FIGURE 1

p95.D series

Hit	Her2-D pep	Her2-hFc	Her2-A pep
D1	0.837	0.563	1.553
D3	2.306	0.089	0.134
D4	3.941	1.683	0.107
D5	3.417	0.094	0.108
D6	0.902	1.720	0.086
D7	2.087	0.115	0.093
D8	3.701	0.219	0.084
D9	3.460	0.311	0.084
D10	3.120	0.087	0.094
D11	3.392	0.103	0.081
D12	3.871	4.000	0.092
D13	1.189	0.092	0.096
Her2 Ab5		3.929	
A3			3.130

FIGURE 2

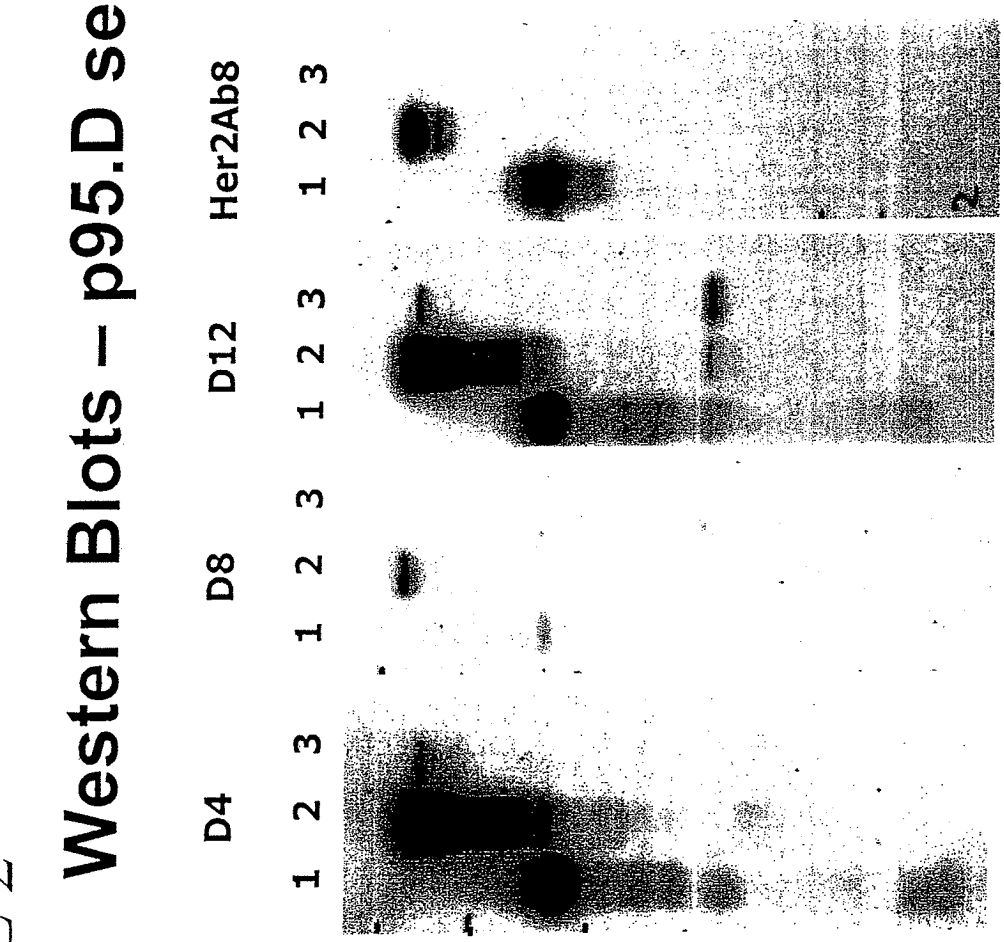

Western Blots – p95.D series
Antibody:
D4
D8
D12
Her2Ab8
1 2 3 1 2 3 1 2 3 1 2 3
191 kDa
97 kDa
64 kDa
Lane 1- pcDNA 6myc/hisA M611p95 (0.1 ug/lane)
Lane 2- SKBr3 lysate (5 ug/lane)
Lane 3- 293T lysate (5 ug/lane)

FIGURE 3a p95.D series - native FACS stain

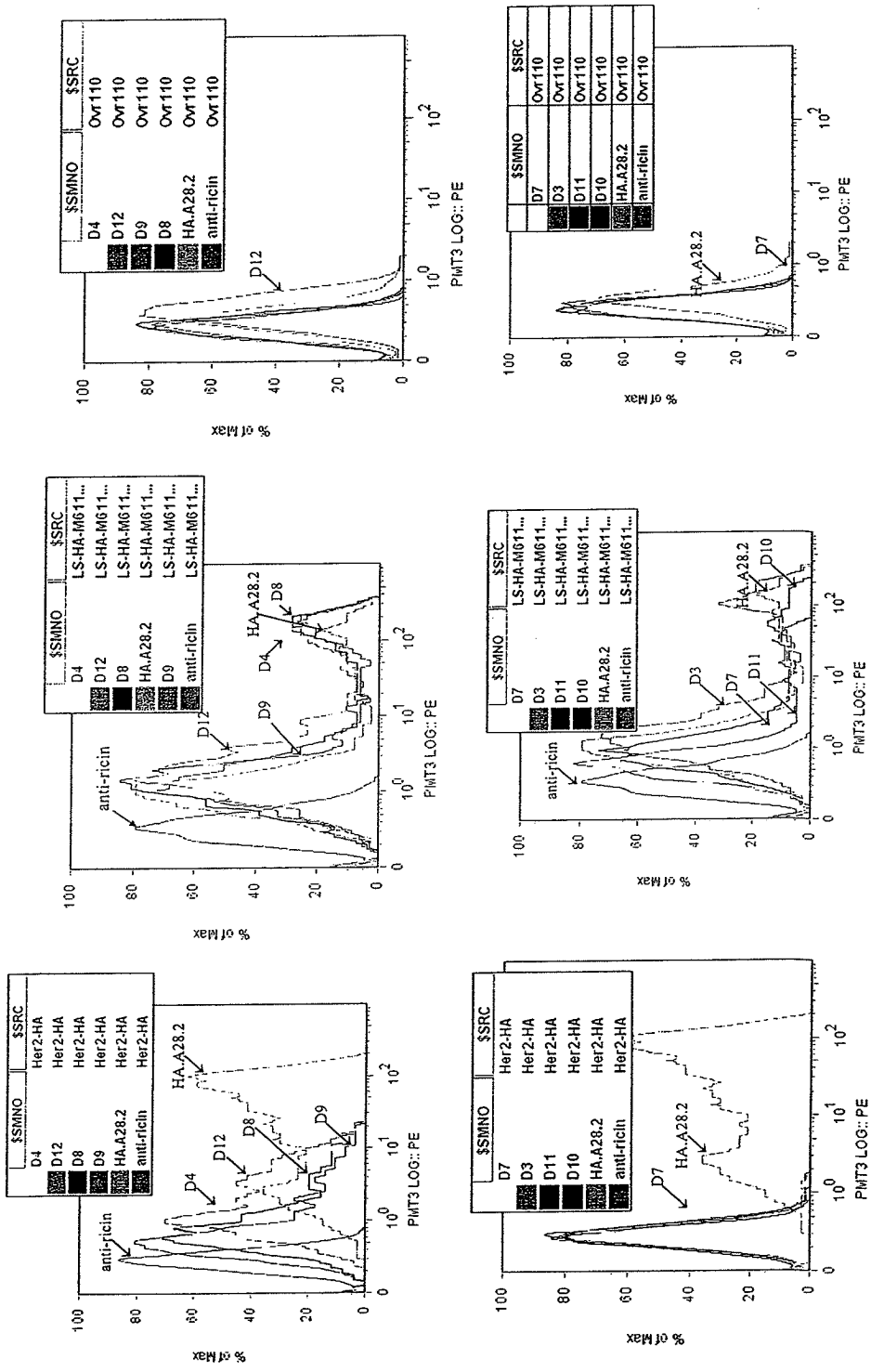


FIGURE 3b p95.D fixed / perm FACS stain

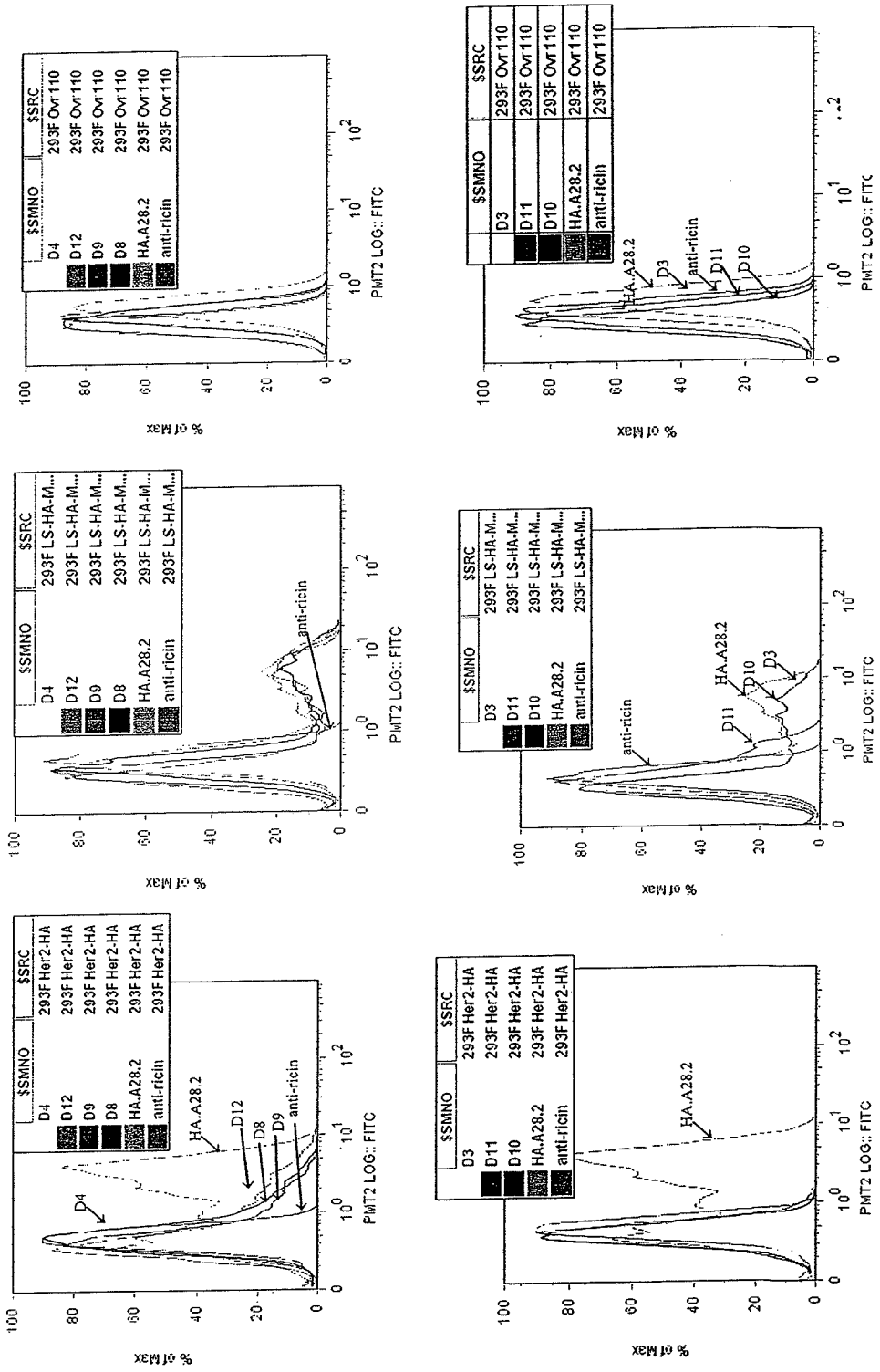


FIGURE 4

p95 and HER2 Expression

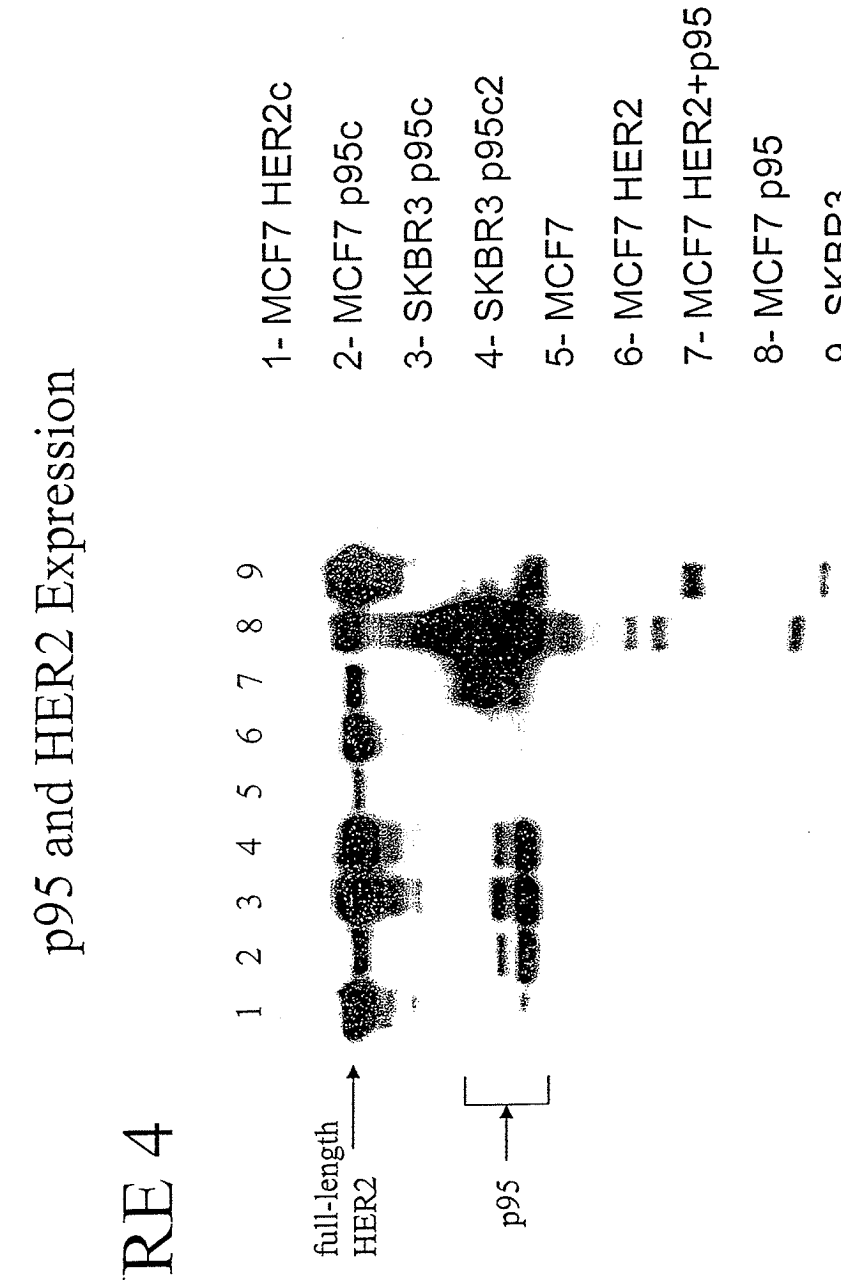


FIGURE 5a

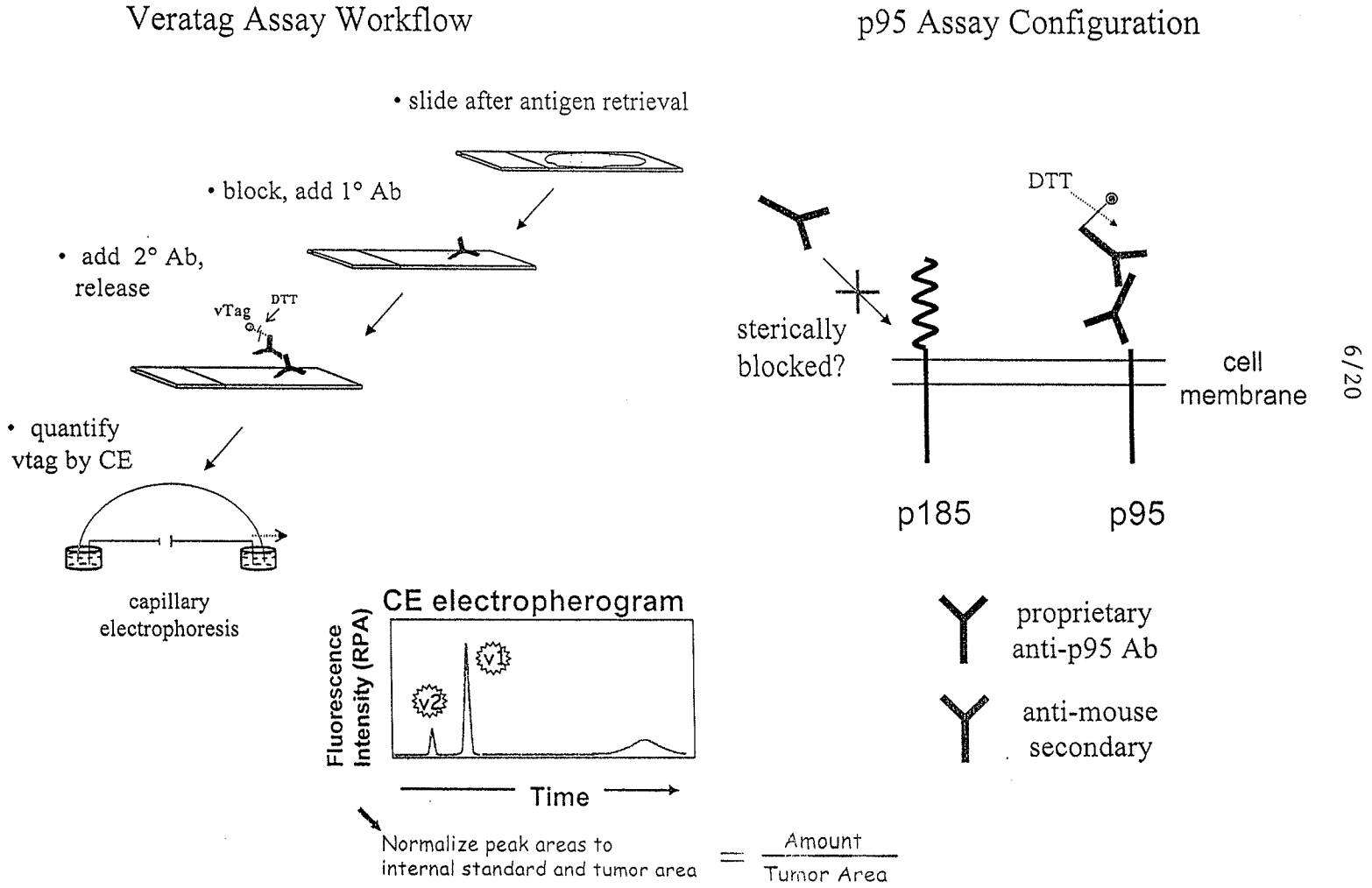
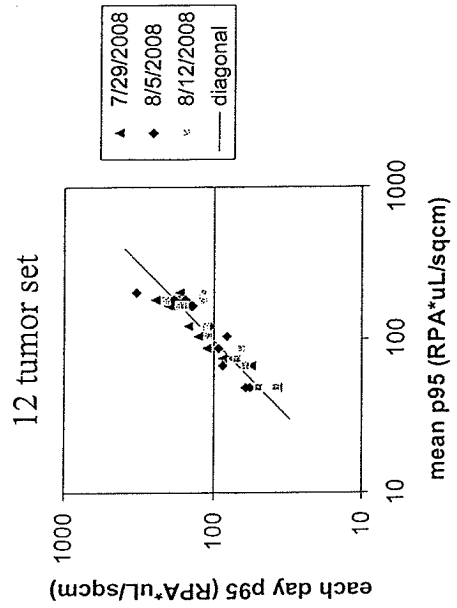
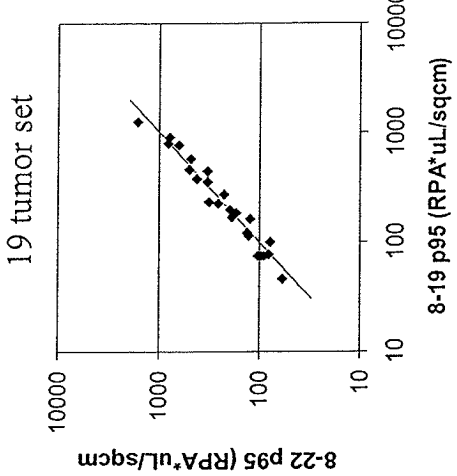

Veratag Assay Workflow
• slide after antigen retrieval
• block, add 1° Ab
• add 2° Ab, release
vTag
DTT
• quantify vtag by CE
capillary electrophoresis
CE electropherogram
Fluorescence Intensity (RPA)
V1
V2
Time
Normalize peak areas to internal standard and tumor area
= Amount / Tumor Area
p95 Assay Configuration
DTT
sterically blocked?
cell membrane
p185
p95
proprietary anti-p95 Ab
anti-mouse secondary

FIGURE 5b Tumor Reproducibility





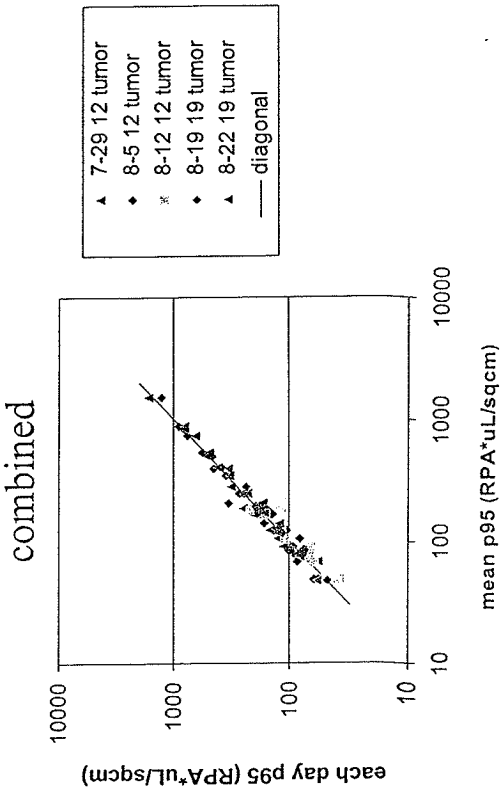
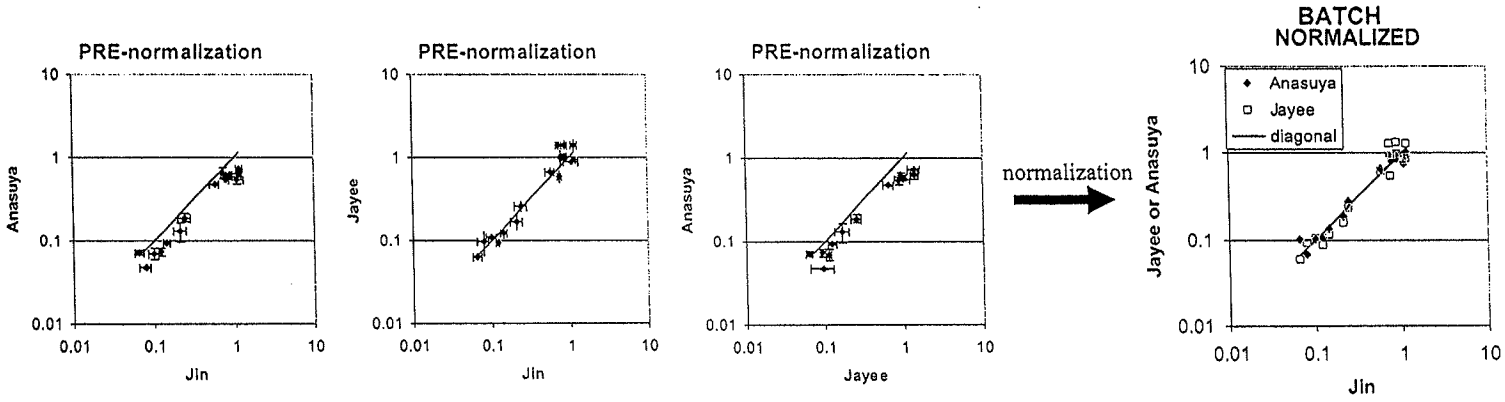


FIGURE 5c

Use of Standards to Achieve Consistency Over Time

- Batch to batch consistency achieved using normalization standards
- Here a range of p95 and ADAM10 transfectants were tested representing a range of p95

3 two-way comparisons between operators:



CV's <30% for a range of batches/operators over entire dynamic range

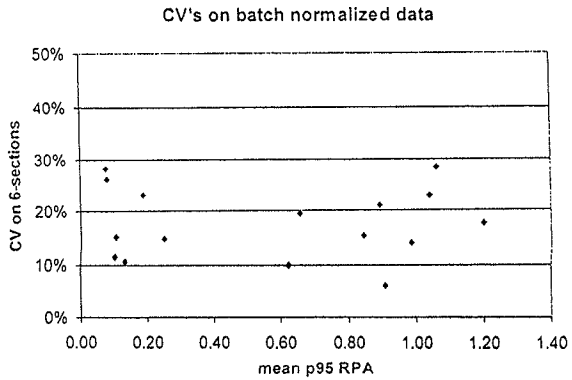


FIGURE 6

Screening of Purified Antibodies

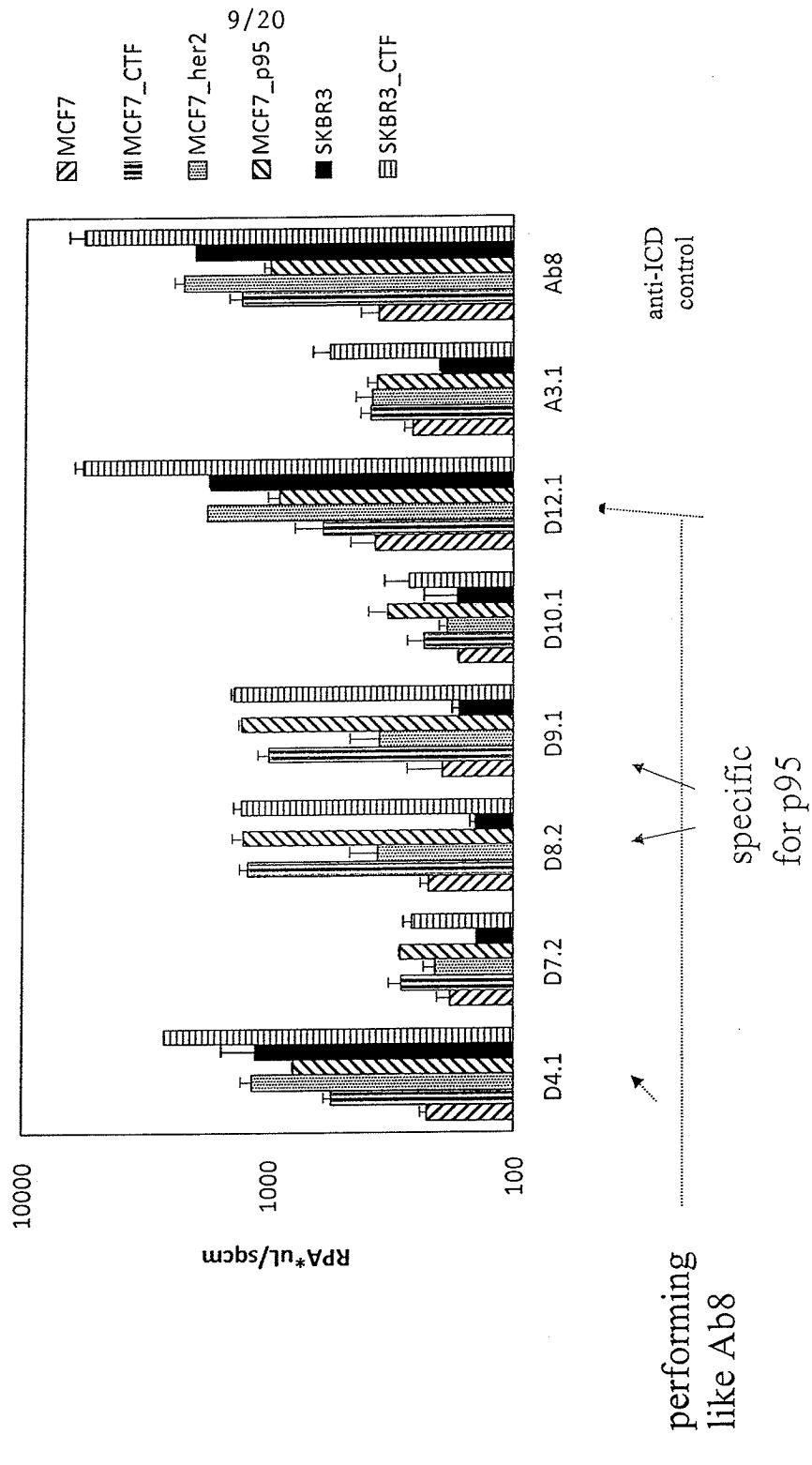


FIGURE 7a-c

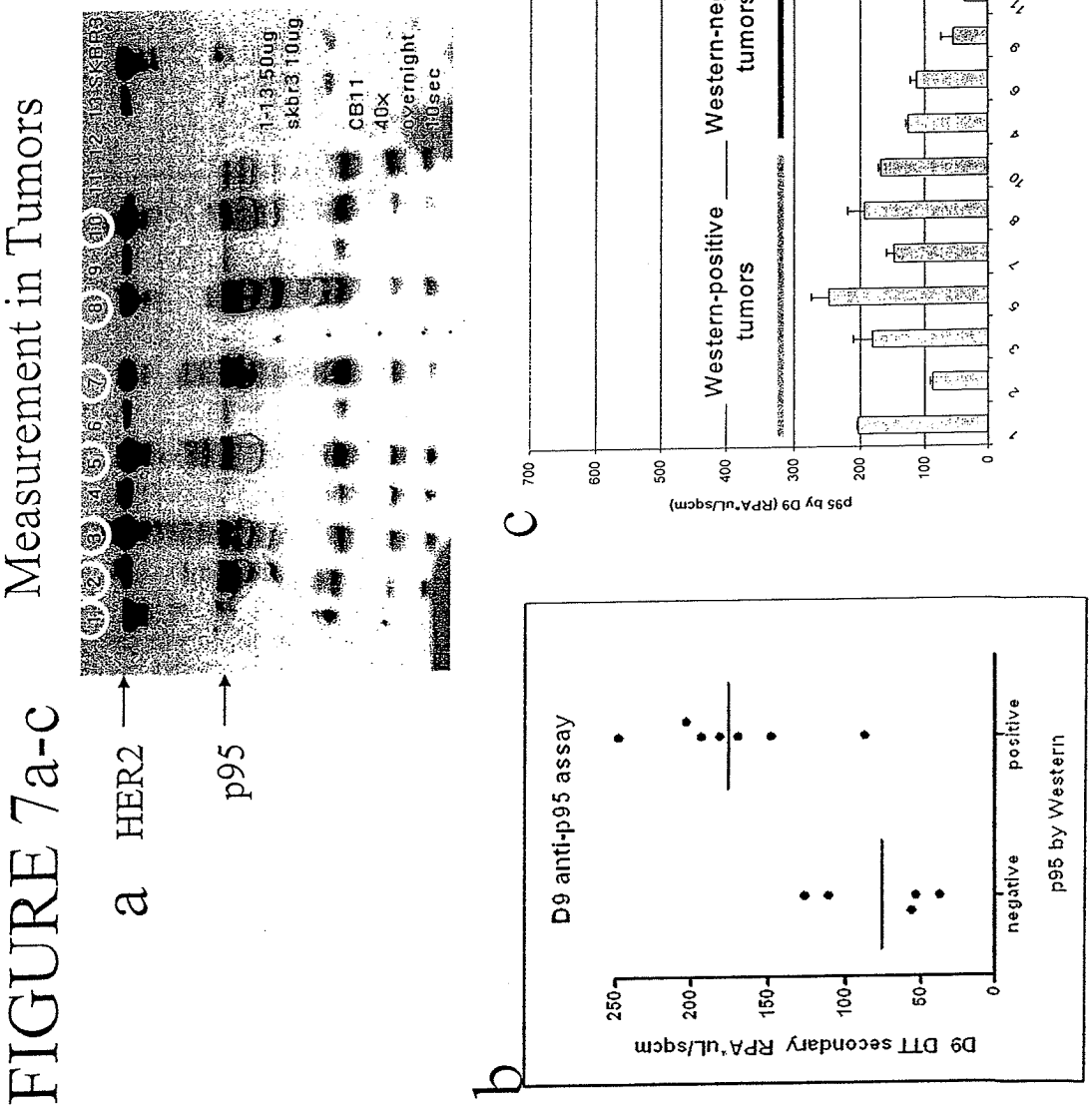

a
HER2
p95
1-13 50ug
skbr3 100g
CB11
40x
overnight
10sec
b
D9 anti-p95 assay
D9 DTT secondary RPA*uL/sqcm
negative
positive
p95 by Western
c
p95 by D9 (RPA*uL/sqcm)
Western-positive tumors
Western-negative tumors
MCF7
MCF7_p95
MCF7_CTF
MCF7_HER2
SKBR3
SKBR3_CTF

FIGURE 8a-c

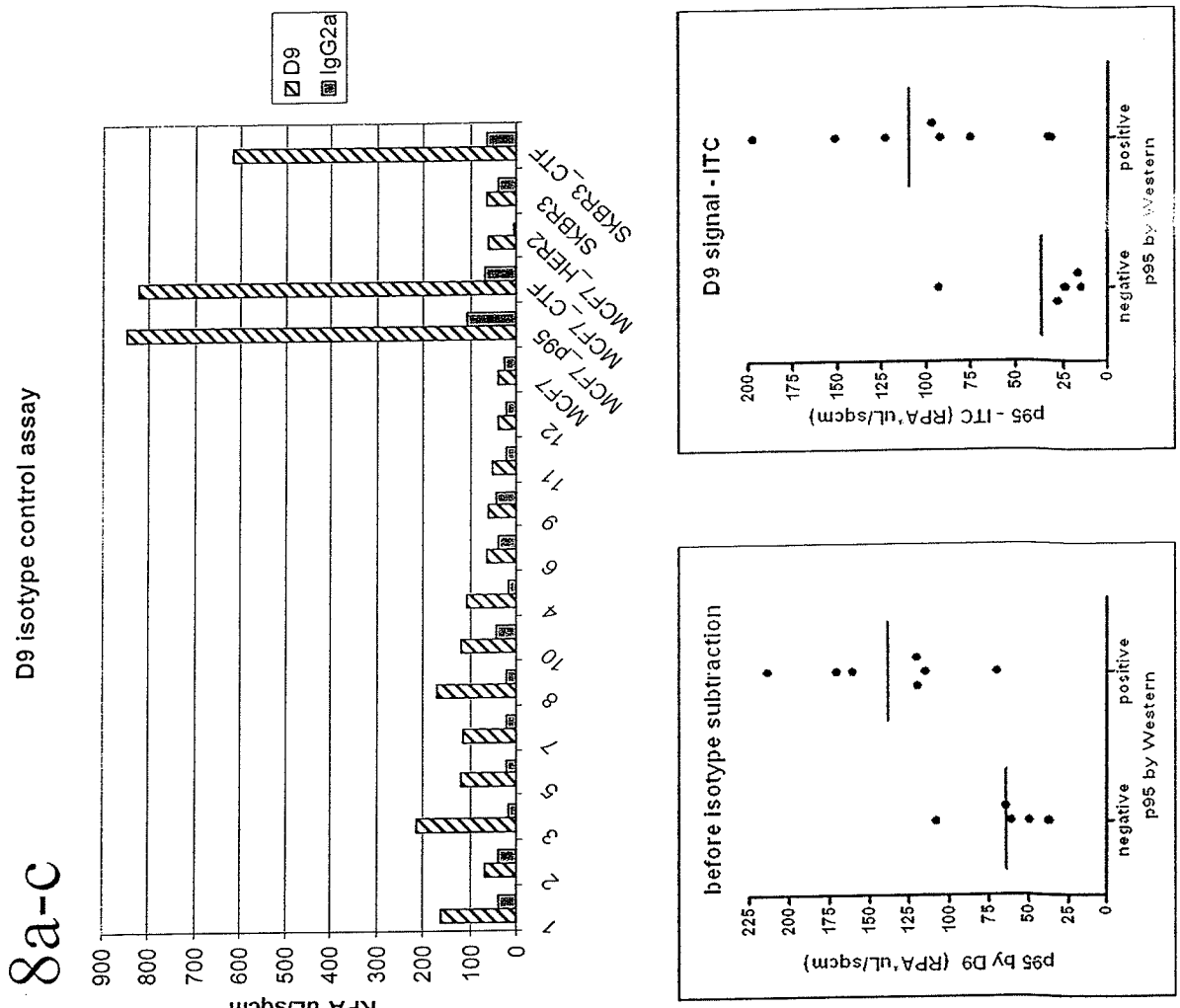

D9 isotype control assay
D9
IgG2a
RPA*uL/sqcm
0
100
200
300
400
500
600
700
800
900
1
2
3
5
7
8
10
4
6
9
11
12
MCF7
MCF7_p95
MCF7_CTF
MCF7_HER2
SKBR3
SKBR3_CTF
before isotype subtraction
p95 by D9 (RPA*uL/sqcm)
negative
positive
p95 by Western
D9 signal - ITC
p95 - ITC (RPA*uL/sqcm)
negative
positive
p95 by Western

FIGURE 9

Ultra-High H2T Enriched in p95+ ?

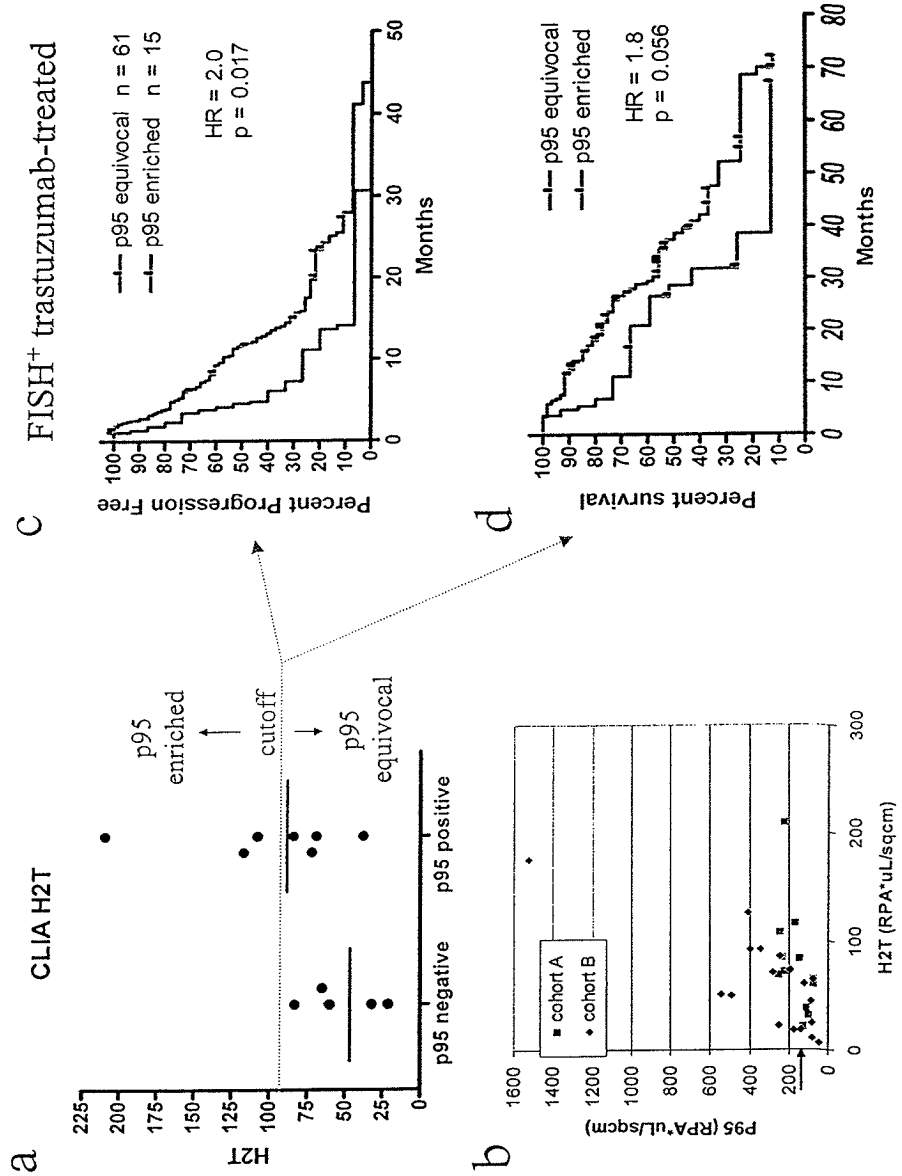


FIGURE 10a

IHC with anti-p95 clone D9.1 in cell lines

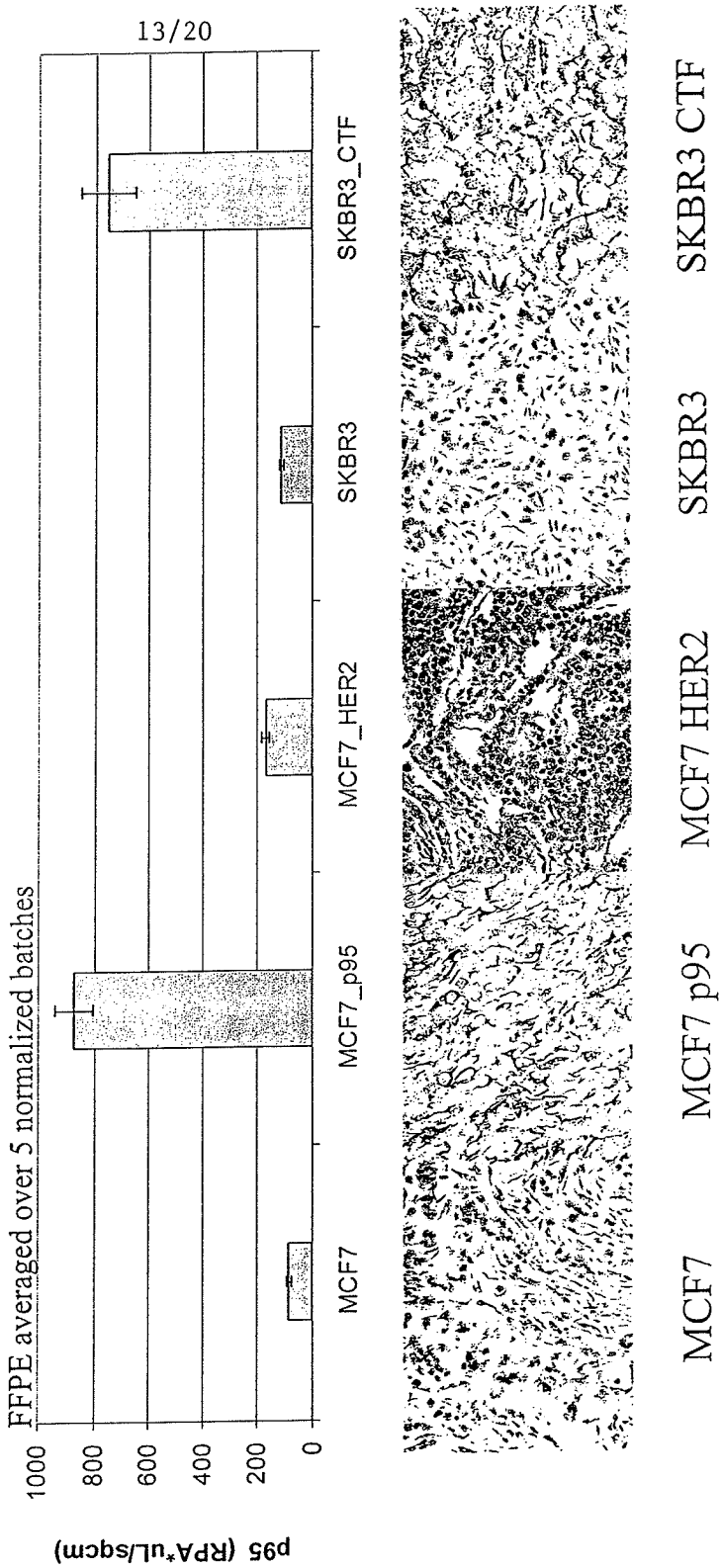
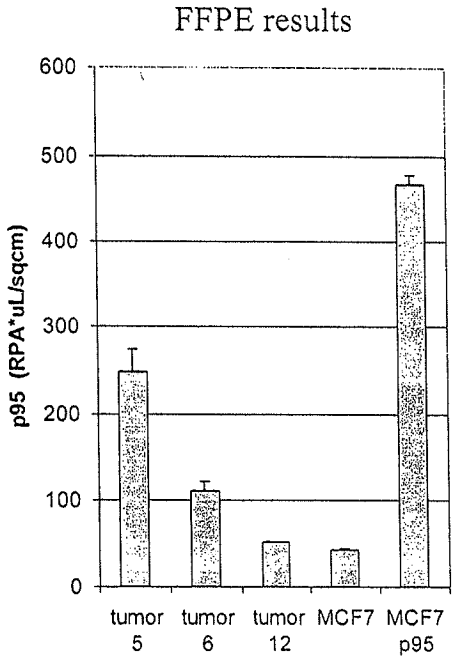
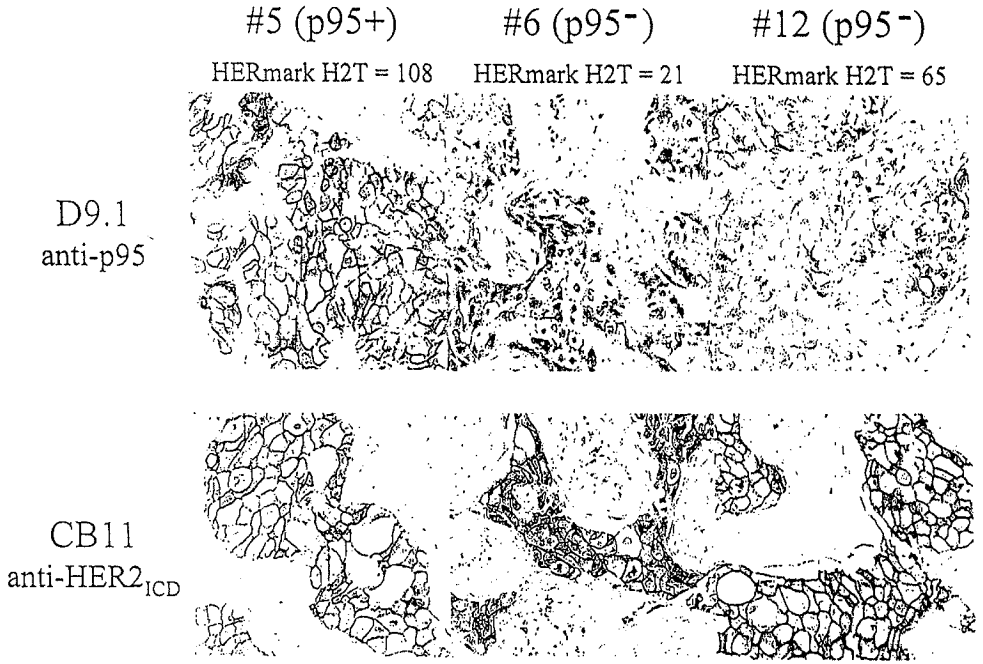


FIGURE 10b

IHC with anti-p95 clone D9.1 in tumors



Strongest p95 FFPE signal (#5) compared against two HER2-positive but p95-low or negative



images from the 12 tumor set

FIGURE 11

Screening of vTag-Labeled Antibodies

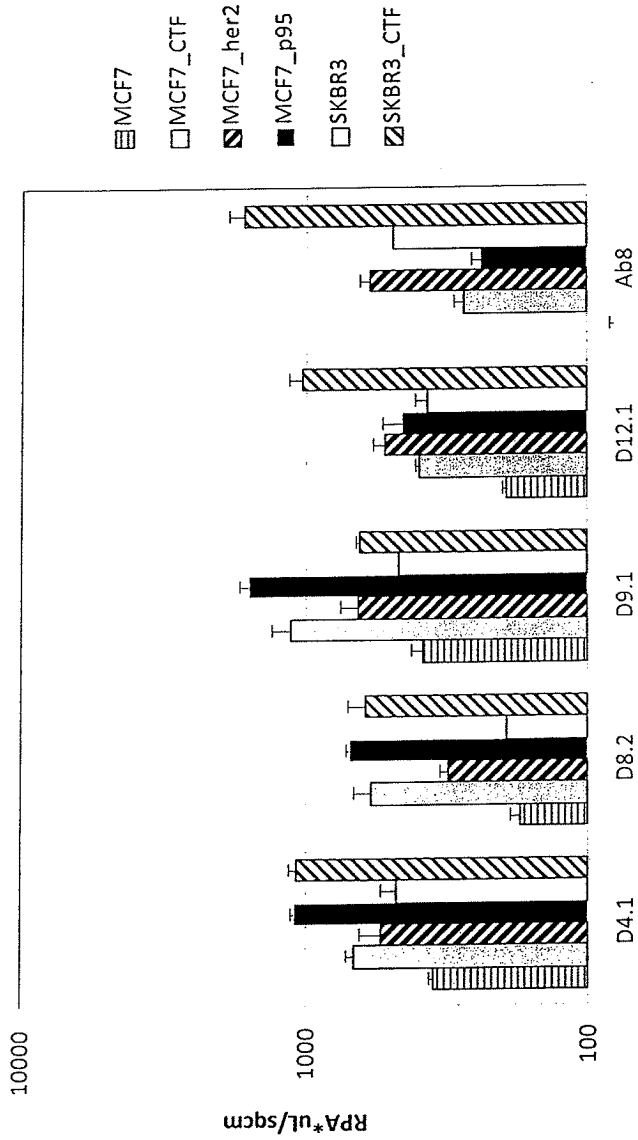


FIGURE 12

Measurement in Tumors

Cell Line Standards

Tumors

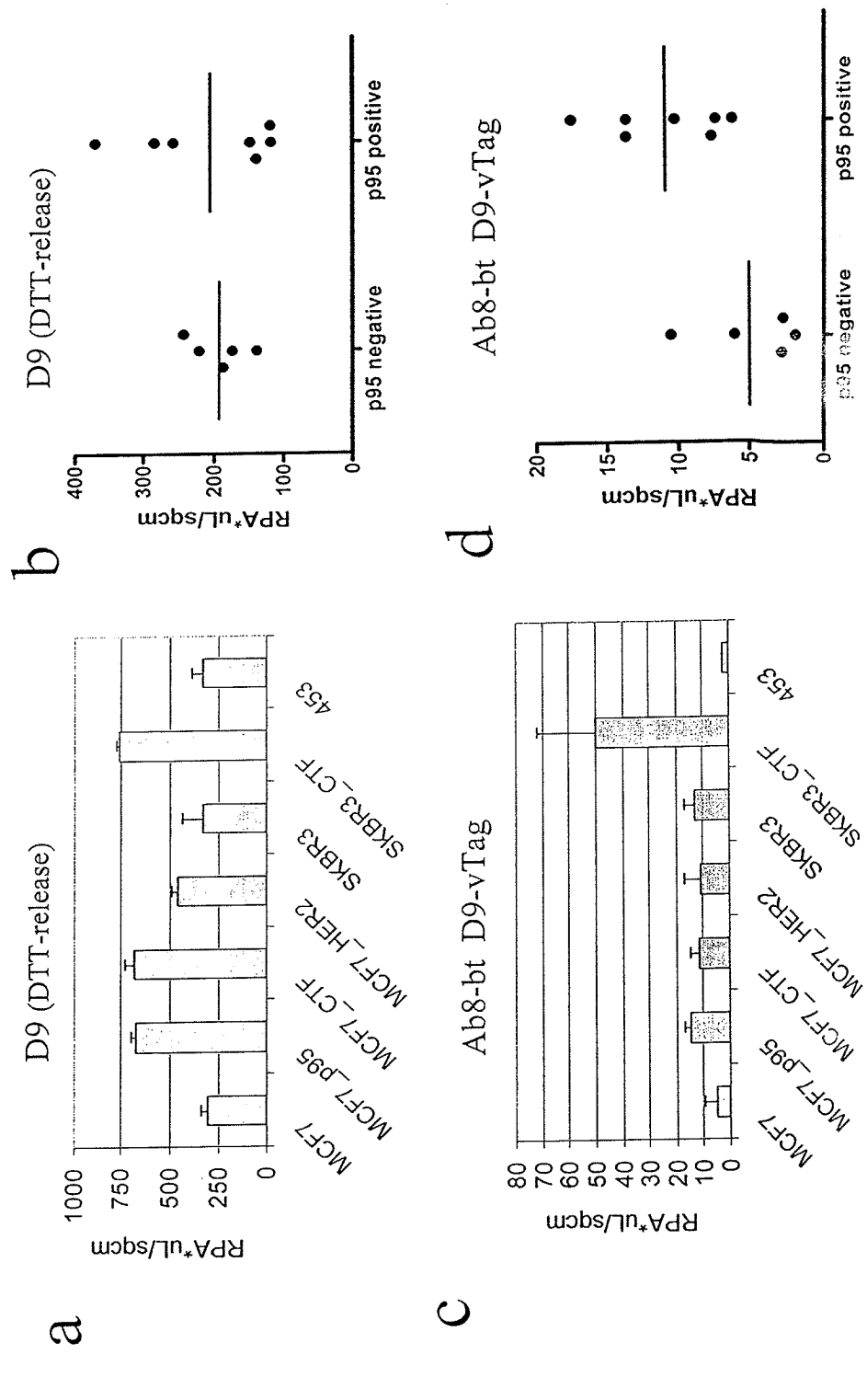


FIGURE 13

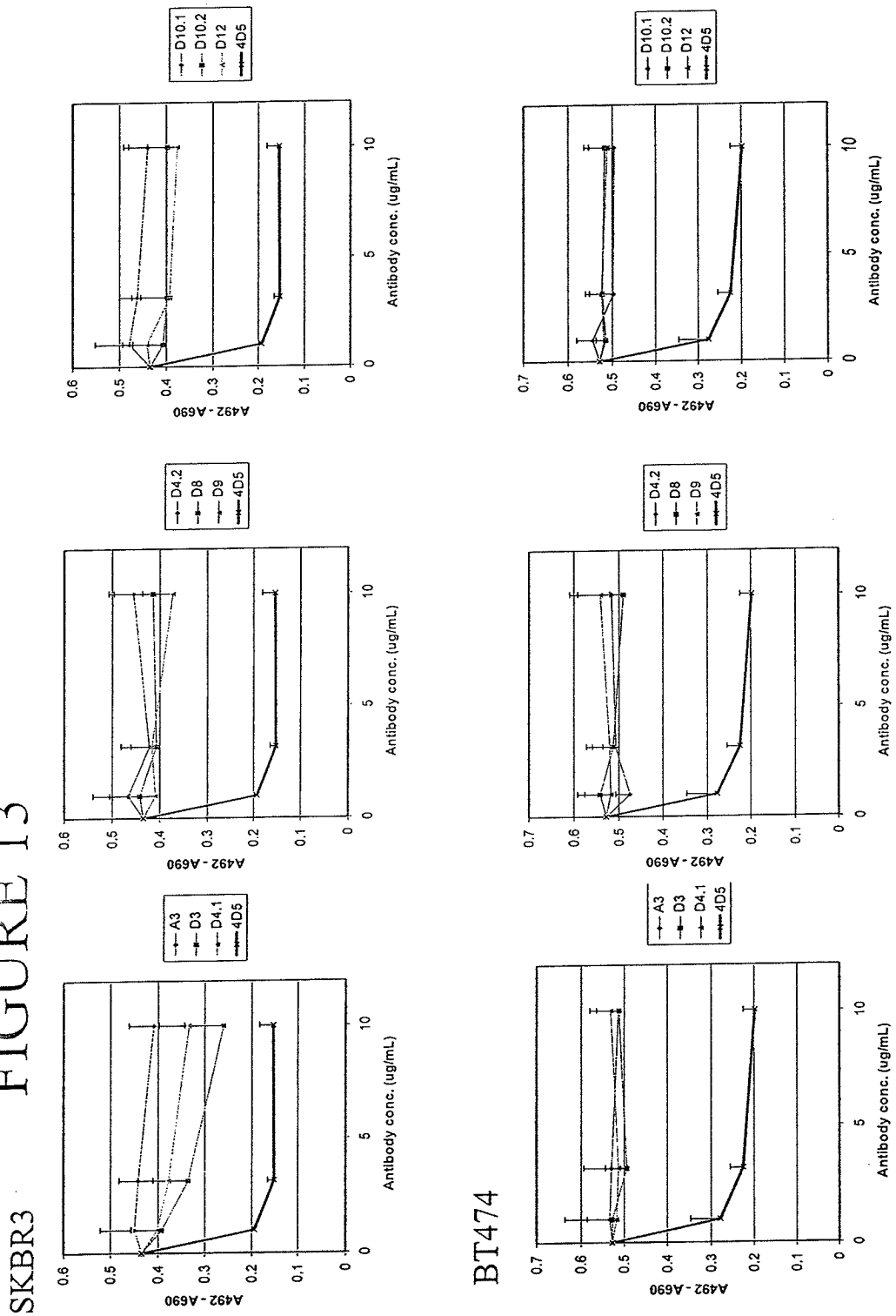

SKBR3
BT474
A492 - A690
Antibody conc. (ug/mL)
A3
D3
D4.1
D4.2
D8
D9
D10.1
D10.2
D12
4D5

Figure 14

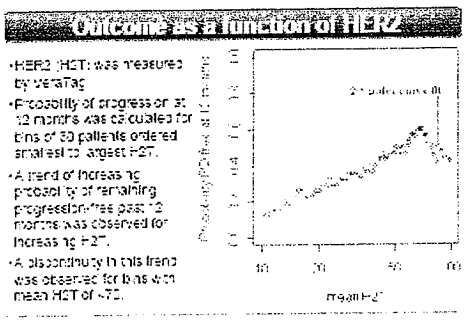
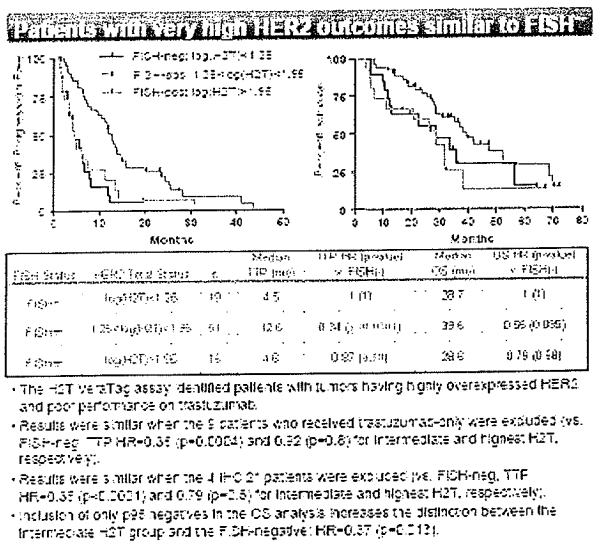


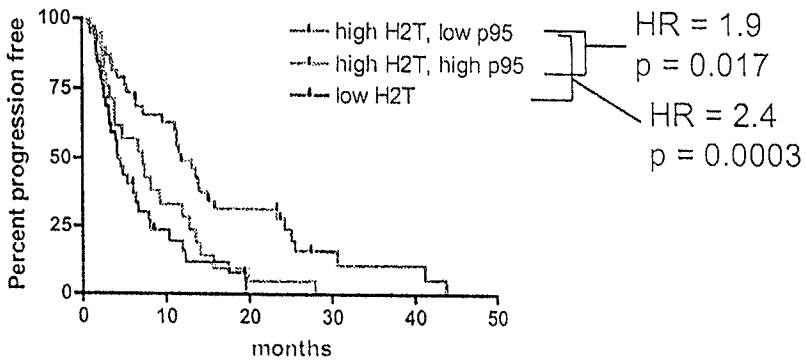
Figure 15



FISH Status	HER2 Total Score	n	Median TTP (mo)	TTP HR (p-value) vs FISH+	Median OS (mo)	OS HR (p-value) vs FISH+
FISH+	log(H2T)>1.38	19	4.5	1 (1)	28.7	1 (1)
FISH−	1.38>log(H2T)>1.55	51	12.6	0.34 (p=0.0004)	39.6	0.66 (0.085)
FISH−	log(H2T)<1.55	16	4.6	0.87 (p=0.8)	28.6	0.79 (0.58)

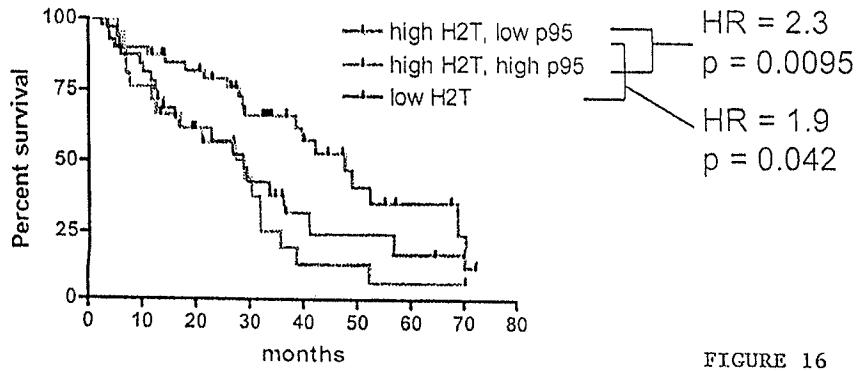
- The H2T VeriTag assay identified patients with tumors having highly overexpressed HER2 and poor performance on trastuzumab.
- Results were similar when the 5 patients who received trastuzumab-only were excluded (vs. FISH-neg: TTP HR=0.35 (p=0.0004) and 0.62 (p=0.8) for intermediate and highest H2T, respectively).
- Results were similar when the 4 IHC 2+ patients were excluded (vs. FISH-neg, TTP HR=0.35 (p<0.0001) and 0.79 (p=0.8) for intermediate and highest H2T, respectively).
- Inclusion of only pos negatives in the OS analysis increases the distinction between the intermediate H2T group and the FISH-negative: HR=0.37 (p=0.012).

TTP



Subgroup (n)	Median TTP
high H2T, low p95 (n=40)	11.7 months
high H2T, high p95 (n=21)	7.2 months
low H2T (n=29)	4.4 months

OS



Subgroup (n)	Median Survival
high H2T, low p95 (n=40)	48 months
high H2T, high p95 (n=21)	29 months
low H2T (n=29)	29 months

FIGURE 16

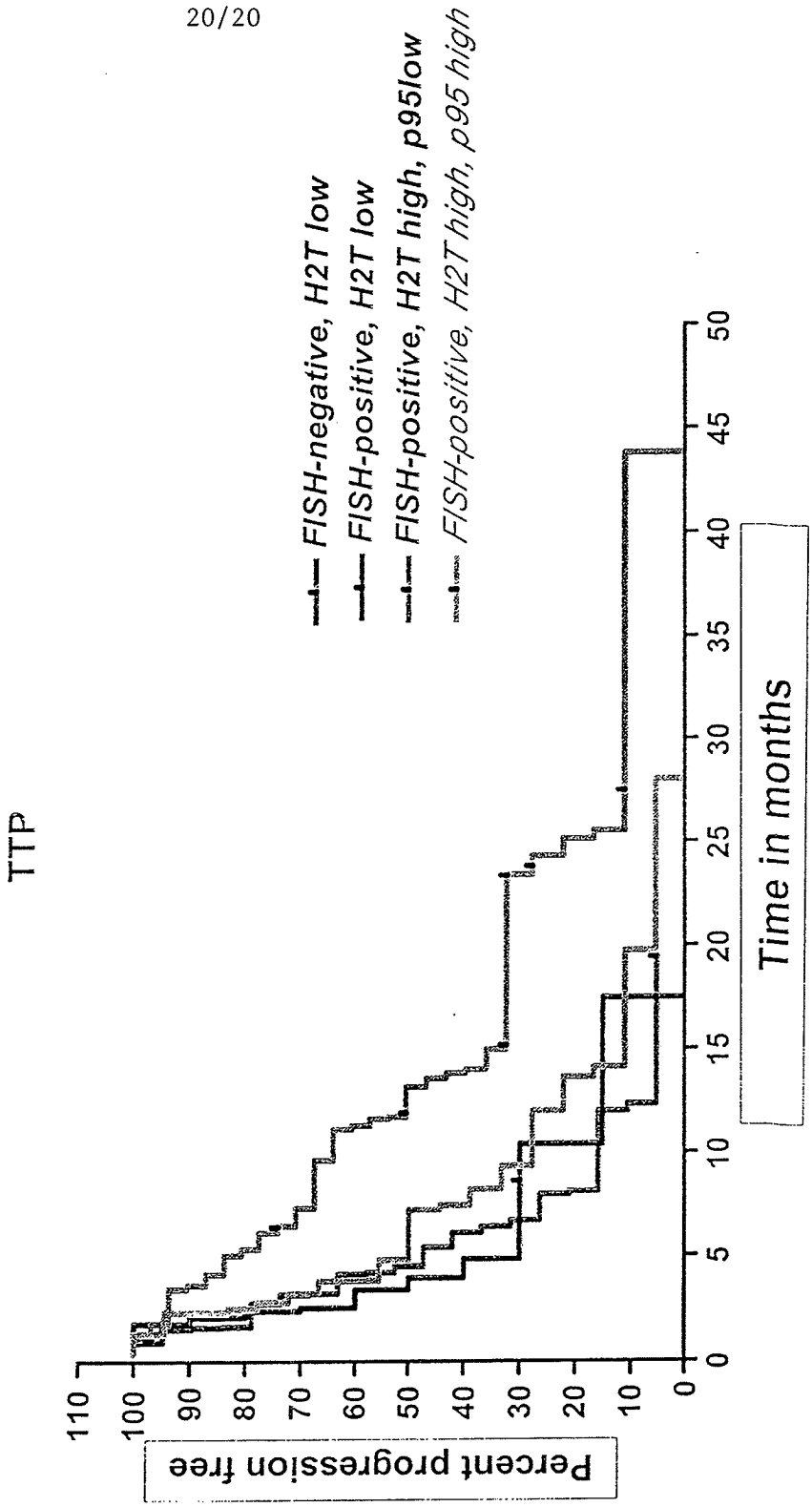

TTP
FISH-negative, H2T low
FISH-positive, H2T low
FISH-positive, H2T high, p95low
FISH-positive, H2T high, p95 high
Percent progression free
110
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
Time in months

FIGURE 17