CELsignia
Multi-Pathway Test | ovarian

Hyperactive c-Met and ErbB signaling detected in a sub-group of ovarian patient tumors: Patient sub-group may benefit from c-Met and pan-HER combination therapy

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Background

Sub-groups of breast, lung, and colon cancer patients with genetic variants associated with dysregulated ErbB (HER) signaling benefit from treatment with ErbB targeted therapies. No sub-groups of ovarian cancer (OvC) patients with actionable ErbB genetic variants have yet been found. Measurement of biological factors other than genetic variants, such as ErbB signaling activity, may identify ovarian cancer patients likely to benefit from ErbB targeted therapies. We have previously reported studies detecting dysregulated ErbB and c-Met signaling activity in 20%-25% of HER2-negative breast cancer patient tumors using the CELsignia Multi-Pathway (MP) Signaling Function Test. To determine whether ErbB or c-Met signaling dysregulation is involved in ovarian cancer, the CELsignia MP test was adapted to analyze ovarian tumor cells.

The current study set out to:

1) characterize c-Met and ErbB family signaling activity in ovarian patient tumor cells and ovarian tumor cell lines;

2) evaluate in vivo response to pan-HER and c-Met inhibitors in an ovarian tumor xenograft model.

Methods

Cell Lines: The ovarian cancer cell lines used in this study (listed in Table 1) were maintained according to ATCC recommendations and authenticated by ATCC.

Tissue Specimens: A training set of de-identified ovarian cancer tissue specimens was obtained from 19 patients (patient characteristics are listed in Table 2). Methods for tissue extraction and primary cell culture were based on (Laing et al. 2016, 2017).

Table 1. List of cell lines

Cell line	OvC type
CaOV-3	High Grade Serous
CaOV-4	High Grade Serous
COV362	Endometrioid(HGS
HEY-T30	High Grade Serous
KURAMOCHI	High Grade Serous
MES-OV	Serous Cystadenocarcinoma
OV-90	Adenocarcinoma
OVCAR-3	High Grade Serous
OVCAR-4	High Grade Serous
OVKATE	High Grade Serous
OVMANA	Clear Cell Adenocarcinoma
OVSAHO	High Grade Serous
SK-OV-3*	Serous Cystadenocarcinoma
TOV-112D	Endometrioid
TOV-21G	Clear Cell Adenocarcinoma
UACC-1598	Serous Cystadenocarcinoma
UACC2727	Serous Carcinoma
UWB1.289	Ovarian carcinoma

* Reported to have Her2 amplification

Table 2. List of OvC patient specimens

Sample	Age	Ethnicity	Primary Site	Removal Site	Type	Grade	Stage
C1113.01.38	51	Caucasian	Ovary	Ovary	Serous	High grade	pT3c N1
C1468.01.18	65	Caucasian	Ovary	Ovary	Serous	High grade	pT3c N1
C1497.01.10	58	Caucasian	Ovary	Ovary	Serous	High grade	pT3c N1
C1504.01.10	56	African Amer.	Ovary	Ovary	Serous	High grade	pT2 N0
C1508.01.13	48	Caucasian	Ovary	Ovary	Endometrioid	Grade 2	pT3a Nx
C1620.01.21	42	Caucasian	Ovary	Omentum	Serous	High grade	NA
C1774.01.11	48	Caucasian	Ovary	Omentum	Serous	High grade	pT3c N1
C1928.01.4	67	African Amer.	Ovary	Ovary	Serous	High grade	pT3b N0
C1944.01.3	52	Caucasian	Ovary	Ovary	Serous	High grade	pT3c N1
C1966.01.47	58	Caucasian	Ovary	Ovary	Serous	High grade	pT3c N1
C2032.01.4	78	Caucasian	Ovary	Omentum	Serous	High grade	NA
C2040.01.9	49	Asian	Ovary	Ovary	Serous	High grade	pT3c N1
C2060.01.1	59	Caucasian	Ovary	Ovary	Serous	High grade	pT3c N0
C2064.01.1	70	Caucasian	Ovary	Omentum	Serous	High grade	NA
C2096.01.1	63	Caucasian	Ovary	Ovary	Serous	High grade	pT3c Nx
C2332.01.10	66	Caucasian	Ovary	Omentum	Serous	High grade	NA
C2408.01	59	Caucasian	Ovary	Ovary	Serous	High grade	pT1c3 N1a
C2427.01.5	48	Caucasian	Ovary	Omentum	Serous	High grade	pT3c NX
C2457.01.1	64	Caucasian	Ovary	Ovary	Serous	High grade	pT3c Nx

Flow cytometry: Flow cytometry on disaggregated tissue and cultured cells was performed on Agilent Novocyte 3005. Extracellular staining with indicated antibodies was performed by standard procedures. DNA staining for DNA index calculation was performed with FxCycle violet.

Sequencing: Genomic DNA was extracted from tissue or cells and provided to contract research organizations for targeted next-generation sequencing to an average depth of 1000x. Sequencing was targeted to panels of 500 – 1000 genes frequently mutated in solid tumors with enrichment (Nimblegen SeqCap or Novogene NovoPM 1.0). Reads were aligned to the reference genome with BWA. Tumor vs. normal comparisons were performed with VarScan or Mutect™ programs

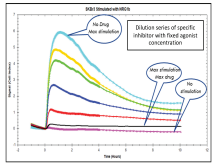
Xenografts: 40 female NSG™ mice were injected with two million OVCAR-4 cells. Mice were randomly assigned to either a control group that received Captisol excipient or a treatment group that received either neratinib, tepotinib, or neratinib and tepotinib for 16 days.

CELsignia analysis: Real-time live cell response to specific ErbB and c-Met agonists (3 nM NRG1b, 0.3 nM EGF, and 4 ng/mL HGF) with or without an antagonist (2C4, a HER2 dimerization inhibitor; neratinib, a pan-HER inhibitor; or tepotinib, a c-Met kinase inhibitor) was measured and quantified using an xCELLigence impedance biosensor (Agilent Technologies). Signaling activity above a previously established cut-off value (250 signaling units) was used to identify abnormal levels of EGFR, HER2, and c-Met signaling activity. A HER2 Signaling Score, reflecting NRG and EGF responses that can be attenuated by 2C4, was calculated as was previously described (Laing et al. 2016, 2017).

Figure 1. CELsignia Analysis Uses Biosensor to Quantify Signaling Activity in Real-Time in Live Cells

- CELsignia analysis leverages connections between cell adhesion, impedance, and cell signal transduction
- Live cells are attached via ECM to a microelectrode on the bottom of a 96-well impedance biosensor plate. Additionally, the tumor epithelial cells in the wells form adhesion-based gap junctions.
- The cells attached to the biosensor impede the flow of electrons when mV AC current is applied and changes in impedance (mΩ) are recorded
- Signaling activity causes cell adhesion changes that affect impedance levels recorded by the biosensor

Example Impedance vs. Time Data Set



Pathway specific ligands and inhibitors used to turn on/off signaling pathways

Very subtle cell adhesion changes are measured

Response quantified as signaling units over time to characterize signaling activity

Determine Signaling Score for each pathway tested

Results

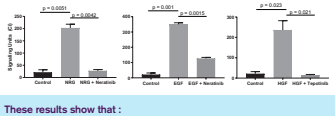
Figure 2. CELsignia analysis identifies OvC cells lines with hyperactive c-Met and ErbB signaling

A

Cell line	Receptor Expression in EpCam+ cells (FACS)			Pathway signaling activity (CELsignia analysis)			
	HER1	HER2	HER3	HER1	HER2	HER3	c-Met
OVMANA	79	7	9	55	368	460	204
OVCAR-4	72	5	2	486	302	238	545
OVCAR-3	86	24	7	788	278	526	304
UWB1.289	39	5	5	32	168	349	46
TOV-21G	30	10	6	73	61	58	71
OVKATE	148	15	14	53	46	85	5
KURAMOCHI	31	5	5	29	37.2	34	5
OVSAHO	28	8	1	4	17	6	30
CaOV-4	16	3	1	-2	13	10	5
SK-OV-3	105	854	6	-68	12	11	11
COV362	385	4	1	34	1	25	221
HEY-T30	170	5	3	-24	0	-9	-49
CaOV-3	198	3	2	-208	0	-91	-173
MES-OV	40	2	2	-9	NA	56	30
OV-90	19	4	1	4	NA	3	21
TOV-112D	NA	NA	NA	4	NA	2	3
UACC-1598	NA	NA	NA	1	NA	6	8
UACC2727	11	3	2	107	NA	55	12
MDA-MB-231	130	13	4	← HER2 IHC 0			
MDA-175	14	66	9	← HER2 IHC 1+			
SKBR3	111	408	31	← HER2 IHC 3+			

(A) Table showing HER receptors expression (FACS stain index) and CELsignia responses: HER1 signaling (EGF activation), HER3 signaling (NRG activation), HER2 signaling (CELsignia HER2 Test score), c-Met (HGF activation). The breast cancer cells lines MDA-MB-231 (with HER2 expression "0" by IHC), MDA-175 (with HER2 "+1" by IHC) and SKBR3 (with HER2 "+3" by IHC) are shown as comparison.

B



(B) CELsignia response to NRG, EGF, and HGF alone or in combination with neratinib or tepotinib in OVCAR4 cells with hyperactive c-Met and ErbB signaling.

These results show that :
1. 3 of 18 OvC cell lines (OVMANA, OVACR3, OVCAR4) have hyperactive c-Met and/or ErbB signaling
2. Hyperactive ErbB signaling does not correlate with HER1-4 expression
3. Neratinib significantly attenuates NRG and EGF response
4. Tepotinib significantly attenuates HGF signaling response

Figure 3: Neratinib and tepotinib reduce xenograft tumor growth in OvC cells with hyperactive c-Met and ErbB signaling

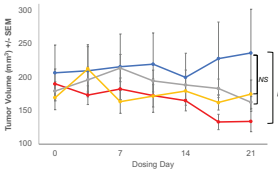
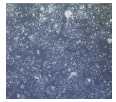


Chart showing OVCAR4 xenograft tumor size in response to neratinib, tepotinib, or neratinib + tepotinib administered for 21 days; p<0.001 (t-test) between vehicle and neratinib + tepotinib group.

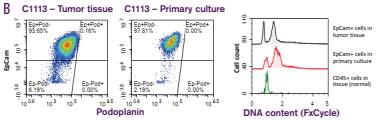
These results show that neratinib and tepotinib induce significant tumor reduction in an OVCAR4 xenograft model found by the CELsignia test to have hyperactive ErbB and c-Met signaling.

Figure 4: Establishment of ovarian cancer primary cultures for CELsignia analysis

A C1113 – 3 Day culture



B C1113 – Tumor tissue C1113 – Primary culture



(A) Representative culture of primary cells from a digested tumor biopsy showing epithelial cells with a tight cobblestone structure.

(B) Representative FACS analysis of an initial disaggregated tissue and the resulting cultured cells showing similar % of epithelial (EpCAM+) and stromal cells (EpCAM-/Podoplanin+) and similar hyperploid DNA content.

(C) Genetic analysis confirms that cultured cells are enriched for mutations present in cancer tissue (selected mutations are shown).

(D) Screening of 19 OvC patient specimens (see Table 2) showing that primary cultures are enriched for epithelial (EpCAM+) cells with a DNA index similar to what found in the digest tissue. FACS reference ranges for HER2 expression: SKBR3 (IHC 3+; 408); MDA-175 (IHC 1+; 66); MDA-231 (IHC 0; 13).

C

Gene	Mutation	COSMIC	Prediction	Variant Allele Frequency		
				Normal tissue	Tumor Tissue	Tumor Culture
TP53	P87fs*	-	pathogenic	0	0.90	0.86
MSI2	V201M	COSV52356527	pathogenic	0	0.89	0.88
TET2	Y1315H	-	-	0	0.93	0.89
ARID1A	D1354N	COSV61381688	pathogenic	0	0.43	0.44
IGF2R	G601A	-	-	0.01	0.91	0.85

D

Sample	% EpCam+ cells		Her2 expression in Ep+		DNA index in Ep+	
	Digest	Culture	Digest	Culture	Digest	Culture
C1113.01.38	97.3%	86.0%	5.9	1.8	1.57	1.65
C1468.01.18	29.4%	78.4%	NA	8.9	1.24	1.40
C1497.01.10	80.4%	85.6%	4.0	4.5	1.29	1.38
C1504.01.10	70.6%	92.1%	7.9	4.7	1.65	1.70
C1508.01.13	50.1%	79.5%	0.8	0.1	1.01	1.09
C1620.01.21	78.9%	86.8%	7.0	9.8	1.04	1.15
C1774.01.11	74.5%	89.4%	13	16	1.35	1.66
C1928.01.4	79.6%	90.2%	5	4	1.96	2.00
C1944.01.3	64.9%	94.8%	8	15	1.28	1.59
C1966.01.47	97.5%	93.7%	3.3	2.9	1.03	1.03
C2032.01.4	74.5%	95.9%	26	29	1.42	1.68
C2040.01.9	98.4%	82.8%	53	29	0.86	0.98
C2060.01.01	76.9%	90.9%	13	11	0.93	1.21
C2064.01.01	85.0%	98.1%	42	23	1.48	1.75
C2096.01.1	78.0%	76.8%	27	8	1.73	1.86
C2332.01.10	51.4%	73.7%	7	7	1.54	1.64
C2408.01.3	30.3%	73.6%	18	6	1.72	1.84
C2427.01.5	78.3%	92.0%	13	7	1.37	1.51
C2457.01.1	81.6%	76.3%	13	10	1.31	1.76

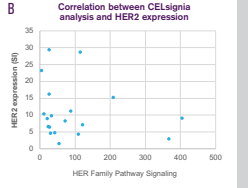
These results show that :
1. Primary cultures are enriched for epithelial cancer cells present in the original OvC tissue.
2. HER2 is expressed at low levels in all specimens tested.

Figure 5: CELsignia analysis identifies OvC patients with hyperactive c-Met and ErbB signaling

A

Ovarian Cancer patient sample	Receptor Expression in EpCam+ cells (FACS)			Pathway signaling activity (CELsignia analysis)			
	HER1	HER2	HER3	HER1	HER2	HER3	c-Met
C1508.01.13	32	9	5	816	406	578	446
C1966.01.47	15	3	2	317	367	576	410
C1944.01.3	26	15	1	166	209	266	190
C2332.01.10	69	7	2	278	122	94	86
C2032.01.4	35	29	5	89	119	168	70
C1928.01.4	26	4	4	185	110	125	134
C2060.01.1	52	11	7	58	89	115	65
C2096.01.1	33	8	3	170	72	127	137
C1113.01.38	12	2	1	67	55	60	19
C1504.01.10	24	6	1	72	43	89	22
C1620.01.21	11	10	3	29	34	50	34
C1497.01.10	5	5	1	14	31	41	26
C2408.01.3	6	6	3	48	29	72	26
C1774.01.11	23	16	5	14	26	46	8
C2040.01.9	61	29	8	41	26	31	38
C2427.01.5	18	7	3	6	24	38	12
C1468.01.18	11	9	3	22	22	48	19
C2457.01.1	8	10	3	22	12	12	2
C2064.01.1	6	23	3	14	5	0	14

B



(A) Table showing HER receptors expression (FACS stain index) and CELsignia responses: HER1 signaling (EGF activation), HER3 signaling (NRG activation), HER2 signaling (CELsignia HER2 Test score), c-Met (HGF activation). FACS reference ranges for Her2 expression: SKBR3 (IHC 3+; 408); MDA-175 (IHC 1+; 66); MDA-231 (IHC 0; 13).

(B) Plot showing lack of correlation between HSF score and HER2 expression (FACS stain index).

These results show that :
1. 2 of 19 OvC patient samples have hyperactive c-Met and ErbB signaling.
2. Hyperactive ErbB signaling does not correlate with HER1-4 expression.

Summary of Results

- **The CELsignia Multi-Pathway Signaling Function Test identified 3 of 18 (17%) OvC cell lines and 2 of 19 (11%) OvC patients with hyperactive c-Met and/or ErbB signaling.**
- **FACS and sequencing analyses confirmed that OvC primary cultures maintained the epithelial cancer phenotype present in the original tissue.**
- **Hyperactive ErbB signaling did not correlate with HER1-4 expression.**
- **Neratinib and tepotinib significantly attenuated ErbB signaling and c-Met signaling in the CELsignia analysis.**
- **In vivo studies showed that mouse xenograft tumors developed from an OvC cell line with hyperactive ErbB and c-Met signaling significantly decreased in size after treatment with a combination of tepotinib and neratinib.**

Conclusions

These findings suggest that a significant sub-group of ovarian cancer patients have abnormal ErbB and c-Met signaling activity that may respond to treatment with a combination of ErbB and c-Met inhibitors. A clinical trial to evaluate treatment response of this patient sub-set to combined c-Met and pan-HER inhibitors is warranted.

References

1. Laing, et al. A functional signal profiling test for identifying a subset of HER2-negative breast cancers with abnormally amplified HER2 signaling activity. Oncotarget. 2016. 7(48):78577-78590.

2. Laing, et al. Development of a test that measures real-time HER2 signaling function in live breast cancer cell lines and primary cells. BMC Cancer 17:199.

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