What is the appropriate sample (s) on which to perform sequencing for mutational analysis to guide the selection of targeted therapy?

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ABSTRACT

BACKGROUND: **Success of targeted therapy requires expression of the protein. Tumor tissue source can include diagnostic biopsy, surgical samples from initial or follow-up surgeries, fluids e.g. pleural or ascites and circulating tumor cells (CTC). The goal of using CTCs was 1. To determine whether CTC can be used as a "liquid" tumor biopsy and enable gene sequence information at the single cell level and 2. To determine the heterogeneity represented in the circulation compared to that seen in solid tumor by examining single cells (or a small cluster of cells) for the presence of a specific mutation which was detected in tissue tumor source.**

METHODS: **We performed sequencing for mutational analysis on tissue(s) from patients with inflammatory breast cancer (IBC). Tumor sources varied from mastectomy tissue, metastatic site(s) e.g. liver or skin from chest wall disease, pleural fluid and CTC isolated into pure single cell populations (or groups of cells) using Silicon Biosystems DEPArray. Ampli1™ WGA kit was used for CTC amplification. Of the 22 patients sequenced, mastectomy primary tumor was examined in 3, metastatic site skin chest wall disease in 15, other metastatic site in 4, pleural fluid in 2 and CTC collected to investigate p53 mutations in 8.**

RESULTS: **To date 35 patients have had mutational data performed, 23/35 had mutations in p53, 6/35 in RB1 and BRAC, 9/35 in PI3K and 5/35 in ERBB2, 2/35 in Notch 1 and 1/35 in each of, ATM, KRAS, MEN1 and ESR1. Numerous amplifications were noted including AKT, RPTOR, MLC1, MYC, CCND1, AURKA, MDM2, FGFR1 and ERBB2. For one patient's chest wall biopsy compared to two single CTCs and a cluster of 10 CTCs the same TP53C229fs*10 mutation was detected revealing the same 2bp deletion. No 2bp deletion was found in single white blood cells. Whereas, another patient which showed a TP53 S215G mutation in her skin biopsy of chest wall disease, only amplifications of AURKA, CCND1, IGF1R, MDM2 and SRC in pleural tumor cells were detected and no mutations in three single CTC, two single pleural tumor cells and in single white blood cells were seen. Primary tumor tissue is being sort for both of these patients. Mutational data reviewed to date suggest that IBC is not one disease but a multiplicity of diseases, revealing a variety of target(s). Aberrations are not consistent across tissue source.**

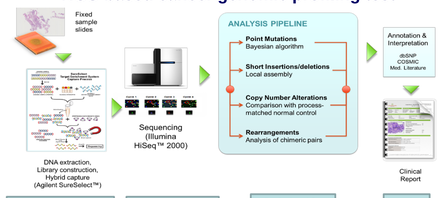
CONCLUSIONS: **Successful treatment outcomes using standard of care chemotherapy combined with target therapies will require not one, but a panel, of tissue sources for sequencing to guide the selection of appropriate targeted therapies.**

OBJECTIVES

- To detect genomic abnormalities with potential for therapeutic targeting.
- To compare mutations/amplifications in tissue from various disease sites and compare to circulating tumor cells (CTCs).
- To improve the understanding of the molecular drivers of metastasis in IBC and metastatic breast cancer.

METHODS & RESULTS

TISSUE SEQUENCING *(Foundation Medicine, Inc.)*
NGS-based cancer genomic profiling test



Mutation Frequency (Tissue)

P53	65.7%
PI3K, PI3KR1,pI3KCA	25.7%
RB1	17.1%
BRAC1, BRAC2	17.1%
Notch1	5.7%

Amplification Frequency (Tissue)

MYC1	28.6%
CCND1, CCNE1	20%
MCL1	14.3%
ERBb2	14.3%
FGFR1	8.6%
MDM2	5.7%
AURAK	5.7%
AKT1, AKT2, AKT3	5.7%

Concordance Between Multiple Tissue Samples

Patient ID	Disease	Tissue source	Mutation	Amplification	CTCs (TP53 analysis) (DEPArray captured)
0156/1106	IBC	Breast bx 2011	TP53 R273H, PIK3CA E545K, RB1 R661W	Kras, MCL1, MYC	
		Breast 2012	TP53 R273H, PIK3CA E545K, RB1 R661W	Kras, MYC	
3198/1440	IBC	Pleural fluid 2012	AKT1 E17K, TP53 D259Y, RB1 E693* CDH1 S337_L343del+splice		
		Abdominal skin punch 2012	AKT1 E17K, TP53 D259Y, RB1 E693* CDH1 S337_L343del+splice		
0097/3768	IBC	Pleural fluid 2011	PTEN D107Y, BRAC1 truncation		8 single CTC (N.D.)
		Chest wall 2011	PTEN D107Y, BRAC1 truncation BRAC2 truncation		
1321/0105	IBC	Breast 2011	TP53 R110fs*13	MCL1, MYC, JUN	1 of 1 single CTC (*showed same TP53 mutation*) 1 pool CTCs 3 WBC controls (*no mutation*)
		Chest wall 2012	TP53 R110fs*13	MCL1, MYC	
3304/3293	IBC	Chest wall 2012	TP53 S99fs*44, RB1 L779*	MYC, MDM4	3 of 3 single CTC(*showed same TP53 mutation*) 1 WBC control (*no mutation*)
		Pleural fluid 2012	TP53 S99fs*44, RB1 L779*	FGFR1,MYC, MDM4	

Discordance Between Multiple Tissue Samples

Patient ID	Disease	Tissue source	Mutation	Amplification	CTCs (TP53 analysis) (DEPArray captured)
6155	IBC	Chest wall 2008	PIK3CA H1047R, CDKN2A H98T	CCND1	5 single CTC (N.D.)
		Chest wall 2012	PIK3CA H1047R, ESR1 D538G	CCND1	
1167/3867/ 0121	IBC	Breast 2009		CCND1, IGF1R, MDM2	0 of 5 single CTC 0 of 1 cluster CTC (*no TP53 mutation seen*) 5 WBC controls
		Chest wall 2012	TP53 S215G	CCNE1	
		Pleural fluid 2012		AURKA, CCND1, IGF1R, MDM2, SRC	
1439/3116	IBC	Right breast bx 2012	TP53 R156fs*14, PIK3CA E8_L15>G	MCL 1, MYC	
		Left breast 2012	TP53 R156fs*14	MCL 1, MYC	
3108	IBC	Breast 2010	TP53 A159V, PAX5 I301T	ERBB2	
		Pleural fluid 2012	NF1 truncation exon28, PAX5 I301T		
1186/1073	IBC	Breast 2010	TP53 P98fs*18, SOX10 A361V	RAF1	
		Chest wall 2011	TP53 Q104fs*19	ERBB2	
2673/0155	IBC	Breast 2009	TP53 R110fs*13, BRAC2 A1327fs*4, RB1 F721>*		3 of 3 single CTC 2 of 2 clusters (*same TP53 mutation*) 8 WBC controls (*no mutation*)
		Chest wall 2012	TP53 R110fs*13, BRAC2 A1327fs*4, RB1 F720*	CCNE1, MYC	

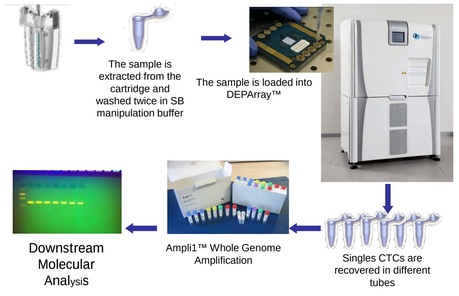
Single Tissue source analysis

Patient ID	Disease	Tissue source	Mutation	Amplification	CTCs (TP53 analysis) (DEPArray captured)
2192	IBC	Lymph node	TP53 splice site 782+1C>T, RB1 P777fs*33		
0102	IBC	Chest wall	TP53 G245S,G245D		
6146	IBC	Chest wall	TP53 C229fs*10, ERBB2 V777L, ERBB2 S310F, PIK3CA K111E		4 of 5 single CTC (*same TP53* 1 of 1 cluster CTC *mutation*) 5 WBC controls (*no mutation*)
6143	IBC	Chest wall	BRAC2 L1768fs*5		
3866	IBC	Chest wall	RB1 splice 607+1G>C		15 single CTC (N.D.) WBC controls (N.D.)
3833	IBC	Chest wall	TP53 H179R PIK3R1 441N_452YdelINIEAVGKKLHEY	ERBB2	
0099	IBC	Breast		CCND1, CDK4, MDM2	
0002	IBC	Brain bx		ERBB2	
3865	IBC	Liver bx	TP53 P190_H193>*E, BRCA1 E23fs*17	AKT1, RPTOR,MCL1, MYC	2 of 2 single CTC(*same TP53 mutation*) 4 WBC controls(*no mutation*)
0034	IBC	Chest wall	TP53 R248Q, MEN1 E496*	CCND1	
0053	IBC	Breast	TP53 splice site 993+1 C>T	CCND1, MYC	
0004	IBC	Chest wall	TP53 K132N, PIK3CA H1047R, EGFR L858R	ERBB2	
1110	IBC	Chest wall	TP53 S241fs*23	AKT2	
1938	IBC	Chest wall	Kras G12D, NOTCH1 E424K		
1939	IBC	Lymph node	TP53 W146*, ATM E672fs*31	CCND1, MCL1, MYC, NKX2-1	
3080	IBC	Chest wall	PIK3R1 K567_L570del, ARID1A A2097fs*39, CARD11 N184S		
1370	IBC	Chest wall		AKT1, AURKA, FGFR1, MYC	
2771	IBC	Chest wall	NOTCH1 loss		
3770	IBC	Chest wall	TP53 R342*	FGFR1	
3296	IBC	Bone marrow	TP53 G245C, BRAC1 R1583fs*39, EPHA3 E237K	AKT3,JAK2	
4836	Breast CA	Breast	BRAC2 S1982fs*22, PIK3CA H1047R	MYC	CTC filter captured FISH results
0933	Breast CA	Lymph node	TP53 Q317fs*28		10 single CTC (N.D.) WBC controls (N.D.)
5510	Breast CA	Parietal scalp	PIK3CA E454K, RUNX1 Q213*		
5507	Breast CA	Lung lesion	TP53 R342*, FANCA truncation		

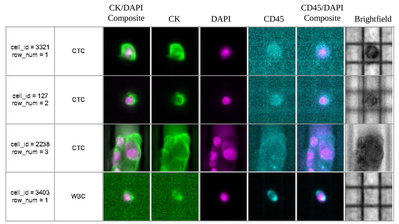
CTC analysis *Ampli1™ WGA (Silicon Biosystems)*

Patient	p53 mutation (tissue/biopsy)	# of cells with the mutation	Mutated sequences
1 (2673/0155)	R110fs*13	3/3 single CTCs 2/2 clustered CTCs 0/8 single WBCs	GCTACGGTTTCC-TCTGGGCTTCTTGC GCTACGGTTTCCGTCTGGGCTTCTTGC
2 (1321/0105)	R110fs*13	1/1 single CTC 9/9 sequences from a CTC pool 0/3 single WBCs	GCTACGGTTTCC-TCTGGGCTTCTTGC GCTACGGTTTCCGTCTGGGCTTCTTGC
3 (3865)	P190_H193>*E	2/2 single CTCs 0/4 single WBCs	GGTCTGGCC------TAAGAGCTTATC GGTCTGGCCCCTCCTCAGCATCTTATC
4 (1167/3867/ 0121)	S215G	0/5 single CTC 0/1 clustered CTC 0/5 single WBCs 0/5 sequences from a WBC pool	None observed
5 (6146)	C229fs*10	4/5 single CTCs 1/1 clustered CTCs 0/5 single WBCs	GTTGGCTCTGAC--TACCACCATCCAC GTTGGCTCTGACTGTACCACCATCCAC
6 (3304/3293)	S99fs*44	3/3 single CTCs 0/1 single WBCs	TGTCC----------CAGGG TGTCCCTTCCCAGAAAACCTACCAGGG GGACC----------TGGAGG GGACCTGGAGGGCTGGGGACCTGGAGG
		*This 16 bp del within the 109 bp intron 3 sequence was detected in the CTCs but not in the WBC.	

DEPArray- Samples Processing Flow *(Silicon Biosystems)*

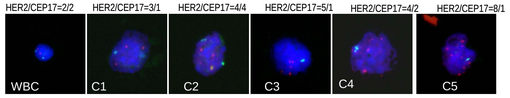


DEPArray Images of cells recovered from Pt. 6146



CTCs were enriched using Veridex CellSearch technology which uses anti-CK-PE, DAPI and anti-CD45-APC to fluorescently label cells. Single CTCs, clustered CTCs and single WBCs were selected and isolated on the Silicon Biosystems DEP Array. Representative cells are shown.

HER2/neu Status in CellSieve™ (CreatvMicroTech) Filter-Captured Cells via FISH in Pt. #4836



Average ratio in 5 unusual cells

HER2/CEP17=24 /9=2.67

Typical WBC - 2 copies of HER2 and 2 copies of CEP17

Aberrant copy number of CEP17

CONCLUSIONS

- **Genomic sequencing in MBC is feasible and has identified potential therapeutic targets.**
- **Tissue selection should represent the current disease sites e.g. chest wall, bone, lymph node, pleural fluid and CTCs.**
- **All sites of current disease, if feasible, should be sought.**
- **Targeted treatment modalities should address the gene mutation and/or gene amplifications detected in the tissue(s) analysis.**