3593
13. Partin AW, Yoo J, Carter HB, et al. The use of prostate specific antigen, clinical stage and Gleason score to predict pathological stage in men with localized prostate cancer. *J Urol*. 1993;150:110–114
14. NCCN Clinical Practice Guidelines in Oncology v2.2007. Available at [http://www.nccn.org/professionals/physician\\_gls/default.asp](http://www.nccn.org/professionals/physician_gls/default.asp)
15. European urology – prostate cancer nomograms: An update. *UroToday*. 2006;50:914–926. Available at [http://www.urotoday.com/european\\_urology/review\\_articles\\_november\\_2006/european\\_urology\\_\\_prostate\\_cancer\\_nomograms\\_an\\_update.html](http://www.urotoday.com/european_urology/review_articles_november_2006/european_urology__prostate_cancer_nomograms_an_update.html)
16. Kattan MW, Eastham JA, Stapleton AMF, et al. A preoperative nomogram for disease recurrence following radical prostatectomy for prostate cancer. *J Natl Can Inst*. 1998;90:766–771
17. Kattan MW, Wheeler TM, and Scardino P. Postoperative nomogram for disease recurrence after radical prostatectomy for prostate cancer. *J Clin Oncol*. 1999;17:1499–1507
18. Steyerberg EW, Roobol MJ, Kattan MW, et al. Prediction of indolent prostate cancer: validation and updating of a prognostic nomogram. *J Urol*. 2007;177:107–112
19. Kattan MW, Zelefsky MJ, Kupelian PA, et al. Pretreatment nomogram for predicting the outcome of three-dimensional conformal radiotherapy in prostate cancer. *J Clin Oncol*. 2000;18:3352–3359
20. Kattan MW, Potters L, Blasko JC, et al. Pretreatment nomogram for predicting freedom from recurrence after permanent prostate brachytherapy in prostate cancer. *Urology*. 2001;58:393–399
21. Stephenson A, Scardino P, Eastham J, et al. Preoperative nomogram predicting the 10-year probability of prostate cancer recurrence after radical prostatectomy. *J Natl Cancer Inst*. 2006;98:715–717
22. Saidi O, Cordon-Cardo C, and Jose Costa. Technology insight: will systems pathology replace the pathologist? *Nature Clin Practice*. 2007;4:39–45
23. Cordon-Cardo C, Kotsianti A, Verbel, D, et al. Improved prediction of prostate cancer recurrence through systems pathology. *J Clin Invest*. 2007;117:1876–1883
24. Donovan M, Hamann S, Clayton M, et al. A systems pathology approach for the prediction of prostate cancer progression after radical prostatectomy. *J Clin Oncol*. 2008 (in press)
25. Tabesh A and Teverovskiy M. Tumor classification in histological images of prostate using color texture. *Proc. Asilomar Conf. Signals, Systems, and Computers*. 2006
26. Teverovskiy M, Kumar V, Ma J, Kotsianti A, et al. Improved prediction of prostate cancer recurrence based on an automated tissue image analysis system. *IEEE International Symposium on Biomedical Imaging: From Nano to Macro Conference Proceedings*. 2004
27. Teverovskiy M, Vengrenyuk Y, Tabesh A, et al. Automated Localization and Quantification of Protein Multiplexes Via Multi-spectral Fluorescence Imaging. *ISBI Conference*. 2008

## **Aureon Laboratories**

Aureon Laboratories is a life science company founded in 2001 dedicated to enabling the advancement of predictive and personalized cancer treatment. Aureon employs a unique Systems Pathology approach and provides personalized test results by analyzing a patient's existing biopsy sample using molecular diagnostics, sophisticated tissue-based image analysis and advanced mathematics. The resulting information is objective, predictive and personalized and allows the patient and their doctor to make more-informed treatment decisions.

Aureon's technology fits smoothly with existing clinical and pathological processes. Aureon examines formalin-fixed, paraffin-embedded (FFPE) specimens that are routinely obtained as tissue biopsies. Many existing methods need to destroy the tissue to examine protein or genetic biomarkers, losing critical biological information in the process. Vitaly important, Aureon's approach retains the biological information embedded in the patient's tissue, resulting in improved test performance.

In addition to prognostic clinical tests, Aureon also collaborates with bio-pharmaceutical partners to apply our innovative platform to drug discovery and clinical development.

Aureon was co-founded by leading physician-scientists at Memorial Sloan-Kettering Cancer Center, Yale Cancer Center, and Albert Einstein College of Medicine.