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14. ABSTRACT

This is a study of vitamin D therapy of women with breast cancer (BCa) in the neoadjuvant period. The goal of the study is to determine whether vitamin D changes the expression of important target genes that may predict BCa prognosis. Thus far we have recruited 48 patients of the planned total of 50 subjects. Several subjects are not included in this report since they have not yet had their surgery or we have not yet received their biopsy or surgical specimens. These data will eventually be added to the current analysis. The women were stratified according to their circulating vitamin D status (serum 25(OH)D concentration) receiving daily doses of vitamin D3 of 2000 IU, 4000 IU or 6000 IU or no therapy. We have analyzed the biopsy samples (pre-intervention) and compared results with the surgical specimens (post-intervention) to determine whether dietary vitamin D supplements can regulate the gene expression profile in the BCa cells and normal cells. The goal is to determine whether vitamin D deficiency is associated with a poor prognosis gene profile and whether vitamin D intervention converts the profile toward a more normal profile of gene expression, perhaps representing a better prognosis.

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vitamin D, calcitriol, breast cancer, vitamin D receptor, estrogen receptor, antiproliferation, COX-2, prostaglandins, aromatase

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Table of Contents

INTRODUCTION

Although the grant was supposed to start 9/1/07 the DOD human subjects approval process greatly delayed the start date. We finally received human subjects approval in mid-July 2008 and our Notice of Award was received on 3/13/08. We actually initiated work on the grant and began to charge effort to the grant after human subjects approval. We have requested and received a No Cost Extension to work on this project until June 30, 2010, approximately 2 full years, which was the original time projected to complete the study. Since the last specimens are still being obtained and the data are not fully analyzed, I have requested a postponement of the deadline for this report. However approval of a delay in reporting has not been forthcoming. Therefore this analysis is incomplete but the final patients will eventually be added to our data and a complete analysis will eventually be submitted for publication.

The work to be accomplished as described in the SOW.

1. To recruit patients undergoing core needle biopsy for breast abnormalities so as to accrue 50 evaluable patients with breast cancer to study.
2. To analyze their vitamin D, PTH, and calcium status in blood samples.
3. To characterize the classical prognostic and predictive characteristics of the breast cancer at time of diagnostic biopsy.
4. To analyze the cancer biopsy specimens for a limited gene expression profile focusing on BCa genes and vitamin D-regulated genes.
5. To determine whether the prognostic and predictive factors differ between vitamin D deficient or insufficient patients and vitamin D sufficient patients.
6. To treat the vitamin D deficient and insufficient patients with vitamin D during the interval between biopsy and definitive breast surgery.
7. To reanalyze the vitamin D status after intervention and before surgery.
8. To analyze the surgical cancer specimens for their gene profile and compare the findings to the core biopsy gene profile to determine whether vitamin D therapy changes the profile.
9. To compare the results of treated deficient/insufficient patients to sufficient patients to determine whether vitamin D intervention normalized the gene profile.
10. To analyze the data.

BODY

Patient enrollment and treatment schedule

Recruitment of subjects with breast cancer is almost complete. At this time we have enrolled 48 patients. Their vitamin D [25(OH)D], $1,25(OH)_2D_3$ and other hormone levels as well as demographic data are shown in the accompanying table (Appendix, Table 1). The patients have been stratified to receive vitamin D therapy depending on their initial 25(OH)D levels: 14 were untreated, 19 received 2000 IU, 11 received 4000 IU and 4 received 6000 IU. The table also details for each subject: the dose of vitamin D and the duration of therapy, race/ethnicity, breast cancer status, ER and PR status, baseline and treated presurgery 25(OH)D and $1,25(OH)_2D_3$ serum levels, serum calcium and parathyroid hormone (PTH), type of surgery, vitamin D dose and duration of therapy and BMI.

Breast Cancer Pathology

In Appendix Table 2 we detail the pathology data for the cohort of patients listed by ID number. Each patient's cancer is described: age of patient, whether the core or surgical sample was obtained in or outside of Stanford hospital, tumor location, histology of the surgical sample, grade, size, node involvement and pathology TNM status, whether DCIS was present, ER and PR status by intensity, percent of cells staining positive and a combined Allred score both from the clinical pathologist reading and the research pathologist. Similar readings are provided for KI-67 protein, and Her 2 status. Finally some comments are made for some of the specimens.

Gene Expression Array
The gene array that we used was custom made to our specifications by the SABiosciences Company. The genes in our array are shown in Table 3. The method employed is a polymerase chain reaction (PCR) technique that uses amplification to increase the amount of mRNA. Some genes have been or will be validated by direct PCR measurement.

Gene Expression Profiles
At this time we have measured the gene expression profiles of 38 of the subjects in both biopsy and surgical specimens. Not all have ben analyzed for this report. There are 17 treated patients, 3 patients have 2 separate cancer specimens. There are 11 untreated patients, 1 with 2 separate cancers. Other patients have been analyzed but either the biopsy or the surgical sample is not yet available. We expect to finish 50 patients with as much information as possible before we write our paper.

Some genes are expressed at very low levels and we have omitted these genes from some of the analyses because the values are too low to have confidence in their reliability. The gene expression analysis of 4 genes of most interest are singled out here for analysis. These genes include IGFBP-3, p21, MMP-11 and Ki-67.

a. IGFBP-3 Insulin-like growth factor binding protein 3, is encoded by the *IGFBP3* gene. IGFBP-3 is the carrier protein for IGF-1 the major stimulator of proliferation. By binding IGF-1, IGFBP-3 is anti-proliferative. But, IGFBP-3 has important IGF-independent actions that are anti-proliferative, pro-apoptotic and anti-inflammatory making it an important anti-cancer gene. We have previously shown it to be directly regulated by calcitriol and to be a critical component of the anti-cancer activity of vitamin D.

b. p21. Also called WAF1 is known as cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1 is encoded by the *CDKN1A* gene located on chromosome 6 (6p21.2). The protein is a major inhibitor of the cell cycle and is represents a major pathway for vitamin D to be anti-proliferative. We have shown this gene to be responsive to therapy in prostate and it is often used to predict prognosis.

c. MMP11 Stromelysin-3 (SL-3) also known as matrix metalloproteinase-11 is an enzyme that is encoded by the *MMP11* gene. Proteins of the matrix metalloproteinase family are involved in the breakdown of extra-cellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and cancer metastasis. Inhibition of this protein suggests activity that would inhibit invasion and metastasis of cancer.

d. Ki-67. Antigen KI-67 also known as MKI67 is encoded by the *MKI67* gene. The Ki-67 protein is a cellular marker for proliferation. It is strictly associated with the proliferative phase and is absent from resting cells. It is routinely used as a marker of proliferation in breast cancer pathology analysis. The fraction of Ki-67-positive cells (the *Ki-67 labelling index*) is often correlated with the clinical course of cancer especially in breast cancer. The prognostic value for survival and tumor recurrence have repeatedly been proven in various analyses.

The data for these genes in our patients are shown according to treatment compared to no treatment in Figure 1. The expression of the same genes, analyzed according to 25(OH)D levels achieved, are shown in Figure 2.

Heat maps of expression for all genes comparing treated to untreated, biopsy to surgery and normal to cancer are provided in several panels in Figure 3. These data are shown according to treatment dose that is indicated by the gray bar at the top. The intensity of the gray color indicates no treatment, 2000 IU, 4000 IU or 6000 IU administered. Increasing red color indicates increased gene expression.

In the analysis of the gene expression data we compare the gene expression profile in the diagnostic biopsy with the surgical specimen obtained 2 to 5 weeks later depending on the interval until surgery takes place. We also compare normal regions in the surgery specimen to cancer regions. The gene expression data are very complex.

There are multiple genes, several treatment doses, variable treatment duration and variable final concentration of 25(OH)D levels achieved. The patients vary by untreated to low, medium and high dose. But the treatment interval varies for each patient. It is unclear what the major parameter for analysis should be: treated *vs.* untreated, change in (delta) 25(OH)D levels, or final 25(OH)D level achieved. Some of the data analyses we provide compare patients treated with vitamin D to control patients that were untreated as shown in Figure 1 for 4 critical genes IGFBP-3, p21, MMP-11, KI-67. In Figure 2 these same genes are analyzed based on changes in 25(OH)D levels. There seems a clear trend in most patients that vitamin D stimulated an increase of expression of protective genes IGFBP-3 and p21 and inhibition of the genes suggesting a poor outcome Ki-67 and MMP-11.

Heat Maps

Figure 3 shows the Heat Maps of all of the genes according to vitamin D therapy. The genes are listed on the right hand margin, the patient ID number on the bottom and the patients are clustered by dosage indicated by the gray bar along the top.

3A. Biopsy specimens. Among the genes that show increased expression include ESR1 (estrogen receptor) ERBB2, TFF1, CCND1, CDKN1A (p21). IGFBP-3 is expressed fairly high, as is p21 (CDKN1A*)*. MMP-11 is mixed among the subjects but fairly high while KI-67 has mixed to low expression. Many genes show very low expression including aromatase (CYP19A1), hairless (HR), and the vitamin D enzymes (CYP24A1 and CYP27B1) which we feel are too low for valid detection by our methods.

3B. Surgery specimens – cancer. The pattern is somewhat similar to the biopsy but many more genes show increased expression. IGFBP-3 and p21 were already high and they are further increased, especially in the treated group. MMP-11 and Ki-67 are mixed.

3C. Surgery specimens – normal. The normal specimens have many genes expressed at a lower rate that the cancers. MMP-11 and KI-67 are decidedly low as would be expected in normal tissue.

3D. Surgery minus biopsy. This map shows changes from biopsy to surgery during treatment. Many genes show increased expression. Ki-67 and MMP-11 remain mostly at low expression while IGFBP-3 and p21 are mostly higher in the patients who received vitamin D. There is much individual variation.

3E. Cancer minus normal, This is a comparison of malignant to normal specimen. The map has become uniformly low. MMP-11 is a standout of increased expression showing it as a gene highly expressed in the cancer compared to normal. Ki-67 shows a similar but more variable pattern.

Problems

A potential problem that surfaced during the early days of the grant was the extremely small amount of tissue left for our study after the pathologist has completed the clinically relevant analysis of the diagnostic biopsy material. We have found that the yield of RNA from this residual amount of tissue was not sufficient to perform individual PCR analyses on the full complement of genes that we had planned to study. We therefore worked with a company (SABioscience) to tailor-make a gene array (described in the appendix) and to develop an RNA amplification strategy to generate enough mRNA for the full analysis. This technique appears to be successful except that several low expressing genes are not being adequately detected. So although our array has 40 target genes plus 5 housekeeping genes and various controls, we are obtaining useful data on fewer than 40 genes and only 4 housekeeping genes are being used in the analysis.

A second potential problem is that more of our subjects than expected from literature data were found to have adequate 25(OH)D levels and so they are not being treated with vitamin D. This means that we are finding fewer subjects that qualify to receive high doses of vitamin D intervention, even with the more aggressive strategy that we adopted when the protocol was changed during the grant. Currently 14 patients have no treatment, 19 have 2000 IU, 11 have 4000 IU and 4 received 6000 IU. Since surgery is generally scheduled very soon after the diagnosis is made, the treatment period is quite short. The combination of lower doses of vitamin D and shorter treatment times has resulted in somewhat smaller changes in the 25(OH)D levels than we had hoped to achieve. The delta between initial and final 25(OH)D levels and/or the ultimate level of 25(OH)D achieved are the likely parameters that will result in effective changes in gene transcription. Thus we are

somewhat concerned to find that the dosage schedule we are using, drawn up years ago when concerns about potential toxicity were greater, may have been structured too conservatively in order to avoid toxicity from too much vitamin D when in fact higher doses may have been more efficacious and still safe. We will have to wait until the gene expression data are fully analyzed but we are sorry that our concerns for the safety of our subjects, as well as IRB concerns, may have led us to be too conservative in the vitamin D dosage used in the trial.

Other problems include the variable time the subjects were treated, the small number of 25(OH)D values that achieved a high level, and the absence of untreated participants with very low levels of vitamin D that we felt it was unethical not to treat. So the data are quite variable.

After all patients are recruited and assayed, a final analysis with statistical measurements will be performed.

KEY RESEARCH ACCOMPLISHMENTS

Recruitment of 48 subjects to the study out of planned 50. Analysis of specimens from many of these subjects is completed. The remaining specimens are in the process of being assayed. Selected genes seem to show a pattern of vitamin D therapy increasing protective genes and inhibiting poor prognosis genes. However, the overall pattern is variable. The technical difficulties of measuring gene expression in the limited amount of tissue available from biopsies has been overcome although some genes are expressed at too low a level for valid measurement by this assay.

REPORTABLE OUTCOMES

Many genes have been analyzed. The genes of most interest are IGFBP-3, p21, MMP-11 and Ki-67. When all specimens have been analyzed the findings will be evaluated and a paper prepared for publication.

CONCLUSIONS

The recruitment of patients went well. The gene expression analysis has been completed in many subjects. There are fewer vitamin D deficient patients than we expected based on published ratios of vitamin D levels in the population. Thus many subjects are not being treated (control group) and we are finding that many women qualified only for the low dose of vitamin D and many fewer that qualified for the middle and high dose. We anticipate that patient recruitment will be successful to end with 50 subjects. We have optimized the strategy to evaluate the profile of gene expression in the breast cancer biopsies and surgical specimens and these studies are moving forward toward completion.

REFERENCES

N/A

APPENDICES

Table 1. Patient data showing 48 recruited subjects including vitamin D levels and intervention.

Table 2. Pathology analysis of patient cancer specimens.

Table 3. Description of the gene array and listing of the genes on the array.

Figure 1. Analysis of 4 genes comparing treated to untreated

Figure 2. Analysis of the same 4 genes by achieved 25(OH)D levels

Figure 3. Heat Maps of all of the genes
- 3A. Biopsy specimens
- 3B. Surgery specimens - cancer
- 3C. Surgery specimens – normal
- 3D. Surgery minus biopsy (change from biopsy to surgery during treatment)
- 3E. Cancer minus normal (comparison of malignant to normal specimens)

TABLE 1

Vitamin D Study - Patient Log

							Ref Ranges	**25-80 ng/ml**	**18-78 pg/ml**	**8.5-10.5 mg/dl**	**10-80 pg/ml**						
							Vitamin D 25		Vitamin D 1,25		Calcium		PTH				
# Elig on study	ID	Age	Race/ Ethnicity	Consent date	Breast Cancer Status	ER status	PR status	Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery	Type of Surgery	Vitamin D intervention	Days betw draws	BMI
1	1	49	White/Non Hispanic	4/28/08	Invasive Lobular Carcinoma	Pos	Neg	45	45	23	36	8.7	8.8	27	21	Mastectomy	None	8	23.5
2	2	53	Asian/Non Hispanic	4/28/08	Invasive Ductual Carcinoma	Neg	Neg	35	36	38	44	9.5	9.2	27	51	Lumpectomy	None	16	24.4
3	3	45	White/Non Hispanic	4/29/08	Invasive Ductual Carcinoma	Pos	Pos	26	36	75	62	9.2	9.1	30	31	Mastectomy	One Capsule for 36 days	37	24.2
4	4	47	Other/ Hispanic	5/9/08	Invasive Lobular Carcinoma	Awaiting results	Awaiting results	50	Not Done	58	Not Done	9.5	Not Done	11	Not Done	Mastectomy	None	na	28.3
5	5	46	Asian/Non Hispanic	5/20/08	Invasive Mixed ductal and micropapillary	Pos	Pos	14	26	36	57	9.5	9.5	31	33	Mastectomy	Two Capsules for 12 days	14	24.2
6	6	35	White/Non Hispanic	6/5/08	Invasive Ductual Carcinoma	Neg	Neg	33	35	98	45	9.7	9.5	5	21	Mastectomy	None	34	20.5
7	7	47	Asian/Non Hispanic	6/26/08	Invasive Ductual Carcinoma	Pos	Pos	27	35	45	48	9.1	9.6	20	18	Mastectomy	One Capsule for 14 days	18	21.4
8	8	50	White/Non Hispanic	6/27/08	Invasive Ductual Carcinoma	Pos	Pos	19	37	41	62	8.5	8.7	82	59	Lumpectomy	One Capsule for 48 days	54	28.7
9	9	50	White/Non Hispanic	7/17/08	Invasive Ductual and Tubular Carcinoma	Pos	Pos	39	41	63	71	9.8	8.9	25	29	Lumpectomy	None	12	28.9
10	10	37	White/Non Hispanic	7/17/08	Invasive Ductual, Tubular Carcinoma	Pos	Pos	38	26	60	56	9.7	7.9	36	25	Mastectomy	None	32	23.4
11	11	57	White/Non Hispanic	7/29/08	Invasive Ductual Carcinoma	Pos	Pos	32	29	47	47	10	9.5	76	45	Mastectomy	None	42	29.2
12	12	71	White/Non Hispanic	7/30/08	Invasive Mucinous Carcinoma	Pos	Pos	11	20	27	31	9.5	9.0	73	78	Lumpectomy	Two Capsules for 15 days	19	34.7
13	13	54	White/Non Hispanic	8/7/08	Invasive Ductual Carcinoma	L-pos R-Neg	L-pos R-Neg	49	38	62	58	9.1	9.3	31	32	Mastectomy	None	46	21.8
14	14	56	Asian/Non Hispanic	8/26/08	Invasive Ductual Carcinoma	Pos	Pos	51	47	44	47	9.4	9.3	42	43	Mastectomy	None	16	23.5
15	15	40	Asian/Non Hispanic	10/6/08	Invasive Ductual Carcinoma	Pos	Pos	22	27	48	51	9.6	9.6	27	26	Lumpectomy	1 capsule for 15 days	16	26.1

* Blood drawn 7 days AFTER surgery.

TABLE 1 (contd)

Vitamin D Study - Patient Log

# Elig on study	ID	Age	Race/ Ethnicity	Consent date	Breast Cancer Status	ER status	PR status	Vitamin D 25		Vitamin D 1,25		Calcium		PTH		Type of Surgery	Vitamin D intervention	Days betw draws	BMI
								Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery				
16	16	55	White/Non Hispanic	11/17/08	Invasive Ductual Carcinoma	Pos	Pos	14	21	60	61	9.3	9.0	70	75	Lumpectomy	2 capsules for 6 days	8	39.1
17	18	63	White/Non Hispanic	3/26/09	IDC and DCIS	Pos	Pos	17	26	27	29	9	8.5	38	50	Matectomy	1 capsule for 27 days	47	27.5
18	20	61	Other/ Hispanic	3/31/09	Invasive Ductual Carcinoma	Pos	Pos	43	43	66	38	10.1	9.6	33	24	Mastectomy	None	56	29.7
19	22	51	White/Non Hispanic	5/1/09	Invasive Ductual Carcinoma	Pos	Pos	23	30	48	44	9.1	8.6	30	25	**bilateral lumpectmy**	1 capsule for 30 days	38	25.2
20	23	39	Asian/Non Hispanic	5/15/09	Invasive Ductual Carcinoma	Pos	Pos	17	28	60	77	8.5	9.1	72	21	Mastectomy	1 capsule for 12 days	14	21.5
21	24	51	White/Non Hispanic	5/22/09	invasive Ductal Carcinoma	Pos	Neg	33	31	47	55	9.1	9.1	35	39	Mastectomy	None	18	26.1
22	25	56	White/Non Hispanic	6/1/09	invasive Ductal Carcinoma	Pos	Pos	32	31	53	43	9.6	9.4	39	31	Bilat Mastectomy	None	17	20.8
23	26	65	White/Non Hispanic	6/4/09	invasive Ductal Carcinoma	95%	95%	36	35	58	59	9.6	9.1	40	56	Lumpectomy	None	49	21.9
24	27	43		6/4/08	Invasive Lobular Carcinoma	31%	22%	25	27	62	49	9.3	9	27	18	Lumpectomy	1 capsule for 12 days	18	33.1
25	28	63	White/Non Hispanic	6/24/09	invasive Ductal Carcinoma	95,95%	50/90%	35	42	62	70	9.1	9.4	25	18	Bilat Mastectomy	1 capsule for 34 days	50	34.5
26	33	60	White/Non Hispanic	7/10/09	invasive Ductal Carcinoma	100%	90%	33	29*	42	na	8.9	8.6	46	16*	Bilat Mastectomy	1 capsule for 20 days	na	20.7
27	35	71	White/Non Hispanic	7/21/09	invasive Ductal Carcinoma			33	37	68	75	8.6	8.8	70	32	Mastectomy	1 capsule 35 days	41	19
28	38	46	Other/ Hispanic	7/31/09	invasive Ductal Carcinoma	90%	95%	17	48	82	56	9.1	9.3	94	84	Lumpectomy	3 capsules for 50 days	60	24.6
29	40	50	Pacific Islander	8/14/09	Invasive Ductal Carcinoma	90%	80%	19	42	58	48	8.9	9	41	38	Mastectomy	3 capsules for 73 days	79	34.4
30	41	27	White/Non Hispanic	8/18/09	Invasive Ductal Carcinoma			40	39	58	36	9	9.2	35	39	Bilat Mastectomy	1 capsule for 15 days	23	22.3
31	42	59	White/Non Hispanic	8/27/09	ID C			27	45	63	89	9.4	9.3	45	32	Lumpectomy	2 capsules for 33 days	39	22.6
32	44	63	White/Non Hispanic	10/21/09	IDC	95%	80%	41	37	65	54	9.2	9	16	34	Lumpectomy	none	76	22.1
33	45	54	Asian/Non Hispanic	10/30/09	IDC	>95%	neg	30	39	47	59	9.7	9.8	26	27	PAMF pt bilat mx	2 capsules for 30 days	32	26
34	46	73	Asian/Non Hispanic	11/5/09	IDC and DCIS	3+	3+	39	43	37	34	10	9.3	19	45	Mastectomy	1 capsule for 53 days	55	20.5
35	47	89	White/Non Hispanic	11/12/09	IDC			34	58	26	31	10.5	10.6	113	108	lumpectomy	1 capsule		27

* Blood drawn 7 days AFTER surgery.

TABLE 1 (contd)

Vitamin D Study - Patient Log

# Elig on study	ID	Age	Race/ Ethnicity	Consent date	Breast Cancer Status	ER status	PR status	Vitamin D 25		Vitamin D 1,25		Calcium		PTH		Type of Surgery	Vitamin D intervention	Days betw draws	BMI
								Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery	Baseline	Presurgery				
36	48	65	White/Non Hispanic	11/13/09	IDC	3+	3+	39	50	68	54	9	8.7	44	36	Mills pt	1 capsule for 30 days	33	
37	49	54	White/Non Hispanic	1/8/10	IDC	90%	90%	17	40	65	21	9.1	9.1	158	64	Lumpectomy	3 capsules for 80 days		45.8
38	50	68	White/Non Hispanic	1/15/10	IDC			21	na	56	na	9.1	na	46	na	John Muir Pt	2 capsules		25.5
39	51	58	Other/ Hispanic	1/22/10	IDC	55%	10%	27	44 (S)	69	80	9.6	9.6	31	27	Lumpectomy	2 capsules	51	21.9
40	52	71	Asian/Non Hispanic	1/28/10	ILC	95%	10%	28 (S)	63(S) 73(M)	58	56	8.9	9.6	58	42	Lumpectomy	2 capsulres	39	28.2
41	53	47	White/Non Hispanic	1/29/10	IDC	90	5	24 (S)	36(S) 38(M)	91	65	8.7	9.2	91	65	biopsy	2 capsules for 13 days		23.8
42	54	37	White/Non Hispanic	2/12/10	IDC	95	95	32(M)	28(M)	38	32	9.4	9.5	54	38	Mastectomy	1 capsule for 16 days		24.1
43	55	66	White/Non Hispanic	2/25/10	ILC	95	95	31	37	53	39	9.5	9.8	61	47	Lumpectomy	1 capsule for 21 days	28	22
44	56	67	White/Non Hispanic	3/19/10	ILC	90	60	17	47	38	43	9.8	9.9	71	70	Lumpectomy	3 capsules 33 days		31.2
45	58	52	White/Non Hispanic	4/5/10	IDC	90	50	29	38	29	29	9.5	9.7	25	25		2 capsules	36	41.2
46	59	58	White/Non Hispanic	4/6/10	IDC	95	95	33	34	68	53	9.6	9.7	32	22		1 capsule	40	23.8
47	60	49	White/Non Hispanic	4/30/10	IDC			35		52		8.7		40		sched 7/26	1 capsule		
48	62	45	Asian/Non Hispanic	6/15/10	IDC			21	30	66	55	8.9	9.2	19	24	Lumpectomy	2 capsules	31	

* Blood drawn 7 days AFTER surgery.

TABLE 2 Pathology analysis of patient cancer specimens

ID number	Age	Out_core?	Out_res?	Tumor_side	Tumor_site	Histology (resection)	Grade (resection)	Size (resection)	Nodes	PathTNM	In situ present?	Stage	Core_ER_int
1	49	Yes	No	Left	2 o'clock	Lobular	2	4.8 cm	0/4	pT2pN0(i-)pMX	No		3
2	53	No	No	Left	3:30	Ductal	3	2.4 cm	0/3	pT2pN0(i-)pMX	No		0
3-A (1)	45	Yes	No	Right	11:00-12:00	Ductal	2	2.4 cm	1/4	same as above	No		2
3-B (2)	45	Yes	No	Right	Central	Ductal	1	2.5 cm	1/4	pT2pN1mi(sn)pMX	Yes (DCIS)		2
4	47	Yes	Yes	Right	Unknown	Lobular	1-2	6 cm	1/4	pT3pN1mi(sn)pMX(?)	Yes (LCIS)		
5	45	No	No	Right	UOQ	Ductal and micropapillary	2	3.9 cm	2/21	pT2pN1pMX	Yes (DCIS)		2
6	35	Yes	No	Right	11:30	Ductal	3	1.5 cm	1/15	pT1cpN1mipMX	Yes (DCIS)		
7	50	Yes	No	Right	9:30	Ductal	2	2.3 cm	0/5	pT2pN0(i-)pMX	Yes (DCIS)		
8	47	Yes	No	Left	Subareolar	Ductal	3	3.5 cm	2/29	pT2pN1apMX	Yes (DCIS)		
9	50	Yes	No	Left	11:00	Ductal	1	0.4 cm	0/2	pT1apN0(i-)pMX	No		
10	36	Yes	No	Right	12:00	Ductal	3	3.5 cm	0/12	pT2pN0(i-)pMX	Yes (DCIS)		0
11-A (1)	57	No	No	Right	3:00	Ductal	1	2.1 cm	0/4	pT2pN0(i-)pMX	Yes (DCIS)		2
11-B (2)	57	No	No	Right	6:00	Ductal	1	0.4 cm	0/4	same as above	No		N/A
11-C (3)	57	No	No	Right	6:00	Ductal	1	0.6 cm	0/4	same as above	Yes (DCIS)		N/A
12	71	No	No	Left	retroareolar	Ductal with mucinous features	1	1.9 cm	0/1	pT1cpN0(i+)pMX	No		1
13-A (1)	54	Yes	No	Left	1:00	Ductal	2	0.7 cm	0/3	pT1cpN0(i-)pMX	Yes (DCIS)		
13-B (2)	54	Yes	No	Left	10:00	Ductal	2	1.8 cm	0/3	same as above	Yes (DCIS)		
13-C (3)	54	No	No	Left	Lateral lower quadrant	Lobular	1	0.9 cm	0/3	same as above	Yes (LCIS)		N/A
14	56	Yes	No	Left	10:00-12:00	Ductal	1	3.5 cm	0/4	pT2pN0pMX	Yes (DCIS)		3
15	40	Yes	No	Right	2:00 subareolar	Ductal	3	1.8 cm	3/20	pT1cpN1apMX	Yes (DCIS)		2
16	55	Yes	No	Left	12:00	Ductal	2	1.2 cm	0/16	pT1cpN0(i-)pMX	Yes (DCIS)		
18	62	Yes	No	Left	3:30-4:00	Ductal	3	5.4 cm	1/10	pT3pN1pMX	Yes (DCIS)		3
20	62	Yes	No	Right	7:00	Ductal	2	0.4 cm	1/4	pT1apN1mipMX	No		3
22-A (Lt)	51	No	No	Left	6:00	Ductal	1	0.7 cm	0/3	pT1bpN0(i-)pMX	Yes (DCIS)		3
22-B (Rt)	51	No	No	Right	9:30	Ductal	3	2.5 cm	0/4	pT2pN0(i-)pMX	Yes (DCIS)		3
23	39	Yes	No	Left	2:00	Ductal	3	2.8 cm	1/8	pT2pN1mipMX	Yes (DCIS)		1
24	51	Yes	No	Right	UOQ	Ductal with metaplastic differentiation	3	2.7 cm	0/8	pT2pN0(i-)pMX	Yes (DCIS)		
25	56	Yes	No	Right	11:00	Ductal	2	1.2 cm	0/4	pT1cpN0(i+)pMX	Yes (DCIS)		
26	65	Yes	No	Right	Lower breast	Ductal	1	1.5 cm	0/5	pT1cpN0(i-)pMX	Yes (DCIS)		3
27	43	No	No	Left	LOQ	Lobular	1	5.7 cm	2/25	pT3pN1apMX	Yes (LCIS)		3
28-A (Lt)	63	Yes	No	Left	Not known	Ductal	1	Not known	0/4	pTXpN0(i-)pMX	Yes (DCIS)		
28-B (Rt)	63	Yes	No	Right	11:00	Ductal	2	Not known	0/3	pTXpN0(i-)pMX	Yes (DCIS)		
33	60	Yes	No	Right	9:00	Ductal	1	0.9 cm	0/4	pT1bpN0(i-)pMX	Yes (DCIS)		
35	71	No	No	Right	5:00	Ductal	2	1.2 cm	0/1	pT1cpN0(i-)pMX	Yes (DCIS)		3
38	46	Yes	No	Left	11:30	Lobular	2	1.9 cm	1/15	pT1cpN1apMX	Yes (LCIS)		
40	50	Yes	No	Left	12:00	Ductal	1	1.2 cm (mammo)	0/1	pT1bcpNo(sn)pMX	Yes (DCIS)		
41	27	Yes	No	Left	3:00	Ductal	2	2.2 cm	0/4	pT2pN0(i-)pMX	Yes (DCIS)		
42	59	Yes	No	Right	10:00	Ductal	2	0.8 cm	0/3	pT1bpN0(i-)pMX	Yes (LCIS)		
44	63	No	No	Left	4:00	Ductal	2	1.8 cm	N/A	pT1cpNXpMX	No		
45	54	Yes	Yes	Right	Lateral	Ductal	2	3.0 cm	4/18 (ECE)	pT2pN2pMX	Yes (DCIS)		
46	73	Yes	No	Left	2:00	Ductal	1	0.45 cm	0/1	pT1apN0(sn)pMX	Yes (DCIS)		
47-A (Lt)	89	No	No	Left	11:00	Ductal	2	1.8 cm	N/A	pT1cpNXpMX	Yes (DCIS)		
47-B (Rt)	89	No	No	Right	8:00	Ductal	1	1.0 cm	N/A	pT1cpNXpMX	No		3
48	65	Yes	Yes	Right	1:00	Ductal	1	1.3 cm	0/1	pT1cpN0(sn)pMX	Yes (DCIS)		
49	54	Yes	No	Right	10:00	Ductal	1	0.8 cm	0/3	pT1bpN0(sn)(i-)pMX	No		
50	58	Yes	Yes	Right	12:00	Ductal							
51	58	Yes	No	Left	Not known	Ductal	2	2.8 cm	0/3	pT2pN0(sn)(i-)pMX	Yes		
52	71	No	No	Left	9:00	Lobular	1	3.2 cm	0/1	pT2pN0(sn)(i-)pMX	Yes (LCIS)		3
53	47	Yes	Yes	Right	10:00-2:00	Ductal	2						2-3
54	37	Yes	No	Right	12:00	Ductal	2	5.5 cm	0/9	pT3pN0(i-)pMX	Yes (DCIS)		
55	66	Yes	No	Right	7:00	Lobular	1	2.7 cm	0/1	pT2pN0(sn)(i-)pMX	No		
56	67	Yes	No	Left	Outer	Lobular	2	5.1 cm	1/16	pT3pN1apMX	Yes (LCIS)		
58	52	No	No	Left	Lower outer quadrant	Lobular	2	1.5 cm	2/34	pT1cpN1apMX	Yes (LCIS)		
59	58	No	No	Right	3:00	Ductal	1	0.7 cm	0/4	pT1bpN0(sn)(i-)pMX	Yes (DCIS)		3
60-A (Lt)	49	Yes		Left	4:00 and 6:00								
60-B(Rt)	49	Yes		Right	Axillary tail and 9:00								
61	47	Yes		Left	3:30								
62	45	Yes		Left	10:00								

TABLE 2 (contd)

ID number	Core_ER_pct	Core_ER_Allred	Core_PR_int	Core_PR_pct	Core_PR_Allred	Core_Ki67_pct	Core_HER2	Res_ER_int	Res_ER_pct	Res_ER_Allred	Res_PR_int	Res_PR_pct	Res_PR_Allred	Res_Ki67_pct	Res_HER2
1	80	8	2	50	6	<5		3	95	8	3	95	8	20	0.98
2	0	0	0	0	0	80		0	0	0	0	0	0	80	1.07
3-A (1)	95	7	3	95	8	10		2	50	6	3	50	7	20	1.02
3-B (2)	80	7	2	60	6	<5		2	80	7	3	95	8	10	1.44
4															
5	90	7	3	95	8	30	1.17								
6								0	0	0	0	0	0	35	1.07
7								1	30	4	2	95	7	<5	
8								2	95	7	3	15	6	15	1.38
9								3	95	8	1	30	4	<5	
10	0	0	0	0	0	80		0	0	0	0	0	0	40	
11-A (1)	70	7	1	50	5	<5		3	95	8	2	10	4	<5	1.07
11-B (2)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3	95	8	3	30	6	<5	1.09
11-C (3)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3	95	8	3	95	8	<5	1.05
12	30	4	2	20	5	15		2	95	7	3	95	8	10	1.05
13-A (1)								2	80	7	3	95	8	<5	0 (IHC)
13-B (2)								2	95	7	3	95	8	<5	0 (IHC)
13-C (3)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2	20	5	3	95	8	<5	0 (IHC)
14	95	8	0	0	0	5-10									0.97
15	25	5	1	5	3	30		2	80	7	3	80	8	<5	3.42
16								3	95	8	2-3	90	7-8	5-10	0.94
18	100	8	2	5	4	85	0.53								
20	80	8	3	40	7	<5		3	95	8	2	5	4	10	
22-A (Lt)	95	8	3	95	8	5-10	1.26								
22-B (Rt)	95	8	3	95	8	30		3	95	8	3	95	8	70	1
23	80	6	1	20	4	20		1-2	50	5-6	2-3	70	7-8	20	0.84
24															
25								3	95	8	3	<1%	4	5	0.98
26	95	8	2	95	7	5		3	95	8	3	95	8	<5	0.98
27	95	8	3	95	8	5		3	95	8	3	95	8	<5	
28-A (Lt)															
28-B (Rt)															
33								3	95	8	3	70	8	0	1.37
35	95	8	0	0	0	5		3	95	8	0	0	0	10	1.55
38															
40								3	95	8	3	30		No inv	Not done
41								3	95	8	3	95	8	10	0.91
42															
44								3	95	8	2-3	80	7-8	10	1.3
45															
46															
47-A (Lt)								3	95	8	2-3	30	5-6	5-10	0.99
47-B (Rt)	95	8	3	95	8	<1	1.12								
48															
49															
50															
51								3	95	8	0	0	0	5	1.04
52	95	8	3	10	5	10	1.02								
53	90	7-8	1-2	5	3-4	20	0.97								
54								3	95	8	3	95	8	5	1
55															
56															
58								3	70	8	3	80	8	10	1.05
59	95	8	2-3	95	7-8	5	1.2								
60-A (Lt)															
60-B(Rt)															
61															
62															

TABLE 2 (contd)

ID number	Comments
1	Size garnered from addition of two lumpectomies and subsequent completion mastectomy
2	
3-A (1)	
3-B (2)	
4	
5	
6	
7	
8	
9	
10	
11-A (1)	Only tumor one tested by Feldman lab with matching core/resection pair
11-B (2)	
11-C (3)	
12	Isolated (less than 10) keratin-positive cells on keratin stain only
13-A (1)	
13-B (2)	
13-C (3)	Not tested by Feldman lab
14	
15	
16	To date (12/15/09), outside core needle biopsy block cannot be found
18	
20	Four separate foci ranging from 0.2 cm to 0.4 cm
22-A (Lt)	
22-B (Rt)	Smaller focus 0.9 cm, not tested by Feldman lab
23	
24	Smaller focus 0.9 cm, not tested by Feldman lab
25	Isolated (less than 10) keratin-positive cells on keratin stain only
26	
27	Additional 6 lymph nodes showed keratin-positive cells
28-A (Lt)	No residual infiltrating cancer at resection in either breast
28-B (Rt)	No residual infiltrating cancer at resection in either breast
33	Multifocal, size is largest focus
35	
38	
40	No residual infiltrating cancer at resection
41	Second smaller (0.4 cm) focus found in ipsilateral breast
42	
44	Prior ipsilateral cancer; axillary lymph nodes dissected at that surgery
45	Simultaneous contralateral cancer (IDC/DCIS)
46	
47-A (Lt)	
47-B (Rt)	
48	
49	
50	
51	DCIS in right breast
52	
53	Participating in PARP trial, "resection" is a core biopsy post-vitamin D therapy
54	
55	
56	
58	
59	
60-A (Lt)	
60-B(Rt)	
61	
62	

TABLE 3 Description of the gene array and listing of the genes on the array

	1	2	3	4	5	6	7	8	9	10	11	12
A	ACTB	GAPDH	RPLP0	GUSB	TFRC	ESR1	ESR2	PGR	CYP19A1	TFF1	ERBB2	GRB7
B	EGFR	MKI67	AURKA	BIRC5	CCND1	CCNA1	TP53	MMP11	CTSL2	BAX	BCL2	VDR
C	CYP24A1	CYP27B1	HR	SNAI2	MYC	PTGS2	HPGD	PTGER4	DUSP10	IL6	TGFB1	TNF
D	CDKN1A	IGFBP3	SPP1	AR	PTHLH	AMH	FABP5	PPARG	GSTM1	GDC	RTC	PPC
E	ACTB	GAPDH	RPLP0	GUSB	TFRC	ESR1	ESR2	PGR	CYP19A1	TFF1	ERBB2	GRB7
F	EGFR	MKI67	AURKA	BIRC5	CCND1	CCNA1	TP53	MMP11	CTSL2	BAX	BCL2	VDR
G	CYP24A1	CYP27B1	HR	SNAI2	MYC	PTGS2	HPGD	PTGER4	DUSP10	IL6	TGFB1	TNF
H	CDKN1A	IGFBP3	SPP1	AR	PTHLH	AMH	FABP5	PPARG	GSTM1	GDC	RTC	PPC

GDC: Genomic DNA contamination control

RTC: Reverse transcription control

PPC: Positive PCR control

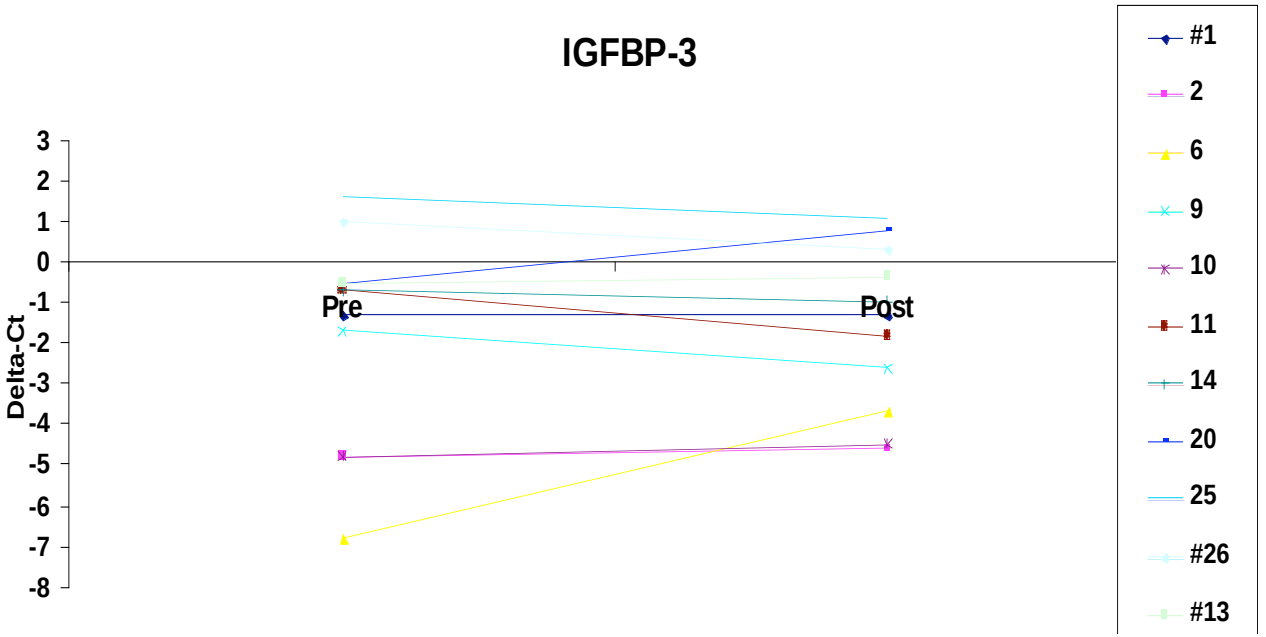
TABLE 3 (contd) Listing of the genes on the array

PCR Array Catalog #:		Feldman		
Position	**UniGene**	**RefSeq**	**Symbol**	**Description**
A01	Hs.520640	NM_001101	ACTB	Actin, beta
A02	Hs.544577	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
A03	Hs.546285	NM_001002	RPLP0	Ribosomal protein, large, P0
A04	Hs.255230	NM_000181	GUSB	Glucuronidase, beta
A05	Hs.529618	NM_003234	TFRC	Transferrin receptor (p90, CD71)
A06	Hs.208124	NM_000125	ESR1	Estrogen receptor 1
A07	Hs.443150	NM_001437	ESR2	Estrogen receptor 2 (ER beta)
A08	Hs.32405	NM_000926	PGR	Progesterone receptor
A09	Hs.654384	NM_000103	CYP19A1	Cytochrome P450, family 19, subfamily A, polypeptide 1
A10	Hs.162807	NM_003225	TFF1	Trefoil factor 1
A11	Hs.446352	NM_004448	ERBB2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)
A12	Hs.86859	NM_005310	GRB7	Growth factor receptor-bound protein 7
B01	Hs.488293	NM_005228	EGFR	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
B02	Hs.80976	NM_002417	MKI67	Antigen identified by monoclonal antibody Ki-67
B03	Hs.250822	NM_003600	AURKA	Aurora kinase A
B04	Hs.514527	NM_001168	BIRC5	Baculoviral IAP repeat-containing 5 (survivin)
B05	Hs.523852	NM_053056	CCND1	Cyclin D1
B06	Hs.417050	NM_003914	CCNA1	Cyclin A1
B07	Hs.654481	NM_000546	TP53	Tumor protein p53
B08	Hs.143751	NM_005940	MMP11	Matrix metallopeptidase 11 (stromelysin 3)
B09	Hs.660866	NM_001333	CTSL2	Cathepsin L2
B10	Hs.631546	NM_004324	BAX	BCL2-associated X protein
B11	Hs.150749	NM_000633	BCL2	B-cell CLL/lymphoma 2
B12	Hs.524368	NM_000376	VDR	Vitamin D (1,25- dihydroxyvitamin D3) receptor
C01	Hs.89663	NM_000782	CYP24A1	Cytochrome P450, family 24, subfamily A, polypeptide 1
C02	Hs.524528	NM_000785	CYP27B1	Cytochrome P450, family 27, subfamily B, polypeptide 1
C03	Hs.272367	NM_018411	HR	Hairless homolog (mouse)
C04	Hs.360174	NM_003068	SNAI2	Snail homolog 2 (Drosophila)
C05	Hs.202453	NM_002467	MYC	V-myc myelocytomatosis viral oncogene homolog (avian)
C06	Hs.196384	NM_000963	PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
C07	Hs.655491	NM_000860	HPGD	Hydroxyprostaglandin dehydrogenase 15-(NAD)
C08	Hs.199248	NM_000958	PTGER4	Prostaglandin E receptor 4 (subtype EP4)
C09	Hs.497822	NM_007207	DUSP10	Dual specificity phosphatase 10
C10	Hs.654458	NM_000600	IL6	Interleukin 6 (interferon, beta 2)
C11	Hs.645227	NM_000660	TGFB1	Transforming growth factor, beta 1
C12	Hs.241570	NM_000594	TNF	Tumor necrosis factor (TNF superfamily, member 2)
D01	Hs.370771	NM_000389	CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)
D02	Hs.450230	NM_000598	IGFBP3	Insulin-like growth factor binding protein 3
D03	Hs.313	NM_000582	SPP1	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)
D04	Hs.496240	NM_000044	AR	Androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)
D05	Hs.591159	NM_002820	PTHLH	Parathyroid hormone-like hormone
D06	Hs.112432	NM_000479	AMH	Anti-Mullerian hormone
D07	Hs.408061	NM_001444	FABP5	Fatty acid binding protein 5 (psoriasis-associated)
D08	Hs.162646	NM_015869	PPARG	Peroxisome proliferator-activated receptor gamma
D09	Hs.301961	NM_000561	GSTM1	Glutathione S-transferase M1
D10	N/A	SA_00105	HGDC	Human Genomic DNA Contamination
D11	N/A	SA_00104	RTC	Reverse Transcription Control
D12	N/A	SA_00103	PPC	Positive PCR Control

Analysis of 4 genes comparing treated to untreated

FIGURE 1A

Untreated



Treated

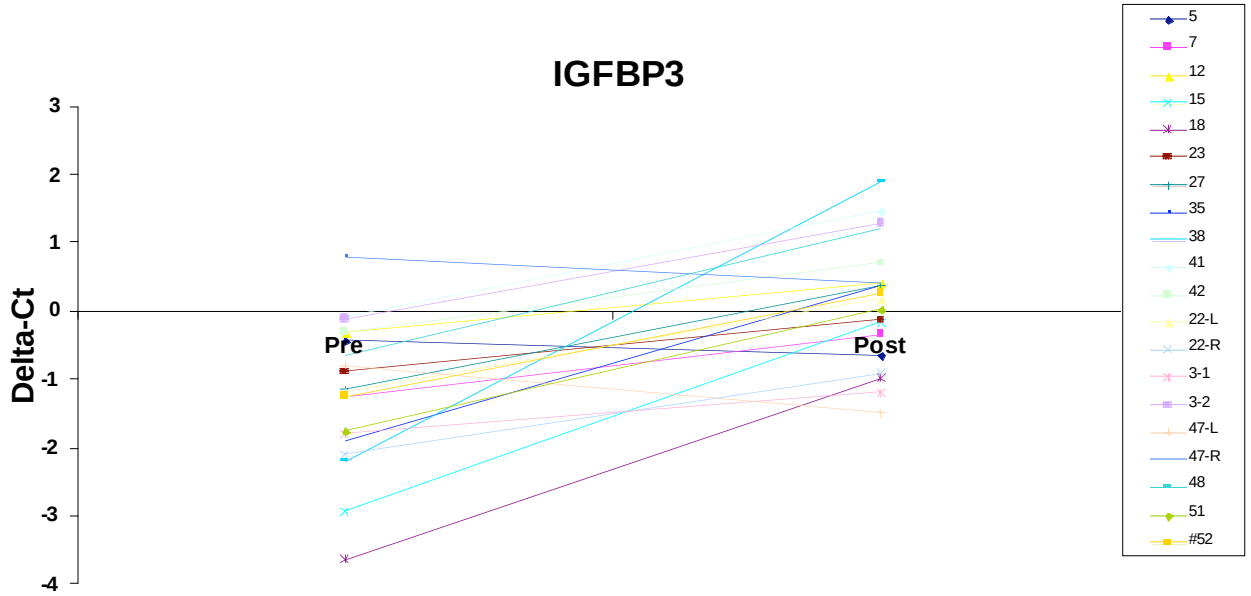
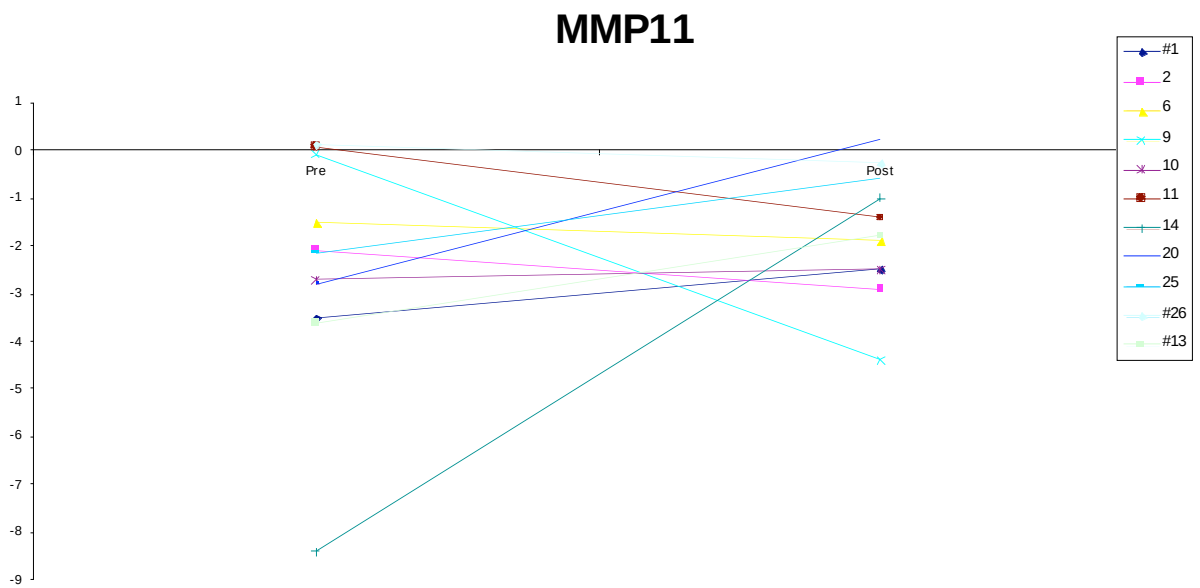
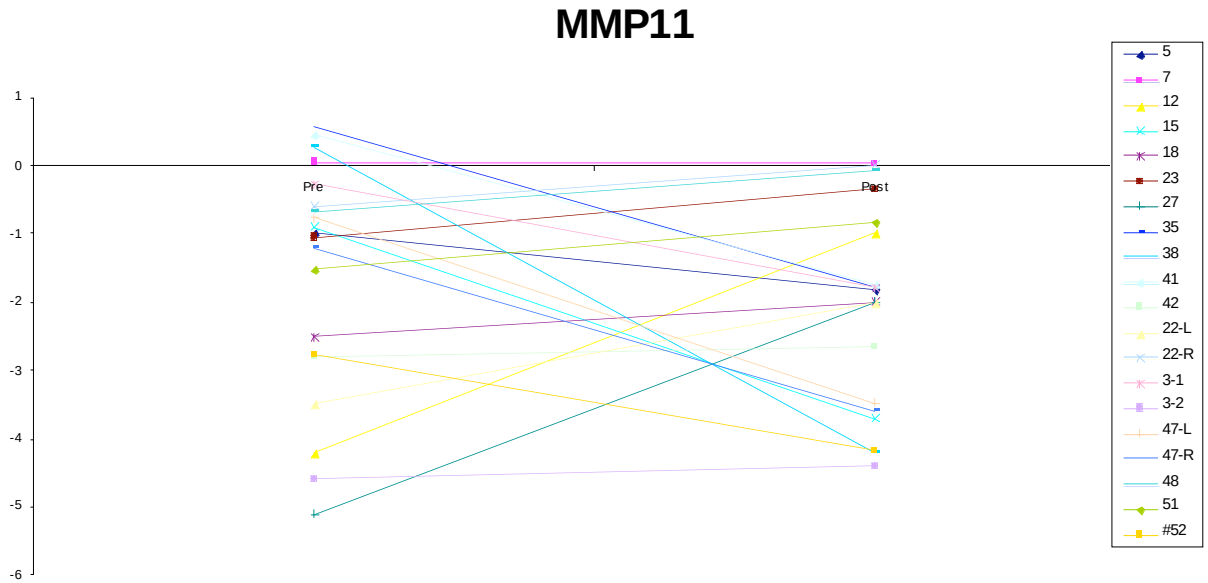


FIGURE 1B

Untreated



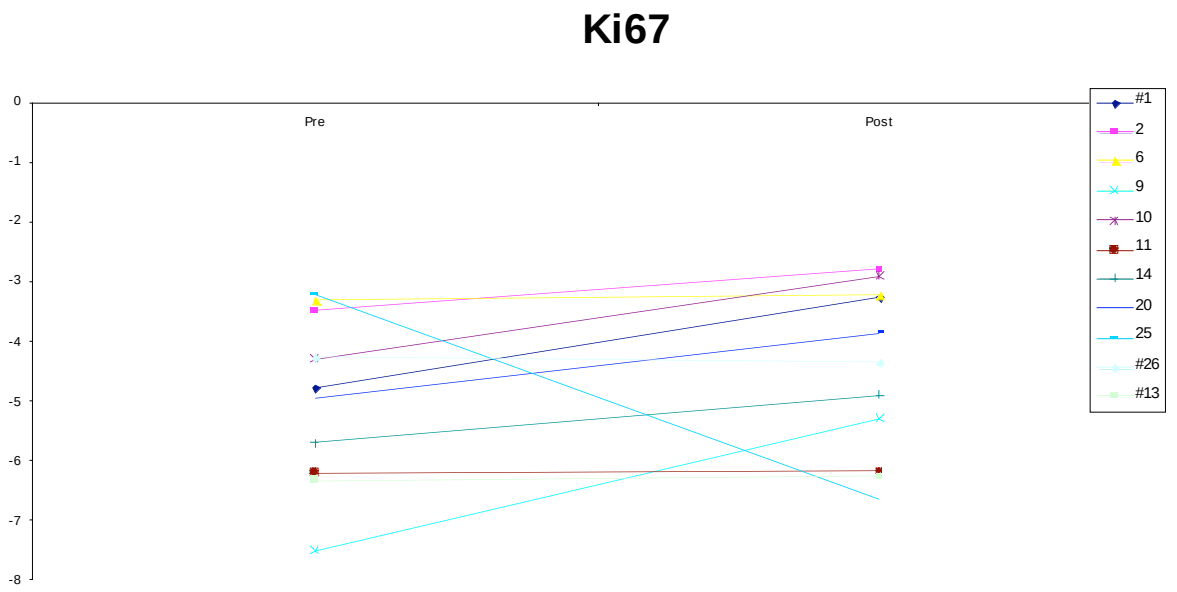
Treated



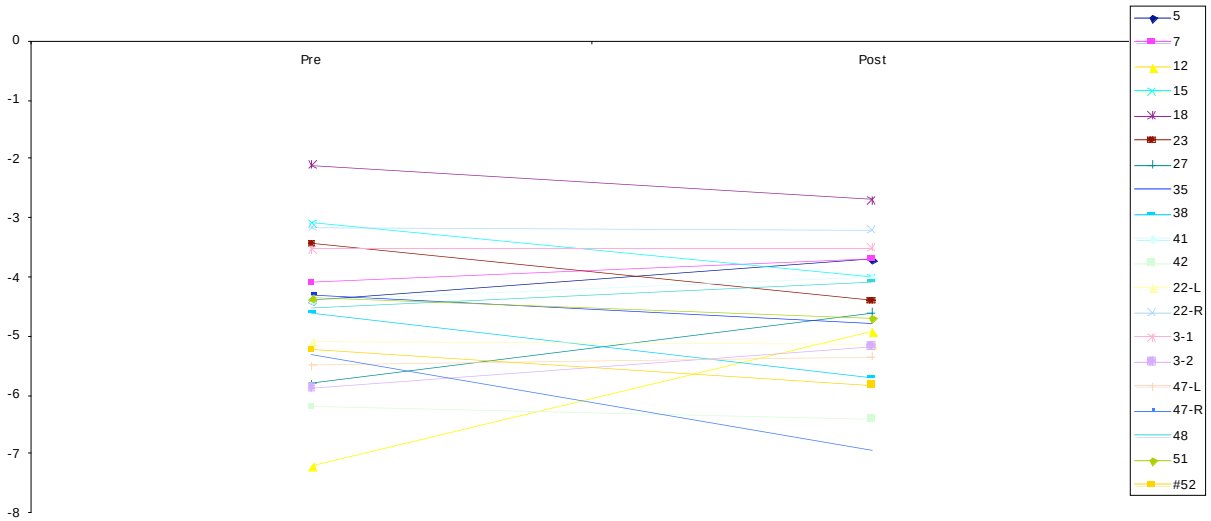
Analysis of 4 genes comparing treated to untreated

FIGURE 1C

Untreated



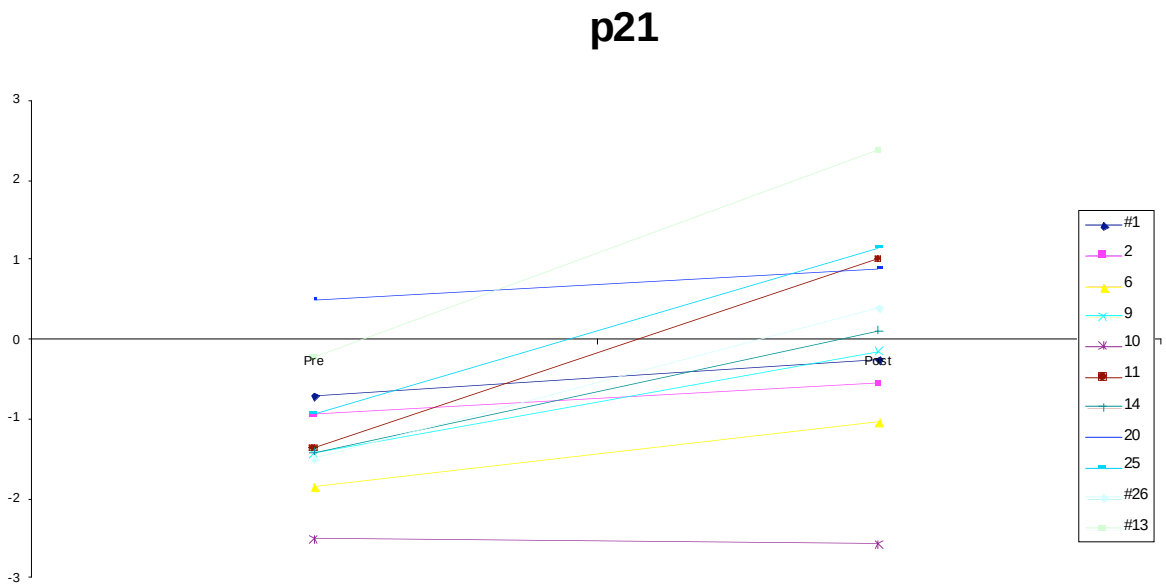
Treated



Analysis of 4 genes comparing treated to untreated

FIGURE 1D

Untreated



Treated

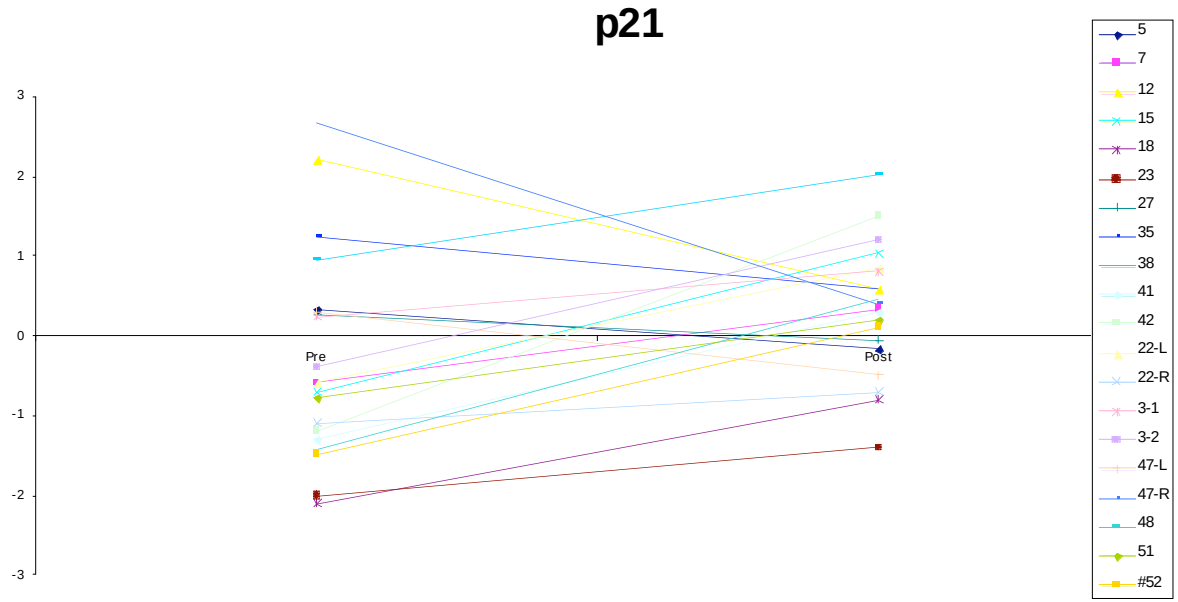
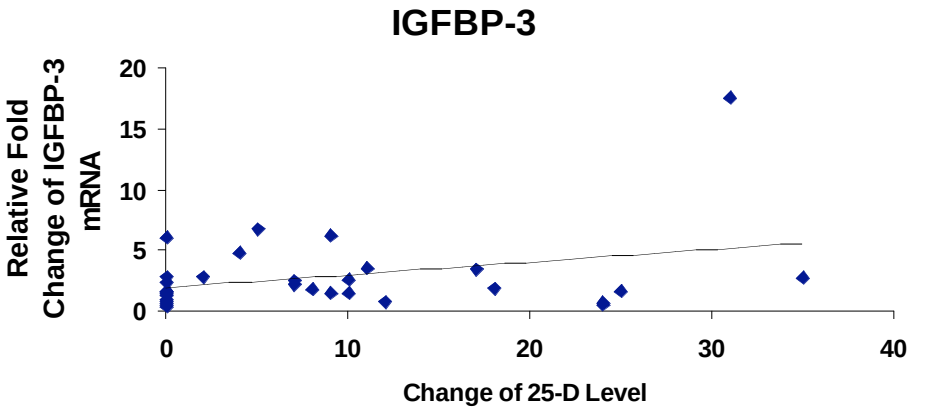
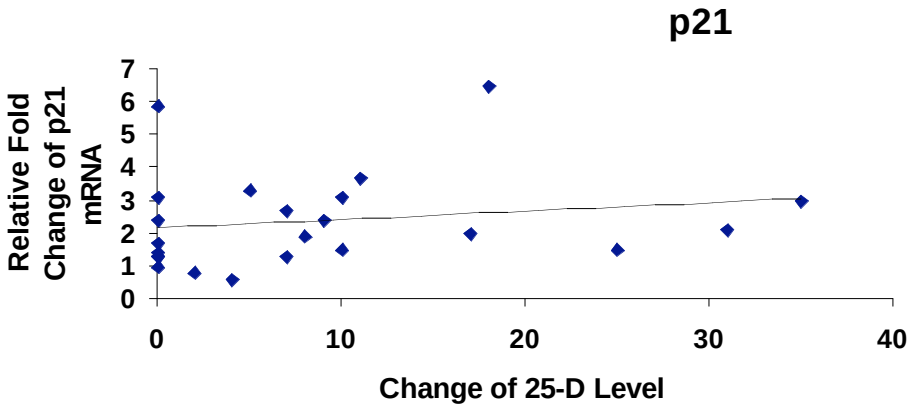
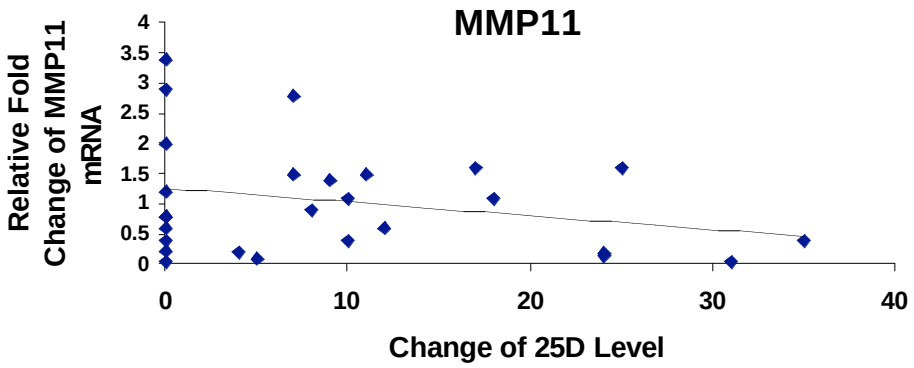
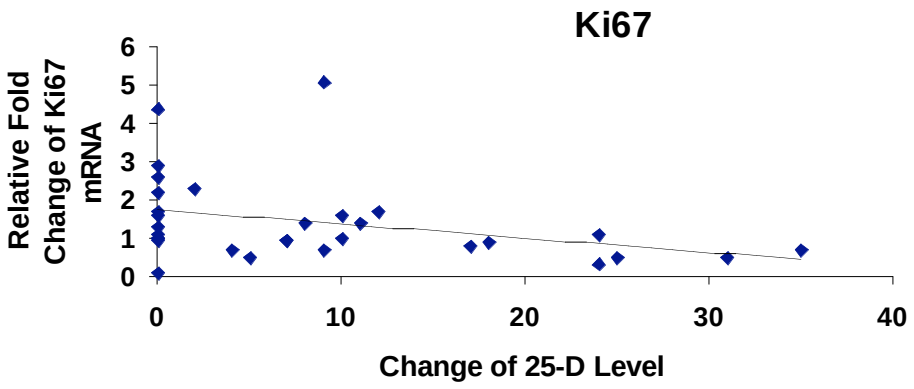


FIGURE 2 Analysis of the same 4 genes by achieved 25(OH)D levels





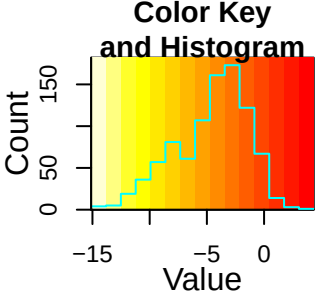


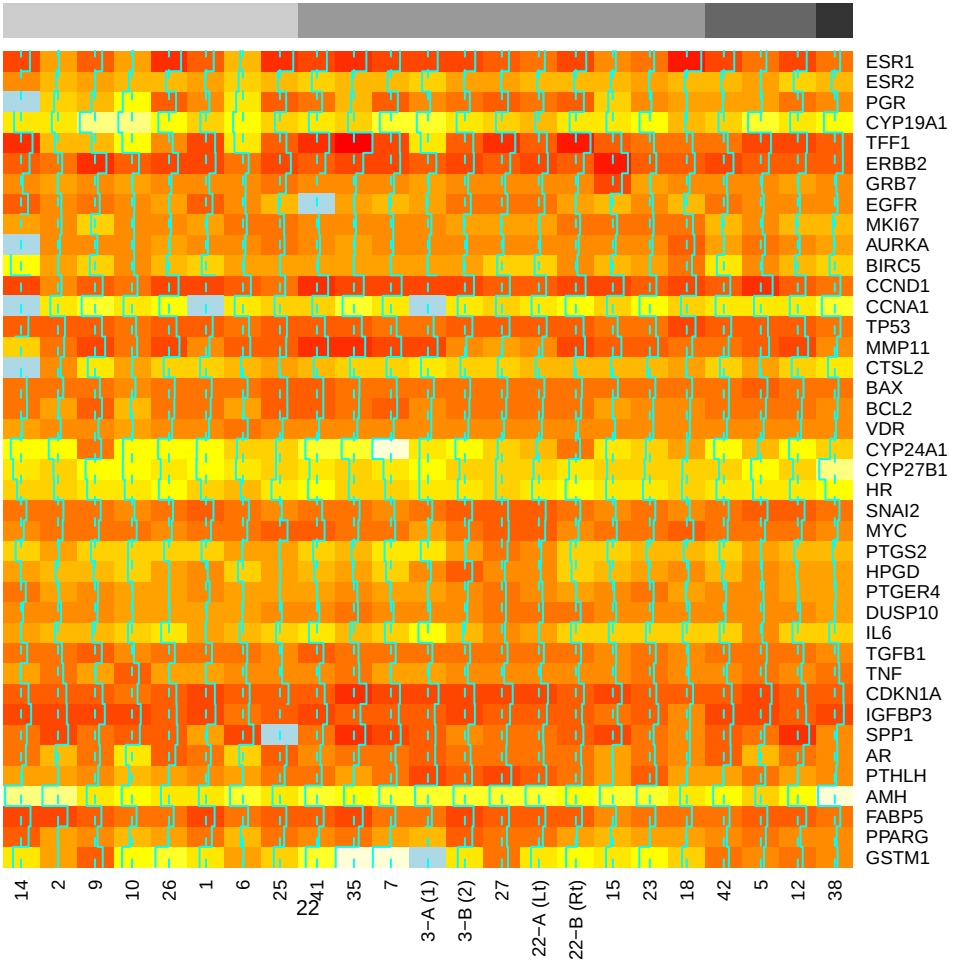


Heat Maps of all of the genes

Biopsy

FIGURE 3A





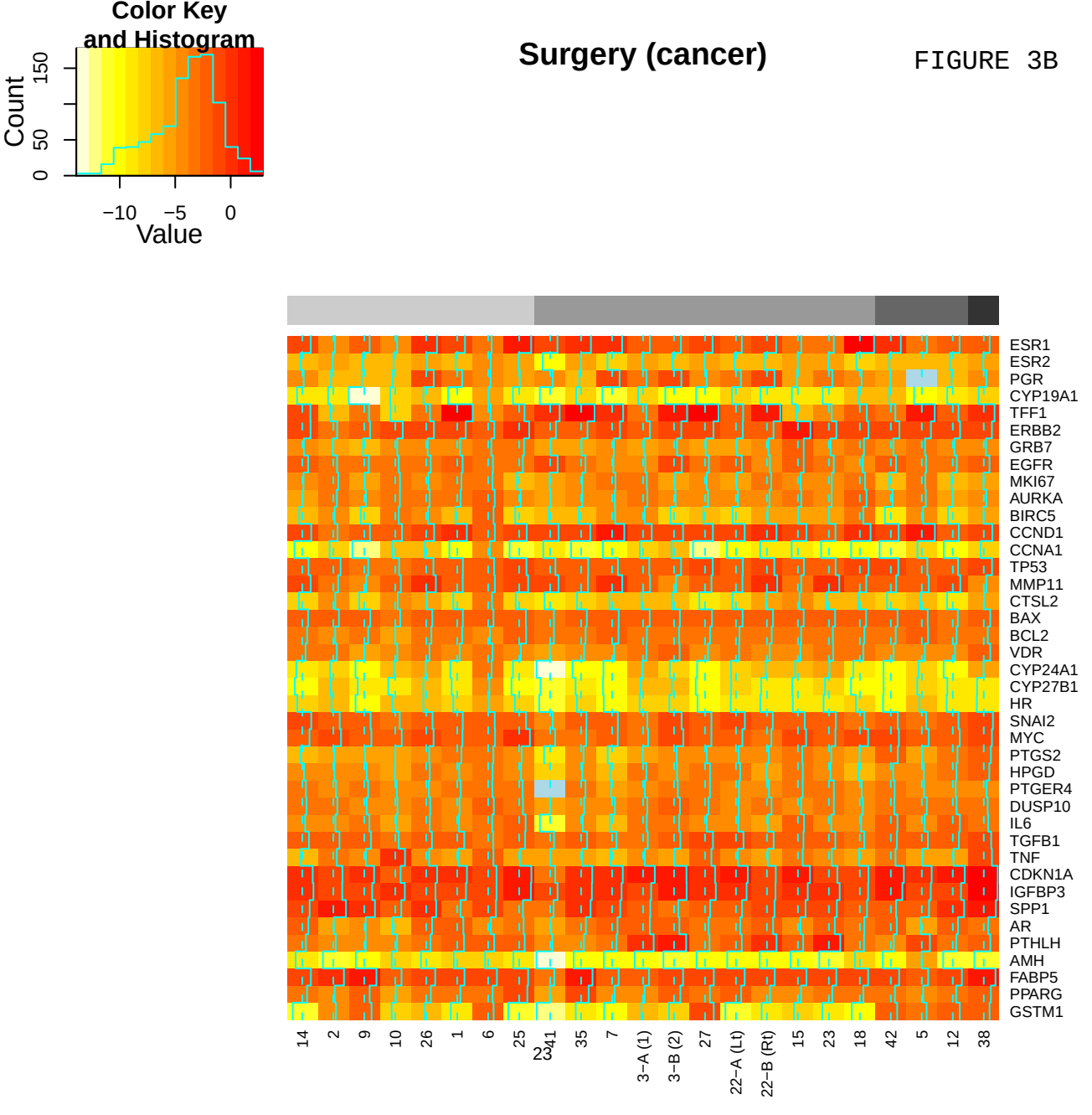

Color Key and Histogram
Count
0
50
150
−10
−5
0
Value
Surgery (cancer)
ESR1
ESR2
PGR
CYP19A1
TFF1
ERBB2
GRB7
EGFR
MKI67
AURKA
BIRC5
CCND1
CCNA1
TP53
MMP11
CTSL2
BAX
BCL2
VDR
CYP24A1
CYP27B1
HR
SNAI2
MYC
PTGS2
HPGD
PTGER4
DUSP10
IL6
TGFB1
TNF
CDKN1A
IGFBP3
SPP1
AR
PTHLH
AMH
FABP5
PPARG
GSTM1
14
2
9
10
26
1
6
25
23
41
35
7
3−A (1)
3−B (2)
27
22−A (Lt)
22−B (Rt)
15
23
18
42
5
12
38

FIGURE 3B

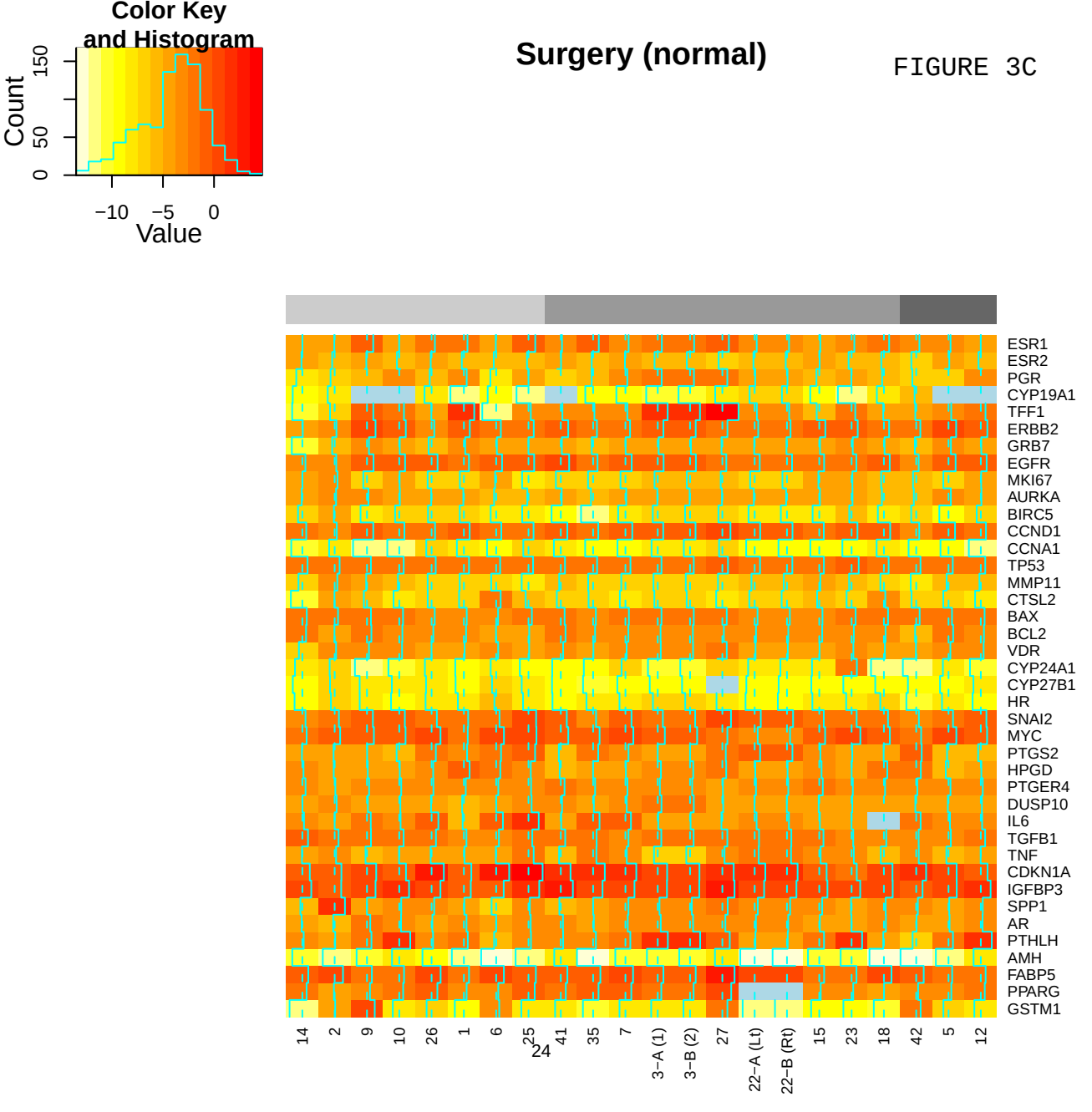
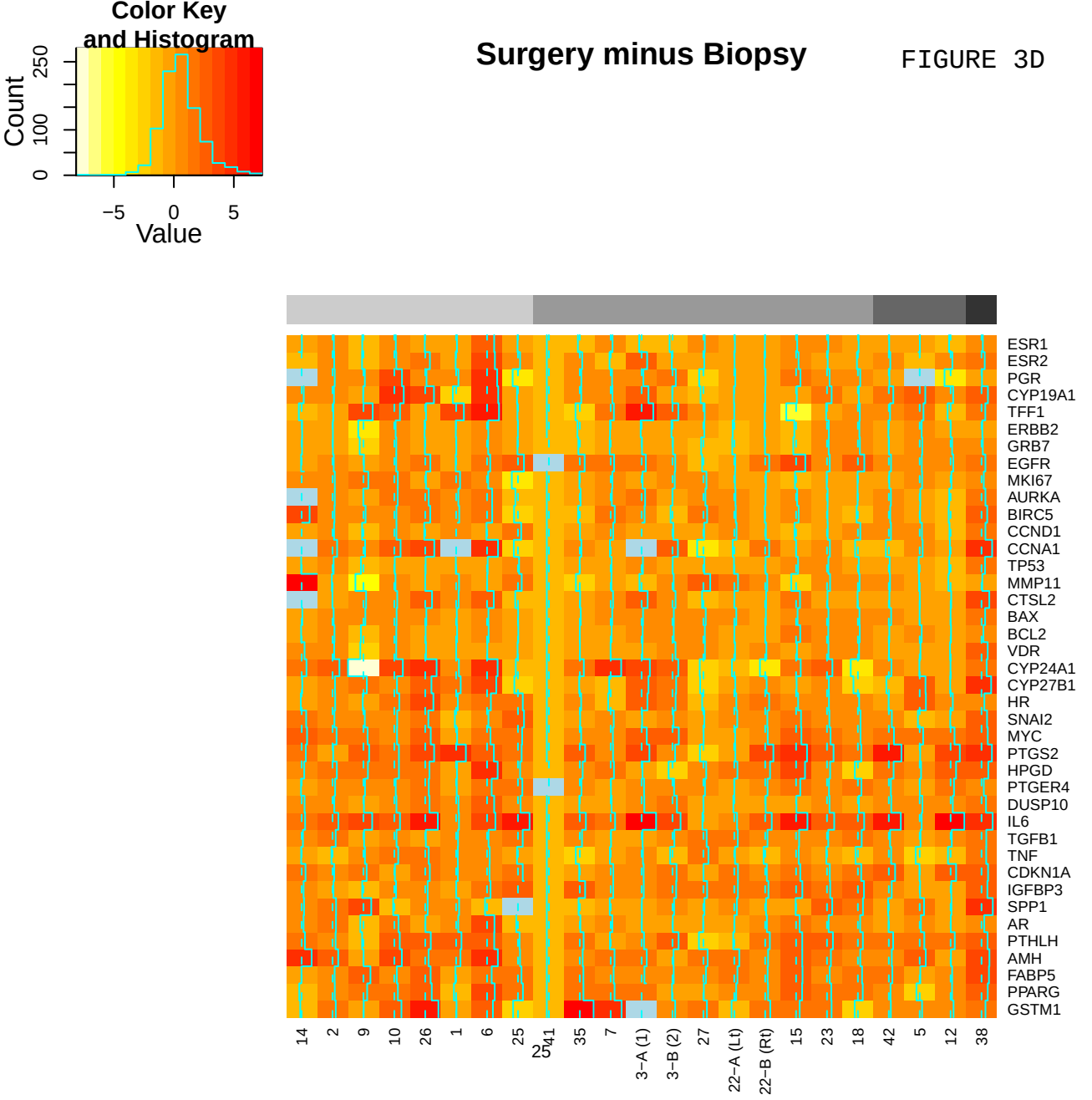
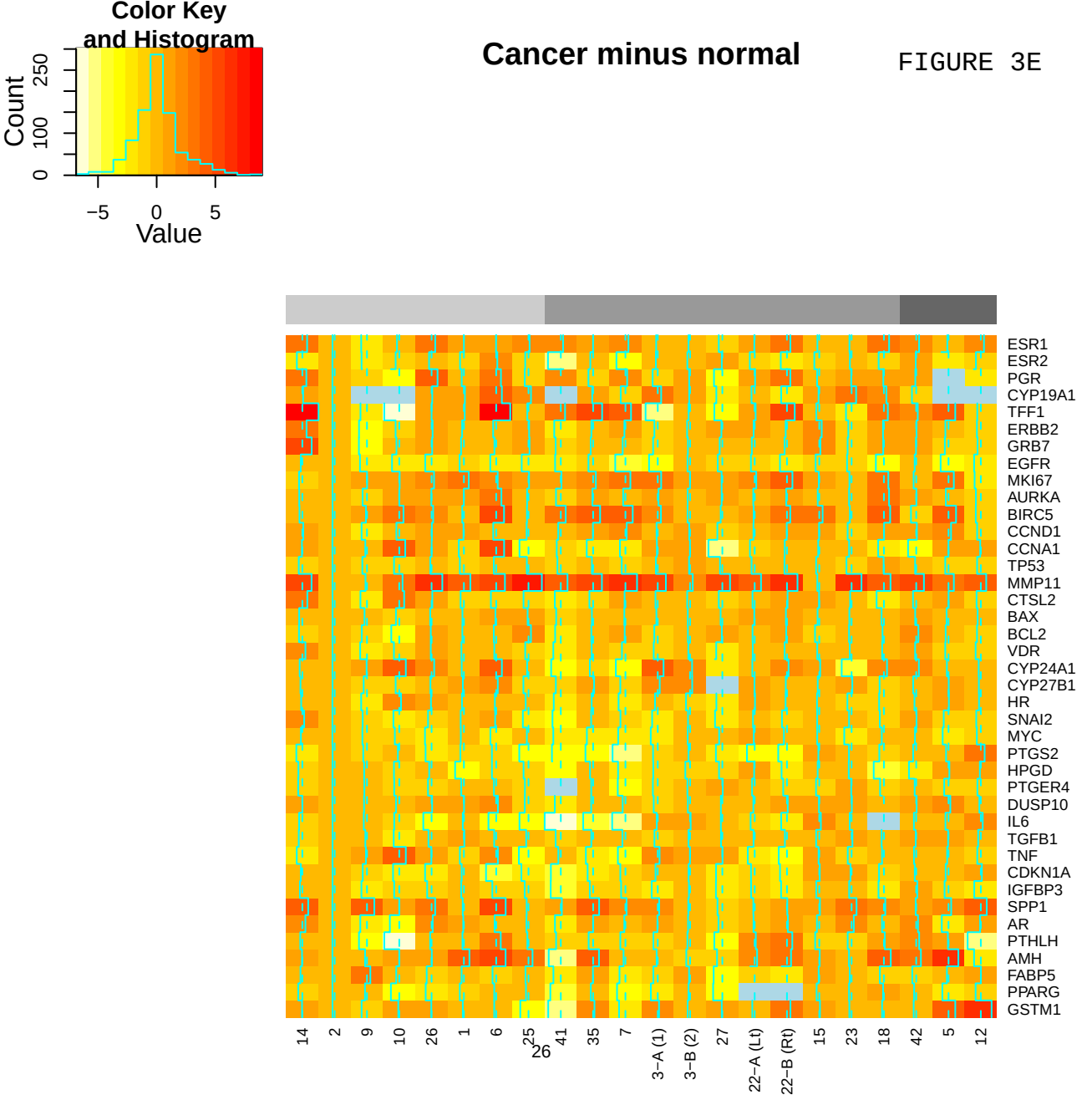

Color Key
and Histogram
Count
150
50
0
−10
−5
0
Value
Surgery (normal)
ESR1
ESR2
PGR
CYP19A1
TFF1
ERBB2
GRB7
EGFR
MKI67
AURKA
BIRC5
CCND1
CCNA1
TP53
MMP11
CTSL2
BAX
BCL2
VDR
CYP24A1
CYP27B1
HR
SNAI2
MYC
PTGS2
HPGD
PTGER4
DUSP10
IL6
TGFB1
TNF
CDKN1A
IGFBP3
SPP1
AR
PTHLH
AMH
FABP5
PPARG
GSTM1
14
2
9
10
26
1
6
25
24
41
35
7
3−A (1)
3−B (2)
27
22−A (Lt)
22−B (Rt)
15
23
18
42
5
12

FIGURE 3C


Color Key
and Histogram
Count
250
100
0
−5
0
5
Value
Surgery minus Biopsy
FIGURE 3D
ESR1
ESR2
PGR
CYP19A1
TFF1
ERBB2
GRB7
EGFR
MKI67
AURKA
BIRC5
CCND1
CCNA1
TP53
MMP11
CTSL2
BAX
BCL2
VDR
CYP24A1
CYP27B1
HR
SNAI2
MYC
PTGS2
HPGD
PTGER4
DUSP10
IL6
TGFB1
TNF
CDKN1A
IGFBP3
SPP1
AR
PTHLH
AMH
FABP5
PPARG
GSTM1
14
2
9
10
26
1
6
25
41
35
7
3−A (1)
3−B (2)
27
22−A (Lt)
22−B (Rt)
15
23
18
42
5
12
38


Color Key
and Histogram
Count
0
100
250
−5
0
5
Value
Cancer minus normal
FIGURE 3E
ESR1
ESR2
PGR
CYP19A1
TFF1
ERBB2
GRB7
EGFR
MKI67
AURKA
BIRC5
CCND1
CCNA1
TP53
MMP11
CTSL2
BAX
BCL2
VDR
CYP24A1
CYP27B1
HR
SNAI2
MYC
PTGS2
HPGD
PTGER4
DUSP10
IL6
TGFB1
TNF
CDKN1A
IGFBP3
SPP1
AR
PTHLH
AMH
FABP5
PPARG
GSTM1
14
2
9
10
26
1
6
25
41
35
7
3−A (1)
3−B (2)
27
22−A (Lt)
22−B (Rt)
15
23
18
42
5
12
26