OncoTAb
Improving the Quality of Cancer Care
9201 University City Blvd., 243 Bioinformatics Bldg., Charlotte NC 28223
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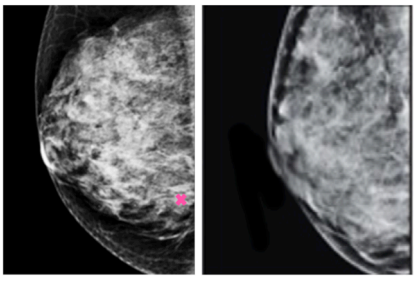
Agkura™ Personal Score

Scientific Background: The Agkura™ Personal Score is a simple blood test that measures the levels of the tumor form of a glycoprotein i.e. a protein with carbohydrates attached to it. This glycoprotein, MUC1, plays a lubrication as well as protective role in normal cells by binding to pathogens [1]. When a cell transforms to be cancerous, the carbohydrates attached to this protein change in structure. OncoTAb's CSO Dr. Pinku Mukherjee, a Mayo Clinic alumna, has studied this protein for close to 20 years and developed a patented antibody (named TAB004) [2-4] that recognizes the tumor form of MUC1 or tMUC1.

tMUC1 is known to be over produced in cancer cells and is present in the tumors of over 90% of breast cancer patients [5]. We have tested for the presence of tMUC1 in breast cancer tissue and have found TAB004 to detect its presence in 95% of tissue samples tested, regardless of breast cancer subtype or receptor status [6]. It is noteworthy that TAB004 did not detect tMUC1 in normal and benign breast tissue. tMUC1 is released from cancer tissue into circulation making it possible to be detected with a simple blood test. The release of tMUC1 into circulation requires a minimum threshold of tMUC1 loading in the cancer cells, following which the release increases exponentially with the amount of tMUC1 in the cancer cells [6].

Over a hundred years ago, it was first proposed that cells are continuously transformed in our bodies and the immune system eliminates these cells before clinical symptoms arise [7]. It took the scientific community more than 80 years to accept this idea, following multiple studies in animals. Now this idea has matured to the concept of cancer immunoediting, which has 3 phases: Elimination, Equilibrium and Escape [8]. In the first two phases, the immune system either eliminates the cancer cells or keeps them in check, preventing them from growing. If the immune response fails to completely eliminate the tumor cells, immune resistant tumor cell variants develop. This phase is known as the escape phase and the tumor starts to grow. Imaging modalities detect breast cancer in the escape phase of cancer immunoediting [7]. **Given that the changes to the MUC1 structure occur with the transformation of normal cells, levels of tMUC1 can be expected even in normal individuals who are in the elimination and equilibrium phases of cancer immunoediting.** However, if the cancer cell does not escape the immune surveillance and grows, this level of tMUC1 released in normal individuals does not increase significantly.

An Unmet Breast Cancer Detection Need: Early detection of breast cancer with regular screening increases the chances of survival as early stage cancers (Stages 1 and 2) are easier to treat compared to later stage disease (Stages 3 & 4). Screening for breast cancer has predominantly been done by mammography with breast MRI's often being used for women at high risk. While routine mammograms have resulted in early diagnosis for many women, screening with mammograms is not perfect. Cancers can be missed, especially in women with dense breast tissue for whom mammograms miss cancer 50% [9, 10] of the time resulting in later stage diagnosis, increased mortality and higher lifetime treatment costs. A major reason for breast cancer's being missed in these women is because radiologists find it difficult to distinguish between tumor and normal dense tissue, both of which appear white on a mammogram. This is illustrated in **Figure 1** that shows two mammogram images of dense breasts – one with a tumor and one without. The pink "x" identifies where the tumor is hidden.



Dense Breast #1 **Dense Breast #2**

Figure 1

The American College of Radiology (ACR) suggests considering supplemental ultrasonography as an option in women with intermediate risk and dense breasts [11]. Compared with mammography, ultrasound has a sufficiently high sensitivity to detect breast cancer regardless of breast tissue density; however, the specificity of ultrasound is low due to higher false-positive detection rates (up to 95%) [9 - 10, 12 - 14].

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OncoTAb's Strategy to Aid Breast Cancer Detection in Women with Dense Breast Tissue: Based on the scientific evidence related to tMUC1 and cancer immunoediting, OncoTAb has used its patented and tumor specific antibody TAB004, to develop the Agkura™ Personal Score. The strategy entails establishing a baseline score for each individual and monitoring for a change to their score over time. The risk of an individual having breast cancer and recommendations for diagnostic imaging are made by:

a. Checking if their Agkura™ Personal Score exceeds a cut-off established using normal volunteers without a breast cancer diagnosis (called "reference interval" < 31.4 µg/ml), and
b. Checking if changes in their Agkura™ Personal Score relative to their baseline on a bi-annual basis exceeds the "normal variation" (described later)

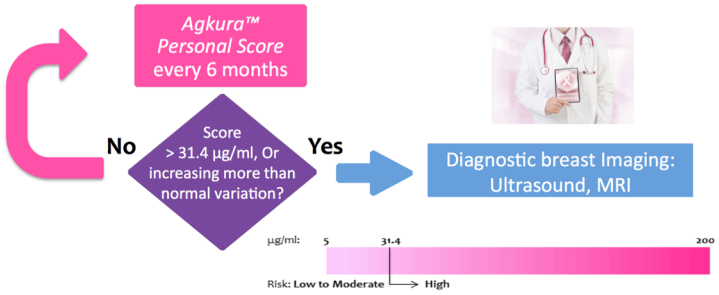

Figure 2

This strategy is illustrated in **Figure 2**. The Agkura™ Personal Score is not meant to be a standalone test to detect breast cancer. If either of the above criteria is met, a recommendation for additional diagnostic imaging is made. ***The Agkura™ Personal Score is a Laboratory Developed Test and its performance has not been cleared or approved by the U.S. Food and Drug Administration.***

Clinical Validation: In order to validate if our strategy could work for women with dense breast tissue, clinical studies were conducted and evaluated to answer the following questions:

1. Does the Agkura™ Personal Score increase with breast cancer progression?
2. Does dense breast tissue affect the levels of tMUC1 measured in circulation?
3. What is the "normal variation" of the Agkura™ Personal Score in women who do not have a breast cancer diagnosis? and
4. Does the Agkura™ Personal Score increase with age in normal women?

In these clinical studies, samples were obtained from the Fox Chase Cancer Center (FCCC), from the Women and Infants Hospital of Rhode Island (WIHRI), and from normal volunteers enrolled at the University of North Carolina at Charlotte. Samples from over 350 women have been tested in these studies. Other than the healthy volunteers, the diagnosis of these women ranged from benign conditions to invasive breast cancer spanning across all stages. The results are described below.

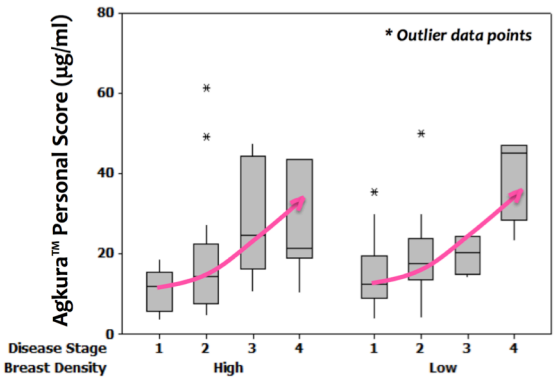

Figure 3

Figure 3 presents data from a subset of the cohort (n = 100) for whom breast density information is available. The figure shows boxplots for each breast cancer stage and across low and high breast tissue densities. There is overlap in the boxplots between the high and low tissue densities corresponding to each stage. Statistical analysis showed no significant difference in the Agkura™ Personal Score between high and low tissue density (t-test p-value = 0.102). **Both high and low tissue density, however, showed an increase in the Agkura™ Personal Score as the breast cancer stage increased (t-test p-value = 0.006 for early stage versus late stage).** ***This clinical data supports the use of the Agkura™ Personal Score for women with dense breast tissue.*** The increasing scores will result in these women being flagged for diagnostic imaging as their cancer progresses.

Figure 4 presents the data from the normal volunteers whose samples were collected 15 days apart. The idea of collecting their samples over a relative short duration span was to capture the variation in their Agkura™ Personal Score for reasons other than cancer progression – in the event there are undiagnosed cancer cases in this cohort. Two measurements were taken on each day and each box plot represents 4 measurements of each of the volunteers. The data was analyzed using an approach described in the literature to capture biological variation [15]. Visually, it is very evident that each volunteer has some variation around their own personal score that can be very different from the personal scores of other volunteers. ***The 95% and 99% confidence levels of acceptable normal variation (measurement and biological combined) were calculated to be 10% change and 14% change respectively***. Additional volunteers are being tested and a 6-month duration time point will also be analyzed.

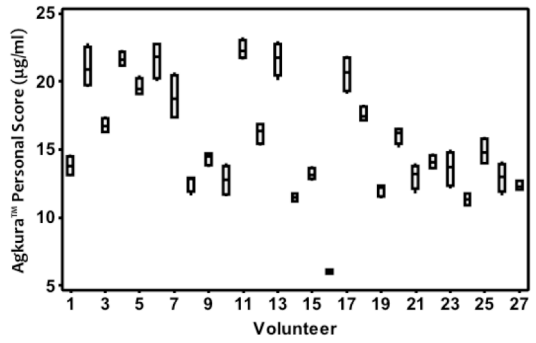

Figure 4

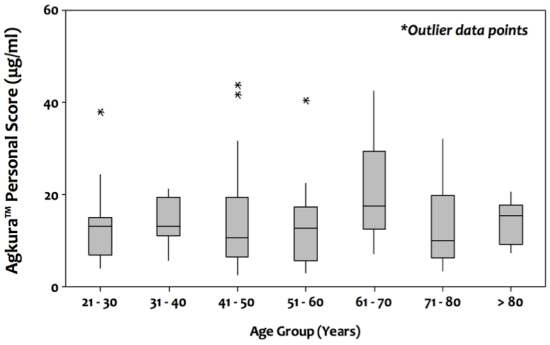

Figure 5

Given the strategy of monitoring changes in the Agkura™ Personal Score of women over time, it was important to understand if there is an age dependence of this score. **Figure 5** presents boxplots of the measured scores from the normal volunteers (n = 122) in the various age groups. While the scores for the women in the age group 61-70 visually appear to be higher, there was no statistically significant difference between the age groups (ANOVA p-value = 0.614).

Clinical Performance: As described earlier, we assess the risk of an individual having breast cancer and recommend diagnostic imaging by:

a. Checking if their Agkura™ Personal Score exceeds the "reference interval" of 31.4 µg/ml, and
b. Checking if changes in their Agkura™ Personal Score relative to their baseline exceeds acceptable "normal variation"

The clinical studies described above from breast cancer patients were only one-time measurements following breast cancer diagnosis and hence allow us to evaluate performance attributes for criteria (a) only. We have conducted a pilot study to evaluate both criteria, however, clinical performance attributes cannot be evaluated from that study since all samples were tested prior to any breast cancer diagnosis. We can only state the results in terms of how the recommendation of diagnostic imaging would have performed. Ultimate diagnosis would still be dependent on the performance of the imaging modality used subsequent to OncoTAb's recommendation.

Criteria (a) Clinical Performance (Stage 2+ breast cancer)

- Clinical Sensitivity (Percent of breast cancer cases with Agkura™ Personal Score above 31.4µg/ml) = 26%
- Clinical Specificity (Percent of normal volunteers with Agkura™ Personal Score below 31.4µg/ml) = 92%
- Positive Predictive Value (Percent of Agkura™ Personal Scores above 31.4µg/ml that had breast cancer) = 66%
- Negative Predictive Value (Percent of Agkura™ Personal Scores below 31.4µg/ml that did not have breast cancer) = 69%

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Pilot Study using criteria (a) & (b)

During the course of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) fifty thousand women volunteers gave blood each year over the course of ten years (from 2001 to 2011). When these women signed up to participate in this trial, they had no cancer diagnosis. During the course of the ten years, roughly 3,625 women were diagnosed with breast cancer. OncoTAb tested 180 samples from UK cohort of 60 volunteer women in a blinded pilot study (i.e. we were provided information on diagnosis only after we shared results with our collaborators):

- 30 breast cancer cases (15 early stage, 15 late stage) at 3 time points:
  - 0-1 year before diagnosis
  - 1-2 years before diagnosis, and
  - 3+ years before diagnosis
- 30 matched controls at 3 time points: no cancer diagnosis for at least 5 years from last time point

The controls were matched by age, sample collection center and time of storage of sample.

Application of criteria (a) & (b) to 30 Breast Cancer Cases

Using the reference interval of < 31.4 µg/ml and the 10% normal variation threshold that gives 95% confidence, the Agkura™ Personal Score would have triggered a diagnostic imaging recommendation as shown in Table 1:

Breast Cancer Stage	Years Before Diagnosis			Total # of Cases	% Cases triggered prior to actual diagnosis
	3 - 4	1 – 2	0 – 1		
1	1	3	0	11	36%
2		1		4	25%
3+		7	2	15	60%

Table 1

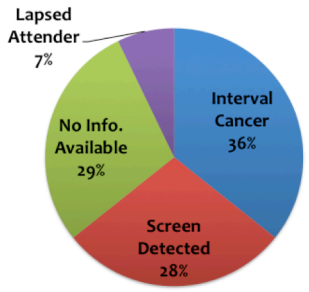

Figure 6

Overall, 47% of all cases and 60% of the late stage cases would have received an earlier diagnostic imaging recommendation – most of them up to two years earlier. The distribution of these cases by mammography screening status is shown in **Figure 6**. More than a third of these cases were interval cancers confirming that they were missed by mammography. Roughly another third were screen detected. Unfortunately, breast density status of this cohort is not known. ***Nonetheless, this data supports the use of the Agkura™ Personal Score in conjunction with mammography as an aid to earlier diagnosis of breast cancers that are missed by mammography.***

Application of criteria (a) & (b) to 30 Controls

At the time of the study, 30 controls were provided to us by our collaborators and were deemed to be cancer free. Since then, we have been informed that 2 controls were recently reported to have had cancer and we have removed them from our analysis. There is no way of confirming if any of the other controls had cancer at the time of blood draw. Since the closure of the UKCTOCS study, the only way we can determine if the control volunteers had cancer is to wait for their names to show up on a cancer or death registry in the UK, a process that has a two year lag in reporting.

Of the remaining 28 controls, the Agkura™ Personal Score would have triggered a diagnostic imaging recommendation for 39% of the controls (using the reference interval of < 31.4 µg/ml and the 10%

allowed normal variation threshold). ***Even if we were to consider these to be true false positives, the performance would compare very favorably with mammography and ultrasounds***. Of the roughly 1.6 million biopsies conducted each year following a suspicious mammogram, roughly 80% result in benign findings [16, 17]. As a screening tool, ultrasounds can have false positive rates as high as 95% [14].

Summary: Mammograms miss breast cancer 50% of the time in women with dense breast tissue resulting in late stage diagnosis and mortality. OncoTAb has developed a simple blood test, the Agkura™ Personal Score and a strategy to aid in the earlier detection of breast cancer in these women. Data from our clinical studies support the use of the Agkura™ Personal Score in conjunction with mammography.

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