

Novel, Widespread Cancer Marker to Improve Cancer Diagnostics

Snapshot 08 / 8 / 2008

Symbol: BOCX.BB

BioCurex, Inc. ("BioCurex" or "the Company") is a biotechnology company developing products for cancer diagnosis, imaging, and therapy that target a global oncology market forecast to reach over \$50 billion by 2009. The Company uses a novel, patented technology based on the RECAFTM cancer marker, discovered by Dr. Ricardo Moro-Vidal, BioCurex's chief executive officer (CEO), president, and founder. RECAFTM is a molecule found on **malignant**^T cells but absent from most normal or **benign** cells. BioCurex believes that the detection of this molecule in tissue or **serum** samples is the foundation for new cancer diagnostic tests and imaging techniques. The RECAFTM marker has been found on every cancer tissue that BioCurex has studied to date, including on many common, severe cancers: breast, lung, gastric, prostate, and leukemia. This characteristic indicates that RECAFTM is capable of detecting more than one type of cancer, which is a limitation of many currently available cancer markers. The Company's RECAFTM technology is reported to have high levels of clinical **sensitivity** and **specificity** (measures of cancer marker accuracy), successfully detecting over 90% of cancer in blood and tissue samples. A serum-based **assay** using RECAFTM recently identified twice as many prostate cancers as the current standard for prostate cancer detection, the **prostate-specific antigen (PSA)** test. A RECAFTM-based test also detected 90% to 93% of **Stage I** and **Stage II** (early stage) breast cancer samples, without any **false positives**. This data supports BioCurex's aim of reducing human suffering through the early detection of cancer or cancer recurrence after treatment. Moreover, due to its ability to differentiate between cancerous and normal cells, RECAFTM technology may facilitate the administration of targeted therapeutics that attack cancer cells but do not damage healthy tissue.

Recent Financial Data

Ticker (Exchange)	BOCX (OTC.BB)
Recent Price (08/8/2008)	\$0.29
52-week Range	\$0.18 - \$0.88
Shares Outstanding Approx	43 million
Market Capitalization	\$12.5 million
Average Volume	76,000
Insider Owners +5%	16.5%
Institutional Owners	<1%
EPS (Yr. ended 12/31/07)	(\$0.08)
Employees*	14

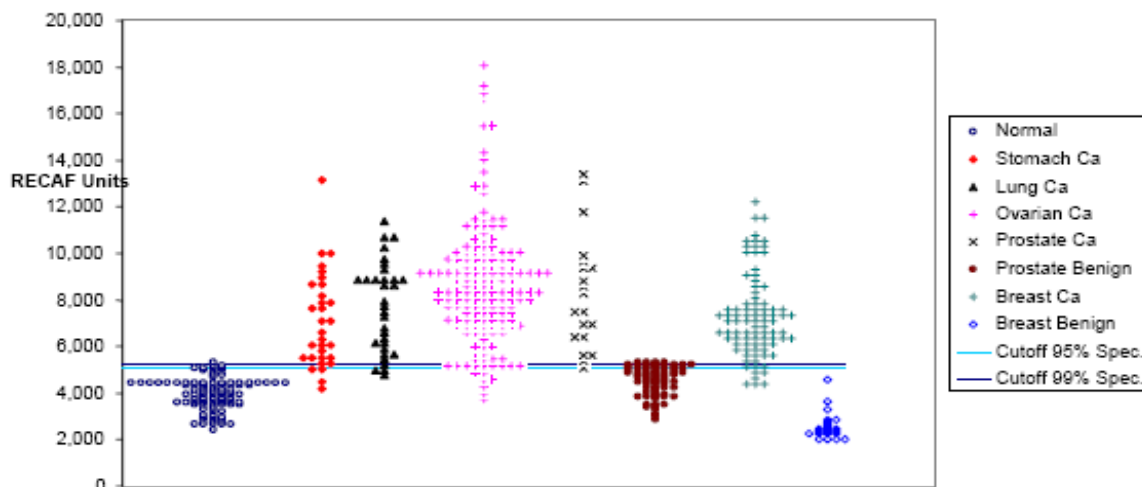


*Includes full time researchers at Pacific BioSciences Research Centre

Key Points

- BioCurex maintains semi-exclusive license agreements with **Abbott Laboratories** (ABT-NYSE) and **Inverness Medical Innovations**, Inc. (IMA-AMEX) to commercialize products using RECAFTM technology. These licenses benefit BioCurex through the payment of milestone fees and royalties, as well as by giving the Company access to independent diagnostic expertise and validation of RECAFTM.
- The Company has developed two diagnostic kits that identify cancerous tissue versus normal or benign tissue by targeting RECAFTM: (1) Histo-RECAFTM (approved in the U.S. as a **Class I Medical Device**); and (2) Cryo-RECAFTM. The Company is also finalizing Serum-RECAFTM, an assay to detect RECAFTM levels in a patient's blood. Other product areas include tumor imaging and the development of a Rapid Test for initial point-of-care cancer detection.
- As of 2005, global laboratory diagnostic tests exceeded \$25 billion each year, with molecular diagnostic testing growing by roughly 20% annually and estimated to be over \$5 billion by 2009. In the diagnostics market, cancer testing, which is dominated by serum-based cancer markers, recently exceeded \$2 billion and is expected to experience some of the greatest growth over the next three to five years.
- BioCurex's management team has brought a research technology toward commercialization in a relatively short timeframe while also negotiating beneficial license agreements. The Company's Scientific Advisory Board (SAB) includes leaders in the global cancer marker field.
- Patents in over 20 countries. Granted patents in the USA, the European Union, Russia and China.

Published in peer reviewed IVD Technology journal. The figure below illustrates the distribution of blood RECAF™ values in a variety of normal, cancer, and benign tumor samples. This figure highlights the variations in RECAF™ expression (RECAF™ units) that enable the identification of “normal” versus “cancer” or “benign.” The horizontal lines represent the cutoff values for 95% and 99% specificity. At the 99% cutoff, only 1% of normal samples fall above the line, whereas at the 95% cutoff, 5% of normal samples are above the line.



BioCurex, Inc.

SENSITIVITY AND SPECIFICITY WITH RECAF™ IN SEVERAL COMMON CANCERS

Cancer Type	Sensitivity with 95% Specificity (%)	Sensitivity with 99% Specificity (%)	Number
Ovarian	96	92	162
Stomach	90	87	31
Lung	91	87	32
Breast	93	90	88
Prostate versus Normal	99	95	20
Total	94	91	333
Breast benign*	0	0	22
Prostate benign*	25	5	77

*At the 95% cutoff value, a small percentage of benign breast and prostate samples were positive. Increasing the cutoff value to include 99% of normal individuals reduces the percentage of false positives, at the expense of a slight decrease in sensitivity.

BioCurex, Inc.

DETECTION OF EARLY STAGE BREAST CANCERS USING RECAF™

Cancer Stage	Sensitivity	Specificity	Number of Samples
Stage I	90%	100%	42
Stage II	93%	100%	45

DETECTION OF EARLY STAGE PROSTATE CANCERS USING RECAF™

Prostate Cancer Patient versus a Healthy Patient			
Cancer Stage	Sensitivity	Specificity	Number of Samples
Stage I and Stage II	75%	100%	84
Stage III	85%	100%	20
Stage IV	86%	100%	28

Poster presented at ISOBM 2007 (Prague)

POINT-OF CARE SERUM TEST FOR CANCER DETECTION BASED ON THE RECAF CANCER MARKER

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INTRODUCTION: RECAF is the receptor for alpha-fetoprotein (AFP). AFP is synthesized mainly by the embryonic yolk sac and liver and at some point, it is the main circulating protein in the foetus [1-5]. After birth, circulating AFP levels drop sharply, virtually disappearing in adult individuals [4]. Hepatomas, teratocarcinomas and some gonad cancers re-express AFP, which has led to develop diagnostic tests and to attempts to delivery anticancer drugs directly to the tumor cells [2,4], [6-8].

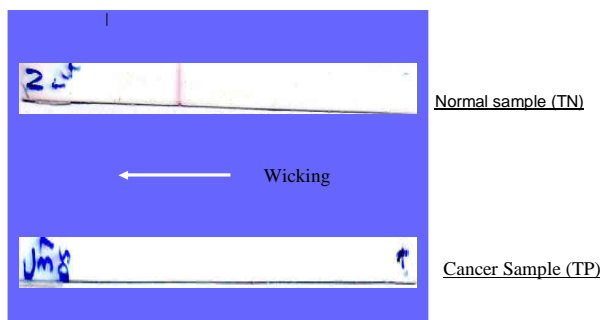
Foetal cells from tissues other than the liver and the yolk sac internalize circulating AFP. This uptake ceases when the foetal cells approach full differentiation, despite the high levels of circulating AFP [9-14] that might be present. Malignant cells regain the ability to internalize AFP [4,7] via a receptor (RECAF) [15, 16]. RECAF behaves like a widespread oncofetal antigen and it can be used for cancer diagnosis on tissue sections and serum samples [17].

In this study, we describe the results obtained with a Rapid Serum RECAF test we developed, on serum samples from normal individuals and from patients with ovarian cancer. It demonstrates that the rapid Serum RECAF test can be used as a point-of care cancer screening tool by physicians.

STUDY OBJECTIVE: To develop a rapid test for an ambulatory cancer detection based on a RECAF cancer marker.

METHODS AND SAMPLES: The lateral flow chromatography device used is based on a competitive RECAF immunoassay that has been previously shown to be highly sensitive and specific for cancer detection in serum samples. A thin line of an anti-RECAF monoclonal antibody at 1 mg/ml in PBS was traced onto a nitrocellulose strip and dried for 1 hour at room temperature. The samples were diluted 1/8 and mixed with 10 ug/ml of RECAF-Gold colloid. 50 ul of the resulting volume was then added to the end of the nitrocellulose strip, from where the wet front advances towards the antibody line by capillary action. One minute later, the presence of a red band over the antibody line means there is no detectable RECAF in the sample (Negative) while the presence of elevated RECAF in the sample is evidenced by the lack of the red band (Positive). The samples studied included 64 Normal controls, 51 serum from patients with ovarian cancer [25 samples at Stage I and II (b-c) and 26 samples at Stage III (b-c)].

	Total No.	TRUE	FALSE
Cancer ovaries	52	41	10
Normal	65	57	8
PPV	83.67		
NPV	84.85		
Sensitivity	80.4%		
Specificity	88%		

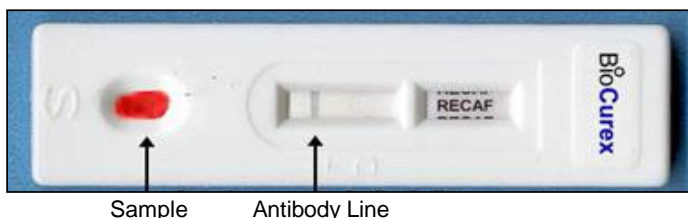


RESULTS: 80.4% of ovarian cancers (Stages I-III) were detected with 88% specificity

CONCLUSIONS: These results show that a rapid lateral flow chromatography test based on the RECAF marker can discriminate well between cancer and normal samples, which is consistent with previous results using a RIA format. When applied to the examples of early-stage ovarian cancer, the rapid test demonstrated better performance than CA-125. Once mechanically improved, the test might prove useful for a first approximation to the diagnosis of cancer at the doctor's office.

Note: A line appears when testing blood from healthy donors, no line using blood from cancer patients. Below: Complete cartridge.

PRELIMINARY RAPID TEST CARTRIDGE



Development of a Chemiluminescent Assay (CIA) for the Receptor of Alpha Fetoprotein (RECAF) to Separate Cancer from Normal Sera

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Abstract

Aims: A radioimmunoassay for the receptor to alpha fetoprotein (RECAF) from BioCurex separates normal specimens from wide variety of cancers. Our aim was to develop a non-radioactive immunoassay for the RECAF for the early detection and screening of cancer.

Methods: The RECAF CIA assay is a competitive assay that utilizes a IgM monoclonal antibody to RECAF (1.4G11) on the solid phase and an acridinium labeled Human Milk Protein RECAF conjugate. We perform the RECAF CIA assay by mixing diluted human sera with acridinium labeled RECAF, and adding the mixture to a 1.4G11-coated microtiter plate. Following incubation, the plates are washed and read in a chemiluminometer. The initial development of the assay focused on the blocking buffer's composition and pH. BioCurex performed initial experiments and confirmation testing. Then, BioCurex shipped the RECAF CIA assay to Abbott Laboratories for confirmatory testing.

Results: Our experiments demonstrated that the separation of cancer from normals were most affected by pH and by Tween 80 concentration. Verification studies using breast, gastric, and other types of cancers (n = 68) and normal samples (n = 52) had an area under the curve (AUC) for the ROC curve of 0.954 with a cancer/normal (C/N) ratio of 1.7. Initial experiments at Abbott Laboratories with prostate cancer (n = 8) and non-cancer samples (n = 16) had an AUC for ROC curve of 0.95 with a C/N ratio of 1.3.

Conclusions: We developed a non-radioactive RECAF CIA assay that separates multiple types of cancer from normal sera with a C/N ratio ranging from 1.3 to 1.7. Our future studies will focus on increasing the cancer/normal ratio to create a manufacturable RECAF CIA assay.

Introduction

AFP is one of the main circulating proteins in the fetus and can be internalized by fetal and malignant cells^{1,2}. Hence, RECAFTM behaves like a widespread oncofetal antigen³. Therefore, RECAF in tissue and blood can be used for cancer diagnosis⁴. Currently, RECAF is measured with the RECAF radioimmunoassay (RIA) from BioCurex, Vancouver, BC. However, a RECAF RIA assay is difficult to automate. On the other hand, a non-isotopic RECAF assay would allow the assay to be placed on automated instruments and increase the availability of the assay to clinical laboratories.

Objectives of this study were to develop and evaluate the performance of a competitive RECAF chemiluminescent immunoassay (CIA) using acridinium-labeled RECAF antigen. This is a first step toward automating RECAF determination in sera.

Materials and Methods

Assay format: Ninety six (96) well microtiter plates were coated with the 1.4G11 monoclonal anti-RECAF antibody. The plates were lyophilized and stored at room temperature for later use. Affinity purified RECAF antigen was coupled to acridinium (Ac RECAF). Before testing, serum samples were diluted 1:5 in assay diluent. The competitive RECAF CIA assay was carried out as follows: 50 uL of Ac RECAF at 400 ng/mL was mixed with 50 uL of cord serum calibrators or diluted serum samples. The mixture was then transferred to anti-RECAF-coated microtiter plates and incubated for 2 hours at 37°C. The RECAF CIA plates were washed, trigger was added and chemiluminescence was determined. **Figures 1 and 2** demonstrate the assay principle.

Statistics: The RECAF CIA assay results were analyzed using Analyze-It (v 1.73, 2000) for ROC curve analysis and C/N ratio. The MedCalc (v 9.3.2.0, 2007) statistical software was used to generate box and whisker plots.

Assay Diluent Optimization: Our experiments demonstrated that separation of cancer from normal RECAF values were most affected by pH and by detergent concentration.

Experiment 1: In BioCurex facilities (**Figure 3**), the RECAF CIA assay with optimized assay diluent was used to quantitate RECAF values in sera from patients with breast, ovarian, kidney, stomach, lung, thyroid, stomach, intestine, uterus, prostate, cervix, and testicular cancer from Russia (n = 68) and in normal serum samples from Canada and Russia (n = 52).

Experiment 2: At Abbott Laboratories (**Figure 4**), the RECAF CIA assay with optimized assay diluent was used to quantitate serum RECAF levels in stage III prostate cancer (n = 8), benign prostatic hyperplasia (BPH; n = 8), and normal specimens (n = 8). The comparison of Abbott and BioCurex ROC curves are shown in **Figure 5**.

Figure 1: RECAF CIA Competitive Assay Principle with No RECAF Present

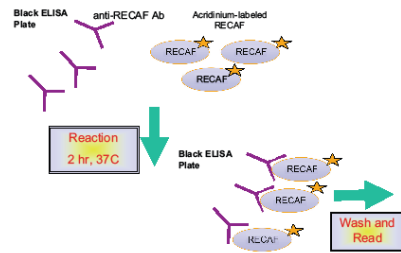
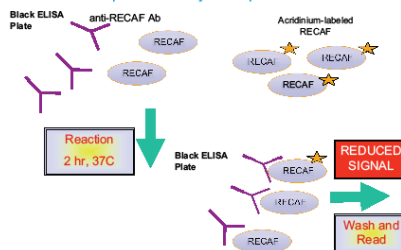
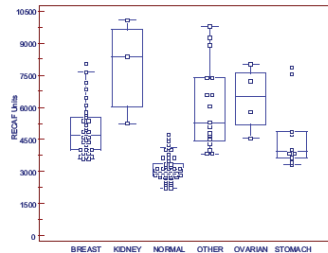


Figure 2: RECAF CIA Competitive Assay Principle with RECAF in Sample



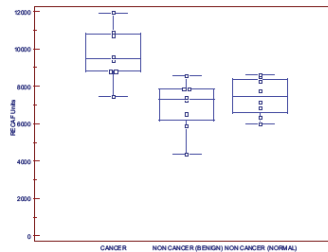
Results

Figure 3: RECAF CIA Assay at BioCurex: Cancer vs Normal Sera



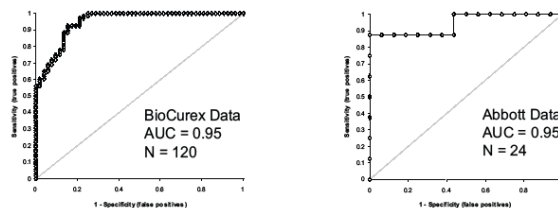
Multiple solid tumor cancers have elevated RECAF values compared to normal samples with a C/N ratio is 1.7. Other cancers include lung, thyroid, stomach, intestine, uterus, prostate, cervix, and testicular cancer.

Figure 4: RECAF CIA Assay at Abbott: Prostate Cancer, BPH, and Normal Specimens



RECAF units for Prostate Cancer samples are elevated compared to BPH and normal specimens. The C/N ratio is 1.3.

Figure 5: ROC Curves from RECAF CIA Assay from BioCurex and Abbott Laboratories



Similar AUC for ROC curves were obtained at both BioCurex and Abbott Laboratories.

Conclusions

The results obtained in both BioCurex and Abbott facilities are consistent. The RECAF CIA assay can discriminate normal from cancer specimens with:

- AUC of 0.95
- C/N ratio ranging from 1.3 to 1.7

The availability of a non-isotopic assay:

- Makes the transfer of the RECAF CIA assay to other sites easier
- Allows for independent validation of our results
- Facilitates the automation of a RECAF immunoassay

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