To: constance.cullen@spcorp.com[constance.cullen@spcorp.com]
Cc: Carolyn Balkenhol[cbalkenhol@theranos.com]; Gary Frenzel[gfrenzel@theranos.com]
From: Elizabeth Holmes[/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=EHOLMES]
Sent: Wed 11/12/2008 9:53:15 PM (UTC)
Subject: Follow up to our call
Theranos_Novartis_Bone Study_Inflammation_Report.pdf
Theranos Evaluation Summary_GSK Biomarker Lab.pdf
Excerpts from Theranos TNONC Angiogenesis System Validation_Final Study Report.pdf
Theranos_Methodology Confidential AML_Sepsis Paper Excerpts.pdf

Connie,

Great to connect. We have attached several reports from programs we have done with other pharmaceutical companies.

We are looking forward to hearing your thoughts on which assays will be most relevant for the inflammatory agents' upcoming trials.

Elizabeth.

Elizabeth Holmes
President and CEO
Theranos, Inc.

650.470.6111
650.838.9804

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Summary Report: Novartis Bone Study

Summary of Study:

- Small amounts of a statistically significant number of blinded archived plasma samples from a Reclast study were sent to Theranos and run on Theranos Systems.
- Information provided to Theranos:
    - sample ID
    - patient initials
    - time points (0, 24 and 72 hours).
- Each sample was run on a multiplexed cartridge customized to run IL-6, CRP, hsCRP, and TNF-α simultaneously across a broad dynamic range.
- Results were summarized in excel and sent back; Novartis shared their results from PBI (a current 'gold standard').

Background on the Theranos System:

- Theranos is a fully integrated point-of-care system designed to work remotely and wirelessly transmit results from a finger-stick of fresh whole blood or assays run on plasma or serum.
- Up to 6 assays are run simultaneously in a multiplex on a single cartridge with on-board controls (the gold standard used by Novartis in this study is not a multiplex and requires considerable amount of sample for each assay).
- Theranos IL-6, TNF-α and CRP assays cover the entire range for high sensitivity and regular sensitivity in one assay system, no need for multiple kits or dilutions.
- Theranos limit of detection is lower than comparable methods.
    - IL-6 average LOD = 0.2 pg/mL, range = 0.2 pg/mL to 1500 pg/mL
    - TNF-α average LOD = 0.65 pg/mL, range = 0.65 pg/mL to 15,000 pg/mL
    - CRP average LOD = 0.06 ug/mL, range = 0.06 ug/mL to 250 ug/mL
- Theranos systems are factory calibrated, no standard curves or in-house calculations are required.
- Theranos systems can be calibrated to Novartis' gold-standard and predicate results using the present data if desirable (the system was calibrated to different standards than Novartis used for this study).

Summary of Results from Theranos Systems and PBI

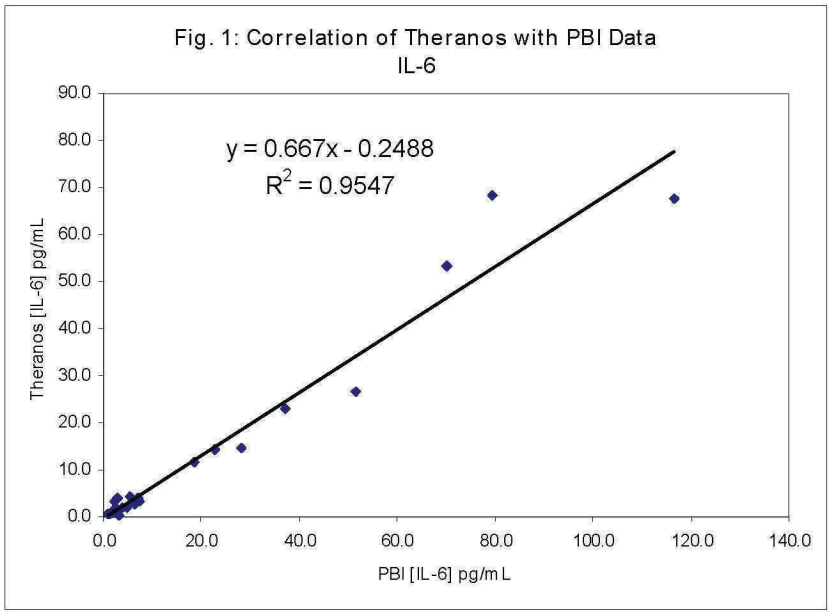
			Theranos			PBI		
Barcode#	Initials	Visit (HR)	pg/mL [IL-6]	pg/mL [TNFa]	ug/mL [CRP]	pg/mL [IL-6]	pg/mL [TNFa]	ug/mL [CRP]
A5673052-6	DMC	72	1.0	1.8	45.8	1.5	3.1	39.1
A5673206-6	NLL	0	0.8	0.8	0.1	1.3	1.9	0.4
A5673050-6	DMC	0	0.8	1.1	4.3	1.1	2.2	3.1
A5673051-6	DMC	24	14.3	5.3	41.0	22.6	8.7	40.2
A5673207-6	NLL	24	1.8	1.2	0.1	2.6	1.7	0.4
A5673208-6	NLL	72	0.5	0.8	0.1	3.3	1.8	0.4
A5803961-6	00011	0	1.5	0.9	11.6	2.1	2.8	13.2
A5803962-6	00011	24	67.7	5.1	56.9	116.6	7.6	80.8
A5851956-6	00011	72	1.9	3.0	70.2	3.7	NES*	89.0
A5851956-6	JCB	24	14.7	2.8	28.8	28.1	4.3	24.7
A5851957-6	JCB	72	1.4	1.1	23.1	2.8	2.3	32.6
A5851955-6	JCB	0	1.1	OOR**	0.8	2.1	1.3	0.8
A5980440-6	NLS	0	2.7	0.9	4.7	6.3	1.6	3.4
A5980441-6	NLS	24	26.7	2.5	18.8	51.5	5.1	17.6
A5980442-6	NLS	72	3.4	1.9	83.6	7.5	3.6	82.7
A5673154-6	SJS	0	0.8	0.8	0.7	2.0	1.5	0.9
A5673155-6	SJS	24	1.5	0.6	1.3	2.4	1.9	0.9
A5673156-6	SJS	72	2.1	2.4	8.3	4.8	1.7	6.9
A5803964-6	SSP	0	1.1	2.6	9.9	2.1	1.8	11.0
A5803965-6	SSP	24	53.5	8.9	49.9	70.0	9.0	76.8
A5803966-6	SSP	72	1.5	2.8	59.8	2.8	4.2	69.5
A5673232-6	GAB	0	3.8	1.9	1.0	2.8	2.8	0.9
A5673233-6	GAB	24	68.3	7.1	25.5	79.4	10.3	24.7
A5673234-6	GAB	72	3.4	3.9	34.7	2.1	4.4	26.8
A5673462-6	GAS	0	0.7	1.9	1.6	1.0	1.3	1.3
A5673463-6	GAS	24	11.5	3.1	16.4	18.6	2.9	20.0
A5673464-6	GAS	72	4.0	2.0	60.7	7.2	2.9	56.2
A5673091-6	MAD	0	1.1	7.9	5.2	1.7	2.1	4.6
A5673092-6	MAD	24	23.1	14.9	33.3	37.3	9.1	45.9
A5673093-6	MAD	72	4.3	12.9	102.2	5.3	4.7	75.4

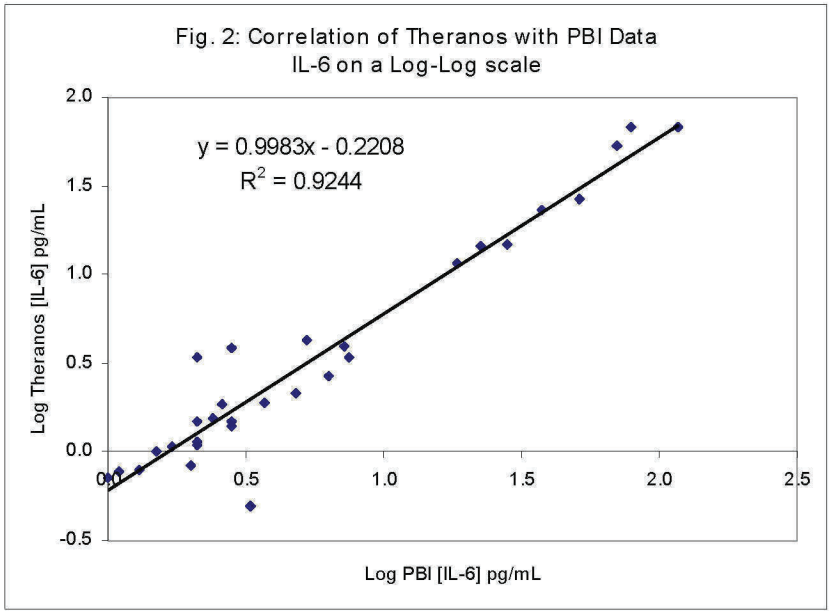
* NES = Not Enough Sample
** OOR = Out of Range (Low)

Analysis of Data

Correlation of IL-6 data from Theranos Systems and PBI

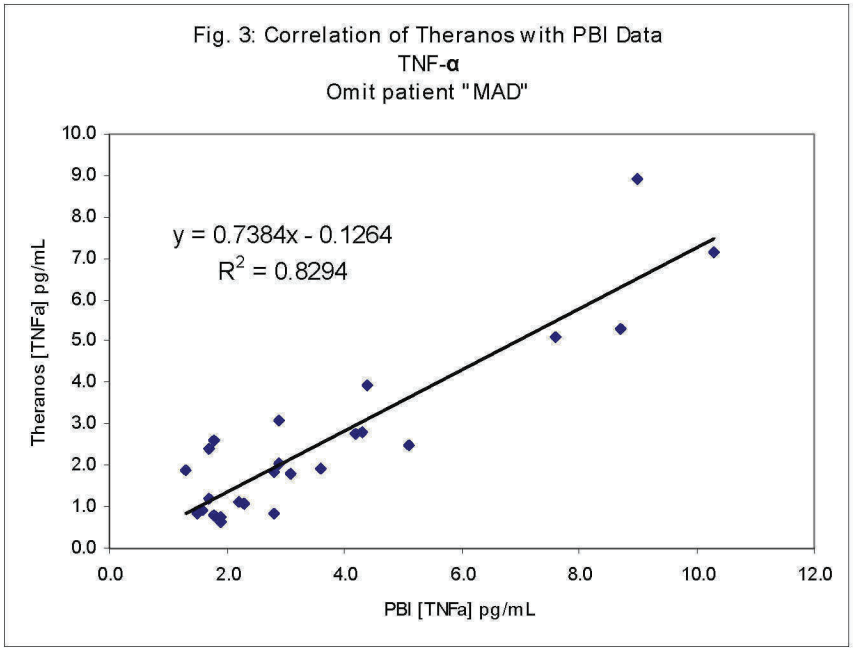
- Correlation between Theranos and PBI results was high (Fig. 1)
- Since the samples spanned a wide range, a log-log plot was used to analyze the correlation across the range of the assay – correlation on the log-log plot was also high (Fig. 2).





Correlation of TNF-α data from Theranos Systems and PBI

- Correlation between Theranos and PBI results was high (Fig. 3).
- TNF-α values for 90% of samples were less than 5 pg/mL.



Correlation of CRP data from Theranos Systems and PBI

- Correlation between Theranos and PBI results was high (Fig. 4).
- Since the samples spanned a wide range, a log-log plot was used to analyze the correlation across the range of the assay – correlation on the log-log plot was also high (Fig. 5).

Fig. 4: Correlation of Theranos with PBI Data
CRP

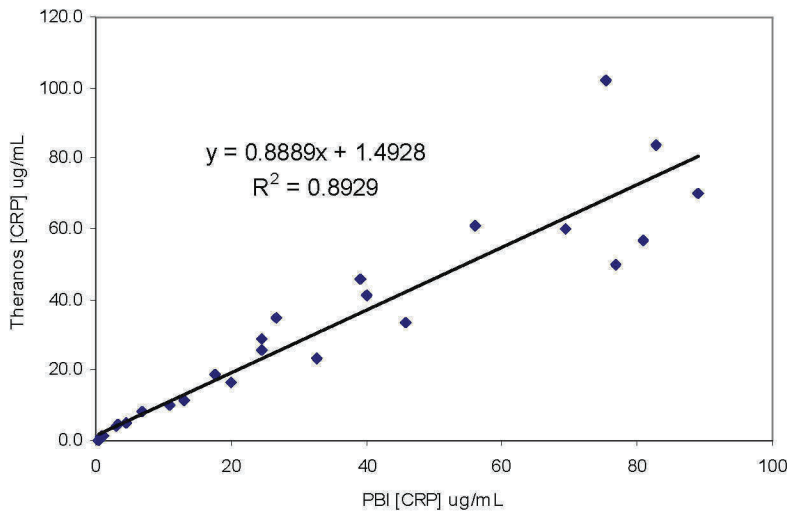


Fig. 5: Correlation of Theranos with PBI Data
CRP on a Log-Log scale

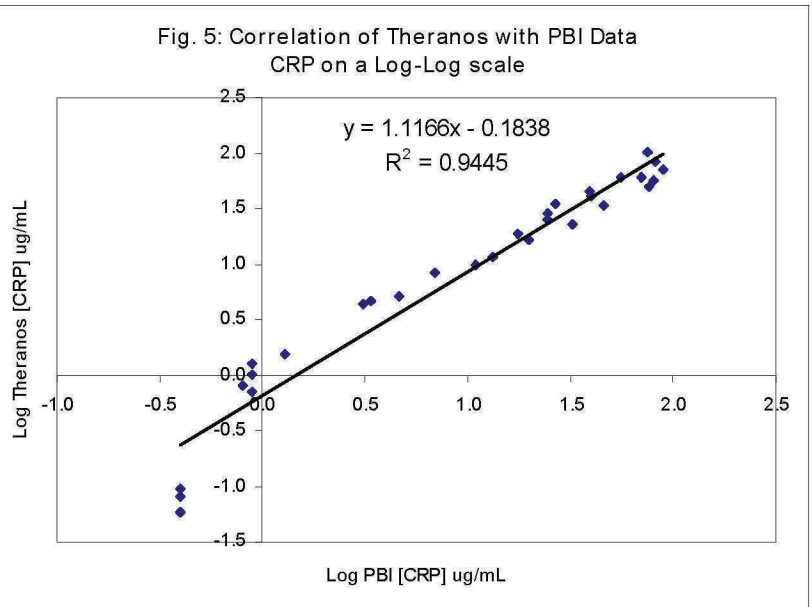


Table 1: IL-6 results from Theranos Systems and PBI for 10 patients at 3 time points

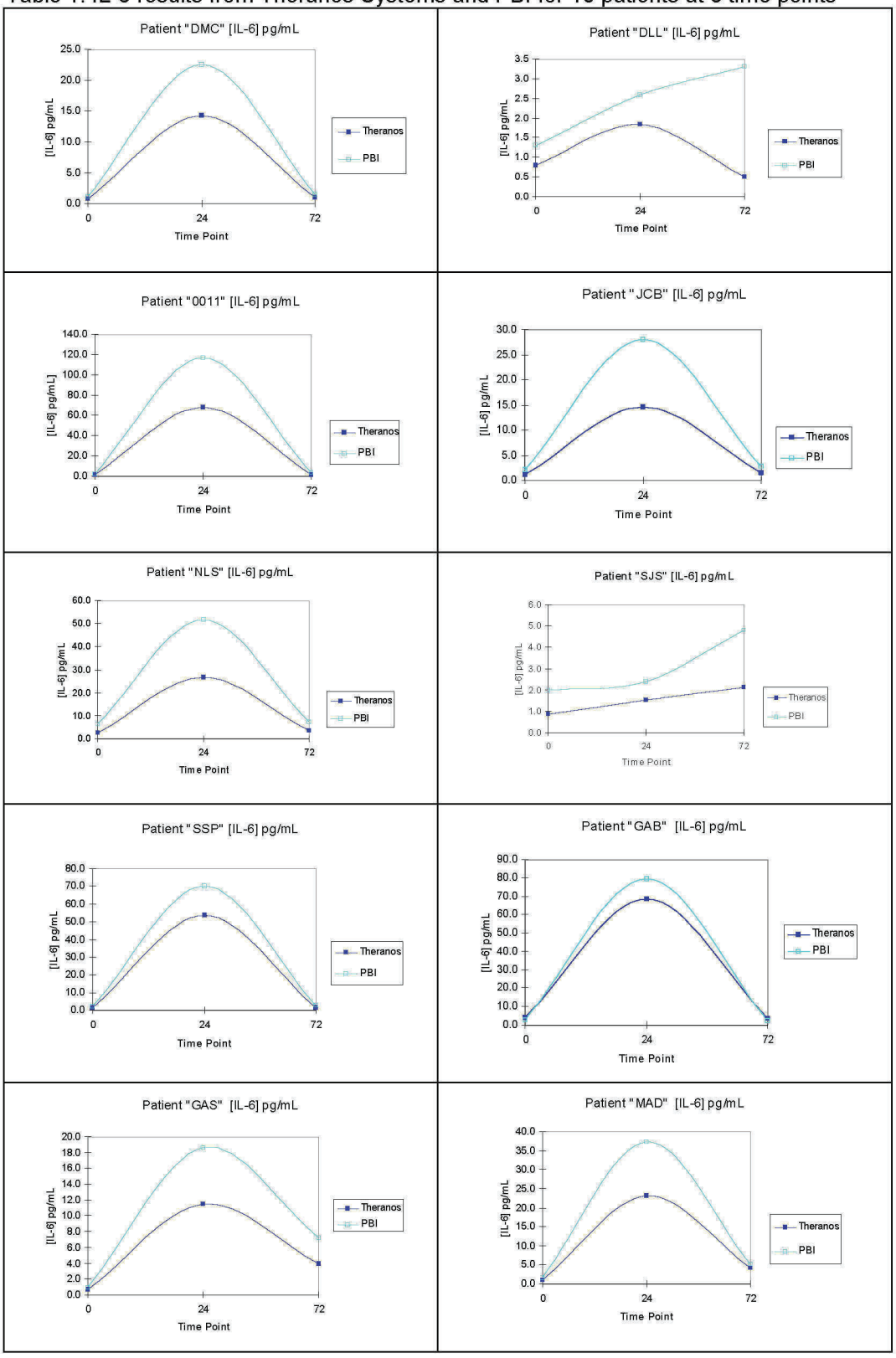


Table 2: TNF-α results from Theranos Systems and PBI for 10 patients at 3 time points

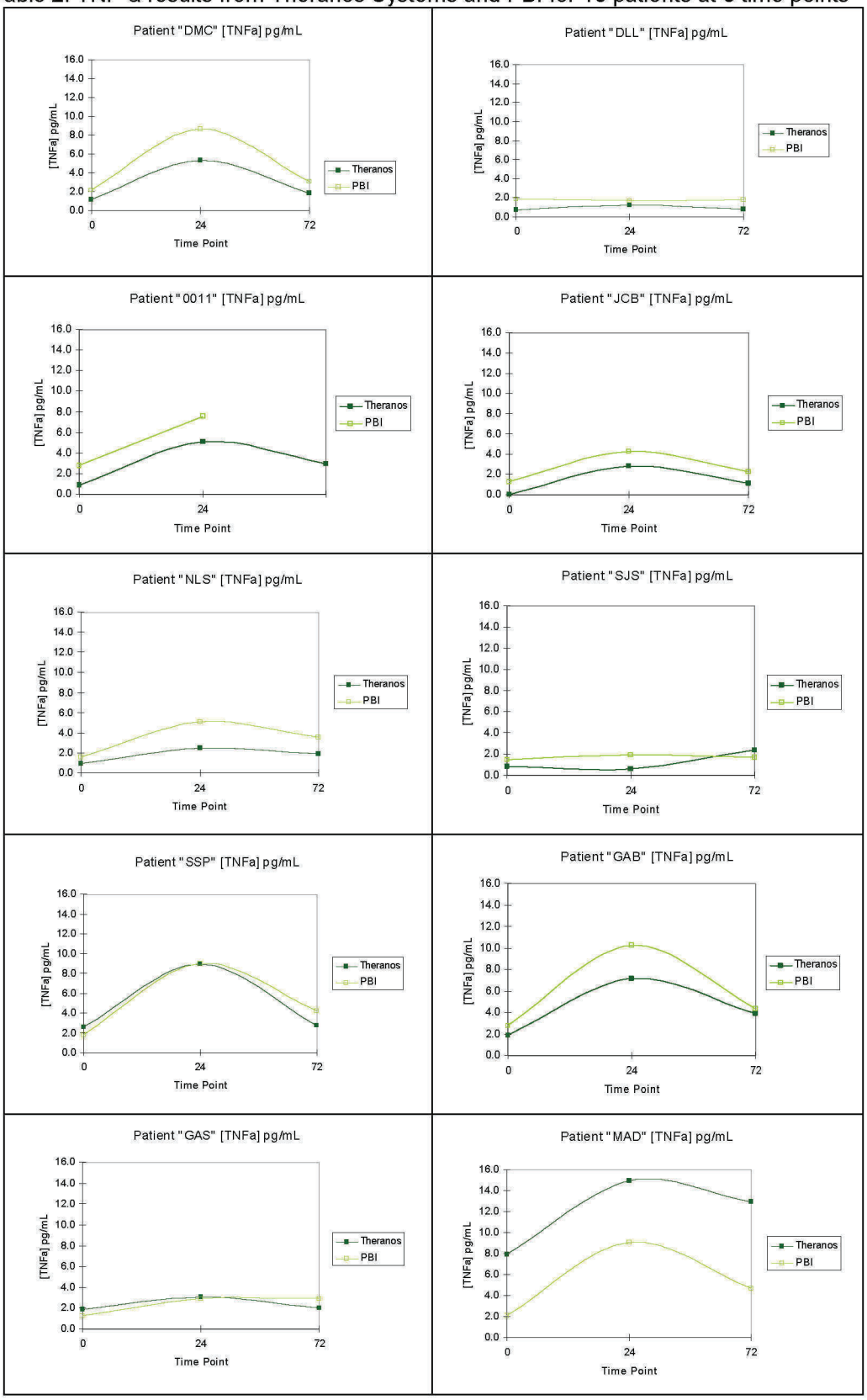
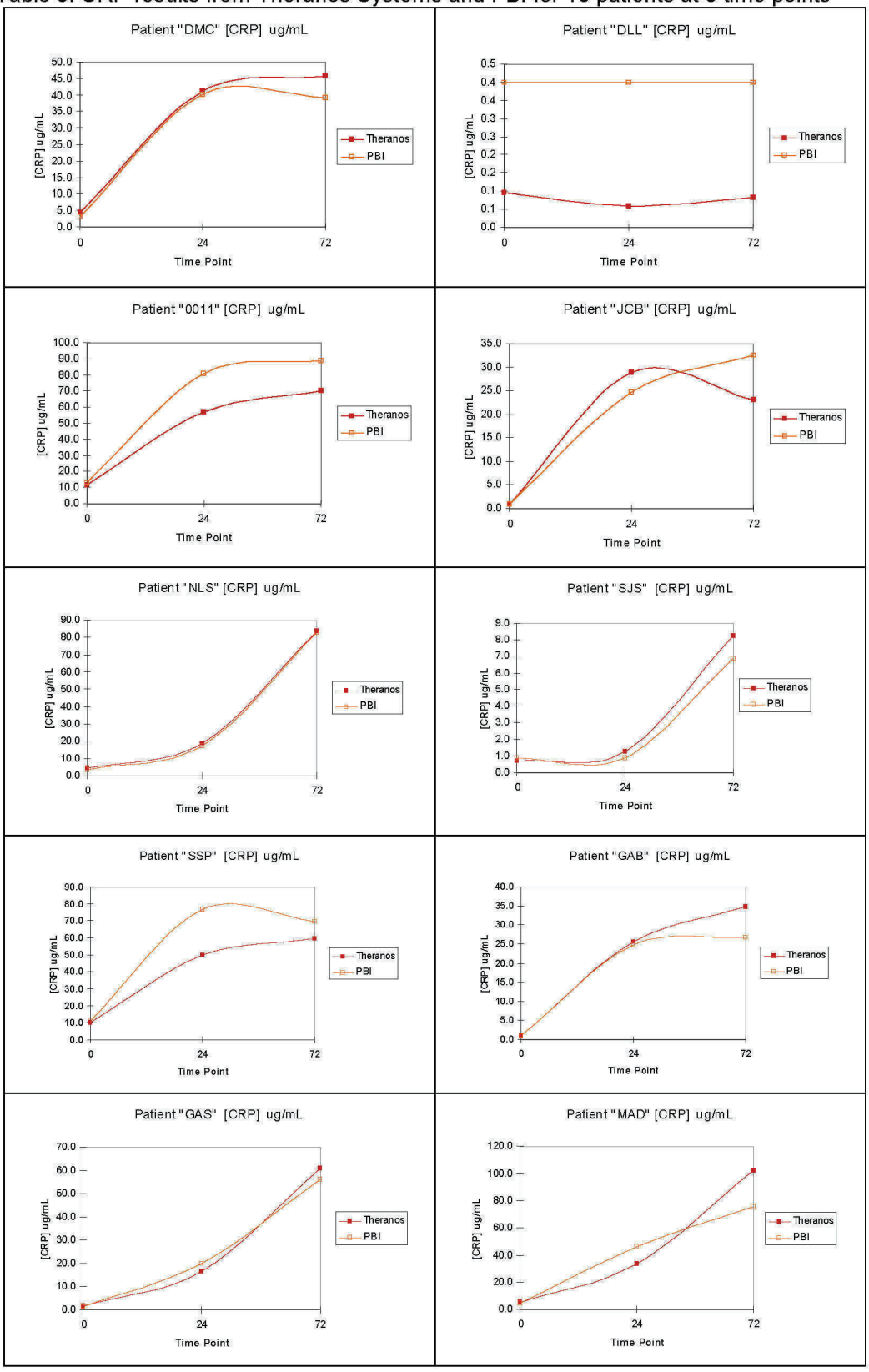


Table 3: CRP results from Theranos Systems and PBI for 10 patients at 3 time points



Excerpts from GSK Metabolic Study Report

Nelson Rhodes, Director GSK Metabolic Biomarker Laboratory
Surekha Gangakhedkar, Theranos Assay Systems Lead

Background information:
The Theranos system was evaluated at GSK to profile active GLP-1 and C-peptide values and these data were compare to "gold standard" ELISAs using frozen human plasma from study XXXXXXX. The key project objectives (found in the attached statement of work) were:

- To assess the performance of the Theranos System in measuring a multiplex for GLP-1 and c-peptide values (the "Cartridge Analytes") as compares to the current gold standard ELISAs (which are not multiplexed).
    - Specifically, the study will assess Theranos' capabilities to detect points that the reference assays failed to accurately detect by running samples with C-peptide values in a standard range (ng/mL) and GLP-1 values between 0-3.2 pM
- To assess the functionality, specificity, reproducibility, accuracy, and precision of the Theranos System.
- Assess the Theranos data reporting and transfer functions

Thirty plasma samples (assayed in duplicate) were chosen based on historical GSK data for total GLP-1 levels from subjects given a mixed meal and two finger prick blood draws were performed. Five Theranos machines were used with active GLP-1 and C-peptide cartridges that required 20µL of plasma. MesoScale Discovery's (MSD) active and total GLP-1, Linco (Millipore) active GLP-1, and Linco (Millipore) C-peptide ELISAs were run as comparator assays.

GSK Metabolic Biomarker Lab comments:

- Data show good correlation
    - $r^2 = 0.90$ for GLP-1 (MSD vs. Theranos)
    - $r^2 = 0.96$ for C-peptide (Linco vs. Theranos)
- Inter-instrument precision (RLU average %CV = 11)
- Machines worked well
- Touch-screen interface was easy to use
- Cartridges were pretty straight forward (easy to handle and load)
- Assays took approximately 1 hour and 15 minutes per cartridge

Overall conclusions:

- The Theranos system eliminates the need for a lab and provided quality data
- The Metabolic Biomarker Lab has a favorable impression of the technology/system and recommends GSK clinical groups to work with Theranos

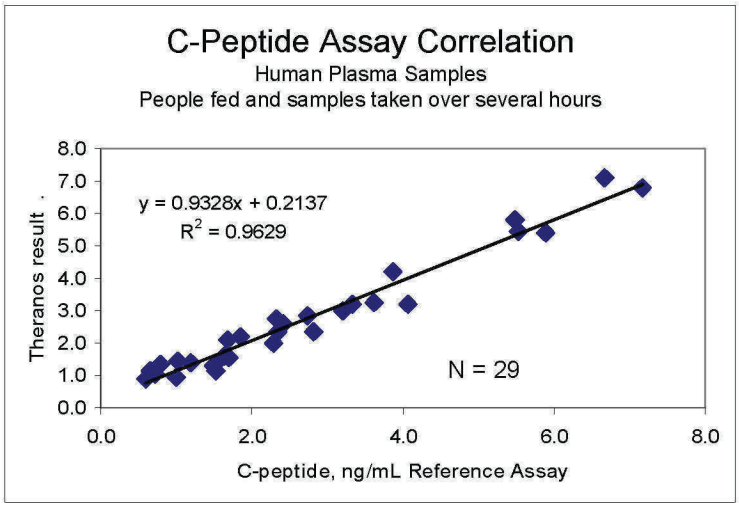
Data:

Study design

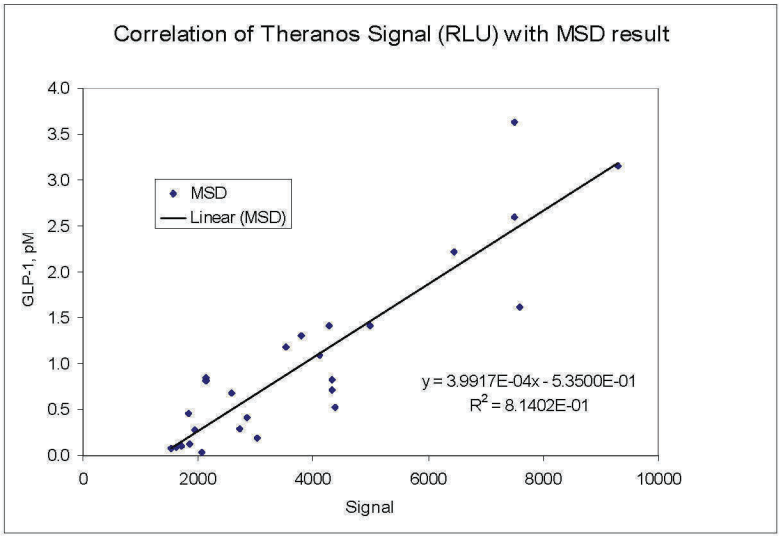
- Human subjects
- Food “challenge”
- Measure GLP-1 and C-Peptide multiplex over 5 time points
  - Linco Assay
  - MSD Assay
  - Theranos Assay

C-Peptide Assay

Averaged results

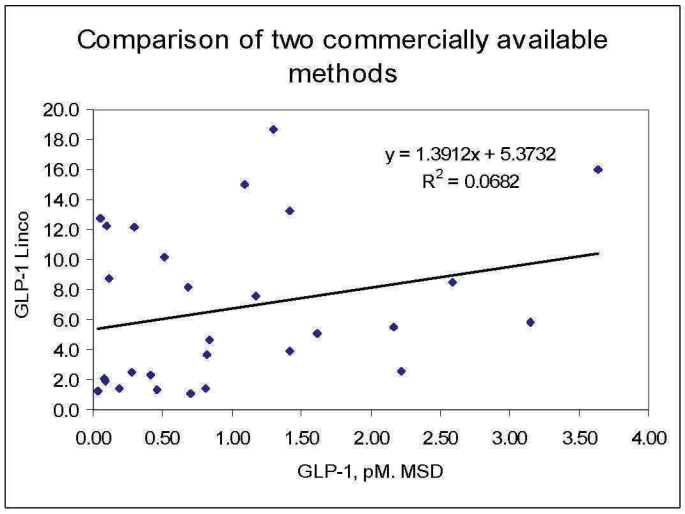


Calibration to GSK matrix



Theranos Confidential

Lack of correlation of predicate methods



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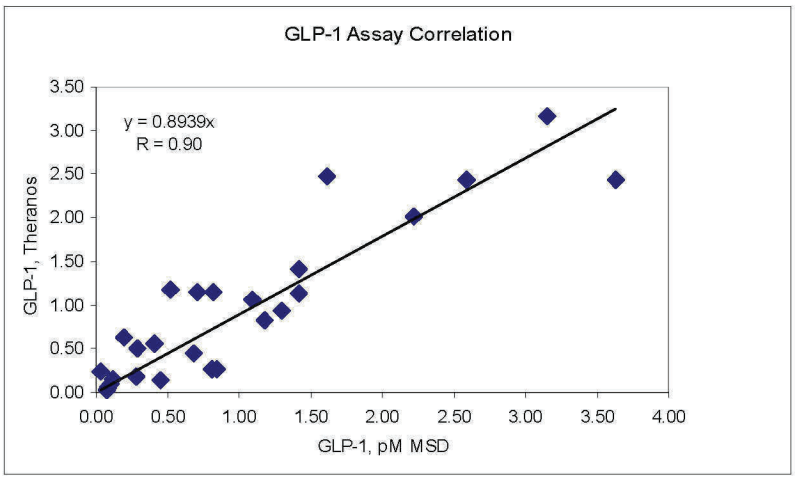
Assay correlation



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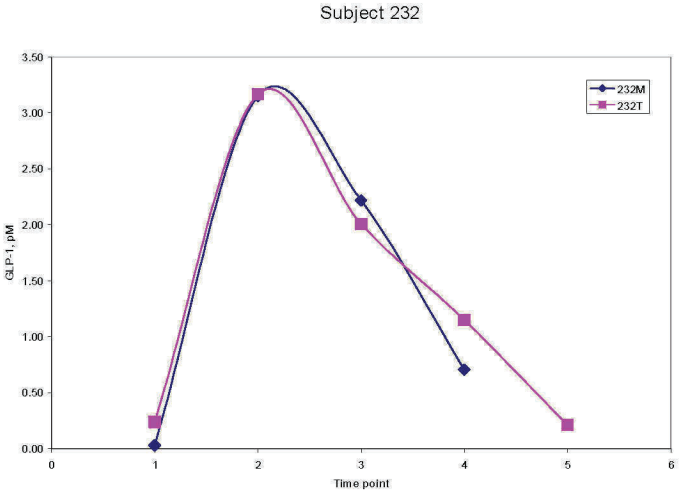
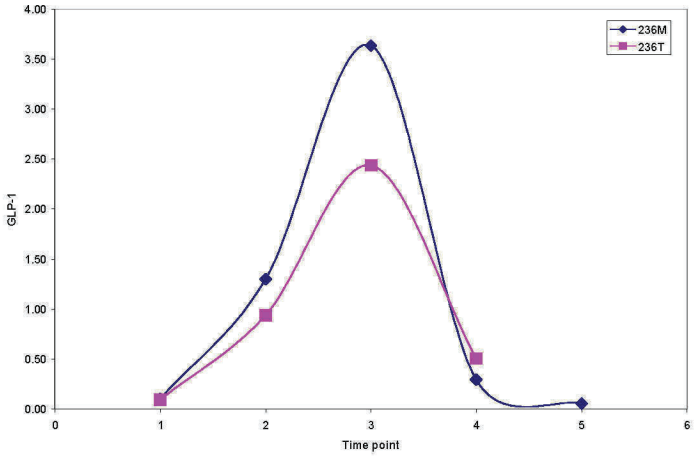
Subject 232



Theranos Confidential

Subject 236



Theranos Confidential

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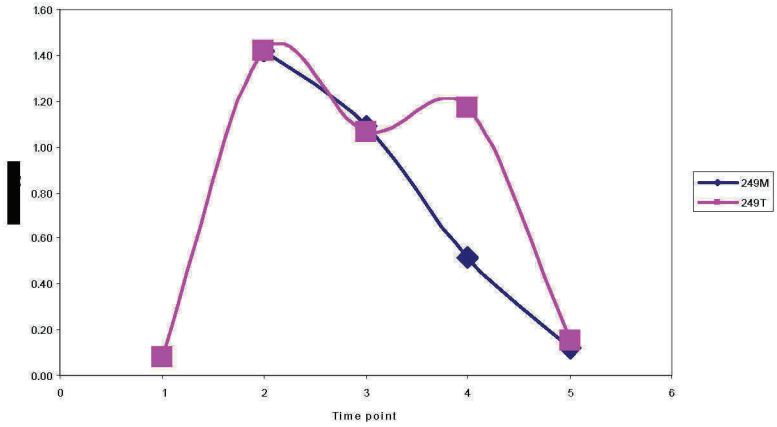
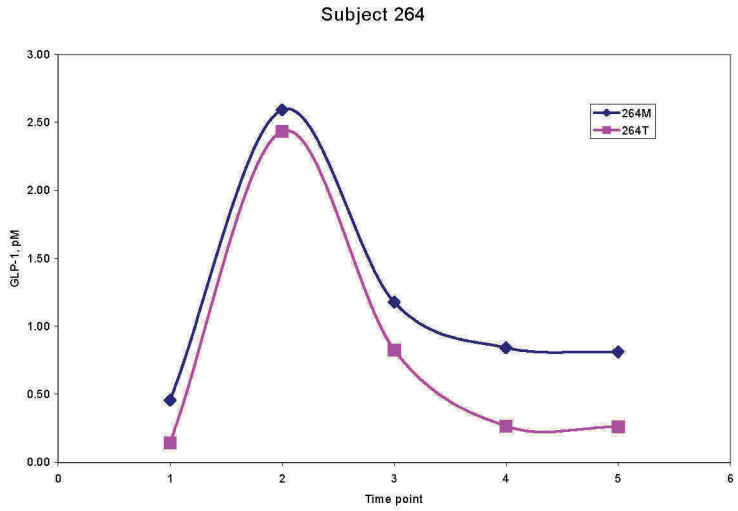


Theranos Confidential

Subject 264



Summary Statistics GLP-1 Comparison

- Theranos LOD = 0.17 pM
- Dynamic range measured: 0-3.2 pM
- Mean = 0.9 pM (Th), 1.0 (MSD)

Excerpts from Theranos Angiogenesis Study Report

Document Outline:

- Angiogenesis Study design
- Theranos System Overview
  - Specifications
  - Theranos System Performance
- Theranos Field Study
  - Field Performance Overview
  - Trial Data
  - Evaluation of time course results from individual patients run through Theranos model
  - Review of generated data, in aggregate by patient ID, sex, cancer type, treatment, etc.
  - Integrated patient information, including date and time of monitoring, medication received, self evaluation of overall health status of each patient and other clinical data in a comprehensive format
  - Assessment of the technical performance of the Theranos System
    - Data transmission % success and mode of transmission used
    - General performance information as logged via the Customer Care line
    - Assessment of patient compliance with protocol
  - Summary of patient assessment of the Theranos System and the Client Solutions team via end-of-study surveys
- Conclusions
  - General
  - Technical
  - Economic

Angiogenesis Study Design:

In July of 2007, the first patient was enrolled in the trial. This trial consisted of very ill late-stage (4^{th} line) cancer patients with various tumor types receiving a variety of therapies at the Sarah Cannon Research Center at Tennessee Oncology (TNONC) in Nashville, Tennessee. The patients in the study typically resided in very remote locations across the eastern US. Almost all patients were not computer literate, and most were from low income families, unable to afford private telephone service.

The Theranos angiogenesis monitoring system was evaluated for clinical efficacy and as a means of more accurately and effectively monitoring cancer therapy and the progression of solid tumor cancers from a mechanism-of-action perspective. 32 patients were enrolled. Various cycles of therapies were monitored as well as physical changes in tumor size.

Patient installations and shipments for this study were done on-demand. As part of the installation procedure, Theranos' client solutions team has performed at-home installations and pick-ups for many weak patients.

For each patient, a total of up to 14 time points were collected during the month-long analysis period, 3-4 time points taken at the clinic and the other 10-11 time points taken in-home. Both finger-stick and venous samples were taken during each clinic visit, while only finger-stick samples were run in-home. The venous draw samples were run on the Theranos System in the clinic at the time of the draw; these samples were also processed so that the plasma and/or serum was analyzed using a reference method.

Venous samples were processed using reference methods and provide an archive of 41 anti-coagulated plasma and serum samples which were frozen and have subsequently been analyzed at Theranos.

Theranos System Overview:

The Theranos System is comprised of consumer-oriented readers, single-use cartridges containing assay chemistry and controls, and a data collection system that communicates through cellular networks with the instrument to provide assay protocols and to compute and display results.

The steps required of a new patient are to 1) take the machine out of the box and 2) plug it into a power source. The touch-screen then walks each patient through the process of poking his/her finger, depositing blood into the cartridge, and placing the cartridge in the reader drawer. The instrument then processes the assays and sends the data through the cellular network in real-time to a secure web-portal.

Theranos Systems allow for quantitative, multiplexed longitudinal time-series measurements to map correlations between the rate of change of blood-borne markers over time to surrogate and clinical end-points.

Specifications:

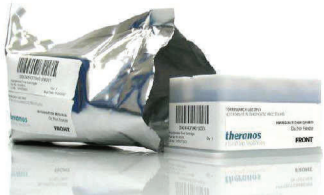
- Designed for at home use. Can also be used in physician's offices, ICU, and laboratories.
- Multiplexed measurement of biomarkers.
- Customizable for different/new assays on demand.
- Average 6 measurements per cartridge
- Serial measurements to comprehensively profile pharmacodynamic response through trends
- Runs fresh whole blood, plasma or serum samples
- Finger-stick – small sample size
- Mix and match selection of analytes on demand.
- Wide measurement range
  - pg/mL – mg/mL (1 billion fold)
- High sensitivity
  - 0.2 pg/mL (2 parts per 10-billion)
- Analyte Recovery: ~100 %
- System CV post-calibration (inter-intra reader, cartridge, and assay): < 10 %
- On-board chemistry controls
- Factory calibration (no user calibration)
- Wireless communication of results to appropriate user through cellular network
- Proprietary algorithms to interpret time trend results

The existence of a technology infrastructure for home, real-time blood monitoring allows collection of information which cannot be obtained using conventional blood testing scenarios:

- Small sample (finger-stick) + more frequent sampling of a small subset of analytes enables:
  - Identification of appropriate analytes (greatly helped by more frequent sampling)
  - Earlier detection of efficacy and safety and acute problems so intervention (for example, dose modification or change in drug type) can be more effective
  - Convenience of monitoring through-out a time-course before an event
- Higher sample integrity; real-time sample analysis on fresh whole blood on a standardized platform which can be deployed at any location (world-wide) eliminates

assay inaccuracy associated with commercially available tests performed on samples which are "old" by the time they are analyzed.

- Elimination of erroneous results (caused by analyte instability) and inherent errors in data and patient correlations (caused by processing data at various contract locations)



For this study, an instrument was deployed in the home of each patient; four others were installed at the Cancer Center.

Three assays were performed simultaneously in multiplex by the system on a finger-stick sample of fresh whole blood. The analytes were Vascular Endothelial Growth Factor (VEGF), soluble VEGF receptor R2 (sVEGFR2, usually referred to as VEGFR2) and Placental Growth Factor (PLGF). Each assay was controlled using within-cartridge control measurements.

The system was calibrated at Theranos. Multiple cartridge lots were produced each with successively more clinically relevant specifications once samples were received from patients in the trial, as samples were not available during assay validation. Each lot was independently calibrated.

Traceability of calibration: Calibration was traced to authentic analytes dissolved at known concentrations in a plasma-like matrix. Calibration materials are prepared as mixed solutions of the three analytes. Assignment of calibrator concentrations is then made to values found for measurements of calibrators using reference assays.

System Performance Goals:

Assay	Reportable low pg/mL	Reportable high pg/mL	Precision CV, %
VEGF	20	10,000	10
VEGFR2	150	15,000	10
PLGF	5	1,000	10

Assay ranges achieved:
The client goals for each assay's dynamic range were achieved. Due to the inability to receive samples for calibration before the beginning of the studies, the upper limit of calibration for VEGF was restricted to 3,000 pg/mL in the first cartridge lots, but then extended[1] to 10,000 pg/mL. For

[1] All three assays have a linear dose-responses extending far above the highest calibrator used.

early cartridge lots the PLGF assay lower limit of sensitivity was set for 50 pg/mL. When the client realized the need to measure below 20 pg/mL, new lots produced which have reportable ranges below 20 pg/mL.

Specificity:
The specificity of the assays depends on the pairs of antibodies chosen for each assay. In the first instance, Theranos relies on the antibody vendor information. Selected pairs are known to have good specificity in ELISA assays. Key issues for these analytes are (1) the structural relationship of VEGF and (2) the fact that VEGF binds to sVEGFR2. We have shown that the Theranos assay system is not affected by the presence of VEGF and VEGFR2 and PLGF in the same samples. In many patients in this study, the drug Avastin is used. This drug is an antibody that binds to VEGF. It is obvious that ELISA assays for VEGF (and perhaps VEGFR2) using antibody pairs are likely to be interfered with by Avastin.

As documented below, Theranos developed and validated assays for VEGF and VEGFR2 for this client which function with minimal interference from Avastin. In contrast, the gold-standard reference assay for VEGF is strongly interfered by Avastin.

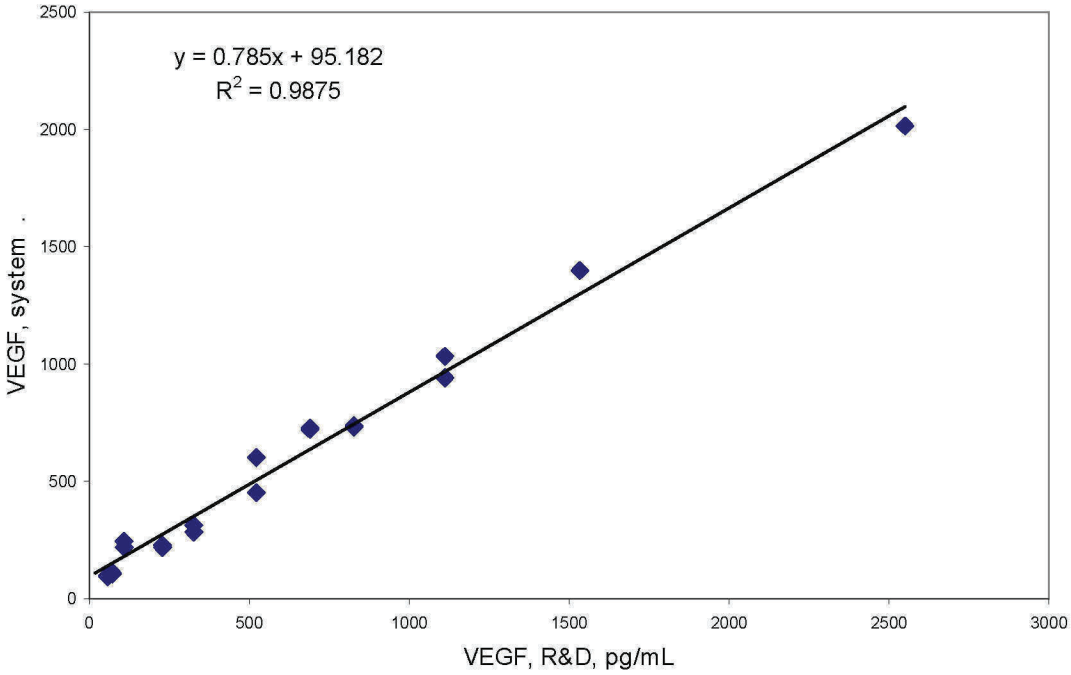
Theranos System Performance:

Assay accuracy:
Accuracy has been evaluated by analysis of clinical samples. Two sets of samples have been used: (1) A set of 12 serum samples from cancer patients (obtained from a commercial vendor), (2) 41 archived serum and plasma samples from this study. Because Avastin was used to treat many of the patients in the TNONC study and this antibody strongly interferes with the reference method, we used the commercially available samples for VEGF assay evaluation.

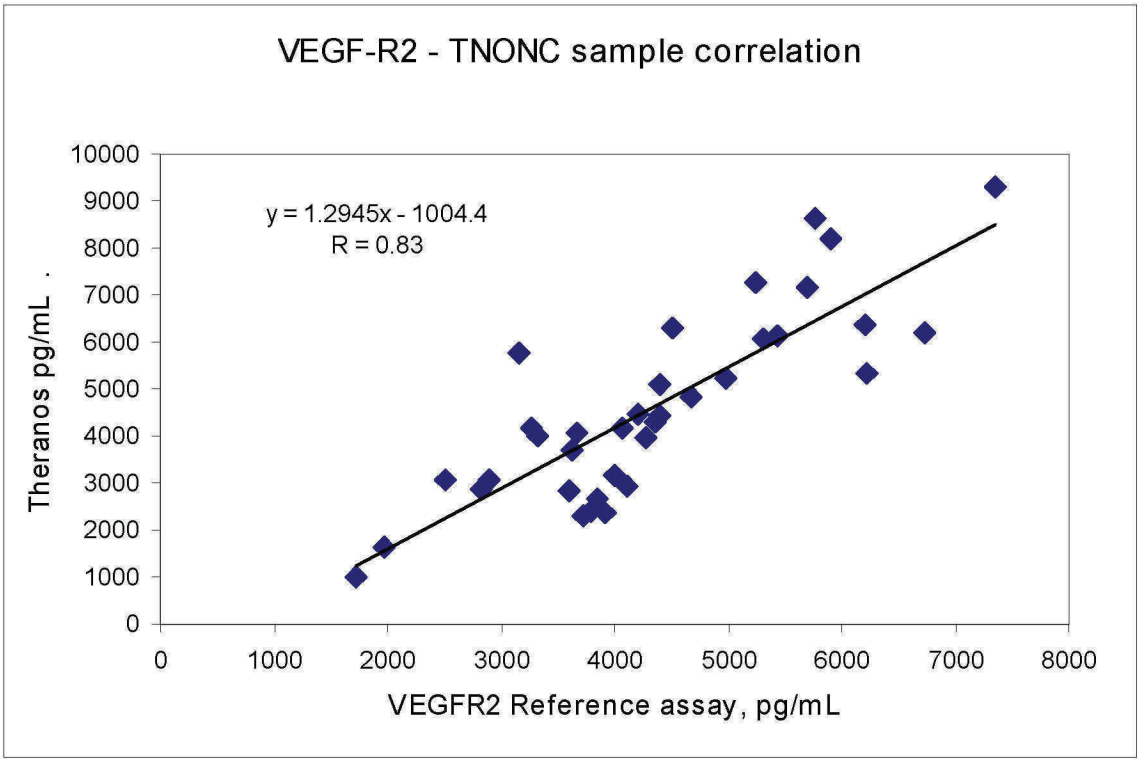
Twelve serum samples were assayed (singlicate) in the Theranos system and in duplicate for the reference method with the following results:

VEGF: y (Theranos) = 0.785 x (reference) + 95.2; R^2 =0.99. Range 96 – 1985 pg/mL. One sample was rejected from the analysis giving very high results in the Theranos system and low results in the reference assay. As detailed in the study data, this patient was being treated with the drug Avastin, which interferes with the reference assay.

Single cartridge clinical results

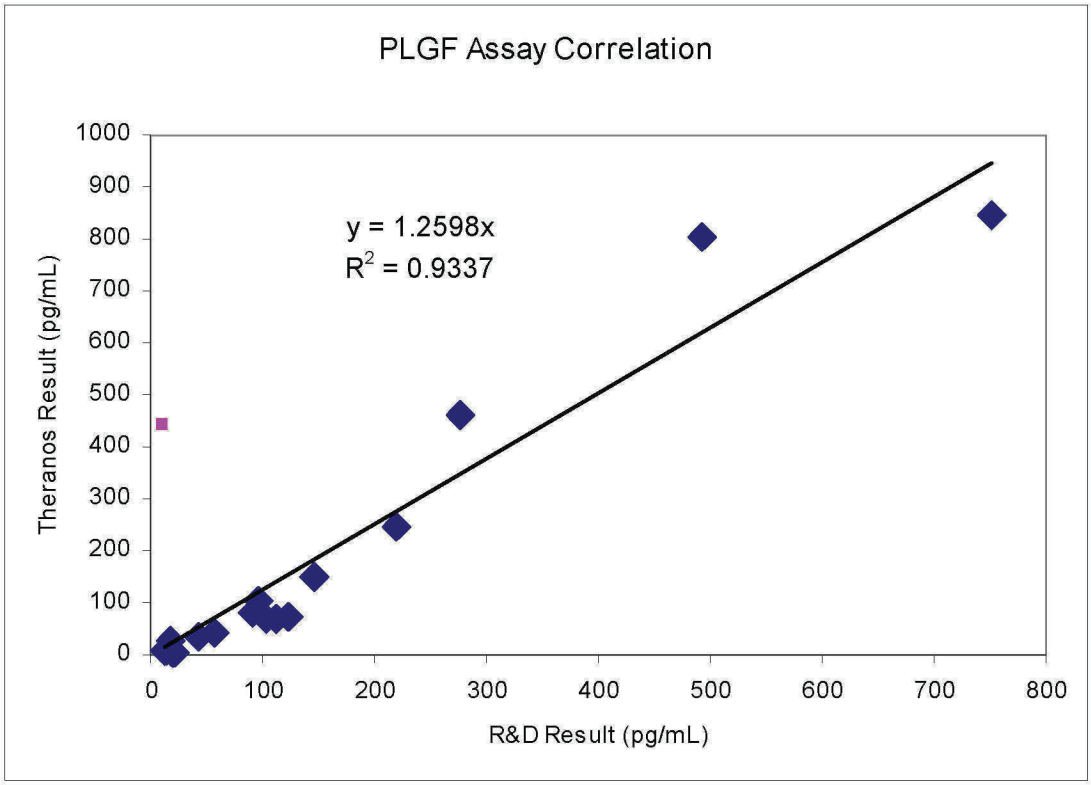


For VEGFR2, 39 TNONC samples were assayed in triplicate in the Theranos system and duplicate for the reference method. The results were: y (Theranos) = 1.29 x (reference) + 1004; R =0.83. Range 1015 – 9285 pg/mL.

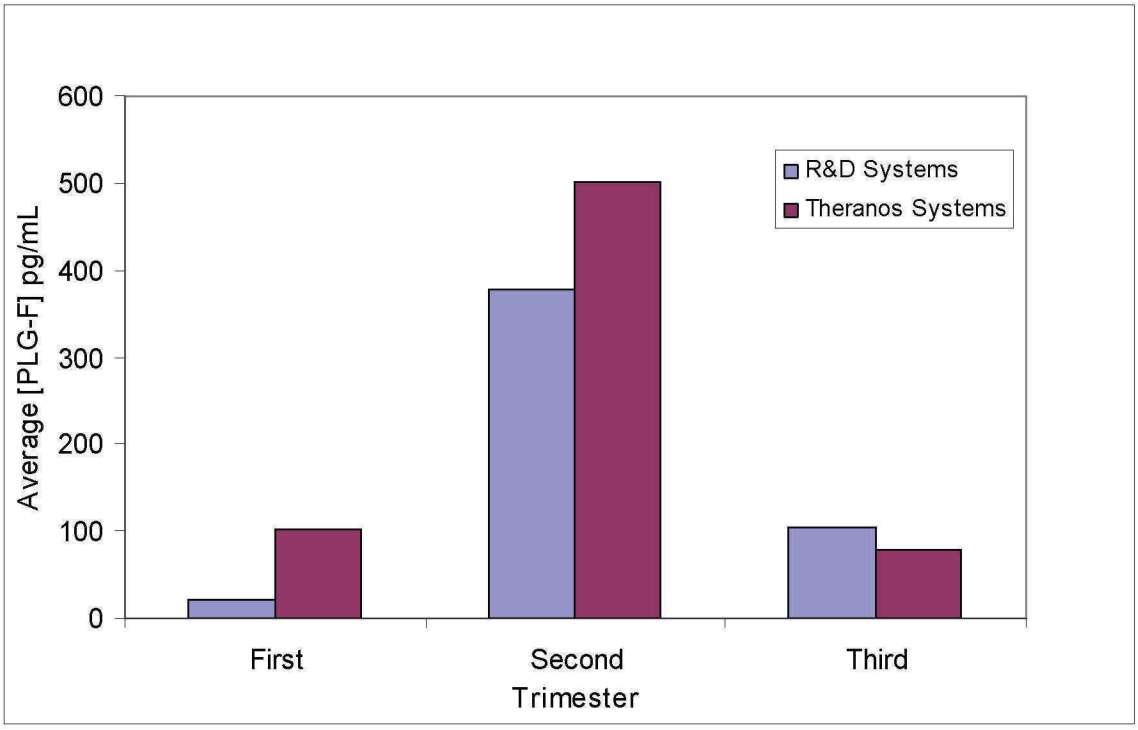


For the initial PLGF samples analyzed by Theranos in the field and with the reference method some results in the undetectable range of the reference methods and Theranos' first lots. Once the Theranos calibration was extended to allow for a lower limit of detection in new cartridge lots, values became detectable from 5-17 pg/mL in the samples.

A significant correlation was achieved during validation on normal serum samples from twenty pregnant women assayed in quadruplicate. They were analyzed on both the Theranos system and the reference R&D Systems kit. The following results were obtained: y (Theranos) = 1.26*x (R&D Systems); R = 0.96. The average within sample CV for the Theranos results was 9%. One sample (shown in pink) below gave discrepant results.



When the results for patients were segregated by trimester and averaged, the concordance shown below was found.



Effect of Avastin on the reference VEGF assay:
Comparison of reference and Theranos VEGF assay results for venous samples were not correlated. Many Theranos results were in the thousands of pg/mL where reference assay gave a low value. Since it was noted that many of the patients had been treated with Avastin which binds to VEGF, Theranos did a study of spike recovery for the reference method. VEGF (400 pg/mL) was added to each sample and the assay repeated. Results are shown below:

Avastin Present	VEGF average, pg/mL Ref	VEGF average, pg/mL Theranos
N	149	588
Y	136	8359

	VEGF spike recovery, %
N	66.5
Y	-1.3

It is evident that Avastin completely blocks the reference assay response. Presumably, Avastin binds at a site on VEGF close to or identical with that recognized by one of the antibodies used in the reference method. The reference assay thus responds only to free VEGF whereas the Theranos assay is not blocked and measures both Avastin-bound and free VEGF.

Assay precision:

Inter-Instrument Precision:
Venous samples from patients were run across four instruments.

Assay	Reportable low pg/mL	Reportable high pg/mL	Precision CV, %
VEGF	20	10,000	8.0
VEGFR2	150	15,000	7.3
PLGF	5	1,000	9.2

Precision in comparison to available reference methods was evaluated during calibration. Singlicate measurements from six instruments were used next to commercially available 'gold-standards'. Theranos adjusted the target range after obtaining clinical samples. Due to the superior performance characteristics of Theranos' assay next to commercial standards, obvious variances are seen where the reference methods report OORL.

Single lot calibration data:

Analyte	Range (pg/mL)	Average CV, %
VEGF (lot 3)	30 – 10,000	12.0
VEGF (lot 1)	30 – 3,000	10.0
VEGFR2 (lot 3)	1,000 – 10,000	4.8
VEGFR2 (lot 1)	50 – 800	17.6
PLGF (lot 3)	5 – 780	26.9
PLGF (lot 1)	50 – 800	9.1

Precision was also measured by analysis of the 41 archived clinical samples in assays and for VEGF 12 commercial samples.

Analyte	Range (pg/mL)	Average CV, %
VEGF	30 – 10,000	16.7
VEGF[2]	96 – 1985	5.7
VEGFR2	1,000 – 10,000	20.4
PLGF	5 – 780	28.7

Dilution linearity:

Data gathered during lot calibration.

VEGF, pg/mL	Recovery, %
10000	(100)
2970	102
990	95
297	105
100	109
30	105
10	101

[2] Commercial samples

VEGFR2, pg/mL	Recovery, %
10560	(100)
7920	92.9
5280	100.9
3960	104.8
2640	97.7
1320	100.8

PLGF, pg/mL	Recovery, %
780	100.0
312	87.6
156	102.8
47	106.3
16	92.4
5	99.4

For all assays, recovery was close to 100 % in the reportable range.

Limit of detection (LOD):
Data gathered during calibration. The LOD is defined at a 95 % confidence level.

Analyte	LOD, pg/mL
VEGF	< 20
VEGFR2	< 200
PLGF[3]	< 20

Theranos Field Study:

The system was deployed to patients' homes and the TNONC study clinic. All units downloaded protocols and uploaded data wirelessly. Some patients used direct telephonic communications (POTs modems) if they were worried about cell reception. Data for every patient was profiled on a secure, client-specific server.

Field Performance Overview:

In this report we document results from:

- 27 patients (41% female and 59% male)
- 13 cancer types
- 38 Instruments
  - 27 instruments deployed to patients' homes
  - 4 instruments deployed to the clinical site in Nashville, TN
- 445 cartridges (approximately 1300 assay results)
  - This number includes cartridges run in-house on archived plasma as well as results gathered in-field

[3] Later stage cartridge lots

Data acquisition has proven feasible in the home setting. There were instruments in the field operating in extreme temperature conditions (from very hot, no A/C to A/C turned to the maximum) as well as in very diverse locations (from RV's to log cabins in the middle of forests), in remote, difficult to reach areas where poor cellular reception is prevalent.

The instruments were deployed across three states: Kentucky, Pennsylvania and Tennessee. As mentioned, turnaround time for installation and patient at-home test was generally less than 24 hours without notice.

In monitoring this multiplex of analytes at far greater frequency than ever before, considerable patient-response variation can be seen across different sub-patient populations, therapies, and cancer types.

When we look at the average results from each patient and the variation seen for each patient, it is evident that the patients vary drastically:

	VEGF Avg., pg/mL	VEGFR2 Avg., pg/mL	PLGF Avg., pg/mL
Maximum	13,584	6,317	410
Minimum	47.5	368	37.3

By evaluating sample statistics such as these, one can identify patients who are anomalous and who may benefit from therapy modification.

For example, of the 13 patients with colon cancer we see one subject with an average VEGF of 13,600 pg/mL and another with an average of 255 pg/mL whereas most of the patients had VEGF values quite closely clustered at 1000 - 5000 pg/mL. Similarly, we see some subjects who show very little variation in analyte values and others with wide variations presumably related to response (high or low) to therapy.

Trial Data:

The following raw trial data was detailed for the client on the server and summarized in the sections below.

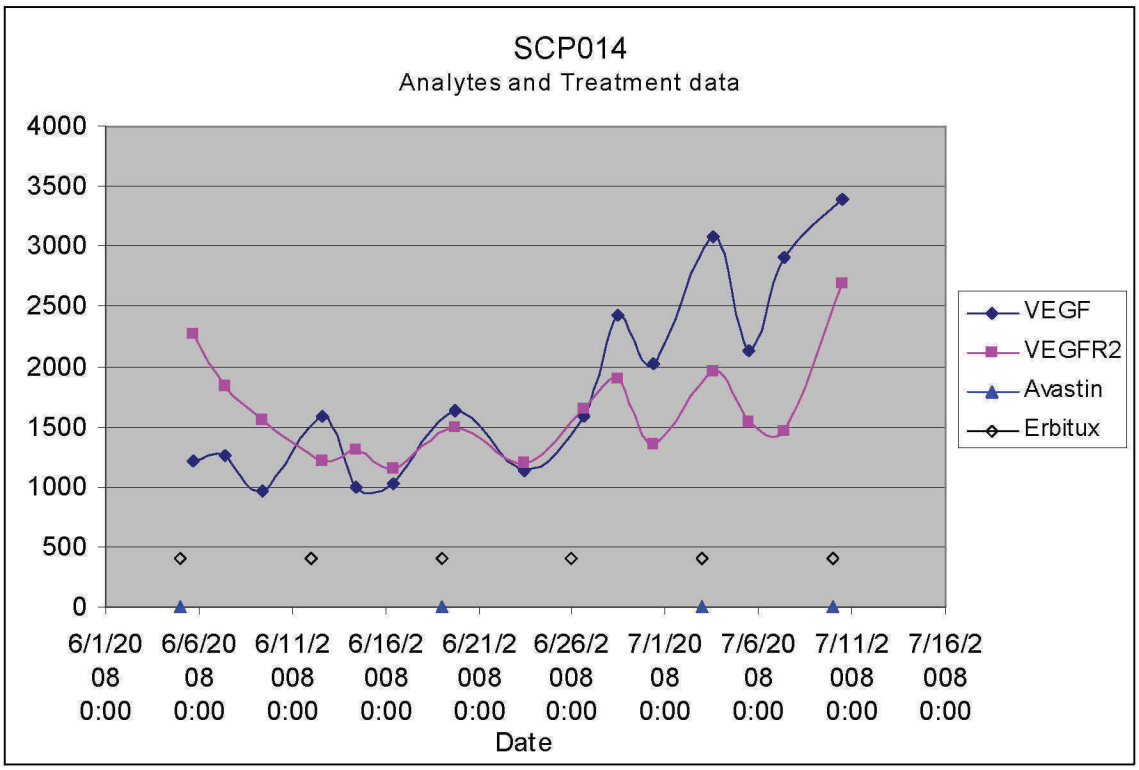
1. Clinic visit diagnostics (Patient characteristics and Clinical assay results)
2. Clinic visit pivot table (clinical results presented as a customizable pivot table)
3. Patient aggregate data (Compliance data, Result averages and CVs by patient and averages by cancer type)
4. All field analyte data results (from the Theranos system presented by patient in a filtered table format [sort-able])
5. Treatment data (drugs used and dosage)
6. Individual end-of-study results (patient evaluation of system)
7. Compilation and summary of end-of-study survey results
8. Data transmission statistics

Evaluation of time course results from individual patients run through Theranos model:

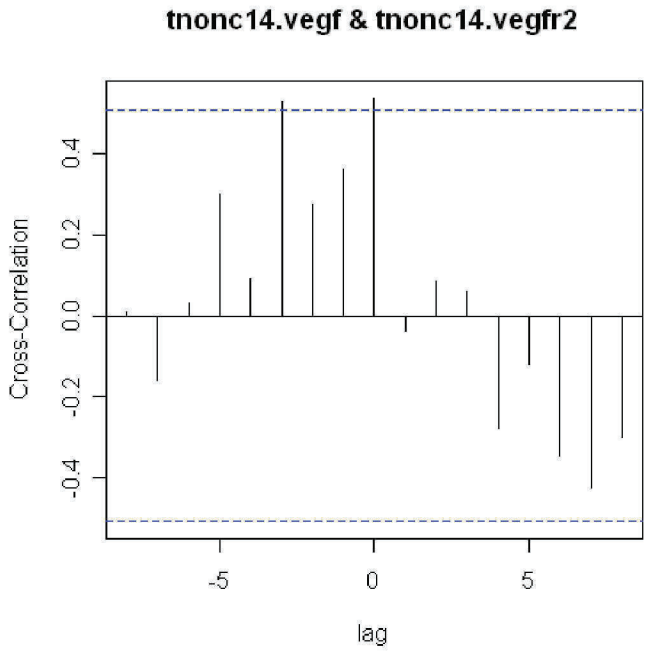
The study data demonstrates that in a statistically controlled study where the endpoint is directly proportional with patient outcome, e.g., a RECIST Score, a correlation between analyte dynamics and patient response to treatment is generated through Theranos models.

Clinical endpoint measurements were accessible to correlate analyte vectors and their rates of change with time to the patient's progression and response to treatment.

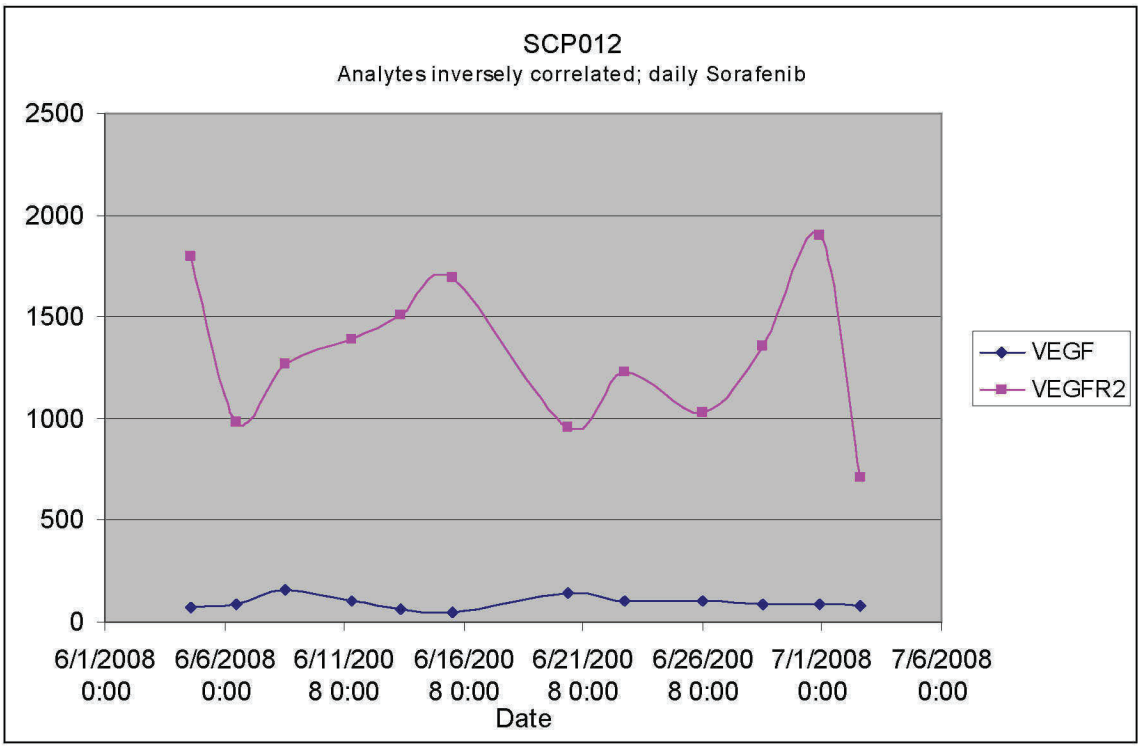
To showcase the ability to profile such predictive correlations between treatment and response profiles, we selected data from two patients -- 14 and 12. Due to patient 14's clinic schedule (first figure below), we were able to collect data following multiple infusion dates, allowing limited statistical analysis to be performed that correlates analyte levels with treatment administration. The data was run through a cross-correlation function in the Theranos model (second figure below) looking at VEGF and VEGFR2 blood levels for patient 14 shows a positive correlation at a cadence of 3 data points. This coincides with the patient's weekly clinic visits during which the patient receives the Avastin infusions.

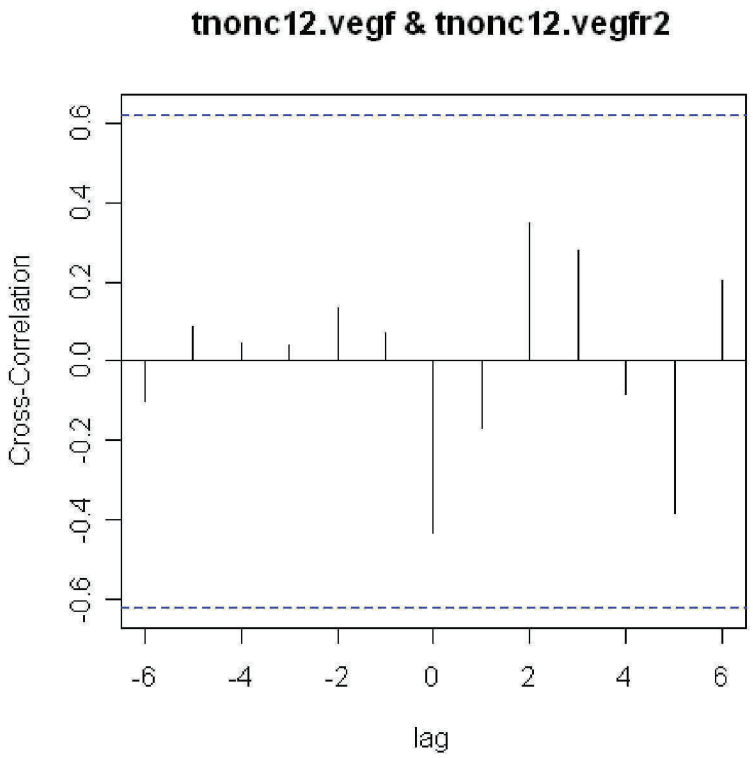


The change in rate of the parameters can be correlated to progress, seen again below in a correlation plot:



For patient 12 (first figure below), we observe an inverse correlation between VEGF and VEGFR2 blood levels. This suggests that the blood analytes behave differently with different drug treatments, pointing at distinct pathways of drug activity (second figure below).





Using Theranos technology makes it possible to generate predictive models for each individual patient to fully characterize the specific disease model, physiology and environmental factors correlating to a clinical outcome or surrogate thereof. The model has the ability to learn, becoming increasingly predictive as more data feeds into it from multiple patients with similar profiles. The model for this program is now powering follow-on studies for rapid label expansion into new indications, where new patient data is being indexed against these stored profiles to get early reads on efficacy in new tumor types.

The Theranos Systems have the capability to generate individual physiological models by allowing frequent and carefully selected critical analyte sampling found in a small amount of a patient's blood.

The integration of such data with clinical history and environmental data yields a model that will then be able to characterize the bio-physiological profile of patients as they converge on one of the model's possible states of responder, non-responder or adverse drug reaction.

The rate with which a particular profile changes and the state towards which it progresses will be early indications of a patient's health and response to treatment. As was shown through data from patients 12 and 14, different treatments may result in very different responses (based on the drugs MOA), which in turn correlates to different surrogate clinical outcomes – for patient 14, the average are increasing, while for the other they remain fairly stable aside from daily variations.

The other data sets that were incorporated into the models are included in aggregate after the actual models to protect client confidentiality and the proprietary nature of their model.

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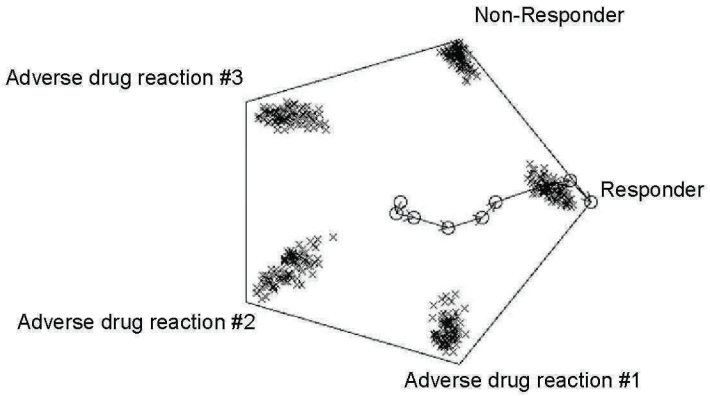
Model enables characterization of responder profiles and prediction of clinical outcomes



All available information is mapped to characterize each responder state

- Genetic information
- Clinical information
- Patient history
- Blood information
- …

x = characteristic data (blood levels, historical data, behavioral information, etc.)

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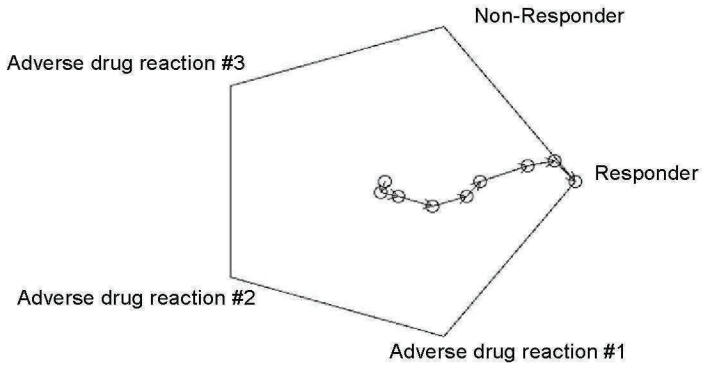
New Patient Data Indexed Against Stored Data to predict new patient response profile.



- Identify who will be a non-responder, when, and what can be done to change that person's 'trajectory' to become a responder.

o = new patient

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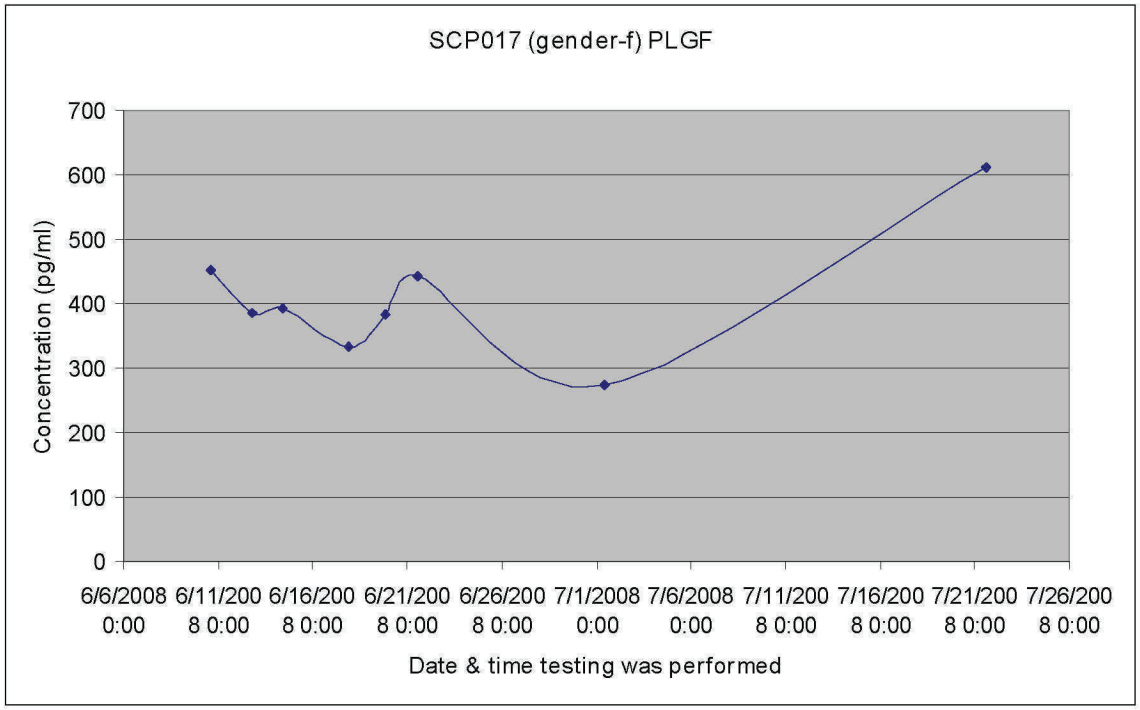
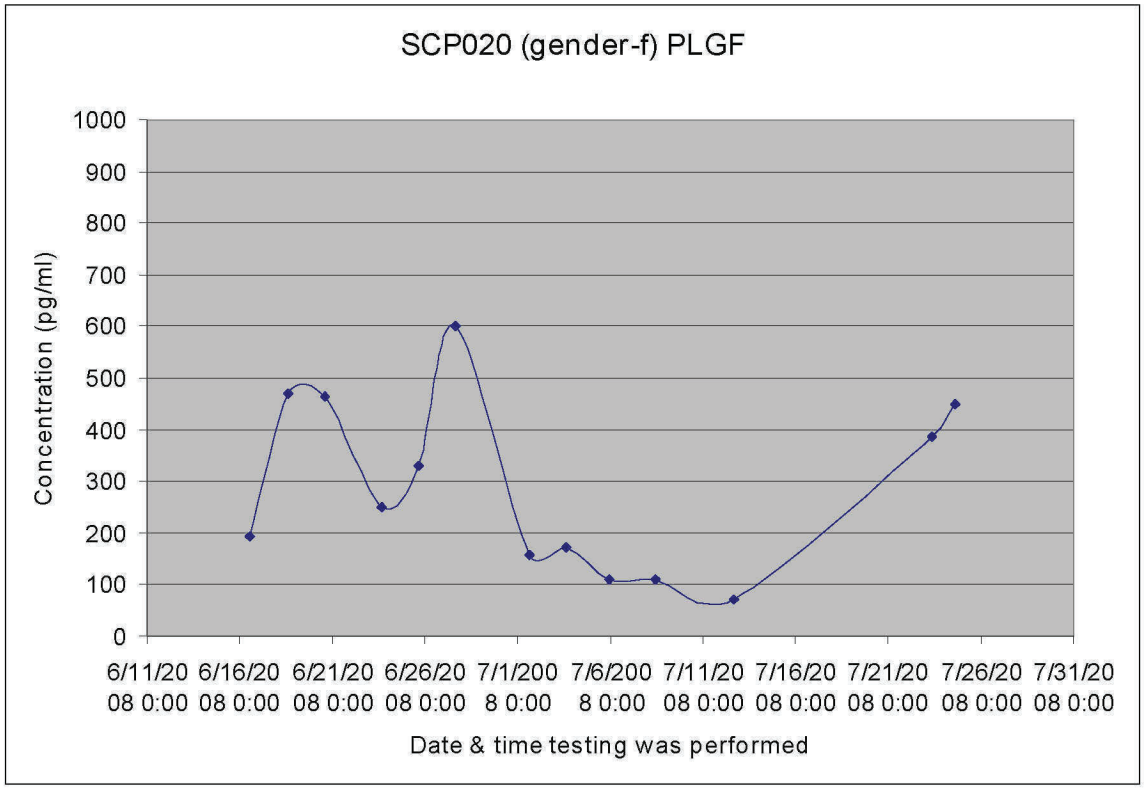
Patient average VEGF and VEGFR2 data by cancer type:

Patient ID	Cancer type	Main Treatment	Average VEGF (pg/ml)	Average VEGFR2 (pg/ml)
SCP001	Adenocarcinoma	Sutent	47.5	2592
SCP006	Breast Cancer	Avastin	2082	2662
SCP010	Breast Cancer	Avastin	2055	3040
SCP008	Breast Cancer	Sorafenib	98	1863
SCP021	Colorectal Cancer	Avastin	4677	3646
SCP027	Colorectal Cancer	Sorafenib	1093	4863
SCP029	Colorectal Cancer	Sorafenib	3612	5658
SCP003	Colorectal Cancer	Sutent	72	2798
SCP007	Colorectal Cancer	Avastin	3860	2350
SCP009	Colorectal Cancer	Avastin	1840	368
SCP022	Colorectal Cancer	Avastin	Patient dropped	N/A
SCP014	Colorectal Cancer	Avastin	1826	1634
SCP019	Colorectal Cancer	N/A	Patient dropped	N/A
SCP016	Colorectal Cancer	Avastin	3006	2143
SCP031	Colorectal Cancer	Avastin	13584	5463
SCP024	Colorectal Cancer	Sorafenib	255	1540
SCP028	Colorectal Cancer	Sorafenib	1274	6317
SCP023	Esophageal Cancer	Avastin	3145	2260
SCP030	Gastrointestinal Stromal Tumor	Sutent	889	2424
SCP012	Liver Cancer	Sorafenib	96	1253
SCP017	Lung Cancer	Avastin	3947	2111
SCP025	Melanoma	Avastin	5399	3294
SCP002	Neuroendocrine carcinoma	N/A	Patient dropped	N/A
SCP026	Ovarian Cancer	Sorafenib	Patient dropped	N/A
SCP020	Renal Cell Carcinoma	Sutent	368	883
SCP004	Renal Cell Carcinoma	Avastin	2316	1057
SCP011	Renal Cell Carcinoma	Avastin	3159	1911
SCP013	Renal Cell Carcinoma	Avastin	3908	770
SCP015	Renal Cell Carcinoma	Avastin	3031	1068
SCP018	Tongue Cancer	Avastin	1457	3074
SCP005	Unknown Primary	Avastin	3099	2980

Selected PlGF time-series plots are shown below.





Patient monitoring times and quality of life by gender:

			Time of day when home monitoring was performed	Quality of life (as measured by on-screen survey)
Patient ID	Cancer type	Gender	(on average)*	(on average)*
SCP001	Adenocarcinoma	f	Morning	N/A (Survey was not yet deployed)
SCP006	Breast Cancer	f	Afternoon	7
SCP010	Breast Cancer	f	Evening	8
SCP008	Breast Cancer	f	Late Evening	7
SCP021	Colorectal Cancer	f	Noon-afternoon	8
SCP027	Colorectal Cancer	f	Afternoon	10
SCP029	Colorectal Cancer	f	Afternoon-Evening	not yet available
SCP003	Colorectal Cancer	f	Morning	N/A (Survey was not yet deployed)
SCP017	Lung Cancer	f	Evening	9
SCP026	Ovarian Cancer	f	N/A	N/A
SCP020	Renal Cell Carcinoma	f	Afternoon	6
SCP005	Unknown Primary	f	Afternoon	9
SCP007	Colorectal Cancer	m	Evening	7
SCP009	Colorectal Cancer	m	Late Evening	7
SCP022	Colorectal Cancer	m	N/A	8
SCP014	Colorectal Cancer	m	Morning	7
SCP019	Colorectal Cancer	m	N/A	N/A
SCP016	Colorectal Cancer	m	Evening	8
SCP031	Colorectal Cancer	m	Afternoon	not yet available
SCP024	Colorectal Cancer	m	Afternoon	9
SCP028	Colorectal Cancer	m	Evening	not yet available
SCP023	Esophageal Cancer	m	Morning	8
SCP030	Gastrointestinal Stromal Tumor	m	Morning	not yet available
SCP012	Liver Cancer	m	Afternoon	10
SCP025	Melanoma	m	Morning	9
SCP002	Neuroendocrine carcinoma	m	N/A	N/A
SCP004	Renal Cell Carcinoma	m	Noon-afternoon	10
SCP011	Renal Cell Carcinoma	m	Morning	9
SCP013	Renal Cell Carcinoma	m	Evening	10
SCP015	Renal Cell Carcinoma	m	Evening	7
SCP018	Tongue Cancer	m	Afternoon	5
* Actual time for each test point and diurnal variations of quality of life can be found online				

Patient compliance with optional on-screen questionnaire was approximately 90%.

Patient clinical visit data by age:

Patient ID	Race	Smoking Status	Alcohol Consumption	Age	Weight (pounds)
SCP029	Caucasian	does not smoke now, positive history	None	36	179
SCP010	Caucasian	never smoked	monthly or less	45	165
SCP018	Caucasian	Smoke daily	None	45	181
SCP007	Caucasian	never smoked	None	46	213
SCP008	Caucasian	smoke occasionally	None	46	180
SCP002	Caucasian	never smoked	monthly or less	49	194
SCP016	Caucasian	smoke occasionally	monthly or less	49	167
SCP012	Caucasian	does not smoke now, positive history	None	53	190
SCP015	Caucasian	does not smoke now, positive history	None	53	174
SCP028	Caucasian	smoke occasionally	None	57	262
SCP001	Caucasian	does not smoke now, positive history	None	61	172
SCP027	African American	never smoked	None	62	167
SCP009	Caucasian	never smoked	None	63	221
SCP011	Caucasian	does not smoke now, positive history	monthly or less	63	305
SCP024	Caucasian	infrequent attempts (never developed a habit)	Every day	64	200
SCP023	Caucasian	never smoked	Every day	65	252
SCP005	Caucasian	does not smoke now, positive history	monthly or less	66	160
SCP021	Caucasian	smoke occasionally	monthly or less	66	198
SCP006	Caucasian	never smoked	monthly or less	68	163
SCP017	Caucasian	does not smoke now, positive history	Every day	69	112
SCP013	Caucasian	never smoked	monthly or less	71	230
SCP020	Caucasian	never smoked	None	72	101
SCP026	Caucasian	never smoked	None	73	132
SCP031	Caucasian	does not smoke now, positive history	None	73	134.5
SCP025	Caucasian	does not smoke now, positive history	None	77	184
SCP014	Caucasian	does not smoke now, positive history	monthly or less	78	217.5
SCP022	African American	never smoked	None	82	178
SCP030	Caucasian	never smoked	None	83	182

Sample of patient clinical blood work by patient ID:

Patient ID	Avg. % Lymphocytes	Avg. Heart Rate	Avg. Total Bilirubin	Avg. Systolic BP	Avg. RBC
SCP001	33.4	67.7	0.7	129.3	3.2
SCP002	34.1	55.0	0.3	161.0	4.3
SCP004	27.8	64.7	0.5	144.7	3.2
SCP005	36.4	75.0	0.2	127.5	3.9
SCP006	29.5	100.7	0.3	112.7	4.3
SCP007	24.0	73.0	0.3	131.3	4.4
SCP008	23.7	84.0	0.4	124.0	5.1
SCP009	25.0	71.5	0.7	133.0	4.5
SCP010	45.3	74.3	0.9	137.8	4.5
SCP011	28.6	82.0	0.6	135.0	4.8
SCP012	28.3	75.5	0.7	122.0	4.0
SCP013	31.1	72.0	0.7	137.0	4.2
SCP014	40.2	81.5	0.4	125.3	4.0
SCP015	35.4	78.3	0.3	147.0	5.0
SCP016	18.0	75.3	0.3	131.3	4.9
SCP017	20.7	89.3	0.4	114.0	4.2
SCP018	23.4	70.0	0.3	133.0	4.8
SCP020	17.9	60.7	0.4	146.0	3.7
SCP021	36.5	91.0	0.4	130.0	4.8
SCP022	23.5	93.5	0.7	123.0	4.0
SCP023	26.3	107.7	0.7	119.7	4.7
SCP024	18.8	83.0	0.7	139.0	3.7
SCP025	33.5	94.0	0.3	143.0	5.2
SCP026	34.6	110.0	0.4	125.0	3.7
SCP027	9.5	70.0	0.7	119.0	3.7
SCP028	21.2	98.0	0.8	125.7	5.2
SCP029	32.6	90.5	0.6	122.8	5.1
SCP030	42.3	72.0	0.4	137.0	3.7
SCP031	16.7	70.0	0.4	145.0	4.3

All individual patient data was profiled as it was generated on the client-specific secure portal at www.theranos.com; raw data can also be found in the attached excel spreadsheet.

Server and Data Transmission

Approximately 361 cartridge results and 203 optional home surveys from the field were successfully transmitted to the Theranos servers. There were less than 5% transmission errors that required the readers to either retry sending the data or wait until they had a better connection to send the data. All data gathered in the field was transmitted to the Theranos servers. For the first two patients, on-screen surveys were not available. The number of surveys received is smaller than the number of cartridge runs due to the above as well as patients filling only one survey for each of their clinic visits (even though they ran two cartridges per visit). Once surveys became available, each cartridge run also asked the user to complete an optional quality of life survey and compliance was very good.

Data distribution by transmission pathway to date		
Direct Internet Connection	Wireless-GSM	Traditional Phone line
5.6 %	90.7%	3.7 %

The only problem encountered with using GSM wireless phone technology was poor signal. The main reasons for poor cellular reception were: dense foliage, metal roofs and poor signal quality due to remote location. In one location (Stewart, TN), there was no cellular coverage at all; therefore the reader used the standard telephone line in order to connect to our servers and report data as it was gathered. All of this patient's logs were received by Theranos servers. In future studies, multiple network providers would be contracted for these areas.

Overall performance of the Theranos System based on Customer Care log:

The customer care line was available to patients 24 hours a day 7 days a week over the course of the entire study (July 07 to October 08). All calls were addressed professionally and all issues were resolved quickly, taking care to minimize the impact on patients and clinical staff.

The types of calls for which patients used the Customer Care line:

- Patient running low on supplies – the solution was to simply ship more of the needed supplies with overnight delivery to make sure patient had enough for the upcoming home tests.
- Patient not knowing how to turn machine on – the solution was to advise the patient over the phone on the procedures outlined in the setup sheet they received and to make sure they have the instrument up and running.
- Patient calling about scheduling an instrument pickup – solution was to schedule one of our representatives to pick up the machine or alternatively to have FedEx pick up the reader if patient was able to place it in the shipping container themselves.
- Patient called about blood transfer question – the solution was to advise the patient to leave the blood transfer device on a flat surface. If this solution was not sufficient, a new batch was shipped to make sure no capillary manufacturer defects were at fault.
- Patient called about instrument not recognizing cartridge – the solution was to ask patient to re-try and call back if problem persisted. The suspicion was that due to poor cellular signal the reader was unable to communicate, and by re-trying it would perform appropriately. There were no subsequent calls from patient.
- Patient called about instrument not being ready due to temperature – the solution was to ask patient to move reader away from A/C units and possible air currents. Patients had moved readers from initial installation location (one moved it to his RV, another into a really hot room) and the temperature extremes affected the readers' ability to maintain desired temperature. The Theranos readers are engineered to control temperature to eliminate variability associated with conventional assays.

The majority of systems deployed in the field performed their duties throughout the entire length of the patient monitoring schedule. One instrument had mechanical issues due to being misused; this happened during new personnel training at TNONC. The instrument was promptly replaced with a new instrument. The other issue was related to the cellular carrier not identifying the instrument. To expedite the process and assure that the clinic was adequately supplied it was decided to replace that instrument with one that was known to work. The problem was later resolved off-line.

Patient Compliance with protocol:

It is hard to estimate the patient compliance with the exact protocol due to the factors out of Theranos' control. In many instances patients re-scheduled their clinic visits and the new appointments were not communicated to us. At the onset of each patient's home monitoring they were provided with a tentative schedule which in many cases changed due to patient's need to travel or inability to keep scheduled appointments. With this in mind, we estimate that patient compliance with protocol was still very good, at approximately 96 % (measured as 80-120% of expected testing completed and received). Given the missing information, a much more accurate derivation would be possible.

Theranos System Assessment by Patients:

Patient end of study surveys were sent out to all participants.

Summary of patients' assessment of the Theranos system:

- 88% of patients surveyed found the Theranos System easy to use; no patients found it "very hard" to use.
- 76% of patients found the written instructions to be very informative, with clear directions; 12% did not read instructions
- 91% of patients scored the training given by their Theranos representative either a 9 or 10 (10 being very good training)
- 76% of patients found the Theranos System takes little time to use (scores between 1 and 4 were tallied, with 1 = very little time and 10 = a lot of time)
- 100% of patients found the optional touch screen survey on the Theranos System easy to use, giving scores of either 8, 9 or 10 (10 = easy to use, 1 = hard to use).
- On a scale of 10 to 1 (10 = least painful, 1 = most painful), only one patient gave the blood drawing experience a score of less than 6. 59% felt almost no pain, scoring either a 9 or 10.
- 100% of the patients that responded to the survey gave Theranos Customer Support an excellent or very good rating
- For the majority of patients, the Theranos System worked very well. The major ways of solving the questions patients had were figuring it out on their own or calling the Theranos Customer Care line.
- In the follow-up survey, 100% of patients that responded said they received excellent or very good technical support over the duration of the study.
- Most patients said they prefer monitoring from home (scored 8 through 10) using the Theranos System; 25% were indecisive (scored 4 to 6) when asked whether they prefer going to the clinic or using the Theranos System; only two patients would rather monitor at the clinic.

From the interactions, the system was:
1. well received and
2. the client solutions team made a very positive impact on the patients through promptitude and professionalism.

Conclusions:

General:

1. The Theranos System performed with superior performance to reference assays while running in a complex ambulatory environment.
2. Theranos built a support infrastructure that enables on-demand home installation and patient training in extremely rural areas.
3. Patients preferred ambulatory monitoring to clinic visits and liked using the Theranos System.
4. Non-computer literate patients liked the interactive reader touch-screen and were able to successfully use it.
5. Data can be run through the Theranos model to extract predictive correlations to end-points or patient response profiles.

Technical:

6. Inter-system accuracy is excellent and was demonstrated on a platform with superior performance specifications to reference methods.
7. Calibrations were updated with access to samples from the trial.
8. Good correlations were seen to various commercially available gold-standards.
9. Avastin does not block the Theranos assay.
10. The Theranos System can measure VEGF both free and bound to VEGFR2 and Avastin to better quantify dose-response.

Economic:

11. With this validation data, the technology will now be applied to significantly cut costs and bring compounds to market faster:

12. More frequent sampling enabled better characterization of longitudinal time-series profiles of angiogenesis protein panels. More accurate insight of the change in rate of those panels over time enables significantly faster and earlier reads on efficacy dynamics.
    a. See efficacy dynamics trends and correlation to end-points in patient time-course profiles run through the Theranos model.
13. Response profiles were seen in this study over 30 day intervals. Historically, these types of correlations have taken up to a couple years to demonstrate, or in some cases, were previously not demonstrable. This time gained facilitates rapid data generation for additions to a compendia and rapid label expansion of existing drugs. Equally, this approach can be used to fast-track approvals of key compounds and at the same time better optimize those compounds with better visibility to achieve the target product profiles.
    a. One of Theranos' pharma partners is publishing a report which estimates the increased time to market is valued at $1M per day – making every month quite substantial.
14. Through Theranos Systems, a client will be able to reduce the number of sites, eliminate shipping costs for samples, processing costs, and analytical costs. Based on data from this program, implementation of these systems enabled ~50% cost savings over current study spending, demonstrated to be $15M of a $30M study budget. Equally, through better insight into pathway dynamics, Theranos is demonstrating the ability to reduce the number of patients required to show statistical significance in future studies by 30-50%.

Confidential excerpts from Theranos studies

Excerpts From a Study Using a Novel Monitoring System to Evaluate Biomarker Levels During Neutropenic Fever/Infection or Sepsis in Patients with Acute or Chronic Leukemias Undergoing Chemotherapy

John Chadwick,[1], Kwesi Mercurius[2], MS Timothy M. Kemp[2], Michael Chapman[2], BS, Ian Gibbons[2], MA, PhD, Seth Michelson[2], MA, MS, PhD, and Jason Gotlib[3], MS, MD
June 18, 2008

Document Summary

This report documents select findings from clinical studies of febrile neutropenic patients conducted in collaboration with the Cancer Center at Stanford University. It describes the analytical performance of the Theranos System and the clinical data derived from the study. In addition, the report highlights how Theranos Systems and their integrated mathematical algorithms enable: (a) earlier and more accurate reads on pathway dynamics than possible using the conventional infrastructure which, in this setting, translates to predicting the onset of sepsis and the optimal time for intervention; and (b) select appropriate analytes for predicting the efficacy dynamics of a therapy or the progression of other diseases.

Leukemia and Neutropenic Fever

Leukemia is a malignancy of the bone marrow involving clonal expansion of early white blood cells. Leukemias are grouped into lymphoid and myeloid subtypes. In treating leukemias, a course of myelosupressive induction chemotherapy is given, and patients are rendered neutropenic (low white blood cell count). These patients thus become susceptible to neutropenic fever/infection compounded by other risks such as the presence of a central venous catheter and chemotherapy induced mucositis.

Neutropenia (low white blood cell count) is a typical complication of chemotherapy during the treatment of leukemias.

- Neutropenia renders patients more susceptible to neutropenic fever/infection, which can compound other risks such as the presence of a central venous catheter and chemotherapy-induced mucositis.
- Neutropenia is the most prominent serious side effect of induction chemotherapy causing increased morbidity, mortality, decreased dose intensity and results in large costs (increased length of stay, therapy etc).
- Neutropenic infection often leads to sepsis.

Treatment strategies involve empirical antibiotics, antifungals and other supportive measures. These strategies are now well established and effective, with empirical therapy taking into account the character of major pathogens, and in-place, evidence-based guidance. Febrile neutropenics, however, are not a homogenous population and although the current empirical approach is effective some issues regarding both the diagnosis and treatment of sequelae remain.

Some patients develop fever due to local infection, medications, allergies and unknown causes. Treatment with antibiotics in these cases can result in increased antibiotic resistance, resulting in

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increased overall costs. Patients who develop fungal infections may initially receive antibiotic therapy, and may not be prescribed antifungal therapy until later in the course of infection. Certain infections remain refractory to initial antibiotic spectra, and thus there is a delay in treating these particular patients.

More completely determining the diagnosis and risk for neutropenic patients presenting with a fever would allow us to triage the patients more effectively, implement earlier customized therapeutic strategies, and improve outcomes in these patients.

- Lower risk patients could receive outpatient therapy.
- Septic patients could be transferred earlier to the ICU
- Antifungal therapies could be introduced earlier.
- Therapeutic strategies using growth factors to ameliorate the inflammatory response may be identified and tested.

The working hypothesis of the present study was that the Theranos System would enable correlation of the rates of change of cytokines and other biomarkers along with other relevant physiological factors to the time to onset of fever and clinical manifestation of sepsis. In doing so, Theranos Systems would elucidate the optimal time for early intervention to substantially improve patient outcomes.

Study design

This pilot study was planned to enrol leukemic patients and to perform repeated sampling (every eight hours) throughout the treatment course, with increased frequency (every 6 hours) during the febrile episodes. The long inpatient stay (approximately thirty days) and the presence of an indwelling catheter line in these patients facilitated the design of the study.

This observational pilot study enrolled 18 patients over a 4 to 6 month recruitment cycle. Thousands of samples were analyzed, showcasing the ability to extract high-powered correlations from fewer patients using the Theranos methodology.

Upon admittance into the hospital, patients were appropriately consented for participation in the study. Standard of care measures were pursued according to the treating physician/health care team, which included complete blood counts, chemistries, liver function tests, blood cultures, urinalysis and culture, chest x-ray, and/or other imaging modalities.

Below is an example protocol for obtaining biomarker analytes in the setting of AML induction therapy, where chemotherapy is administered over the first 4 to 7 days of treatment. Count nadir is typically expected to commence approximately 10 days post initiation of treatment:

1. From day 1 of admission until the time of the first fever, biomarker analysis were obtained q8 hours (3x daily) from the patient's IV or central venous catheter (1 cc each sample).

2. At the onset of first fever, biomarker analysis were obtained up to a maximum frequency of q6 hours (or 4x daily) from the patient's IV or central venous catheter (1 cc each sample), for a total of up to 4 cc daily. This frequency of blood central venous catheter blood draws continued for 2 days after the last fever of $\geq$38.0C (and during the period when the ANC remains < 1000/mm^3).

3. When both the ANC was greater than or equal to1000/mm^3 and fever has been less than 38.0C for more than 24 hours, biomarker analysis frequency was reduced to q8 hours until the time of hospital discharge.

4. Regardless of ongoing fever or clinical circumstances, biomarker analysis was continued for a maximum of 30 days after initiation of inpatient chemotherapy, or for a maximum of 30 days after admission to the hospital..

5. Under this sampling scheme, a patient who had biomarker analysis performed for a maximum of 30 days in which 15 days were characterized by fever, the estimated volume of blood drawn for the study was 15 days x 4cc (q6 hours) = 60 cc, + 15 days x 3 cc (q8 hours) = 45 cc, for a total of 105 cc over a 1 month period, or an average of 3.1 cc/day. It should be noted that in this observational study, some patients were be enrolled just for neutropenic fever after having received therapy on an outpatient basis. For such patients, the hospital stay lasted anywhere from approximately 2 days to 10 days, and therefore the total volume of blood drawn was less (e.g. up to approximately 30 cc).

6. Whole blood was collected in EDTA tubes and stored at 4C. Twenty microliters of whole blood were used in the Theranos System.

7. The samples were then centrifuged, the plasma aliquoted and then frozen.

8. Batch processing of the frozen plasma samples were performed at Theranos and via an external reference lab.

9. In addition to the longitudinal measures of the proteins, the database element of the Theranos System was customized to automatically integrate all relevant clinical data with the blood measurements, facilitating complete contextual analysis of the protein marker trajectories. The 'back-end' of Theranos Systems is an anonymized, HIPAA-compliant database that contains each patient's clinical and laboratory data along with the cytokine measures. This data is stored in a model which dynamically evolves as new field data is collected. This learning engine then powers subsequent studies where the integrated model enables predictive information to be characterized.

10. Data collected includes patient age, gender, diagnosis, history of prior disease-specific therapies, current use of antibiotics and antifungals, use of hematopoietic growth factors (e.g. G-CSF), duration of antibiotic/antifungal use, and source of blood draw. Laboratory data which were collected includes vital signs (temperature, blood pressure, heart rate), white blood cell count, absolute neutrophil count, hemoglobin, platelet count, microbiology (e.g. blood, urine culture results), and radiology results (e.g. presence of infection such as pneumonia or fungal nodules).

The Theranos system was designed to measure multiplexed high and low sensitivity assays. Due to the proprietary nature of the study findings and the status of currently intellectual property filings, this report primarily details analytical results for only Protein-C and CRP.

Reduction in analytical testing noise and ability to extract predictive information from biomarkers is enabled by Theranos Systems:

The ability to extract more accurate information on biomarker and drug levels from a point of care device than possible from a central lab further strengthens Theranos' ability to extract predictive correlations from blood data.

- Fully integrated blood analysis and data modeling allows for better insight into pathway dynamics (early reads on efficacy, safety and disease progression)
- Small sample size (~10uL) enables far more frequent sampling
- No need to ship or export samples
- No risk of losing samples
- No analytes decay from fresh whole blood to plasma
- Reduction in kit-kit CV
- No kit-kit variability
- No site-site variability

- Better dynamic range
- Better sensitivity
- Instant data access – no wait in receiving data back from labs
- Capability to characterize change in rate of analytes through more comprehensive and accurate longitudinal trends characterizing time to efficacy or adverse events
- Ability to measure PK simultaneously with PD parameters in real-time
- Graphical touch-screens on the readers allow for collection of other environmental parameters to profile blood data in context when the data is sent wirelessly to Theranos' web portal (where the relevant models reside)

Theranos Reader and Cartridge Specifications

- Superior performance over current 'gold-standards' successfully validated externally
- Designed for at home use and can also be used in clinics (will be CLIA waived)
- Multiplexed measurement of biomarkers
    - Max 6-8 per cartridge
- Serial measurements to detect trends
- Fresh whole blood, plasma or serum samples
- Finger-stick: Small sample size (10-20 uL)
- Mix and match selection of analytes
- Wide measurement range
    - pg/mL – mg/mL (1 billion fold) simultaneously
- High sensitivity
    - 0.2 pg/mL (2 parts per 10-billion)
- Analyte Recovery: ~100 %
- System CV (inter-intra assay, inter-intra cartridge, inter-intra reader): <10%
- On-board chemistry controls
- Factory calibration (no user calibration)
- Wireless communication of results to appropriate user
- Proprietary algorithms to interpret time trend results

Predictive and dynamic modeling

Theranos' proprietary modeling system marries the feedback of the sampling to the inductive logic of the modeling.

Unlike any other models, Theranos Systems are deductive models which 'close the loop' by automatically and dynamically feeding back a measure and retuning the model. Moreover, Theranos Systems tune models to given sets of target populations specific to a company's compound. Theranos gives exclusive access to these models to its partners to drive future studies.

A model is a mathematical representation of a biological phenomenon that begins with the general dynamics of the system. That general form is then fit to the observed dynamics by assigning values to a number of parameters. Each group of parameters traces out a different

trajectory, and could be thought of as accounting for one possible individual in a population of individuals. The "typical" parameter set fits the average behavior of the population. The strategy followed by other modeling companies is to begin an in silico study by establishing distributions of possible parameters sets, and from them inductively establishing a family of "could be's". Theranos Systems start with the general mathematical model, and using its proprietary sampling technique, establish patient-specific constraints that narrow the world of "could be's" to a much smaller (hopefully 1) set of "must be's". That longitudinal sampling and its incumbent feedback continuously retunes the model for the specific patient after beginning with the general patient.

Operating characteristics:

- The instrument remained powered and available for use at any time.
- The System required 10-20 uL of sample (fresh fingerstick blood, EDTA-blood, EDTA plasma, or serum).
- Reportable ranges[4]:
    - Protein-C 0.1 – 6.0 ug/mL[5]
    - CRP 0.1 – 300 ug/mL
- Specificity:
    - No known significant cross-reactants[6]. Activated Protein-C (Drotrecogin alpha) showed less than 5 % assay response compared with the same concentration of Protein-C. Thus a level of 1 ug/mL of Activated Protein-C would be reported as out of range low.
- Interfering substances
    - Non found
- Total System precision: < 10 % CV across the clinically relevant ranges
- Calibration stability[2]: > 2 months from shipping

Calibration

Calibration is traceable to authentic analytes dissolved in a proprietary matrix designed to emulate human plasma. A set of five calibrators was made and stored frozen. Each was assayed in triplicate over three days on two Theranos instruments. The instruments measure the rate of photon production (expressed as “Relative Luminescence Units” or “Counts/unit time”). Each assay has two replicate measurements and each cartridge has two on-board chemistry controls for each assay. These data are transmitted to a Theranos Sever wirelessly or though a modem or Ethernet connection. The data are interpreted by a calibration algorithm on the Theranos server. Calibration for plasma samples traces directly to the calibration scheme described above. Results for paired blood and plasma samples were used to create a calibration for blood and results are reported as “in plasma” equivalent values.

Assay protocol:

- Remove cartridge from pouch
- Obtain a drop of blood by fingerstick or use an EDTA-venous blood or plasma sample.
- Use TD or pipette to acquire a 20 uL sample
- Inject sample into marked well in the cartridge
- Using GUI screen buttons
    - Open door of instrument
    - Slide cartridge into the instrument
    - Close door of instrument
    - Assay begins automatically
    - Screen prompts user when test is complete

[4] Results are reported in as “in plasma” values for blood and plasma
[5] Protein-C: 100% normal activity is taken as equivalent to 4.0 ug/mL
[6] Work still in progress

Clinical studies

Under institutional review board approval and informed patient consent, eighteen Acute Myeloid Leukemia (AML) patients undergoing in-patient chemotherapy were enrolled and monitored over about four weeks.

Markers were selected based on previous research in the literature and published clinical studies, but this selection had never been monitored at the frequency facilitated by Theranos Systems or in such an integrated (via modeling), systematic fashion.

Blood samples (2 mL) were taken by hospital nurses up to four times per day from a central line and immediately taken to a satellite laboratory and run in the Theranos System by a Stanford University visiting medical student or Theranos staff. Plasma from remaining blood was separated by centrifugation and either analyzed in the System and/or flash-frozen. Approximately twenty instruments and 2000 cartridges were used in the study. Plasma samples were also subjected to analysis by commercially available ELISA kits (both at Theranos at by a reference laboratory[7]).

Performance validation tests:

Protein-C

Accuracy: Blood samples (84) from the AML study were measured (singlicate) in the Theranos System and the corresponding plasmas assayed in a Helena ELISA kit in duplicate with the following result: Theranos (y) = 0.94*Helena (x) + 0.06; R = 0.95. The range of concentration was: 0.3 – 5.6 ug/mL.

Precision: Control materials were run in triplicate over three days on two instruments with the following results:

Protein C ug/mL	Inter-day Precision CV, %	Between Instrument CV, %
6.0	3.2	↑
4.0	5.4	3.9
2.0	4.7	↓
1.0	12.8	
Average	6.5	3.9

Dilution linearity: Control materials were diluted then measured in triplicate in two instruments over three days; results were averaged.

Protein-C ug/mL Target	Protein-C ug/mL Observed	Recovery %
Instrument 1		
6.0	5.93	98.9
4.0	4.15	103.9
2.0	2.04	102.1
1.0	1.01	100.8
Instrument 2		

[7] Work still in progress

4.0	3.93	98.3

Calibration stability and performance of controls: Stability of System calibration was evaluated over one month using within cartridge controls with the following results.

N	351	Count
Average	5.45	ug/mL
SD	0.58	ug/mL
CV	12.2	%

The control value plotted against time did not change significantly over the study.

Sensitivity: The limit of detection (LOD) was determined by measuring signals for the zero analyte and lowest concentration calibrator. LOD of 0.07 ug/mL was calculated as 2*Signal SD [Averaged over the two values]/Slope of (signal vs. conc.).

Clinical Study Analytical Results

Range of analyte values: See above

Accuracy: See above

Precision: Ten blood samples were analyzed in duplicate. The range of Protein-C concentrations was 0.6 – 1.8 ug/mL. Average CV was 8.4 %.

Plasma and blood results compared: Paired blood and plasma samples (N = 72) from four patients collected over a one month period were measured with the following results:
Blood (y) = 0.95*Plasma (x) + 0.12; R = 0.93.

<u>CRP:</u>

Sensitivity: The limit of detection (LOD) was determined by measuring signals for the zero analyte and lowest concentration calibrator. LOD of 0.56 ug/mL was calculated as 2*Signal SD [Averaged over the two values]/Slope of (signal vs. conc.).

Performance was calculated next to reference methods with inferior sensitivity and dynamic range – note page 12.

Precision and calibration accuracy in whole-blood clinical samples:

CRP ug/mL	Inter-day Precision CV, %
224.9	12.3
58.4	4.4
23.7	18.1
Average	11.6

Dilution linearity in calibrator materials:

CRP ug/mL	Target ug/mL	Diln. Ratio	Within inst. Recovery %
224.9	(224.9)	1.00	(100)
118.4	112.4	0.50	112.4
58.4	45.0	0.20	129.8
23.7	22.5	0.10	105.3
		Average	110.1

Clinical Studies

Since CRP values ranged very widely during the clinical study (from 0.1 – >400 ug/mL, a 4,000-fold range) both between patients and over time within each patient, it was decided that the Log of the CRP concentration (ug/mL) is the most useful parameter to report. In the summary data below, we present both linear and log scale results.

Accuracy:
Results obtained in the Theranos system for blood samples were compared with results for paired plasma samples. For 109 samples with CRP concentrations ranging from 0.1 – 371 ug/mL there as a good correlation of log values y (Theranos) = 0.98* x (Reference method) + 0.02, R = 0.98.

Precision: Ten blood samples were analyzed in duplicate. The range of CRP concentrations was 0.7 – 268 ug/mL. The mean value was 62.5 ug/mL. The standard error of the estimate (SEE)/Mean was 13.9 %. When these data were computed for Log CRP values, the SEE/Mean was 7.1 %.

Blood versus plasma: Four patients were evaluated over about one month with the following results from regression analysis of Log values (Log (blood value) [y] = b*Log(plasma value) [x] + a).

Patient ID	Samples tested	Range (ug/mL)	Slope (b)	Intercept (a)	R
001	15	0.1 – 224	0.951	0.027	0.997
004	29	3.8 – 239	0.947	0.052	0.965
005	13	14.8 – 218	0.934	0.224	0.870
006	14	18 – 210	0.951	0.0271	0.997

Results of the Stanford AML/Sepsis Clinical Study

Patients: Sampling and Clinical Course
Patients were enrolled on admission and most were enrolled and sampling started before their first febrile episode. The enrolled patients experienced varied clinical courses, from florid sepsis to isolate fever for only a short period.

Patient	Sex	Age	Diagnosis	Closest draws Pre and Post Fever (hrs)	Infection /Sepsis	Analysis issues?
001	M	39	Pre T ALL	-50:00, +1:28	Positive Blood cultures. Clinically looked septic for a few hours.	Pre fever draw.
002	M	22	AML M4	-13:34, +4 days	Line infection – cellulitis and thrombophlebitis. Deeper tissues not involved	Line removal.
003	F	37	AML	+4:00	Respiratory infection	No pre-fever draws
004	M	59	Pre B ALL	-8:25, +5:25	Septic shock admitted to ITU.	Missed draw pre -fever
005	F	56	AML M5a	-6:39, +1:31	Pseudomonas cultured but no clinical sepsis	
006	M	60	AML(APL)	-5:00, +1:00	ATRA syndrome	
007	M	31	AML	-12:00	Possibly early sepsis?	Line Removal.
008	M	44	ALL (from CML)	-2:33, +3:42		Refused draws.
009	F	39	AML M5 (consol)		Pharyngitis and Pneumonia	
010	M	70	AML(CMML)	-0:00, -6:22, +5:25	?	
011	M	57	AML M4Eo	-1:32, +0:33	?	
012	M	49	AML M7 from(CML)	-0:18, +4:47	Suspected Septic Shock ITU (prior to neutropenia). From Pneumonia?	
013	F	38	AML M5	-7:28, +0:40	Positive cultures	
014	M	60	AML M0	+6:40	?	Fever prior to sampling
015	M	52	AML with multilineage dysplasia	-5:30, +1:51	Clinically septic day. Enteritis on CT.	
016	F	62	AML (bilineage)	No neutropenic spike yet.	Minor fever spikes, no other symptoms.	Missed non neutropenic spike
017	M	52	Pre B ALL	-2:54, +1:36	??	Possible prior spike??

CRP Data

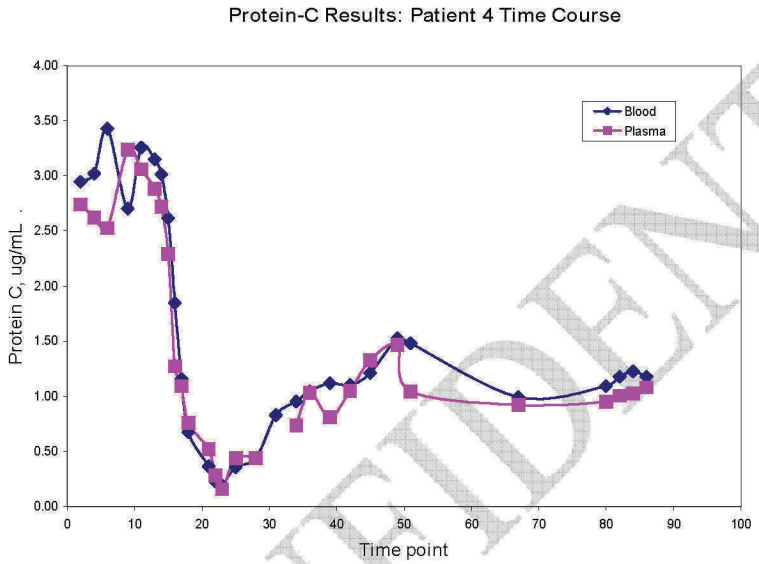
Since CRP values ranged very widely during the clinical study (from 0.1 – 268 ug/mL, a 2500-fold range) both between patients and over time within each patient, they were log-transformed. In the summary data below, we present both linear and log scale results.

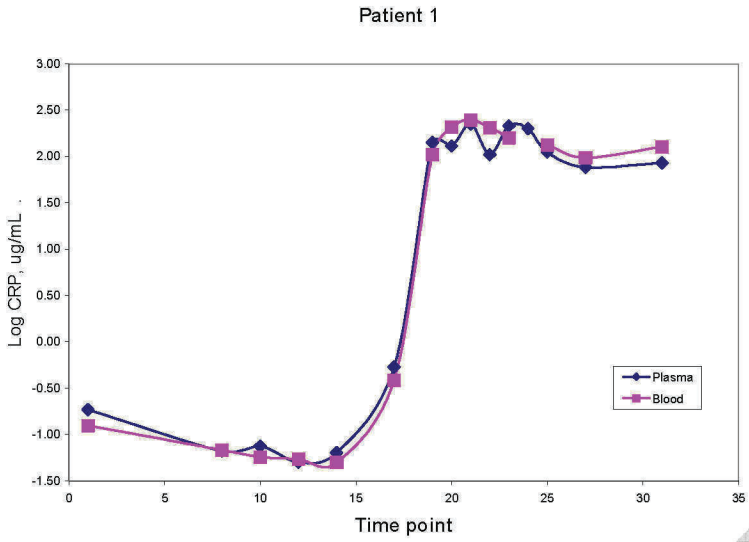
Precision: Ten blood samples were analyzed in duplicate. The range of CRP concentrations was 0.7 – 268 ug/mL. The mean value was 62.5 ug/mL. The standard error of the estimate (SEE)/Mean was 13.9 %. When these data were computed for Log CRP values, the SEE/Mean was 7.1 %.

Blood versus plasma: Four patients were evaluated over about one month with the following results from regression analysis of Log values (Log (blood value) [y] = b*Log(plasma value) [x] + a).

Patient ID	Samples tested	Range (ug/mL)	Slope (b)	Intercept (a)	R
001	15	0.1 – 224	0.951	0.027	0.997
004	29	3.8 – 239	0.947	0.052	0.965
005	13	14.8 – 218	0.934	0.224	0.870
006	14	18 – 210	0.951	0.0271	0.997

Representative samples from two patients comparing whole blood to plasma:

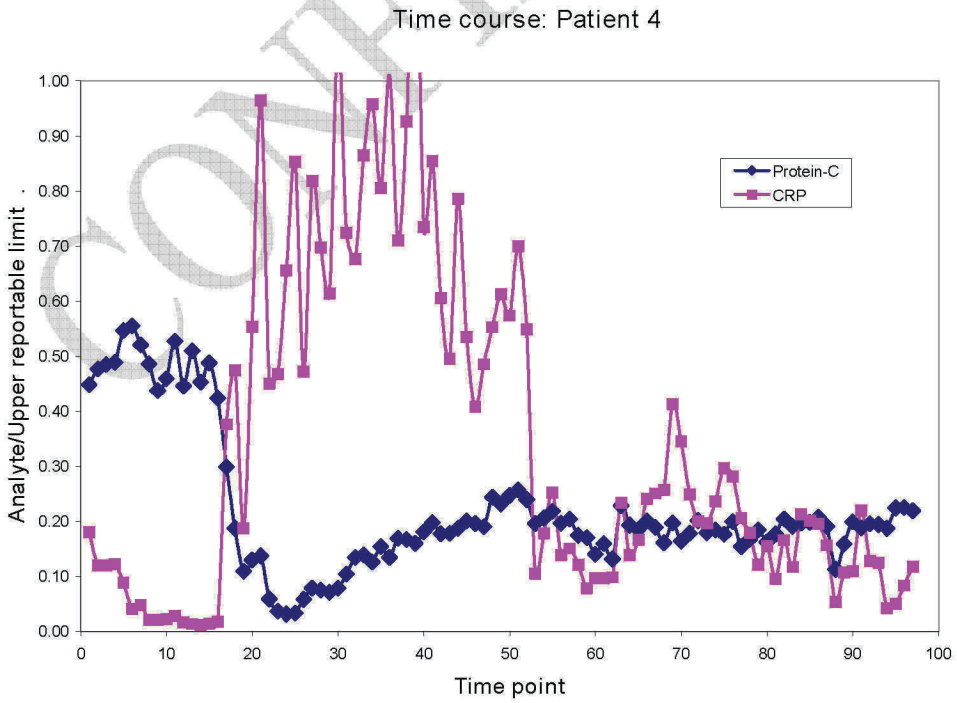




Protein-C Data - Representative time courses for two analytes in one patient:

The system was used to monitor patients over a course of chemotherapy. Time courses for a selected patient is shown below. Other clinical data were gathered as part of this study, and will be include in other publications.

The data for the two Theranos specific analytes are shown below were from a subject who became septic during the study and was moved to the ICU at time point 20. After briefly leaving (time point 50) he was returned to the ICU at time point 65. Protein-C values were initially somewhat low then fell dramatically to less than 0.5 ug/mL as sepsis occurred. In the ICU, levels climbed to 1 - 2 ug/mL. CRP was initially elevated but fell into the normal range until sepsis occurred after which levels spiked to extremely high values only falling after some time in the ICU.



Discussion of the difficulties of multiplexed assays encountered by some methodologies but obviated by the Theranos system.

The reference laboratory used a multiplexed assay method in which all the analytes are measured in a single well. Such methods have intrinsic limitations which caused problems in their assays for CRP and Protein-C. The other biomarkers measured in the multiplex were at low concentrations (pg/mL) so the sample cannot be diluted to any great extent. The multiplexed assays worked well for these low concentration analytes but very poorly for Protein-C and CRP. The Protein-C results correlated poorly with Theranos results and those of a commercial assay (Theranos and the commercial ELISA agreed very well). CRP results were not reportable because they were largely out of range high. It is evident that low and high concentration analytes cannot be combined in the reference method. It is likely that the CRP assay had never been set up for samples with such high concentrations and that the Protein-C assay was at the upper reportable range of the reference assay where precision is poor.

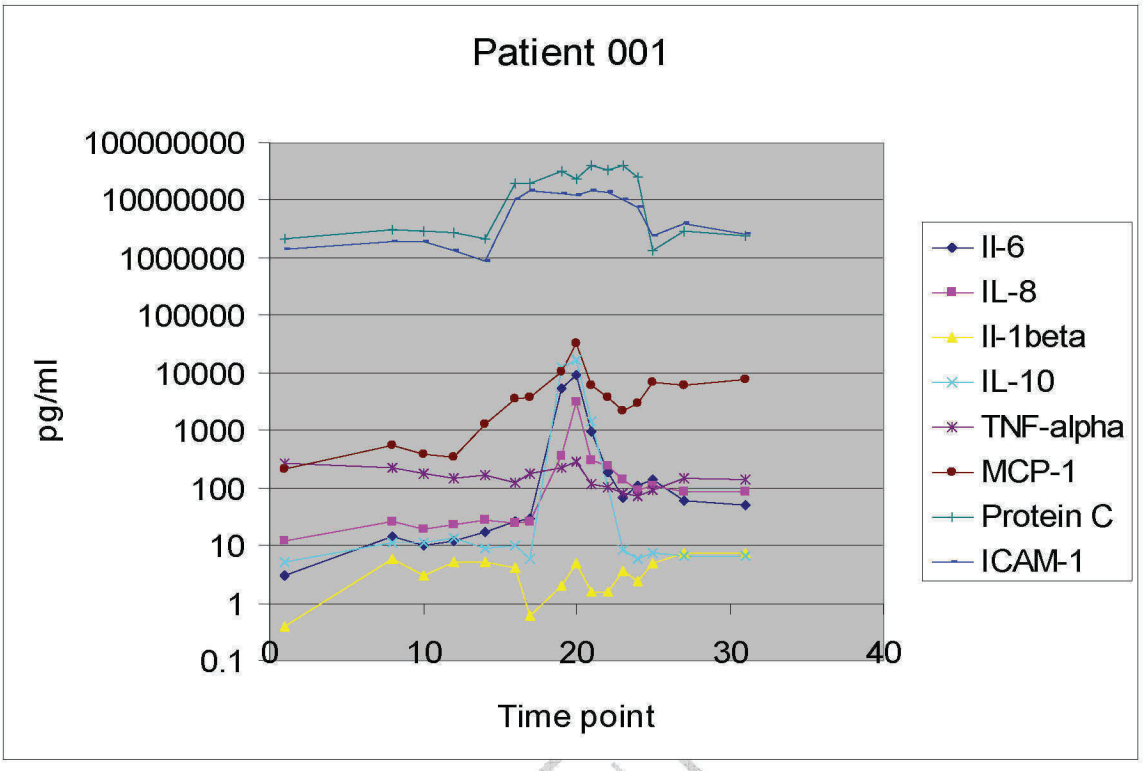
The Theranos System is designed to eliminate such problems. Specifically it can measure analytes at very low and very high concentrations with good precision in the same cartridge. This is achieved by (a) choice of a very sensitive assay method which has a very high dynamic range (10^4 fold) and (b) the ability to use more than one sample dilution level in the same multiplexed assay cartridge (2-fold to 10,000-fold). To illustrate this we show results of a Theranos Multiplexed Assay currently in clinical evaluation. The limit of detection for the high sensitivity assays is less than one pg/mL and the upper reportable concentration of the low sensitivity assay is 250,000,000 pg/mL (7.5*10^6-fold higher).

Analyte	LOD	Highest reportable Range (pg/mL)
IL-6	0.4	1500
TNF□	0.3	7500
CRP	70,000	250,000,000
		Range ug/mL
CRP	0.07	250

Full Data - Representative time courses

One goal of this observational study was to identify the potential most informative biomarkers for predicting the onset of fever and sepsis. Subsequent mathematical and statistical modeling were undertaken to establish novel algorithms for identifying and characterizing the optimal combinations of biomarkers for monitoring such patients. The initial candidate marker set included: CRP, Protein-C, IL-6, IL-8, TNF-α, IL1-β, IL-10, MCP-1 and ICAM-1. These markers were chosen on the basis of previous research in both neutropenic fever and sepsis. They represent both pro- and anti-inflammatory sides of the cytokine response, the acute phase reaction, the Protein C system and adhesion molecule expression patterns. This provides an immunophysiologically broad range of molecule subtypes which are grounded in literature but have not before been tested as a single panel.

Results of the initial analyses for these biomarkers in a subset of samples from all ten evaluable patients are presented below. Representative time course for all analytes are presented below: Patients 1 and 4 became septic at time points 16 and 19 respectively, Patients 5 and 6 did not.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10000000
1000000
100000
10000
1000
100
10
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0.1
pg/ml
0
10
20
30
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Time point
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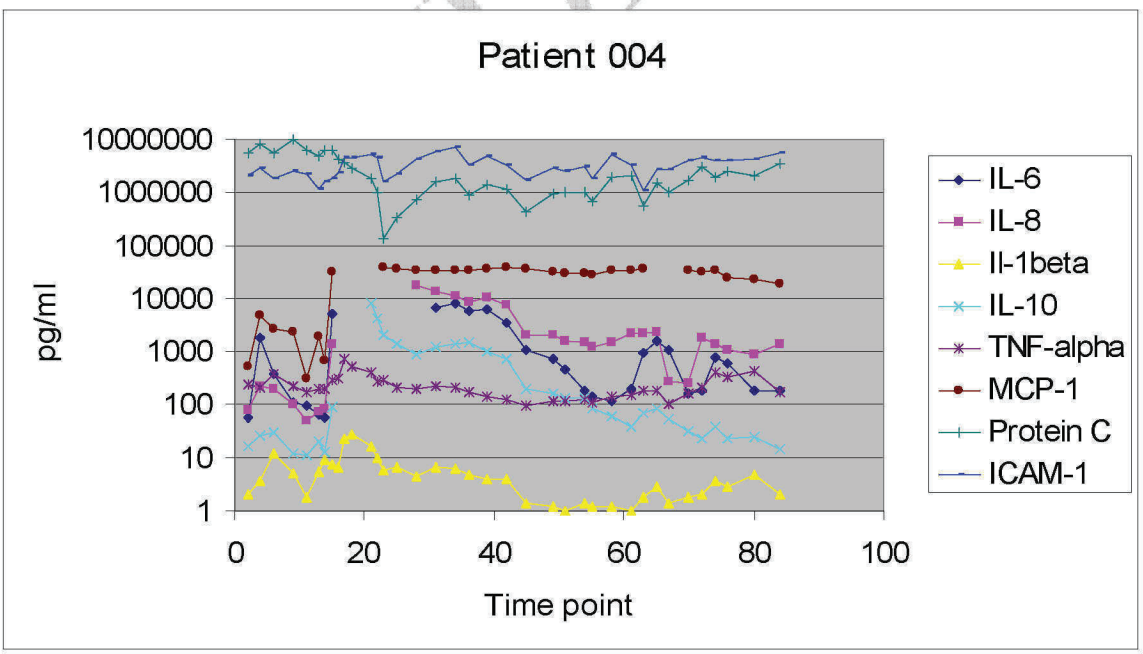
IL-8
Il-1beta
IL-10
TNF-alpha
MCP-1
Protein C
ICAM-1


Patient 004
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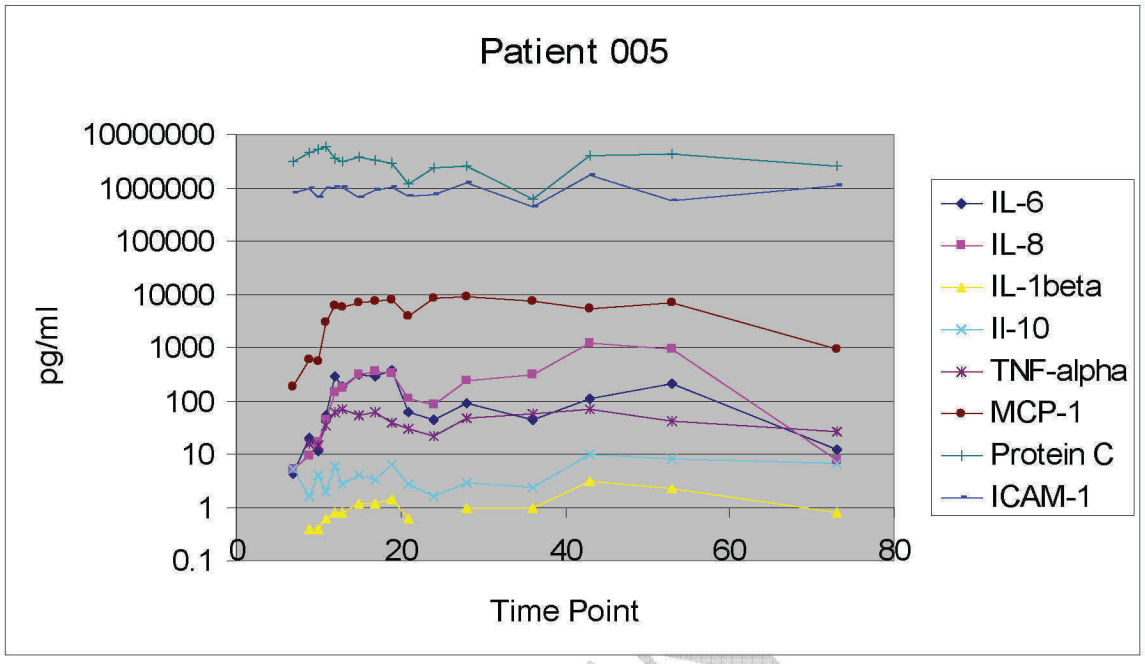
1000000
100000
10000
1000
100
10
1
pg/ml
0
20
40
60
80
100
Time point
IL-6
IL-8
Il-1beta
IL-10
TNF-alpha
MCP-1
Protein C
ICAM-1


Patient 005
10000000
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1
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pg/ml
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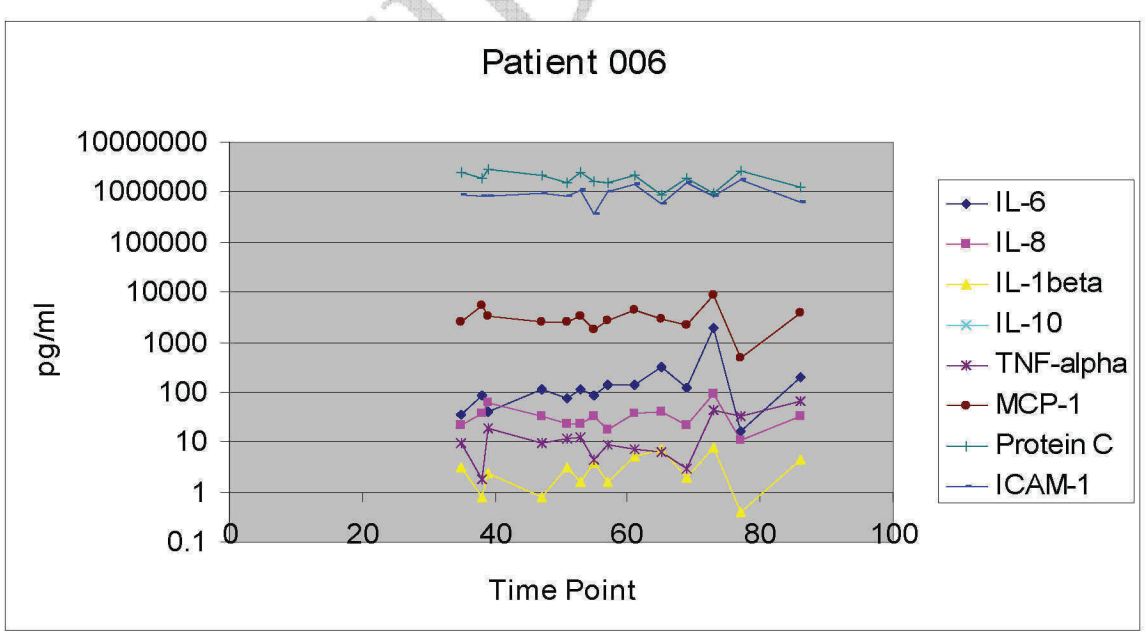
60
80
Time Point
IL-6
IL-8
IL-1beta
Il-10
TNF-alpha
MCP-1
Protein C
ICAM-1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Time Series Analyses and Comparison of Individual Protein Markers

Marker to Marker Correlation

Methods

The time course for each protein marker was plotted and the time series of measures was analyzed. The relationships between pairs of markers were studied using a cross correlation function. This statistical function measures the correlations between protein levels across a spectrum of lag times, essentially, shifting one curve forward or backward in time and testing the translated time series against each other for correlation. The correlation between two series with a lag of 0 (zero), measures how much two protein track each other directly. When one applies a lag time of 1 (one) unit, a significant correlation suggests that one of the proteins precedes the other by one sampling unit, etc.

The output of the analysis is typically presented as histogram of correlation levels (y-axis) versus lag times (x-axis), and is termed a correlogram.

Results

Initial results for only one patient and particular pairs of protein markers are presented below. The patient presented herein is 001, and the relationships studied thus far are:

1. Protein C and ICAM-1
2. Protein C and MCP-1
3. IL-10 and MCP-1
4. IL-6 and MCP-1

These particular protein pairs were chosen because upon visual inspection it was vey apparent that Protein C and ICAM-1 tracked very closely with each other as did IL-10 and MCP-1 and IL-6 and MCP-1. As noted above, if any of these pairs yield a highly significant correlation (> 0.8), then when constructing the final biomarker, one should only choose one of the pair and the choice should be based on criteria such as accessibility, chemical tractability, etc

Further inspection suggested that spikes in MCP-1, IL-10, and IL-6 precede a drop in Protein C, as experienced at a fever spike.

The correlogram for Protein C versus ICAM-1 is given below.

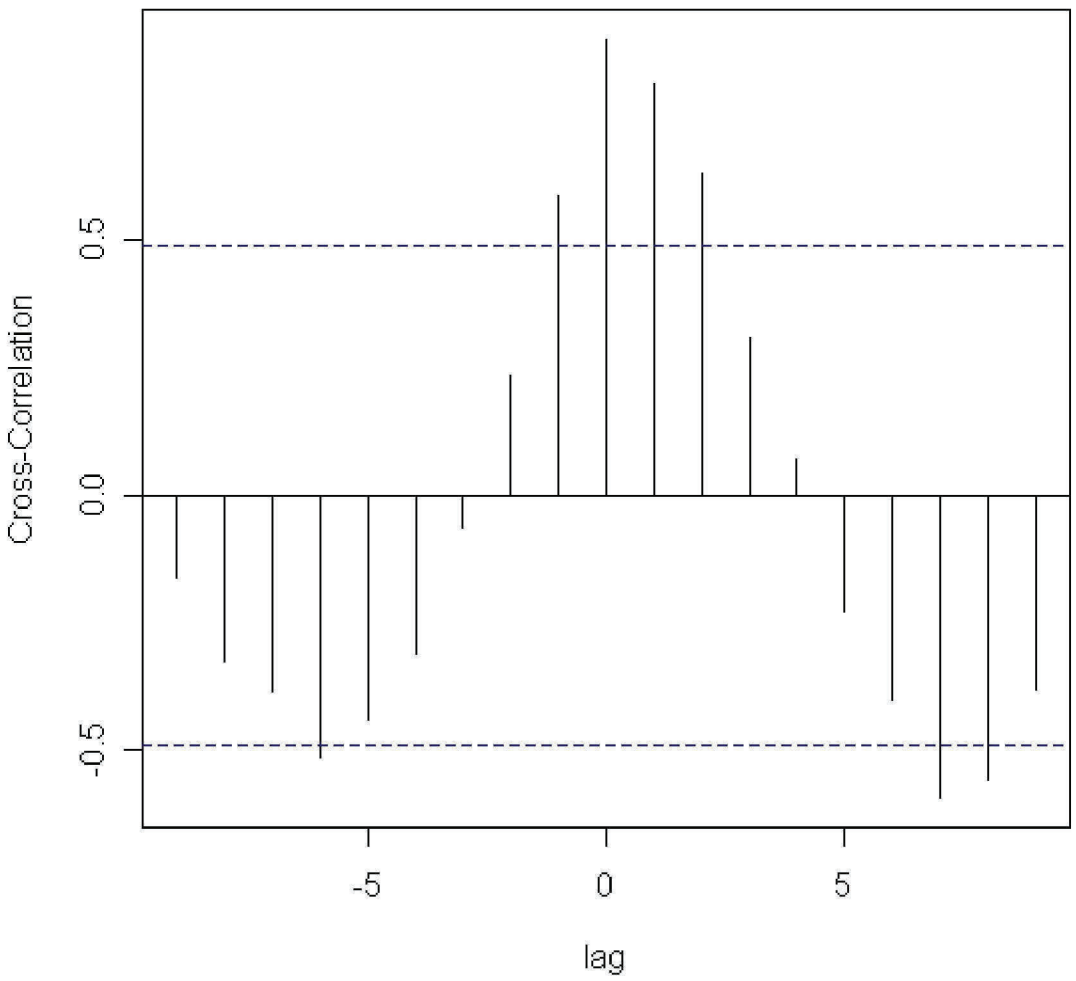

Figure 1 Protein C and ICAM-1

The zero lag correlation between the two marker proteins is 0.89 with significant correlations observed at one and two sampling unit lags (range 0.588 to 0.630). These results suggest that these two protein markers track quite consistently.

Similarly, the correlogram for IL-10 and MCP-1 suggests that the two proteins track quite well (See Figure 2, zero lag correlation = 0.867)

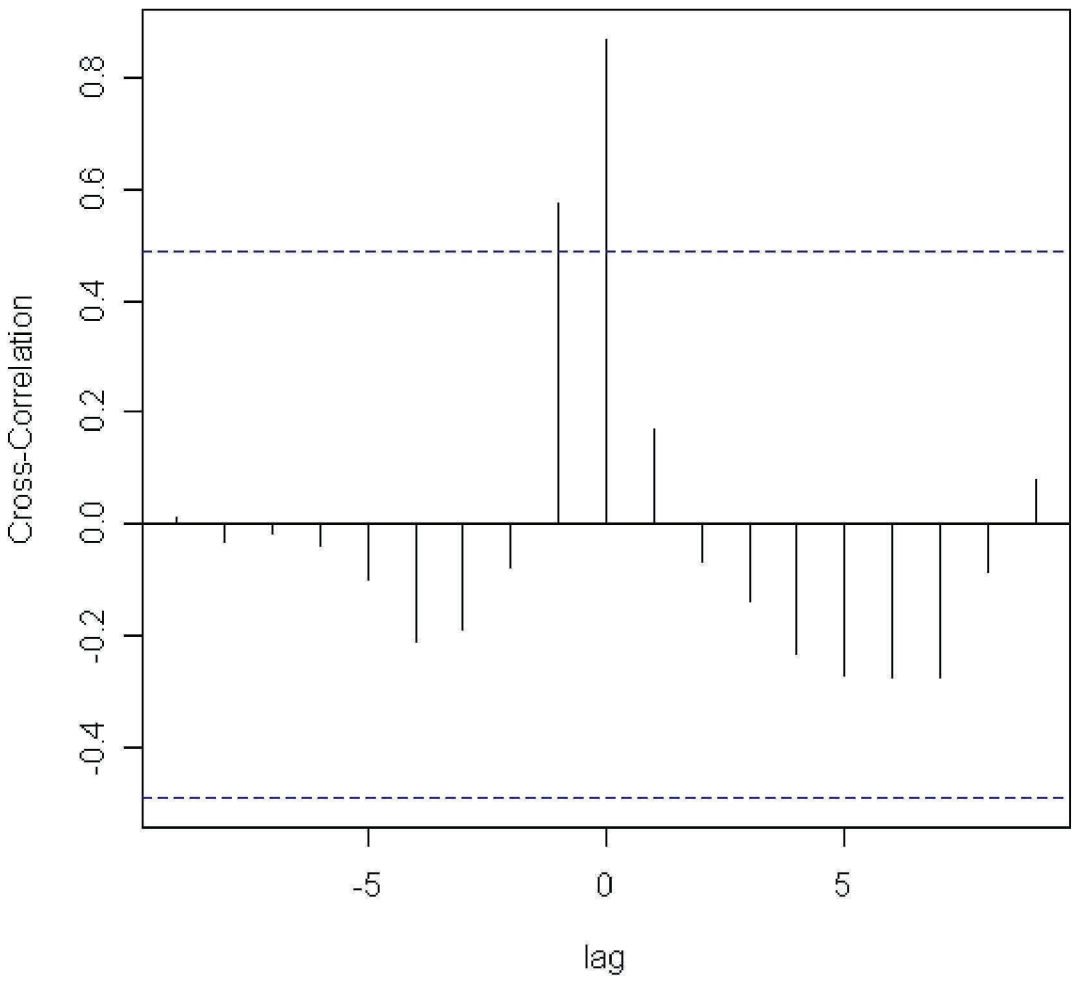

Figure 2 IL-10 and MCP-1

Similar results hold for IL-6 and MCP-1 (See Figure 3, zero lag correlation = 0.911)

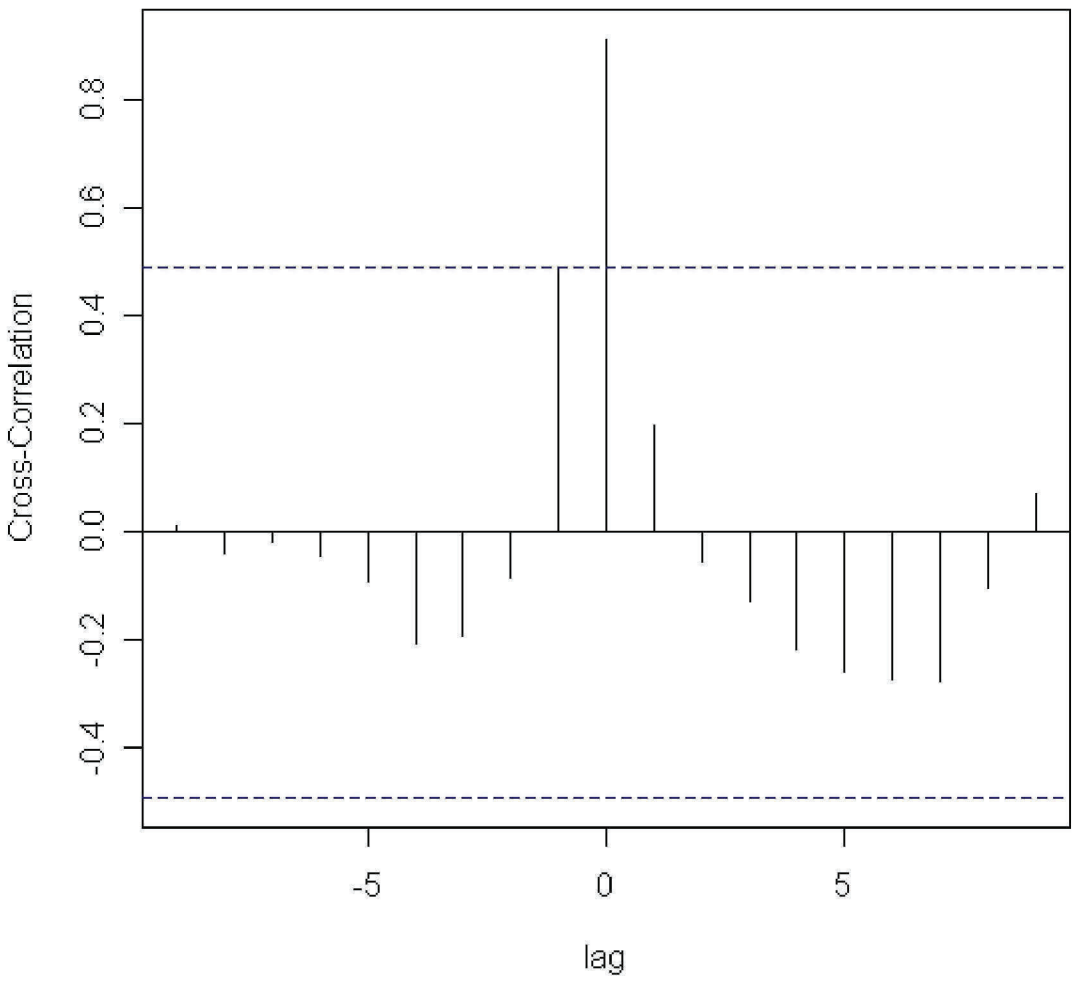

Figure 3 IL-6 and MCP-1

To test whether the Protein C and MCP-1 are temporally related in a “look forward” way, the cross correlation for this pair was also analyzed. The results are presented below in Figure 4.

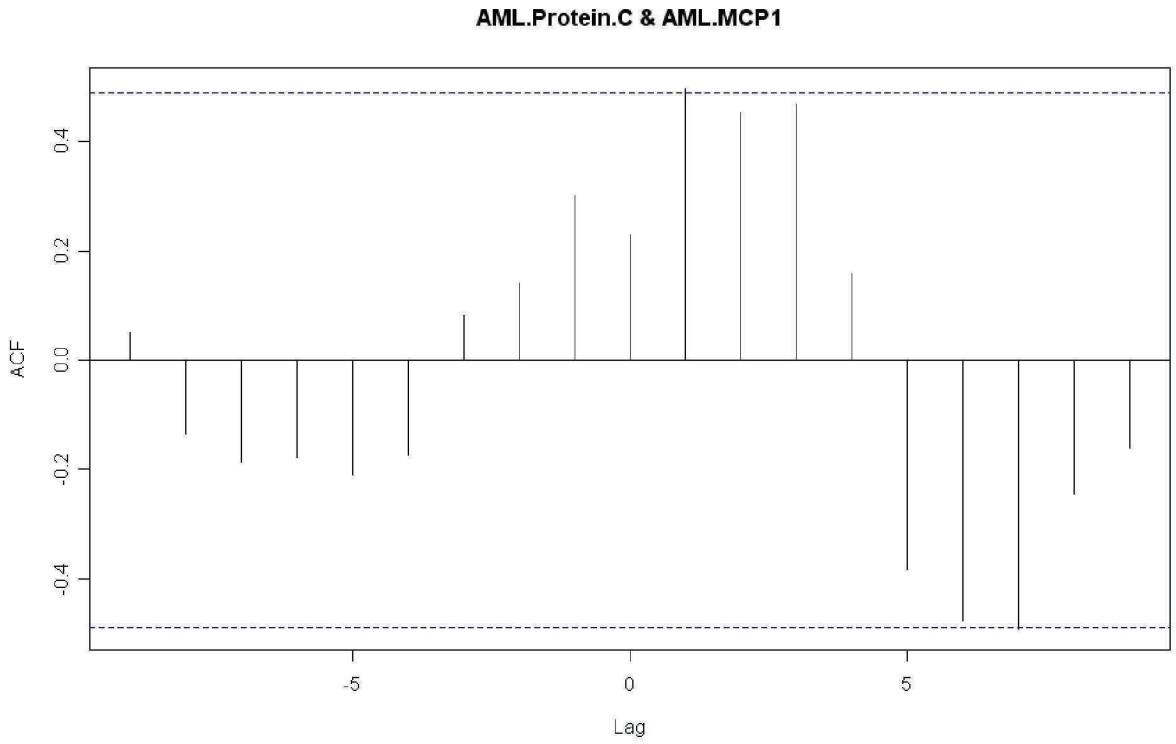

Figure 4 MCP1 and Protein C

In this case a more interesting pattern emerges. The correlations, though significant, are fairly modest (magnitudes ~0.45-0.50), and show two interesting dynamics: a lag of 1 to 3 sampling units between the two protein markers yields marked positive correlations (lag one correlation =0.495, lag 2 correlation = 0.453, lag 3 correlation = 0.467), suggesting that the two markers roughly track each other with a 1 to 3 sample interval offset. More interesting though is that a 7 sampling unit lag yielded a comparable negative correlation (-0.491) suggesting that as one marker goes up (MCP) the other falls about a 56 hours later (Protein C).

Predicting Fever

Fever, as a defense mechanism, results from the effects exerted on the hypothalamus by circulating cytokines, called pyrogens. These cytokines emerge from the immune system during an inflammatory response. The best known of these are IL-6, Il-1beta, and TNF-alpha. The primary goal of this initial effort was to use the time courses for these pyrogens to build a statistical model for the onset of fever.

Methods

A Partial Least Squares (PLS) model was constructed based on a pre-fever spike window of 36 hours. Measures for IL-6, IL1-beta, and TNF-alpha and their sample-to-sample fold changes were included in the saturated model. Concentrations were measured in pg/mL, and sample-to-sample fold changes in concentration were recorded and analyzed as follows:

- The concentration data were first visually inspected for Gaussian normality, and a log transform was applied to correct for log-normality.
- Based on these measures, an initial PLS regression model was built that included all three pyrogens and their fold changes from sample to sample. The dependent variable in the model was time-to-spike (tts) based on when each sample was taken.
- This type of model characterizes the amount of variation accounted for in the covariance structures of the dependent vectors (times-to-spike) and the independent vectors (pyrogen levels).
- In this data set there were more independent variables than degrees of freedom which is termed the overfitting problem. To address the problem standard statistical model building tools like top-down elimination and bottom-up addition were employed. This process is termed feature selection.

Results

Using a bottoms up approach to feature selection, seven submodels were developed. The best model included all three pyrogens (log(IL-6), log(IL-1beta), and log (TNF-alpha)) and accounted for 975% of the total model covariance and 52% correlation with the dependent variable (tts).

In this small observation set, formal confidence intervals are meaningless. However, fully 80% of the predicted tts fell within a ten hour window of the observed tts. Significant patient to patient variation was observed and given a larger, appropriately powered study, a more complete modeling exercise will be undertaken. However, as a proof of principle, these data suggest that it is possible to model the onset of fever as a function of rate of change in the circulating pyrogen levels.

Classifying Sepsis

Methods

Clinical outcomes for the patients were defined as “Fever only”, “Fever plus infection”, and “Sepsis/SIRS”. The underlying hypothesis driving this initial analysis is that the way one progresses to fever (as represented by the candidate pyrogen set) in conjunction with how one reacts after the fever has begun will predict with some statistical integrity the onset of sepsis.

A classifying multivariate analysis was performed using a standard validated technique (Discriminant Function Analysis) on the derived patterns for pre-spike IL-6, Il1-beta, TNF-alpha, and post-spike Protein C and CRP. The data were analyzed as follows:

- At the point of fever spike, the previous 36 hour trajectories for the three candidate pyrogens were analyzed for intra-vector correlations
- Based on these studies an extremely high degree of correlation was observed between the three (Greater than 0.8), and it was decided that the final model building exercise would concentrate on IL-6 and Il1-beta.
- Upon advent of the “fever spike”, the trajectories for the two remaining pyrogen concentration curves were fit to a linear regression starting at time zero (time of spike) and ending at time -36 (36 hours prior to the spike).
- Two derived parameters, slope and intercept, were recorded for each pyrogen for each patient.
- The CRP and Protein C levels for each of the 8 hour intervals following the spike were also recorded, and added to the analysis.
- A Discriminant Function Analysis was performed on the set of 10 evaluable patients from which, a mean vector centroid for each clinical outcome set was determined.
- The Mahalanobis Distance from each patient to each centroid was then calculated,

and a Bayesian probability of class membership was calculated.

Results

10 of the 17 enrolled patients were considered evaluable. Two were determined by the clinical team to have experienced Sepsis/SIRS, three had infection with fever, and 5 had fever alone. The final model included the slope of the Il-6, the slope of the IL-1beta, and the CRP and protein C observed in a window between 8 and 16 hours post-spike. This modeled yielded 100% sensitivity and 100% specificity for assignment to the Sepsis/Sirs group.

Conclusions

This pilot study in acutely ill leukemic patients suggests that repeated measurements of blood markers can detect dynamic alterations and trends which forecast the occurrence of serious events like sepsis. As a result of the integration and modeling of the data, information which previously could not be characterized can be released promptly to medical teams who can implement individualized therapeutic interventions in a more time efficient manner. These findings are being further validated by adequately powered randomized clinical trials.