RECAF, a new broad-spectrum cancer marker.

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Introduction:

Among the diagnostic tools developed in the past few decades, tumor markers provide a unique combination of low cost and accuracy that makes them suitable for cancer diagnosis, monitoring and screening. Despite a great deal of research in the area, only a handful of markers have found their way to clinical use.

In this document, we present data related to a new cancer marker, named RECAF™, which exhibits high sensitivity and specificity on tissue sections and serum of patients with diverse types of malignancies.

RECAF Biology:

RECAF is a receptor present on the surface and in the cytoplasm of fetal cells. It binds and internalizes circulating Alpha-Fetoprotein (AFP), a protein that behaves much like albumin in the early stages of prenatal life. RECAF and AFP should not be confused; the latter is a liver and testicular cancer marker known since 1963[1] whereas RECAF is an emerging broad-spectrum cancer marker. The two are completely different in structure and behavior.

Much like albumin, AFP binds and transports smaller molecules, such as long chain, poly-unsaturated fatty acids[2] (PUFA). Experiments have shown that following binding to RECAF, AFP penetrates the cell via coated-pits[3,4], releases its load of fatty acids and then leaves the cell immunologically intact[5], probably ready to cross again the placenta and fetch another fatty acid molecule from the mother.

The uptake of AFP and hence the expression of RECAF is related to the degree of cell differentiation[6,7,8]: When a given fetal organ or tissue has reached a certain maturity, AFP is no longer taken up.

Since cancer cells are poorly differentiated, it was first postulated[9] and then demonstrated that cancer cells re-express RECAF, thus behaving as an oncofetal antigen[10] (these are molecules expressed by fetal and cancer cells but not by normal adult cells. AFP and CEA [Carcino Embryonic Antigen] are typical oncofetal antigens).

During fetal life, AFP uptake occurs in most organs and tissues[11] and therefore RECAF, which mediates the uptake, is re-expressed in many types of cancers[12,13,14,15,16].

RECAF is a family of molecules including two main soluble cytoplasmic fractions,[17] which are released from the cancer cells and therefore can be detected with a sensitive serum test. These molecules are glycoproteins and they bind AFP via their sugar portion. The antibody used in our assays also recognizes the sugar portion and therefore it "sees" all of the RECAF molecules.

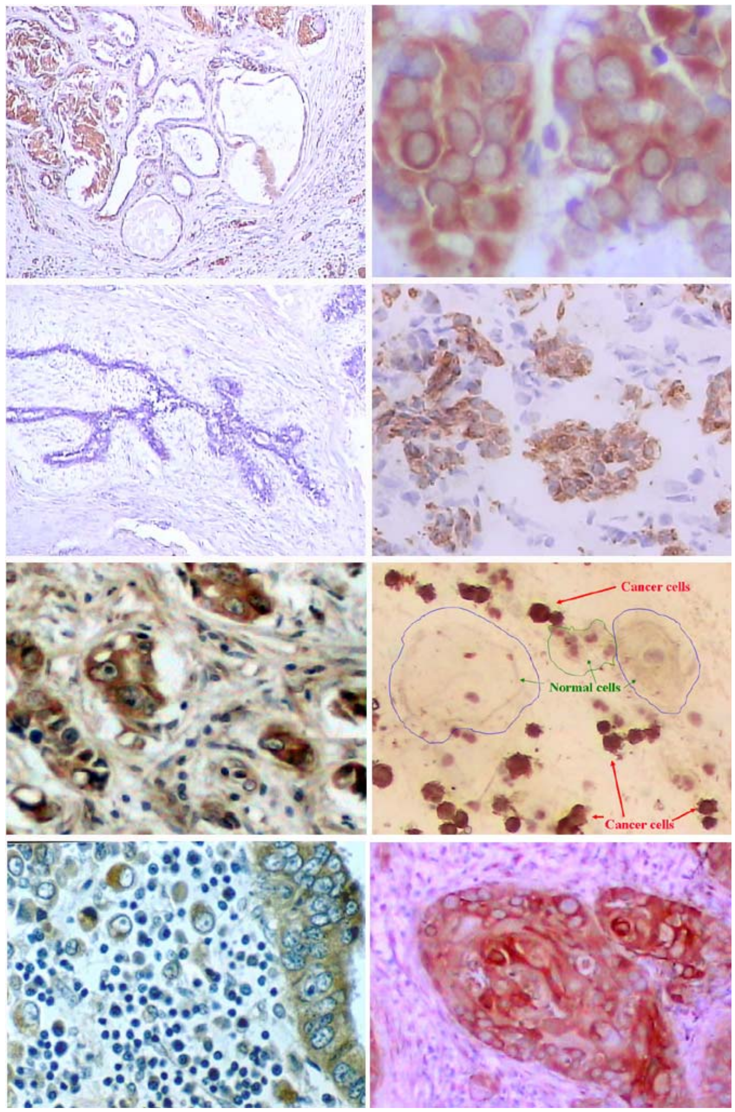
This antibody allows staining and detecting cancer cells under the microscope or measuring the amount of circulating RECAF, which may be increased if the patient has cancer.

RECAF on tissue sections:

The large majority of cancers show RECAF positive staining (brown) with an anti-RECAF antibody. Most normal cells and benign tumor cells are negative.

Figures 1 and 2 show positive breast ductal carcinoma cells (at different magnifications). Fig. 3 corresponds to a negative benign fibroadenoma. Fig. 4 is a frozen section of breast cancer. Figure 5 shows the staining of a prostate carcinoma. Figure 6 is a composite of a normal Pap smear spiked with cervical cancer cells (grown in culture) and then stained for RECAF. While the normal cells are negative, the added cervical cancer cells are strongly positive. Figure 7 shows a stomach cancer and Fig. 8 corresponds to a lung cancer.

The staining is heterogeneous. Nuclear membrane capping is usually present (Fig. 2). For additional information see Reference 18.



RECAF in serum:

Cancer cells release the soluble fractions of RECAF into the blood stream and therefore measuring the circulating RECAF in cancer patients can be used for cancer detection, screening, and monitoring in association with the clinical presentation and/or other cancer markers. Figures 9 and 10 show the distribution of RECAF values for normal individuals, patients with benign lesions and cancer patients. The horizontal lines represent a lower cutoff value - chosen to catch as many cancers as possible – and a higher cutoff value chosen for best discrimination between benign and malignant prostate conditions.

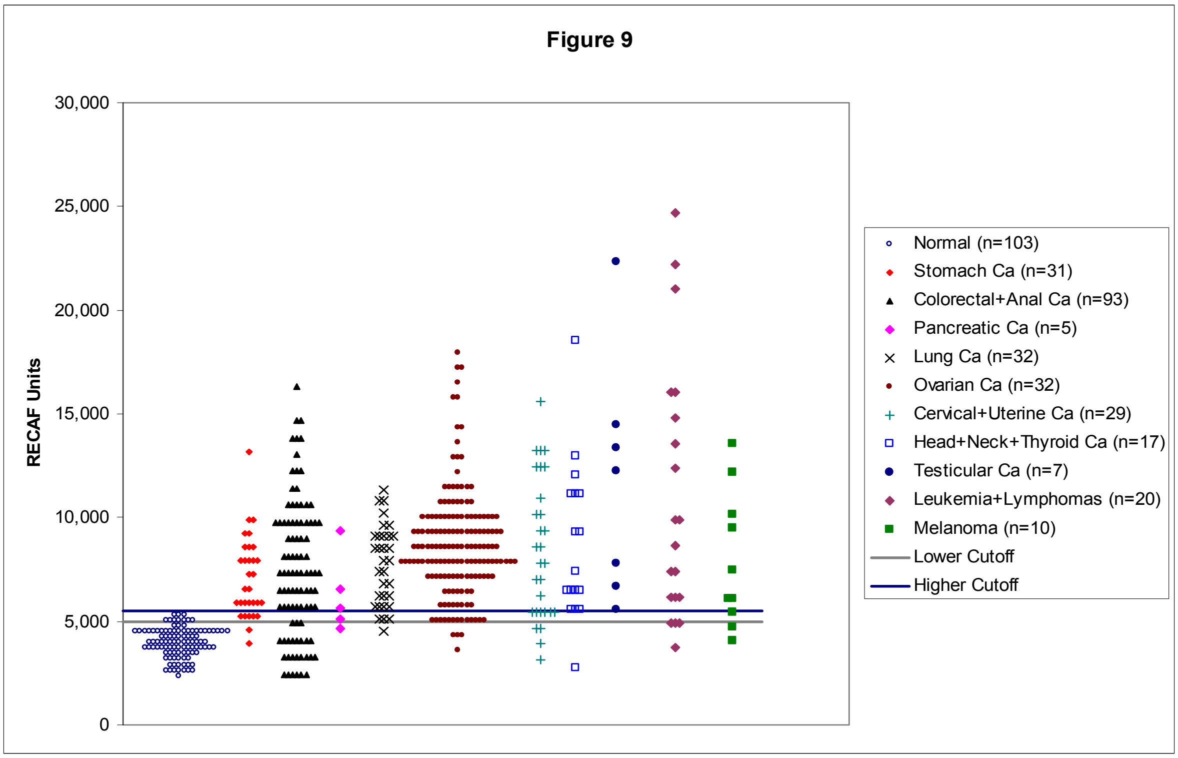


Figure 10

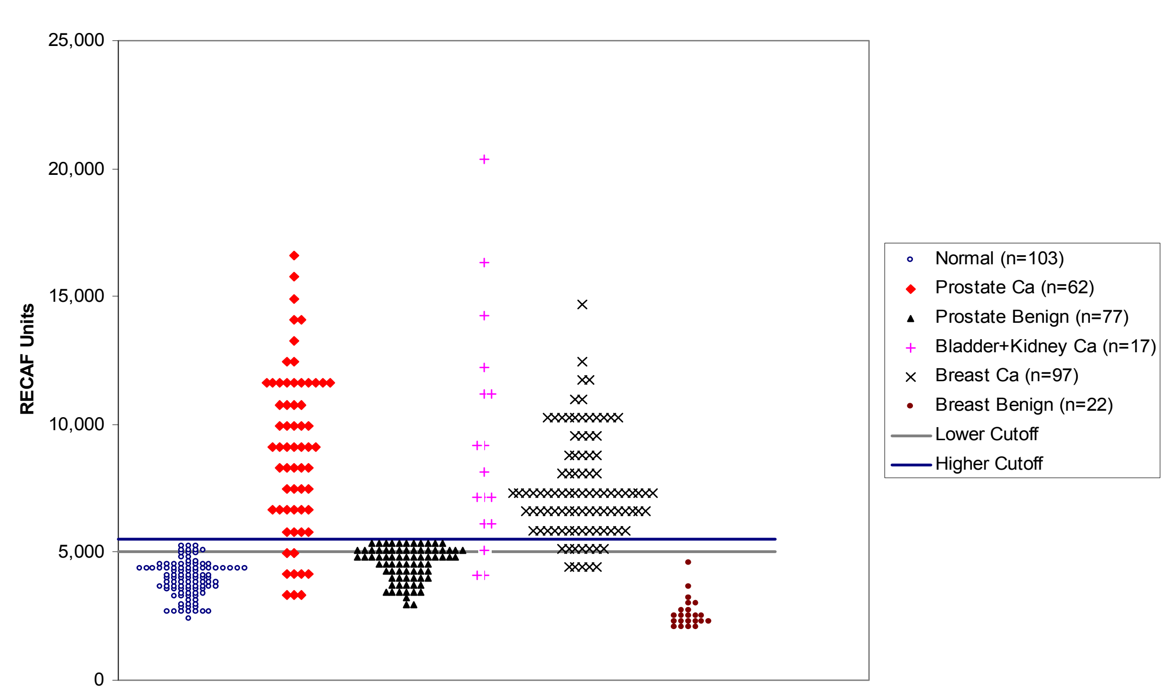


Table I shows the sensitivity, the number and the efficiency for each type of cancer when compared to normal sera, using the two cutoff values:

Table I

CANCER TYPE	n	Sensitivity with 92% Specificity	Sensitivity with 100% Specificity	Efficiency** with 92% Specificity	Efficiency with 100% Specificity
Stomach	31	90.3%	80.6%	91.8%	95.5%
Pancreas	5	80.0%	60.0%	91.7%	98.1%
Colorectal & Anal	93	75.3%	72.0%	84.2%	86.7%
Lung	32	93.8%	84.4%	92.6%	96.3%
Ovarian	162	96.3%	87.7%	94.7%	92.5%
Uterus & Cervical	29	86.2%	79.3%	90.9%	95.5%
Head & Neck & Thyroid	17	94.1%	94.1%	92.5%	99.2%
Testis	7	100.0%	100.0%	92.7%	100.0%
Lymphoma & leukemia	20	80.0%	80.0%	90.2%	96.7%
Melanoma	10	80.0%	70.0%	91.2%	97.3%
Breast	97	93.8%	87.6%	93.0%	94.0%
Prostate	62	88.7%	85.5%	90.9%	94.5%
All Cancers vs. Normals*	565	89.6%	83.4%	90.0%	85.9%

* 103 normal sera were used throughout this study.

** Efficiency is the ability to detect correctly the true positives and true negatives: *Efficiency* = $(TN + TP)(FN + TN + FP + TP)$ *where TP = True Positive, TN = True Positive, FP = False Positive and FN = False Negative*

See Table III for more detailed information.

The RECAF test also differentiates well malignant from benign lesions as shown in Table II:

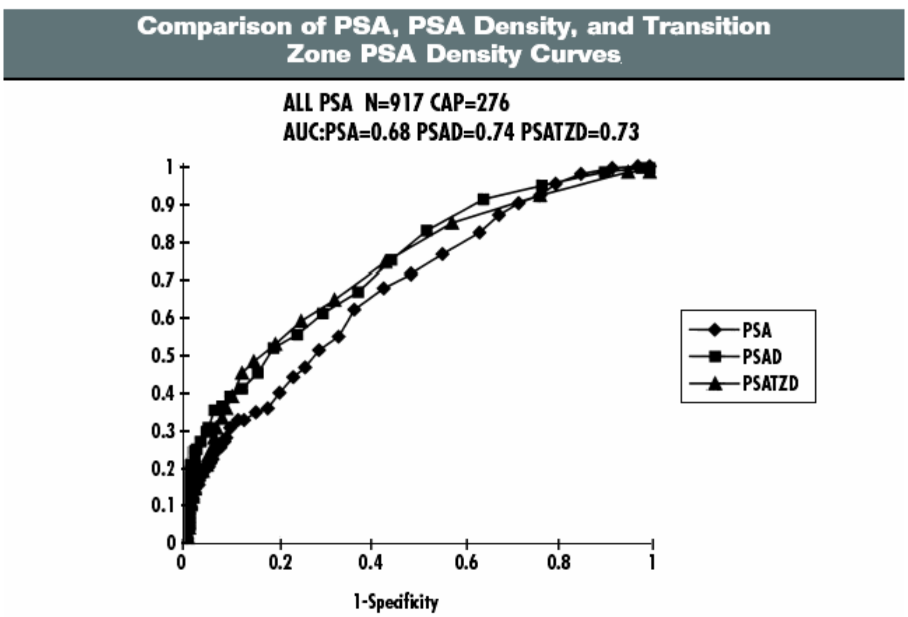
Table II

CANCER TYPE	N*	Sensitivity with 92% Specificity	Sensitivity with 100% Specificity	Efficiency with 92% Specificity	Efficiency with 100% Specificity
Breast Cancer vs. Benign	97C vs 22B*	93.8%	87.6%	95.0%	89.9%
Prostate Cancer vs. Benign	62C vs 77B**	88.7%	85.5%	76.3%	93.5%

* 97 cancer samples were compared to 22 benign tumor samples
** 62 cancer samples were compared to 77 benign prostate hyperplasias (BPH)
To calculate the efficiency, the prevalence was estimated at 0.25 for breast cancers compared to benign tumors and at 0.33 for prostate cancers compared to BPH (see Table III).

It is of particular interest the good discrimination between prostate cancer and BPH; in this study, the test picked up 88.7% of all cancers with 8% false positives among the BPH samples (92% specificity) or 85% of prostate cancers with no false positives among the BPH. It should be noted that PSA detects approximately 80% of prostate cancers with approximately 37% specificity (i.e. of the PSA positive samples, 2/3 are benign lesions).

The Receiver Operator Characteristics (ROC) curves for PSA and its variations are shown below:

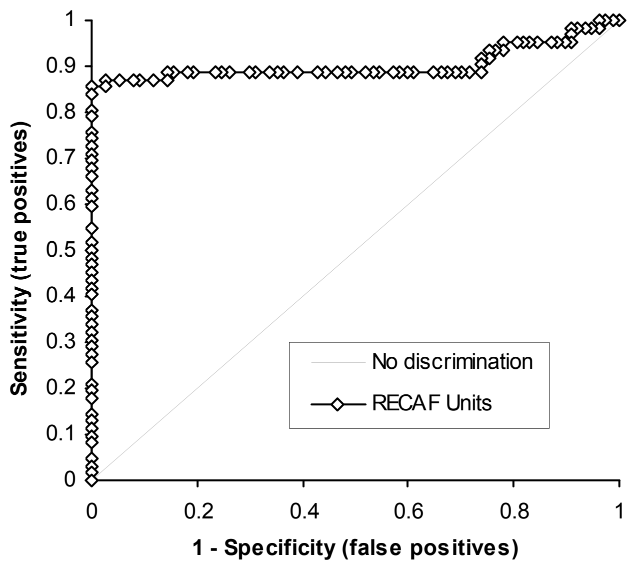


Receiver-operator characteristic curves comparing PSA, PSA density (PSAD), and transition zone PSA density (PSATZD) in the total PSA range. (AUC = Area Under Curve) (CAP=carcinoma of the prostate).

(Source: Prostate-specific antigen: current status M. K. Brawer CA: A Cancer Journal for Clinicians, Vol 49, Issue 5 264-281, 1999)

The ROC curve for the above shown RECAF data presents a significantly better curve (Figure 11). Please note that the closer the points align with a diagonal line, the worse the test discriminates malignancy from hyperplasia. A perfect discrimination is obtained when the curve forms a straight angle on the upper left side, parallel to the axes. The RECAF curve is well aligned with the axes.

Figure 11



In Conclusion:

The RECAF immuno-histology assay is designed as a tool to pinpoint cancer cells under the microscope, particularly when searching for lymph node metastases or in frozen sections and fine needle biopsies. There is a clear difference in the staining of cancer cells as opposed to normal or benign cells. The test is of particular interest for cytology (fine needle biopsies of breast and thyroid) as well as for Pap smears.

In serum, RECAF values were elevated in all the types of cancer studied (breast, prostate, lung, stomach and ovary). Altogether, these represent 50% of all cancers in Occident. We have not yet found a consistently negative type of cancer and therefore it is safe to assume that the assay should also work in other cancers as well. The assay detects approximately 90% of lung and breast cancers which are the two prevalent types of malignancies and for which most current markers perform poorly. The vast majority of the benign tumors studied were RECAF negative, which is advantageous for prostate cancer diagnosis since PSA has the drawback that it tends to be elevated in benign prostate tumors. Thus, the combination of a cancer specific marker such as RECAF with a tissue specific marker such as PSA could improve the specificity of the latter.

The fact that RECAF behaves as a rather sensitive and specific pan-cancer marker makes it a suitable candidate for routine screening and since only one test is required, the cost is minimal. Screening leads to earlier detection and this is, of course, closely linked to survival.

The results shown herein indicate that RECAF has the potential to become a cancer marker of clinical significance. To assess the full extent of that potential, more samples from these and other types of cancer, as well as benign lesions must be studied and therefore we welcome collaborations with colleagues interested in this field.

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