celcuity
EXPANDING TREATMENT OPTIONS

Expanding Treatment Options for Cancer Patients

CELsignia Tests Diagnose the Cancer Driver in Patient Tumors Lacking Actionable Biomarkers

Celcuity Overview

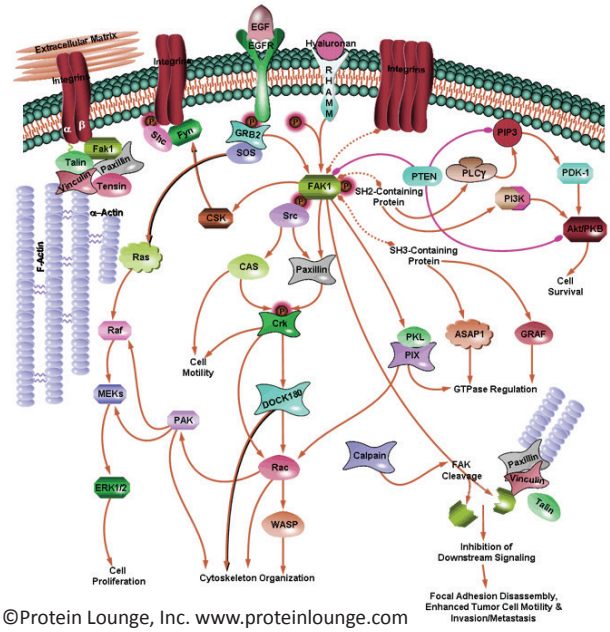
Celcuity develops companion diagnostics that expand the eligible patient populations for targeted therapies by identifying new cancer sub-types molecular-based approaches cannot detect. Instead of identifying genetic variants in fixed tumor cell samples, our diagnostic tests measure dynamic pathway signaling activity in a cancer patient's living cancer cells ex vivo. Patients diagnosed with a hyperactive signaling pathway using our test are expected to respond to matching targeted therapeutics, despite lacking a corresponding genomic biomarker.

The company's first diagnostic, the CELsignia Multi-Pathway Signaling Test, or CELsignia Test, measures dynamic HER2, c-Met, and PI3K-node involved signaling activity in the living tumor cells of HER2-negative breast cancer patients. Analytical validation studies of the CELsignia Test have been completed and two Investigational Device Exemptions have been approved to conduct two Phase II interventional clinical trials to evaluate the efficacy of targeted therapeutics in patients selected with the CELsignia Test. Celcuity expects to collaborate with the manufacturers of targeted therapeutics to obtain FDA approvals for the targeted therapeutics to treat the expanded patient populations selected by the CELsignia Test.

Most cancer patients lack actionable mutations

Advances in molecular tests make it possible to identify increasing numbers of genetic variants in tumor tissue. This has facilitated development of molecularly targeted therapeutics designed to disrupt signaling dysfunction that have significantly improved outcomes for cancer patients. Despite these advances, determining the dysfunction driving most patient's cancer using molecular tests remains elusive. Less than 20% of Americans who died of cancer in 2018 were eligible for a molecular targeted therapy because they lacked what are currently considered actionable genetic or proteomic mutations.[1]

Recent systems biology research reveals that relying solely on static point-in-time proteomic or genetic analyses to diagnose cancer sub-types will not detect patients whose cancer is driven by abnormal signaling not associated with an actionable mutation. This reflects the limitations of using static measurements of proteins or genetic mutations in fixed cells to characterize a dynamic biological system that involves a cascade of interacting components with an estimated 10^{20} permutations of biochemical states. Current tests cannot evaluate either dynamic signaling activity or whether a drug can affect that activity.



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The need for a diagnostic test that identifies the cancer driver in tumors lacking actionable mutations

Measuring dynamic cell signaling activity is an alternative diagnostic approach to identify the cancer driver in patient tumors lacking actionable genomic or proteomic mutations. This approach requires the use of living patient tumor cells as well as technology to quantify signaling activity levels. Efforts to obtain patient tumor cells have previously been limited by the lack of reliable methods to extract and culture cancer cells from patient tumors. Lack of access to living patient tumor cells, in turn, hampered development of technology to analyze dynamic signaling activity.

The CELsignia Platform

To address this unmet need, Celcuity developed the CELsignia platform, a dynamic cellular analysis technology that measures ex vivo cell signal transduction activity in live tumor cells from individual cancer patients. This approach overcomes the limitations of static genetic analyses of fixed cells by determining whether the dynamic signaling activity of important oncogenic pathways is at normal levels or is hyperactive and can be inhibited by a matching targeted therapy.

Table 1: Design Inputs for CELsignia Platform

Design Input	Clinical Need	Status
Specimen Size	Single core (12 gauge)	✓
Sample Yield	>90% from live tissue	✓
Turnaround Time	10-14 days from receipt	✓
Sensitivity & Specificity	>90%	✓
Precision	<10% CV	✓
Process Scalability	>500,000 tests	✓

Design inputs critical to meeting real-world clinical requirements were successfully addressed during the development of the CELsignia platform, including those listed in Table 1.

With the CELsignia platform, we develop diagnostics that measure the cumulative dynamic signaling activity of specific pathways over a four-hour period using a cancer patient's live tumor cells. Tumors lacking actionable mutations found by a CELsignia Test to have a hyperactive signaling pathway represent a new cancer sub-type treatable with a matching targeted therapeutic.

Our studies assessing dynamic signaling activity levels in tumors from cancer patients lacking actionable mutations found that hyperactive signaling pathways are clearly differentiated from those with normal signaling levels. This differentiation is exhibited in the bi-modal distribution of signaling activity levels within the population of cancer patients lacking actionable mutations. The mean level of signaling in the patient sub-group with hyperactive pathways is typically separated by five standard deviations from the mean level of signaling in the patient sub-group with normal signaling activity. This enables CELsignia Tests to report results with 95% specificity and nearly 90% sensitivity. Each test is analytically validated per FDA/CLSI/CAP/CLIA guidelines, and the FDA has approved several Investigational Device Exemptions (IDE) for the CELsignia Test to select patients for enrollment in clinical trials.

The CELsignia Test Diagnoses Three New Cancer Sub-Types

The CELsignia Test is a Laboratory Developed Test (LDT) that measures HER2, c-Met, and PI3K-node involved signaling activity in tumor cells obtained from patients previously diagnosed with HER2-negative breast cancer. Fresh tissue specimens obtained from a biopsy procedure are collected in a Celcuity provided specimen collection kit at the clinical site and then delivered directly to Celcuity's CLIA-certified and CAP-accredited laboratory where the test is performed, and the test report is issued.

Three cancer sub-types are identified with the CELsignia Test:

1. Hyperactive HER2 signaling tumors
2. Hyperactive HER2 and c-Met signaling tumors
3. Hyperactive PI3K-involved signaling tumors

As a companion diagnostic, the CELsignia Test can identify additional cancer patients for targeted therapeutics that inhibit the hyperactive signaling activity detected in a patient's tumor.

CELsignia Technology Overview

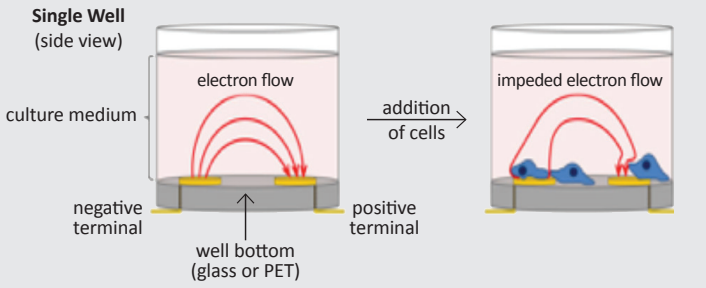
To detect whether HER2, c-Met, or PI3K-involved signaling activity in a breast cancer patient's tumor cells is normal or hyperactive, the CELsignia Test leverages the well-established biologically-linked activities of cell signaling and cell-cell and cell-ECM adhesion processes. Measurement of cell adhesion activities can serve as a metric for cell signaling activity.

Celcuity uses an impedance biosensor instrument (xCELLigence RTCA MP) to quantify dynamic changes in cell adhesion and morphology initiated by signal pathway activation or inhibition in live patient tumor cells. The instrument is comprised of a standard 96-well microplate with thin gold electrode arrays covering the bottom of each well and an analyzer. In one form of cell adhesion, specific adhesion proteins on the surface of the proliferative patient cells attach to extracellular matrix (ECM) that is coated on the microplate well electrodes. When the proliferative cells are placed on top of the well electrodes, the cells act as insulators, leading to an increase in electrode impedance that can be measured.

To obtain a measurement, a small alternating current (I) is applied across the electrode at the bottom of the microplate wells. This results in a potential (V) across the electrodes that is measured by the RTCA Analyzer. The impedance (Z) is determined by Ohm's law Z = V/I. The measurement of impedance is non-invasive and harmless to the cells since only a very weak alternating current is applied to the sensor electrodes.

The schematic in Figure 1 provides an example of impedance measurement in a single-well of the microplate. Activation or inhibition of signaling activity causes changes in cell adhesion. To measure cell adhesion changes in **real-time**, live patient cells are attached to a microelectrode.

Figure 1: Schematic of Impedance Biosensor Well



As cells cover the electrodes, applied current is impeded in a manner related to the number of cells and the manner in which the cells have contacted the ECM. Cell signaling changes modulate cell adhesion and morphological properties near the sensor surface they are attached to in a manner the impedance biosensor is capable of detecting and quantifying. When cells are specifically stimulated with picomolar amounts of agonist to change their signaling, the accompanying cell signaling responses thus alter the electrode impedance. The CELsignia Test is capable of detecting cellular changes in the sub-nanometer to micrometer range.

The electrode impedance change is the analyte of the test and is referred to as a cell attachment signal (CAS), expressed in ohms. CAS can be detected when changes as small as 0.0015 Ω can be measured. The data generated is CAS (impedance change) versus time. The test result is determined using an algorithm that calculates the difference in ΔCAS measured in different test wells containing the patient cells.

CELsignia Test Method

Upon receipt of the patient tumor sample and information provided from the attending physician or pathologist, patient tumor cells are first isolated from fresh breast tumor tissue. The resulting live patient cell samples are seeded in the wells of an xCELLigence 96-well electrode-based microplate.

The cell samples are tested, along with appropriate controls for quality control purposes, with and without specific pathway ligands and antagonists that initiate or inhibit HER2, c-Met, or PI3K involved pathway signaling in tumor cells. To perform this analysis, every CELsignia Test evaluates seven groups of patient cell samples placed in separate wells of the microplate along with control wells.

For each well of patient cells, impedance change, caused by the effect of the pathway agonists or antagonists on the cells, is measured, recorded, and reported over time. For every test, patient cell samples are placed in separate wells of the microplate, tested, and the CAS is measured every minute and analyzed for each well. The sum of the value of the CAS data points for each well is determined and is expressed in "Signaling Units."

Activation of HER2 signaling activity occurs after ligands (EGF and NRG1) bind to HER1 and HER3 receptors and dimerize, or form pairs, with the HER2 receptor. The amount of pathway activity specific to HER2 heterodimerization with HER3 and HER1 receptors is determined using a HER2 dimerization blocker, as highlighted in Figure 2. Activation of c-Met signaling activity occurs after HGF binds to c-Met receptors. Activation of PI3K-involved signaling activity occurs after S1P binds to S1P receptors. A pan-PI3K inhibitor is used to determine the amount of PI3K-involved signaling associated with the S1P activation.

Software quantifies and analyzes the differences between data recorded in different test wells. A sample test output from a CELsignia Test is presented in Figure 3. The Signaling Score for each pathway is compared to a cut-off to determine whether the patient's cell sample demonstrates "normal" or "abnormal" levels of HER2, HER2 and c-Met, or PI3K-involved signaling activity.

Figure 2: CELsignia Test HER2 Signaling Activation and Inhibition

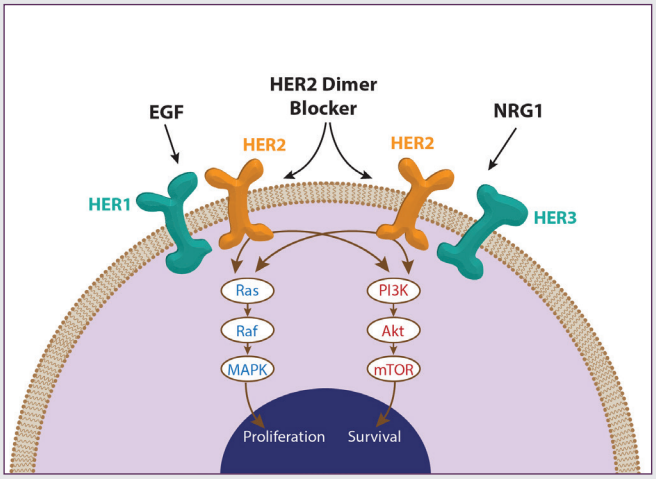
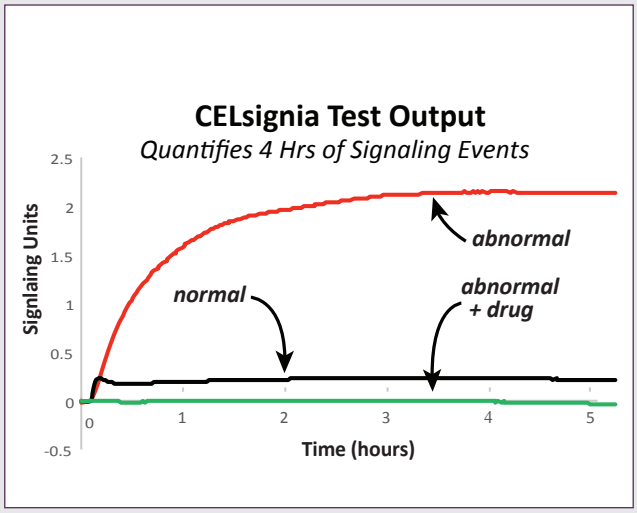


Figure 3: Sample CELsignia Test Output



Analytical Validation Studies

Celcuity has completed analytical validation studies in accordance with applicable FDA guidance and Clinical and Laboratory Standards Institute (CLSI) standards in its CLIA and CAP certified laboratory to characterize the performance of the CELsignia Test. A summary of the results is provided below in Table 2.

Table 2: Results of Analytical Validation Studies for CELsignia Test

Performance Characteristics	Results
Analytical Precision (Qualitative)	
Analytical Sensitivity (95% CI)	95.8% - 100% (88/88)
Analytical Specificity (95% CI)	95.8% - 100% (88/88)
Detection Limits	
Limit of Blank	0.0020 cell attachment units
Limit of Detection	0.0099 cell attachment units
Limit of Quantification	0.1000 cell attachment units
Cut-Off Characterization	250 signaling units

Summary of Published Pre-Clinical Studies

Celcuity has conducted an extensive series of pre-clinical studies using the CELsignia platform to characterize critical test parameters. See Table 3.

Table 3: Characterization of Critical Test Parameters

Critical Test Parameter Evaluated	Key findings
Prevalence of hyperactive signaling in the HER2-negative breast cancer population	Approximately 30% of HER2-negative breast cancer patients have at least one hyperactive signaling pathway not associated with a corresponding variant
Differentiation between hyperactive signaling and normal signal levels	Signaling activity is bi-modally distributed between hyperactive and normal signaling patient sub-groups
Oncogenicity of hyperactive signaling using xenograft models	Hyperactive HER2, HER2/c-Met, and PI3K-involved signaling in tumor cells lacking corresponding variants responded to matching targeted therapies in multiple xenograft models
Specificity of test output using dose response analyses of each agonist and antagonist	EC50 and IC50 values for each pathway derived using the CELsignia platform are consistent with other methods
Detection of pathway cross-talk	Co-activation of c-Met and HER-family receptors and PI3K-involvement in hyperactive GPCR signaling was confirmed

HER2 Signaling Studies

Hyperactive HER2 Signaling Tumors

Dysregulation of Human Epidermal Growth Factor Receptor (HER) family pathways due to aberrant expression or activation of HER-family members can lead to increased proliferation, reduced apoptosis, angiogenesis and invasiveness, which are the hallmarks of cancer. Hence, targeting the HER family receptor tyrosine kinases (RTKs) with small molecule inhibitors or monoclonal antibodies has been a common therapeutic strategy against multiple solid tumor types.

FDA approved drugs targeting HER2, such as trastuzumab, lapatinib, pertuzumab, and neratinib significantly improve clinical outcomes in HER2 overexpressing breast cancer patients. Currently, patients whose tumors were found by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) tests to have overexpressed or amplified HER2 are eligible for HER2-targeted therapies. However, clinical trials evaluating HER2-targeted therapies indicate the correlation between HER2 receptor levels and patient outcomes is less than 50%. In addition, Johnston and colleagues reported that in a trial evaluating lapatinib in combination with letrozole, the progression free survival (PFS) period for a sub-population of HER2-negative patients was comparable to the PFS for the HER2+ patients receiving the same therapies.[2]

Until recently, only patients with HER2 protein overexpression, HER2 gene amplification, or HER2 sequence variants were believed to have dysregulated HER2 signaling pathway activity. However, recent studies published by Celcuity have demonstrated that hyperactive HER2 signaling pathway activity similar to levels found in HER2+ breast tumors is present in a sub-group of patients with normal levels of HER2 receptor.[3] Measurement of HER2 signaling activity represents an opportunity to identify HER2-negative breast cancer patients with hyperactive HER2 signaling. These patients would represent a new cancer sub-type that may benefit from treatment with HER2 monoclonal antibodies such as trastuzumab and pertuzumab, or HER2 tyrosine kinase inhibitors such as neratinib or tucatinib.

Characterization of distribution of hyperactive HER2 signaling in HER2-negative breast cancer patients

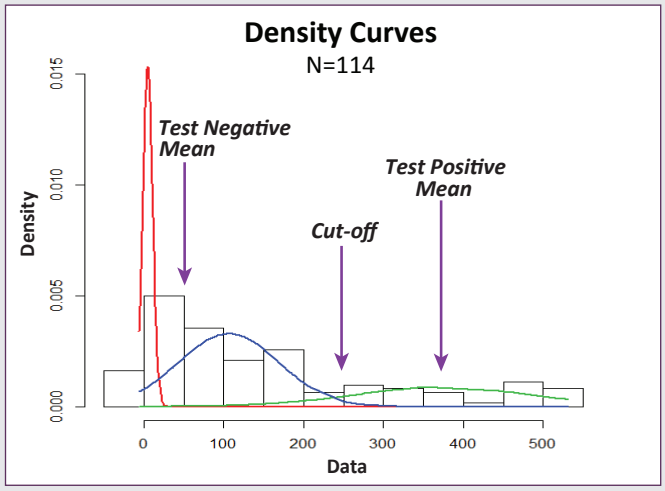
To characterize the prevalence and distinctiveness of hyperactive HER2 signaling within the HER2-negative breast cancer population, fresh tumor specimens were obtained from 114 HER2-negative breast cancer patients. Live cell response to specific HER2 agonists (NRG1b and EGF) and an antagonist (2C4) was measured. The distribution of signaling scores from 114 HER2-negative patient tumors was analyzed using the normalmixEM procedure in the R package mixtools. This analysis revealed that HER2-negative patients fall into three distinct HER2 signaling groups (see Figure 4).

Figure 4: Distribution Analysis of HER2 Signaling Scores

	Groups		
	1	2	3
Mean	4.9	104.4	376.6
Std Deviation	6.3	60.1	114.1
Proportion	0.26	0.49	0.25

Figure 5 shows the three groups arranged according to test score (x-axis) superimposed on a histogram of the data.

Figure 5: HER2 Signaling Score Histogram



The highest HER2 signaling group has a mean CELsignia HER2 Signaling Score of 377 and SD of 114. Hyperactive HER2 signaling was found in 27 of 114 HER2-negative patient samples (24.1%; 95% CI=16%-32%). The two lowest groups combined have a mean CELsignia HER2 Signaling Score of 50 and SD of 69. There are ~5 standard deviations difference in mean between abnormal (Group 3) and normal signalers (Groups 1 and 2), indicating there is a bi-modal distribution of HER2 signaling activity levels within the population of HER2-negative cancer patients. This highly differentiated distribution of HER2 signaling activity is consistent with the hypothesis that hyperactive HER2 signaling activity is oncogenic.

The distribution analysis was then used to construct a ROC curve to distinguish the highest HER2 signaling group (abnormal Group 3) from the normal HER2 signalers (Groups 1 & 2). See Figure 6.

Figure 7 displays the numbers underlying the ROC plot: the sensitivity (blue line) and false positives (red line) as a function of the cutoff used. At a 250 Signaling Score, specificity is >98% (FP<2%) and sensitivity is 85%, indicative of an accurate test.

Figure 6: Inferred ROC Curve for HER2 Signaling Scores

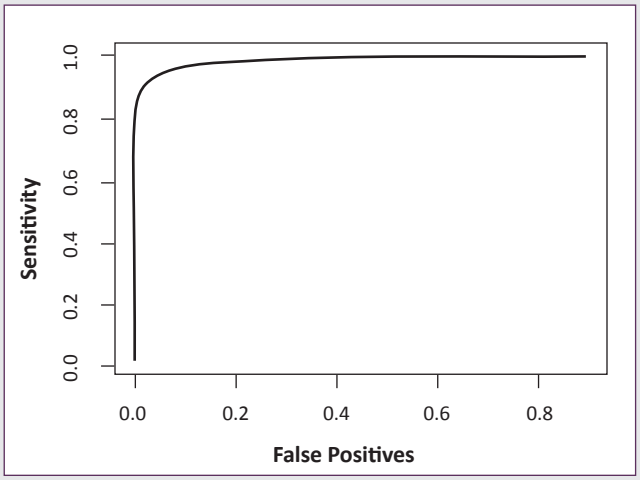
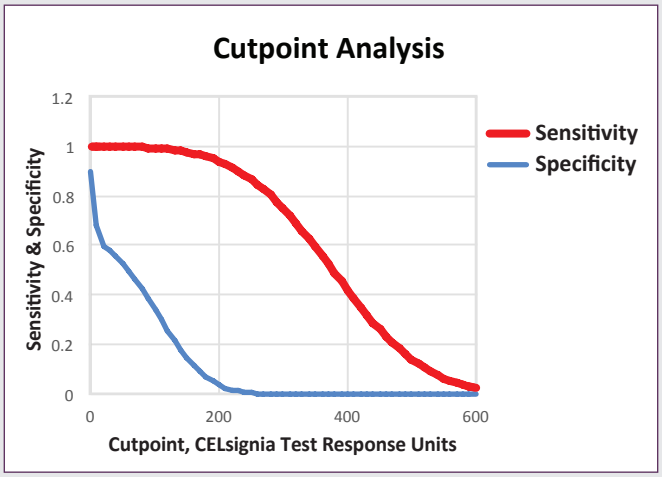


Figure 7: Sensitivity and Specificity Analysis of HER2 Signaling Scores



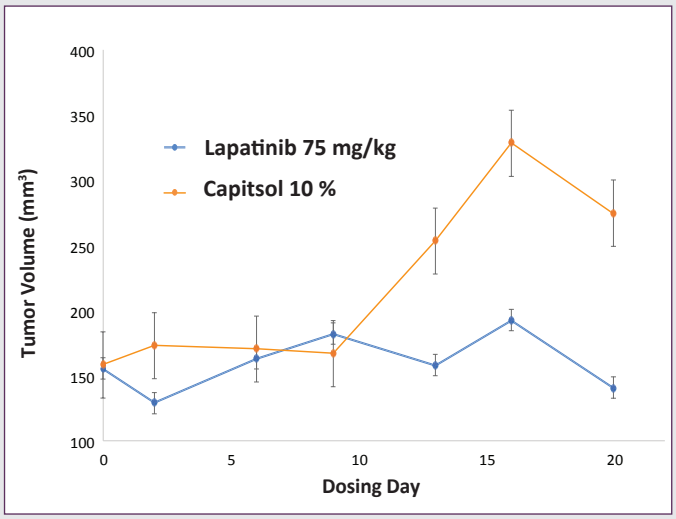
Tumor xenografts using a HER2-negative cell line to evaluate anti-HER2 drug response

The BT483 breast cancer cell line was studied using the CELsignia Test and found to have hyperactive HER2 signaling despite having normally expressed, non-amplified HER2. The cell line was then studied in a xenograft mouse model to evaluate whether hyperactive HER2-driven signaling in a HER2-negative tumor would respond to lapatinib (75 mg/kg), a reversible EGFR and HER2 kinase inhibitor. The study results found that lapatinib was able to inhibit growth of a HER2-negative tumor with hyperactive HER2 signaling (tumor/control Ratio = 0.52, p = 0.01). These findings support the primary hypothesis that HER2-negative breast cancer patients with abnormal HER2-driven signaling may benefit from treatment with anti-HER2 drugs. A summary of the results is provided in Table 4 and Figure 8.

Table 4: Response of BT483 to Lapatinib

	Response
HER2 Receptor Expression (IHC)	0
HER2 Signaling Status (CELsignia)	Hyperactive
Lapatinib Inhibition (Xenograft)	T/C Ratio – 0.52 (p = 0.01)

Figure 8: BT483 Xenograft Response by Dosing Day



Co-involved c-Met and HER2 Studies

Abnormal c-Met Signaling Tumors

Signaling through c-Met, the cognate receptor for Hepatocyte Growth Factor (HGF) is necessary for normal cell maturation and function. Numerous studies have established the significant role of the c-Met pathway in tumor growth and metastasis. Crosstalk between c-Met and HER family receptors is also reported as playing a role in tumor progression and resistance to HER-targeted therapies. Numerous clinical trials have evaluated dual inhibition of c-Met and HER pathways in a variety of tumor types, but they have produced mostly negative results. Since subjects enrolled in these trials were primarily presenting with relatively modest c-Met protein overexpression or gene amplification, other biological factors, such as coincident c-Met and HER signaling activity, are likely more important indicators for identifying patients eligible for c-Met therapies.

Celcuity's recent studies found that a subset of HER2-negative breast cancer patient tumors have abnormal c-Met signaling coincident with abnormal HER2 signaling. The c-Met expression level of each patient studied was normal. Strong evidence was found that c-Met and HER2 signaling is coordinated in a patient sub-group and may explain why a c-Met tyrosine kinase inhibitor is not effective when used as a single targeted therapy. Furthermore, Celcuity's studies with patient breast cancer cell samples have determined that simultaneous inhibition of HER1, HER2, and HER3 signaling with inhibition of c-Met signaling attenuates this dysfunctional signaling activity most effectively ex vivo.[4] These findings suggest that a sub-group of HER2-negative breast cancer patients have coordinated abnormal c-Met and HER2 signaling activity that may respond to treatment with a combination of HER2 and c-Met signaling inhibitors.

Prevalence of hyperactive HER2/c-Met signaling in HER2-negative breast cancer patients

A study and analysis similar to that conducted to characterize HER2 signaling was performed to estimate the prevalence and distinctiveness of hyperactive c-Met signaling within the HER2-negative breast cancer population. Fresh tumor specimens were first obtained from 79 HER2-negative/c-Met-negative breast cancer patients. The CELsignia Test measured real-time live cell response to ErbB and c-Met agonists (NRG1b, EGF, or HGF) alone and in combination, with or without ErbB and c-Met antagonists (2C4, tepotinib, or neratinib). The distribution of c-Met signaling scores from the 79 HER2-negative patient tumors was analyzed using the normalmixEM procedure in the R package mixtools. This analysis revealed that HER2-negative patients fall into three distinct c-Met signaling groups (see Figure 9).

Figure 9: Distribution Analysis of c-Met Signaling Scores

	Groups		
	1	2	3
Mean	-0.06	93.7	446.3
Std Deviation	5.5	74.2	195.4
Proportion	0.32	0.20	0.48

The highest c-Met signaling group has a mean CELsignia c-Met Signaling Score of 446 and SD of 195. Hyperactive and coincident c-Met and ErbB signaling was found in 19 of 79 HER2- patient samples (24.1%; 95% CI=16%-32%). The two lowest groups combined have a mean CELsignia c-Met Signaling Score of 51 and SD of 77. The mean level of signaling in patients with normal c-Met signaling (Groups 1

and 2) is thus separated by five standard deviations from the mean level of signaling in patients with hyperactive signaling (Group 3), indicating there is a bi-modal distribution of c-Met signaling activity levels within the population of HER2-negative cancer patients. This highly differentiated distribution of c-Met signaling activity is consistent with the hypothesis that c-Met hyperactive signaling activity is oncogenic.

Figure 10 shows the three groups arranged according to test score (x-axis) superimposed on a histogram of the data.

The distribution analysis was then used to construct a ROC curve to distinguish the highest c-Met signaling group (abnormal Group 3) from the normal c-Met signalers (Groups 1 & 2). See Figure 11.

Figure 12 displays the numbers underlying the ROC plot: the sensitivity (orange line) and false positives (blue line) as a function of the cutoff used. At 250 signaling units, specificity is >99% (FP<1%) and sensitivity is 84%, indicative of an accurate test.

Figure 10: c-Met Signaling Score Histogram

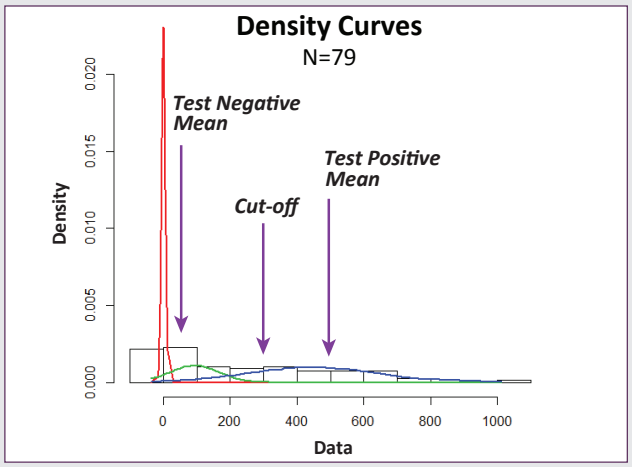


Figure 11: Inferred ROC Curve for c-Met Signaling

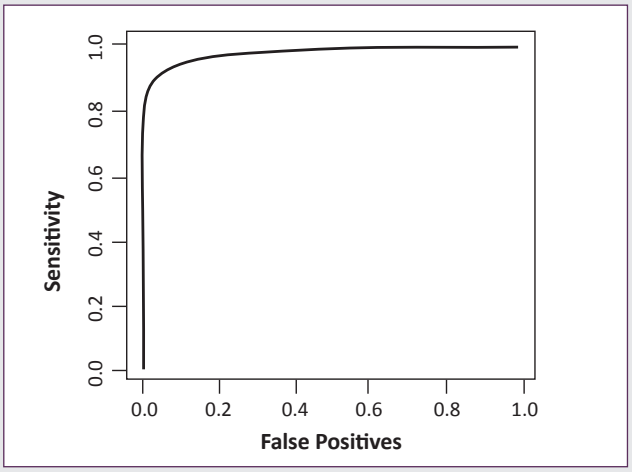
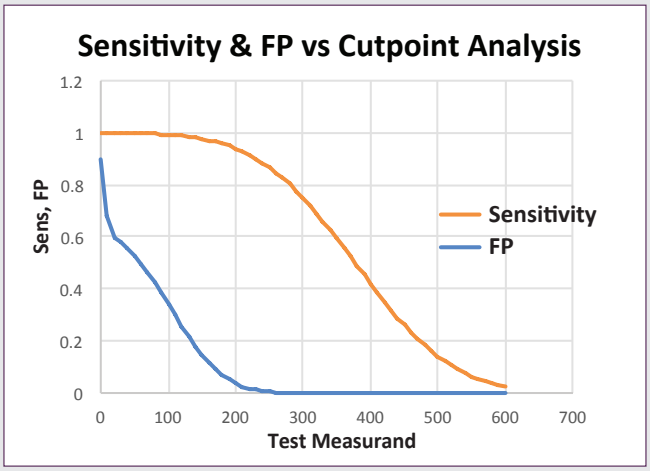


Figure 12: c-Met Signaling Score Sensitivity and Specificity



Tumor xenografts study vs. CELsignia signaling analysis using c-Met and ErbB targeted therapies

The HCC1954 breast cancer cell line was studied using the CELsignia Test to assess the signaling activity levels of the HER1, HER2, and c-Met pathways and then studied in a mouse xenograft model using different single agents and combinations of agents. The goal was to determine the correlation between the ex vivo and in vivo methods of assessing signaling activity and drug efficacy.[10]

First, the CELsignia Test activated HCC1954 cells separately with 0.3 nM EGF, 3 nM NRG, and 30 pM HGF and then inhibited with 2C4 (a HER2 dimerization blocker) and determined that HCC1954 has hyperactive c-Met and EGFR signaling and normal HER3 and HER2-driven signaling. The efficacy of neratinib (a covalent pan-HER tyrosine kinase inhibitor), tepotinib (a selective c-Met TKI), erlotinib (a selective EGFR TKI), erlotinib and tepotinib, or neratinib and tepotinib was then evaluated using the CELsignia Test. Results are summarized below in Table 4:

Table 4: CELsignia Test Results with Different pan-HER and c-Met Inhibitors

Drugs	HER/c-Met Inhibition
Erlotinib (HER1i)	5%
Tepotinib (c-Meti)	5%
Neratinib (pan-HER)	50%
Erlotinib + Tepotinib	48%
Neratinib + Tepotinib	100%

The combination of an EGFR inhibitor and c-Met inhibitor reduced HER family and c-Met signaling activity only 50% as much as a pan-HER inhibitor and c-Met inhibitor, even though only the EGFR and c-Met pathways were hyperactive. In addition, the CELsignia Test found that an EGFR inhibitor

and a c-Met inhibitor as single agents could only inhibit 5% of the signaling activity initiated by the HER-family and c-Met agonists.

A mouse xenograft study was then performed to compare the in vivo efficacy of the same TKI's studied with the CELsignia Test. Mice were randomly assigned to either a control group that received Captisol (10%) or one of five treatment groups that received either neratinib (40 mg/kg), tepotinib (50 mg/kg), erlotinib (25 mg/kg), erlotinib and tepotinib (25 mg/kg and 50 mg/kg), or neratinib and tepotinib (40 mg/kg and 50 mg/kg) for 17 days. Results are summarized below in Table 5 and presented graphically in Figure 13.

Each of the mouse xenograft studies yielded results consistent with the results from the ex vivo signaling and drug efficacy analyses performed using the CELsignia Test. Of particular note was the superior anti-tumor effect of the pan-HER and c-Met inhibitor combination (0.29 T/C ratio), compared to the EGFR and c-Met inhibitor combination (0.49 T/C ratio) or the pan-HER inhibitor alone (0.45 T/C ratio).

Analysis of combined pan-HER and c-Met inhibitors

Since tumor cells are constantly responding to multiple inputs, the effectiveness of a pan-HER inhibitor, neratinib, and a c-Met inhibitor, tepotinib, was evaluated in hyperactive HER family and c-Met signaling patient tumor cells (N=24) simultaneously stimulated with NRG1, EGF, and HGF (the ligands for HER3, HER1, and c-Met which we refer to as "combined GF"). The pan-HER inhibitor and c-Met inhibitor were tested separately at 500 nM, representing a concentration of >IC90 for single GF addition. The pan-HER and c-Met inhibitor combination was also tested at two different concentrations - 500 nM and 50 nM.

When tested individually at 500 nM, the pan-HER inhibitor and c-Met inhibitor reduced the combined GF activated signaling by 57% and 21%, respectively, or 78% on an additive basis. When the two drugs were tested in combination at 500 nM, they reduced the combined GF activated signaling by 100%, significantly greater than the additive inhibition (78%) of the two drugs when tested individually. This data suggests that a pan-HER inhibitor and c-Met inhibitor function synergistically, as was confirmed with subsequent Chou and Talalay analyses. Most strikingly, the combined drugs, when tested at 50 nM, or 10% of the original concentration, inhibited 89% of the combined GF activated signaling test. At 50 nM, the pan-HER and c-Met inhibitor combination was nearly effective (89% vs. 100%) as the combination at 500 nM and more effective (89% vs. 57% and 21%) than the individual drugs at 500 nM. See table 6.

Table 5: Xenograft Study Results

Drug Arm vs. comparator arm	Tumor/Control (T/C) Ratio (t-test)
Erlotinib vs. control	0.95 (p=0.870)
Tepotinib vs. control	0.90 (p=0.680)
Neratinib vs. control	0.45 (p=0.003)
Erlotinib + Tepotinib vs. control	0.49 (p=0.110)
Neratinib + Tepotinib vs. control	0.29 (p=0.0003)

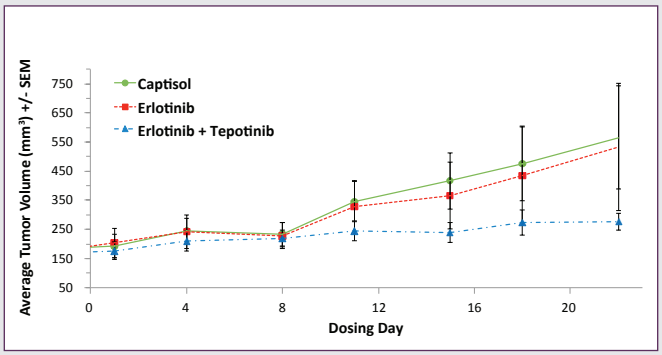
Table 6: Pan-HER and c-Met Inhibitor Synergy Analysis

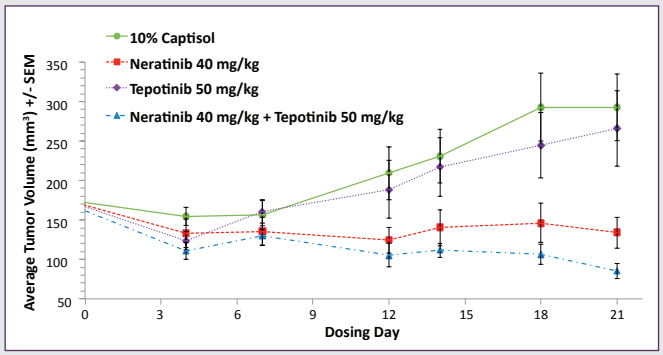
Synergy of Pan-HER and c-Met Inhibitors in Hyperactive HER family and c-Met Signaling Tumor Cells N=24, EGF (0.3nM), NRG1 (3nM), HGF (0.030nM)				
Drug(s) Tested	**Pan-HERi**	**c-Meti**	**pan-HERi + c-Meti**	
Drug Concentration	500 nM	500 nM	500 nM	50 nM
Signaling Inhibited	57%	21%	100%	89%

IC50 values for pan-HER/c-MET inhibitors tested ex vivo in live primary HER2- breast cancer cells

Real-time live cell response to specific ErbB and c-Met agonists (NRG1b, EGF, or HGF) alone and in combination, with or without one of five pan-HER antagonists (neratinib, lapatinib, ibrutinib, dacomitinib, poziotinib) or one of five c-MET antagonists (tepotinib, cabozantinib, crizotinib, capmatinib, or savolitinib) was quantified.

Figure 13: Xenograft Tumor Response to HER and c-MET Inhibitors by Dose Day





Each individual drug's IC50 was determined using a 1000-fold, 5-point, dose response curve with a single fixed concentration of a corresponding agonist. For the drug combination efficacy studies, fixed concentrations of the agonist mixture and clinically relevant concentrations of combinations of the antagonists were used to determine the percentage inhibition of the ErbB and c-MET signaling. The IC50 values for the individual c-MET and pan-HER inhibitors ranged from 3.10nM - 28nM and 2.67nM – 137.27nM, respectively. In the drug efficacy studies, an average of at least 80% of the ErbB and c-MET signaling activated by NRG1, EGF, and HGF co-stimulation was inhibited by each combination of c-MET and pan-HER inhibitors.[4] Table 7 summarizes the results.

Table 7: Pan-Her and c-Met IC_{50} and Inhibition Results

Pan-HER inhibitors	IC_{50} (nM)	Avg Inhibition (%) w/ different c-METi's
Poziotinib	2.67	100
Neratinib	4.81	100
Ibrutinib	13.10	99
Dacomitinib	22.06	100
Lapatinib	137.27	80

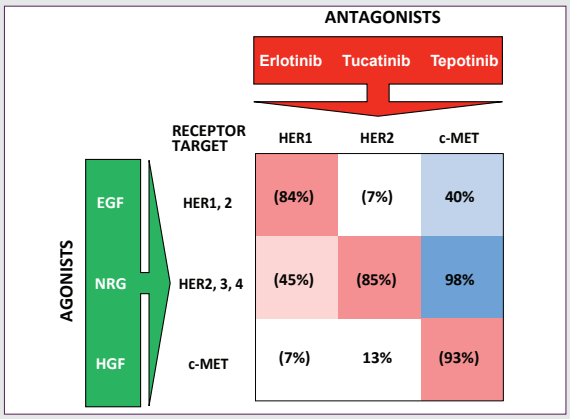
c-MET inhibitors	IC_{50} (nM)	Avg Inhibition (%) w/ different ErbBi's
Capmatinib	3.10	94
Savolitinib	3.56	98
Tepotinib	14.70	96
Cabozantinib	27.36	99
Crizotinib	28.21	100

The CELsignia Test using live cells measures IC50 values comparable to those derived using cell-free methods. Every combination of pan-HER and c-MET inhibitors provided comparably high (at least 80%) levels of inhibitory effect ex vivo. This suggests the sub-group of HER2-negative breast cancer patients diagnosed with coincident hyperactive c-MET and ErbB signaling by the CELsignia Test may respond to virtually any pan-HER and c-Met inhibitor combination.

Drug selection facilitated by receptor interaction analysis

A primary breast cancer sample was treated with one of the agonists, EGF, NRG, or HGF, in combination with each individual specific HER1, HER2, or c-Met receptor tyrosine kinase. A complete matrix of percent-inhibition of each growth factor signal over a four-hour period for each specific antagonist was determined. A positive value in the table below for percent inhibition indicates that the column matched antagonist enhanced the growth factor response. The results are presented in Table 8.

Table 8: Critical Coordinated Receptor Interaction Analysis



Of particular interest for this patient is the increase in EGF and NRG1 agonist response, 40% and 98% respectively, when a c-Met inhibitor (tepotinib) is introduced. This is indicative of complex interactions between c-Met signaling components and the HER-family signaling system.

This example highlights the unique insights available from dynamic signaling analysis of live patient tumor cells. In this patient, as in others with HER2 and c-Met hyperactive signaling tumor, a combination of pan-HER and c-Met inhibitors would likely be most efficacious.

PI3K-Involved Signaling Studies

Abnormal PI3K Signaling Tumors

Class I PI3K isoforms have been shown to transduce signals from GPCRs to regulate signaling and oncogenic transformation. In one significant report, PI3K p110γ and p110β have been shown to be activated directly by GPCR agonist binding. Residues in p110γ and its regulatory isoform, p101, have been shown to be critical for direct interaction with GPCR Gβγ heterodimer driven signaling and oncogenic transformation.[5] Additionally, PI3K activity in epithelial cells is directly linked to adhesion and concomitant cytoskeletal rearrangements.[6]

In breast cancer, only patients with PIK3CA-mutations are eligible for treatment with a PI3K inhibitor. However, recent clinical trial results suggest that factors other than PIK3CA sequence variance status may be important to measure when identifying patients eligible for PI3K inhibitors. Less than 20% of PI3KCA mutated late stage breast cancer patients achieved an objective response in a Phase III clinical trial with alpelisib, a recently approved PIK3CA inhibitor.[7]

Celcuity's studies found that hyperactive signaling of two important GPCR families, S1PR 1-5 and LPAR 1-6, involved PI3K isoforms in a sub-set of HER2-negative, PIK3CA wild-type breast cancer patients. Strong evidence was found that pan-PI3K-isoform inhibitors may provide the most effective attenuation of dysregulated SIP or LPA signaling involving the PI3K-node.[8]

Characterization of PI3K-involved signaling in breast cancer cells in p110α-mutant cell lines

To assess whether PI3K-involved hyperactive phospholipid signaling through S1P and LPA receptors is consistently present in breast cancer cells with PI3KCA mutations, three p110α-mutant breast cancer cell lines, BT20, HCC1954, and CAL-51, were studied. A study using the CELsignia Test with these cell lines had previously determined that a PI3K-α inhibitor (alpelisib) had no appreciable effect on the signaling activity initiated by various RTK agonists. Live cell responses to an S1P agonist (100 nM), an LPA agonist (500 nM), a p110α-isoform specific PI3K antagonist (alpelisib at 333nM) and a pan-PI3K inhibitor (taselisib at 333 nM) were quantified using the CELsignia Test. From these responses, S1P-initiated and LPA-initiated signaling, and the net amount of PI3K-α or pan-PI3K participation in S1P-initiated and LPA-initiated signaling was quantified. A previously determined cutoff was used to identify cell samples with abnormal levels of PI3K-involved signaling activity. Results are summarized in Table 9 below, where the data is reported as a Signaling Score, which represents the signaling activity associated with a pathway and the binding target of the inhibitor.

Table 9: Analysis of PI3K Involvement in S1P & LPA Signaling in Three p110α Mutant Cell Lines

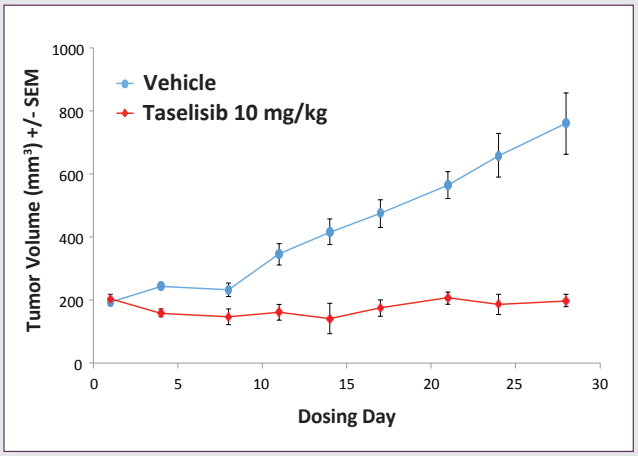
	SIP Activated		LPA Activated	
Cell Line	Pan-PI3K (Taselisib)	PI3K-α (Alpelisib)	Pan-PI3K (Taselisib)	PI3K-α (Alpelisib)
BT20	838	331	1496	517
HCC1954	668	196	1151	143
CAL-51	189	103	147	0

Abnormal activity involving PI3K-α was only found in one of the three cell lines tested, BT20 (in yellow). Two cell lines, HCC1954 and CAL-51, had abnormal S1P and LPA activity involving pan-PI3K-isoforms (in green). The normal level of PI3K-α signaling for the CAL-51 and HCC1954 cell line is consistent with a previously reported xenograft study that found a PI3K-α inhibitor (alpelisib) had no anti-tumor effect on either cell line. These findings provide evidence that signaling activity involving PI3K-α may be more important to measure than the mutational status of PI3K-α when selecting patients for treatment with a PIK3 inhibitor.

Tumor xenograft study using a pan-PI3K targeted therapy

PI3K signaling in the HCC1954 breast cancer cell line was further characterized using the CELsignia Test to assess the involvement of PI3K-α, PI3K-γ, pan-PI3K in HER1, HER3, and S1P and LPA1 agonism. CELsignia analysis found that signaling involving the PI3K-α and PI3K-γ isoforms is low and normal in the four agonist activities, despite the presence of p110α mutations. These results are consistent with previously reported xenograft studies that found alpelisib, a PI3K-α inhibitor, had no anti-tumor effect on an HCC1954 xenograft model.[9] However, hyperactive pan-PI3K activity involving S1P and LPA was detected. The cell line was then studied in a mouse xenograft mouse model with a pan-PI3K inhibitor (taselisib at 10 mg/kg) to assess its anti-tumor effect on hyperactive pan-PI3K involved signaling cells. The goal was to determine the correlation between the ex vivo and in vivo methods of assessing PI3K signaling activity and drug efficacy.

Figure 14: HCC1954 Xenograft Response to pan-PI3K Inhibitor, Taselisib



The study results found that taselisib induces a significant anti-tumor effect in the HCC1954 tumor (tumor/control ratio = 0.21, p = 0.009; t-test). See Figure 14. These results are consistent with the taselisib CELsignia Test data that reported abnormal levels of signaling activity involving pan-PI3K isoforms initiated by S1P or LPA activation. This provides strong evidence that abnormal S1P or LPA signaling through the PI3K node is oncogenic, despite normal levels of PI3K-α involved signaling activity.

Preliminary characterization of PI3K signaling in PIK3CA wild type, HER2- breast cancer patients

To determine whether hyperactive PI3K-involved signaling is present in the PIK3CA wild type, HER2-negative breast cancer population, fresh tumor specimens were obtained from 17 HER2-negative breast cancer patients. Each patient cell sample was confirmed to have no p110-α mutations or variants using Sanger sequencing analysis of the p110-α gene. A CELsignia Test was performed on each sample using

a PI3K-α isoform inhibitor (alpelisib at 333 nM) and a PI3K-γ inhibitor (IPI-549 at 333 nM) to quantify PI3K isoform activity associated with S1P and LPA agonism.

Of the 17 patient cell samples tested, four were found to have total levels of signaling activity involving PI3K isoforms initiated by S1P activation above a previously determined signaling unit test cut-off. These results provide confirmation that hyperactive S1P or LPA activity involving PI3K can occur in PIK3CA WT patient tumor cells.

Interventional clinical trials evaluating breast cancer patients selected with a CELsignia Test

Celcuity is actively collaborating with pharmaceutical companies to evaluate the efficacy of targeted therapies in patients found to have hyperactive signaling pathways using the CELsignia Test. Two clinical trials using the CELsignia Test to select patients for treatment with an anti-HER2 drug regimen are currently in progress. Additional clinical studies are in the planning phase.

FACT 1 (NCT03412643)

An Open-Label Phase II Trial to Evaluate the Efficacy and Safety of Neoadjuvant AC-THP in Early Stage HER2-Negative Breast Cancer Patients Selected with a Test Measuring Live Cell HER2 Signaling Function.

This is a prospective, single arm, open label, multicenter interventional study designed to evaluate the efficacy of neoadjuvant chemotherapy with anti-HER2 antibodies in patients with HER2-negative invasive breast cancer who have abnormal HER2 signaling activity determined by the Celcuity CELsignia Test.

FACT 2 (NCT03812393)

An Open-Label Phase II Trial to Evaluate the Efficacy and Safety of Neoadjuvant Neratinib Followed by Weekly Paclitaxel and Carboplatin Plus Neratinib in Early Stage Triple-Negative Breast Cancer Patients Who Exhibit Enhanced HER2 Signaling by Live Cell HER2 Signaling Transduction Analysis.

This is a prospective, single arm, open label, multicenter interventional study designed to evaluate the efficacy of neoadjuvant chemotherapy with a pan-HER signal inhibitor in patients with triple-negative invasive breast cancer who have abnormal HER2 signaling activity determined by the Celcuity CELsignia Test. The FACT 2 trial uses the same CELsignia Test to screen and select early stage breast cancer patients for neoadjuvant treatment with an approved anti-HER2 therapy as the FACT 1 trial.

Conclusion

By analyzing the signaling activity in a patient's live tumor cells, signaling abnormalities driving a cancer can be revealed that a genomic or proteomic test has not detected. The data presented suggest that three new subtypes of breast cancer, hyperactive HER2 signaling tumors, hyperactive HER2/c-Met signaling tumors, and hyperactive PI3K-involved signaling tumors, may account collectively for approximately 30% of HER2-negative breast cancers. The patients found to have hyperactive signaling via the CELsignia Test thus create an opportunity to expand the patient population eligible for targeted therapies. If the efficacy of targeted therapies treating these hyperactive signaling tumors in patients selected with a CELsignia Test is confirmed in interventional clinical trials, this tumor assessment technology may change the way personalized medicine is practiced in the future.

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