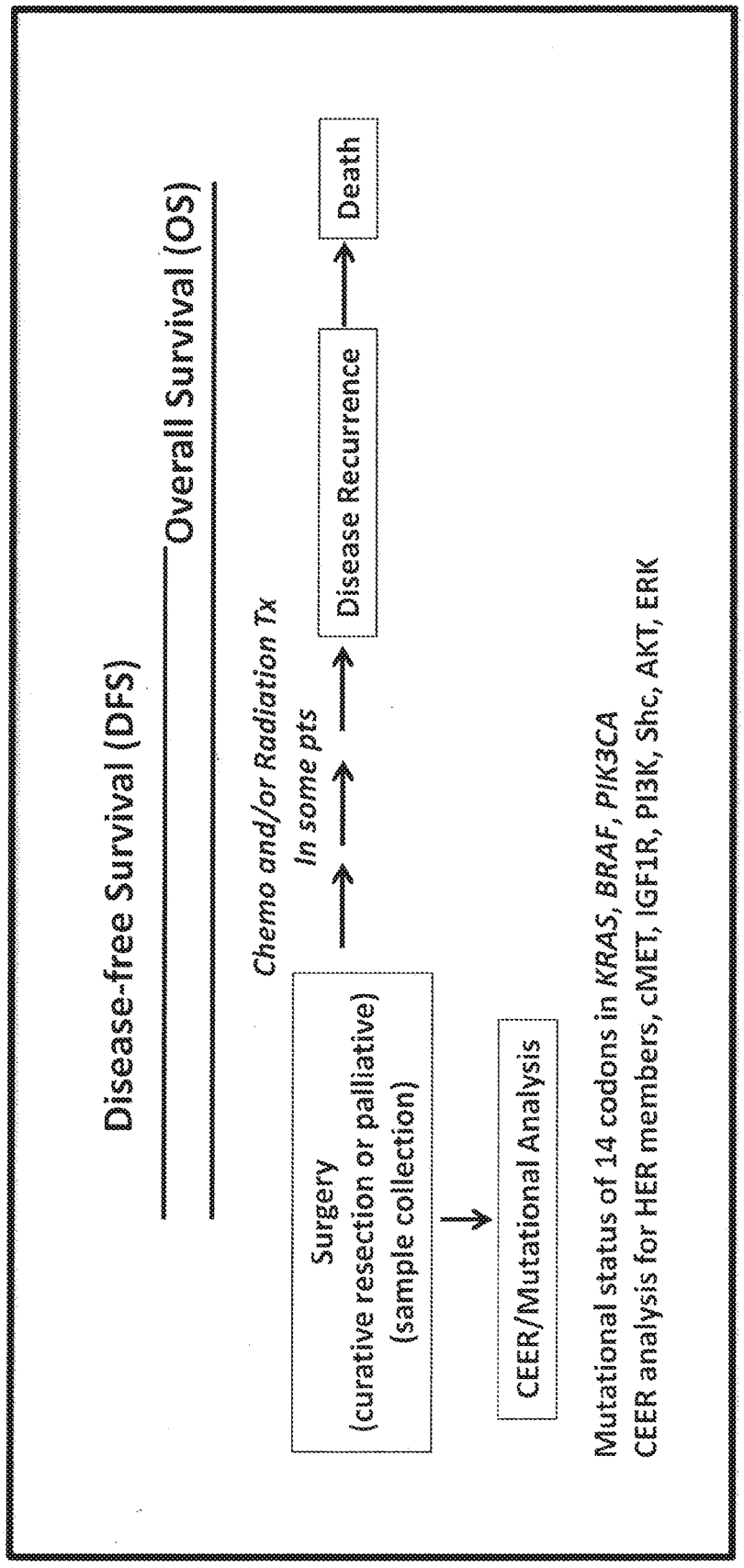

Disease-free Survival (DFS)
Overall Survival (OS)
Surgery
(curative resection or palliative)
(sample collection)
Chemo and/or Radiation Tx
In some pts
Disease Recurrence
Death
CEER/Mutational Analysis
Mutational status of 14 codons in KRAS, BRAF, PIK3CA
CEER analysis for HER members, cMET, IGF1R, PI3K, Shc, AKT, ERK

FIG. 1

Characteristics	(Total N=120)(%)
Age	
Median age(range)	61(31-85)
≤65	68(56.7)
>65	52(43.3)
Sex	
Male	73(60.8)
Female	47(39.2)
Stage	
I	11(9.2)
II	38(31.7)
III	38(31.7)
IV	33(27.4)
Histology	
WD	8(6.7)
MD	99(82.5)
PD	5(4.1)
Adenocarcinoma, not specified	8(6.7)
Primary tumor	
Colon	73(60.8)
Rectum	47(39.2)
Curative resection	
Yes	108(90.0)
No	12(10.0)
Adjuvant chemotherapy(Colon)	
5-FU based chemotherapy	20(27.4)
Capecitabine	18(24.7)
XELOX or XELIRI	13(17.8)
No adjuvant chemotherapy	22(30.1)
Adjuvant chemotherapy(Rectum)	
5-FU+RT	28(59.6)
XELOX+RT	1(2.1)
5-FU based chemotherapy	2(4.3)
XELODA	1(2.1)
No adjuvant chemotherapy	15(31.9)
Tissue specimens (from 120 patients)	
Primary(colon or rectum)	116
Liver metastasis	14(10 paired with primary)
Peritoneal seeding	1 (paired with primary)
KRAS mutational status	
At least one mutation	49 (42%)
KRAS G12S	3
KRAS G12D	18
KRAS G12A	1
KRAS G12V	6
KRAS G12C	7
KRAS G13D	17

WD: Well differentiated; MD: Moderately differentiated; PD: Poorly differentiated.

FIG. 2

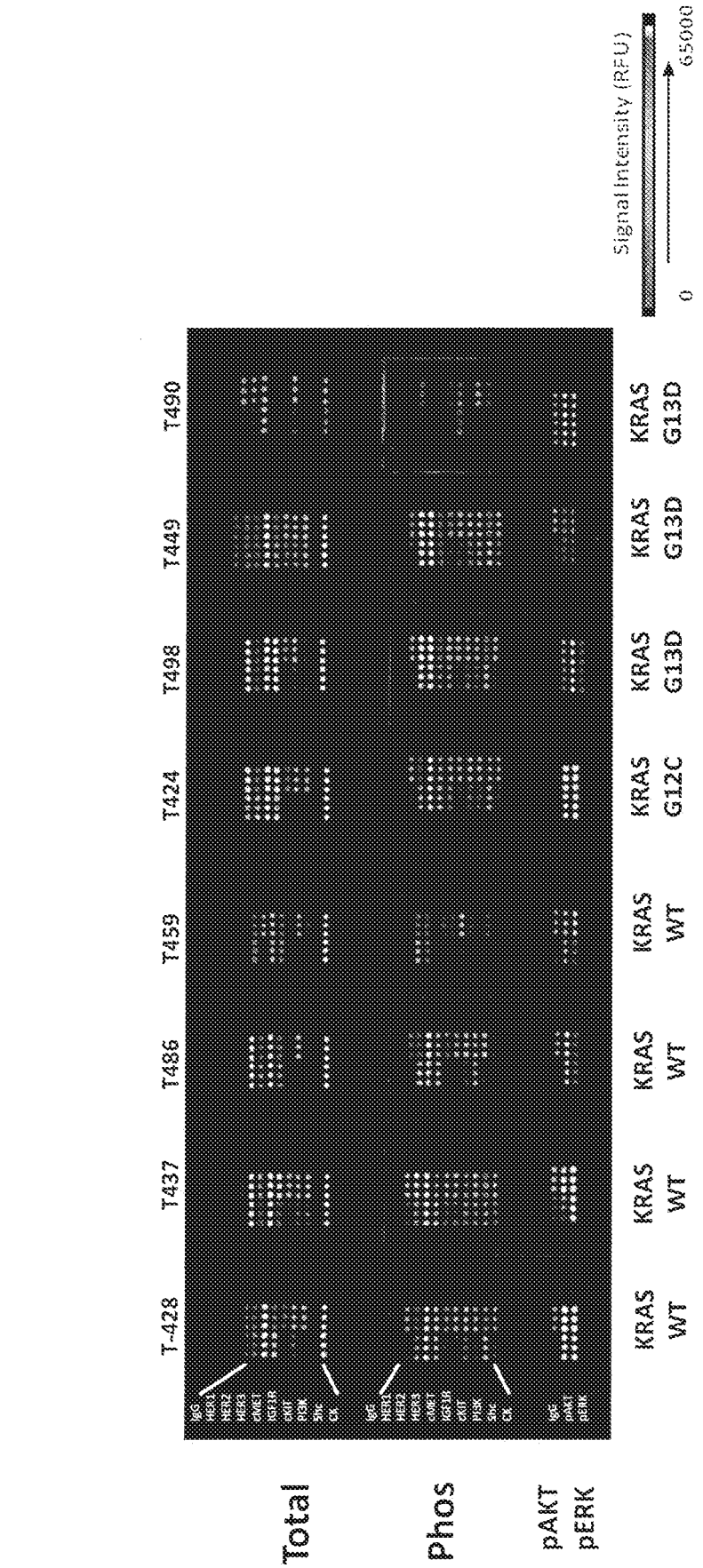

Signal Intensity (RFU)
65000
0
Total
Phos
pAKT
pERK
T-428
T437
T486
T459
T424
T498
T449
T490
KRAS WT
KRAS WT
KRAS WT
KRAS WT
KRAS G12C
KRAS G13D
KRAS G13D
KRAS G13D

FIG. 3

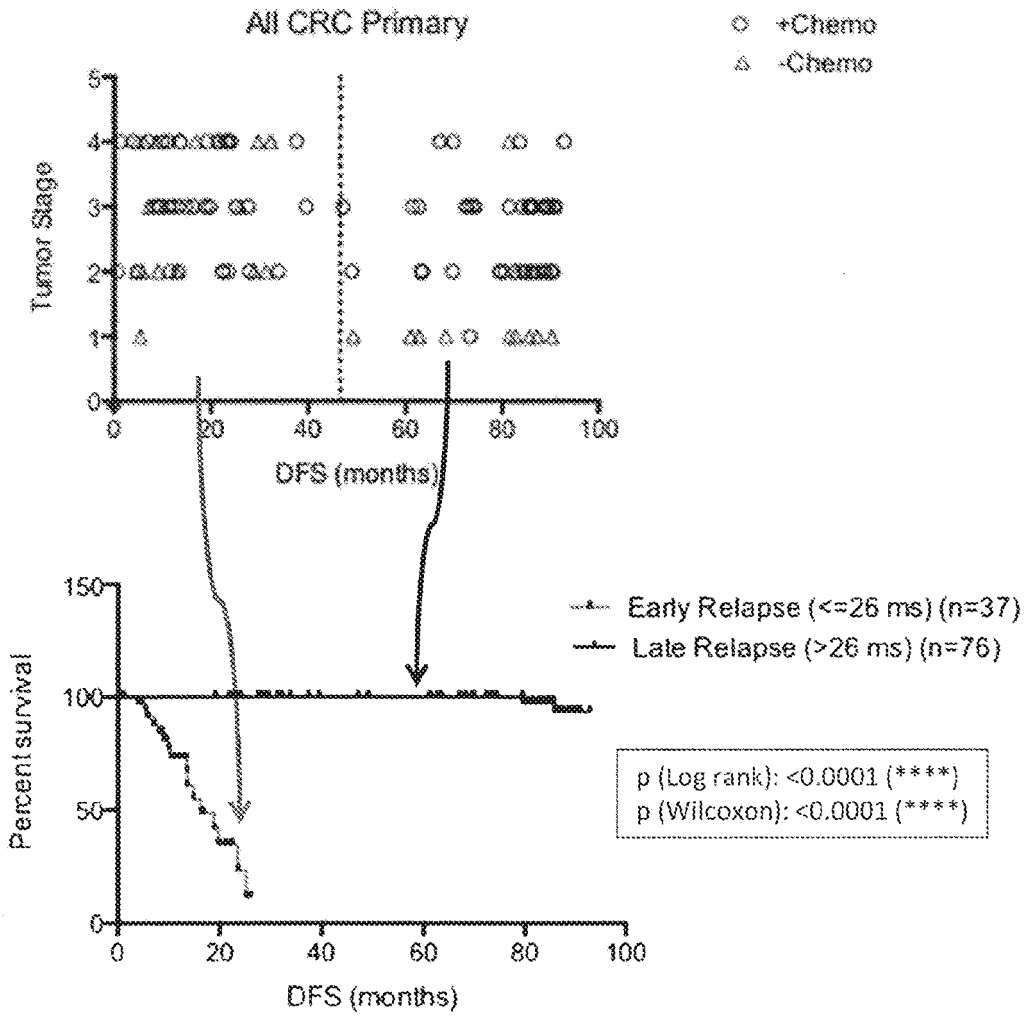

All CRC Primary
+Chemo
-Chemo
Tumor Stage
0
1
2
3
4
5
0
20
40
60
80
100
DFS (months)
Percent survival
0
50
100
150
Early Relapse (<=26 ms) (n=37)
Late Relapse (>26 ms) (n=76)
p (Log rank): <0.0001 (****)
p (Wilcoxon): <0.0001 (****)

FIG. 4

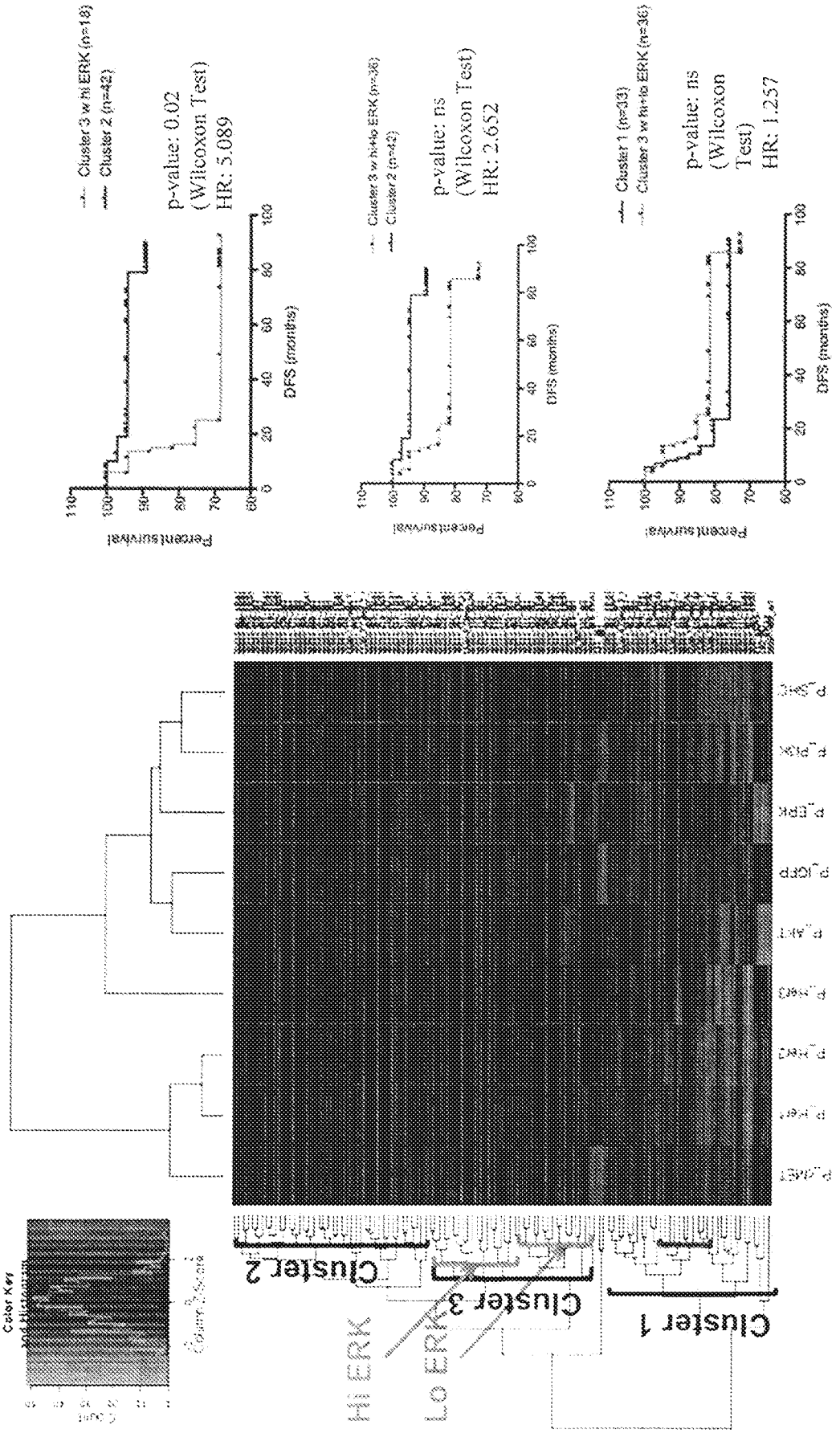

Cluster 3 w hi ERK (n=18)
Cluster 2 (n=42)
p-value: 0.02
(Wilcoxon Test)
HR: 5.089
Percent survival
DFS (months)
Cluster 3 w hi+lo ERK (n=36)
Cluster 2 (n=42)
p-value: ns
(Wilcoxon Test)
HR: 2.652
Cluster 1 (n=33)
Cluster 3 w hi+lo ERK (n=36)
p-value: ns
(Wilcoxon
Test)
HR: 1.257
Color Key
and Histogram
Count
Cluster 2
Cluster 3
Cluster 1
Hi ERK
Lo ERK

FIG. 5

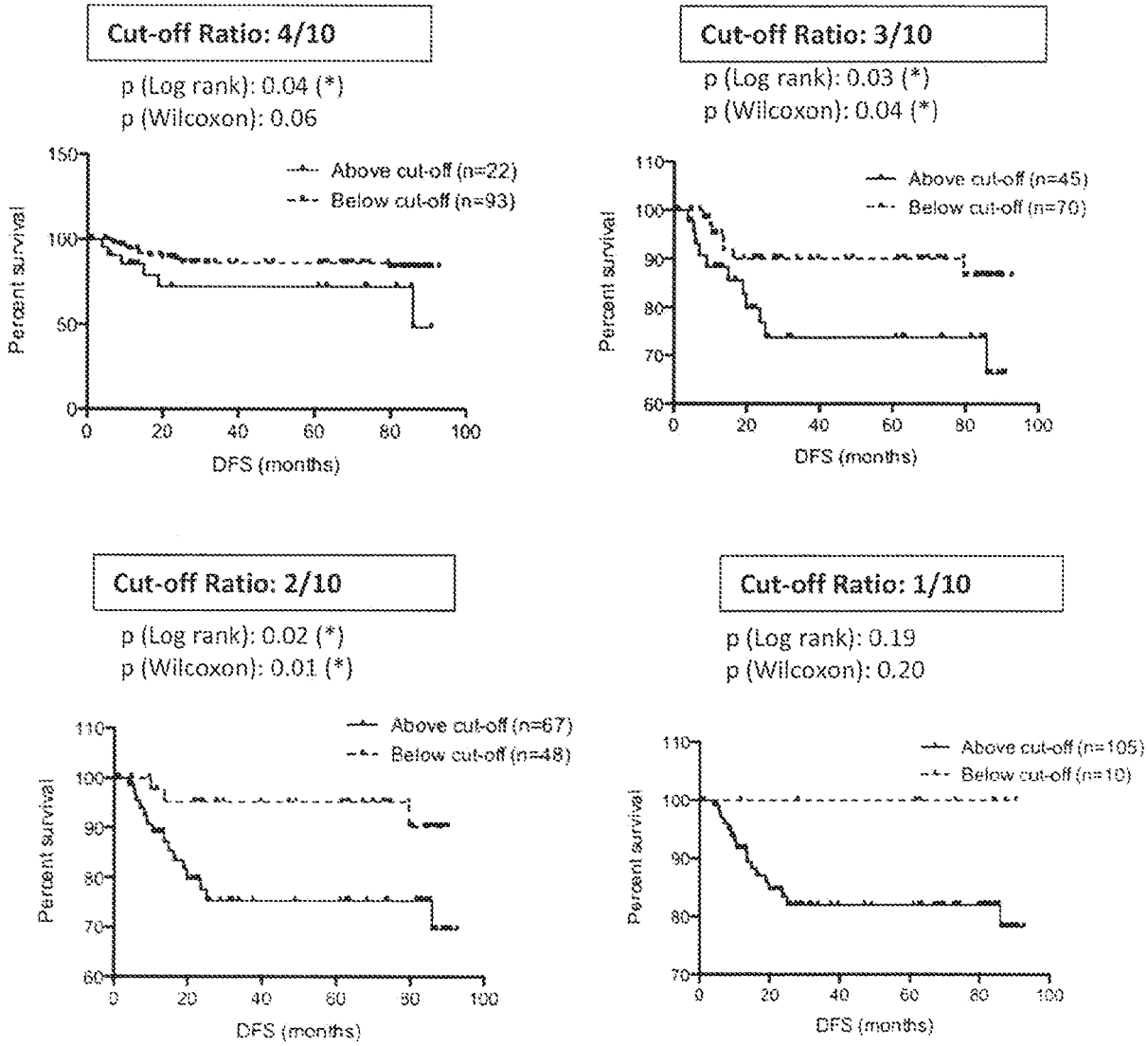

Cut-off Ratio: 4/10
p (Log rank): 0.04 (*)
p (Wilcoxon): 0.06
Above cut-off (n=22)
Below cut-off (n=93)
Percent survival
DFS (months)
Cut-off Ratio: 3/10
p (Log rank): 0.03 (*)
p (Wilcoxon): 0.04 (*)
Above cut-off (n=45)
Below cut-off (n=70)
Percent survival
DFS (months)
Cut-off Ratio: 2/10
p (Log rank): 0.02 (*)
p (Wilcoxon): 0.01 (*)
Above cut-off (n=67)
Below cut-off (n=48)
Percent survival
DFS (months)
Cut-off Ratio: 1/10
p (Log rank): 0.19
p (Wilcoxon): 0.20
Above cut-off (n=105)
Below cut-off (n=10)
Percent survival
DFS (months)

FIG. 6

- KRAS WT (31/48): 65%
- KRAS G13Dmut (8/48): 17%
- KRAS G12mut (9/48): 19%
- Stage IV: 23%, Stage III: 27%, Stage II: 35%, Stage I: 10%
- 77% of the patients received chemotherapy.

[(pPI3K/pHER3)] ratio < 2/10 (n=48)
42% of total Sample cohort
Better Survival

- KRAS WT (39/67): 58%
- KRAS G13Dmut (9/67): 13%
- KRAS G12mut (19/67): 28%
- Majority of the KRAS G12mut tumors (68%), that have the worst prognosis in CRC, lie in this category.
- Stage IV: 22%, Stage III: 37%, Stage II: 31%, Stage I: 9%
- 69% of the patients received chemotherapy.

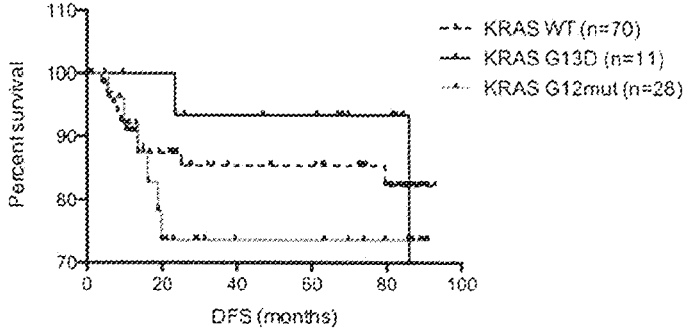
[(pPI3K/pHER3)] ratio > 2/10 (n=67)
58% of total Sample cohort
Worse Survival

FIG. 7

- KRAS WT tumors clearly segregated into 2 subsets with significantly distinct DFS differences based on their pPI3K/pHER3 index ratio.
- KRAS G12mut tumors have a low DFS regardless of their pPI3K/pHER3 ratio. Aggressive all the way.
- ***pPI3K/pHER3 expression is a novel prognostic factor in CRCs.***

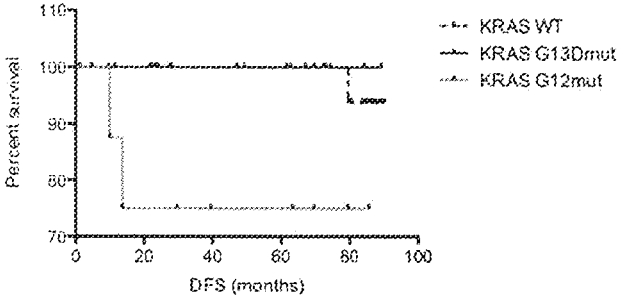
FIG. 8

p (Log rank): 0.46
p (Log rank test for trend): 0.83



All CRC

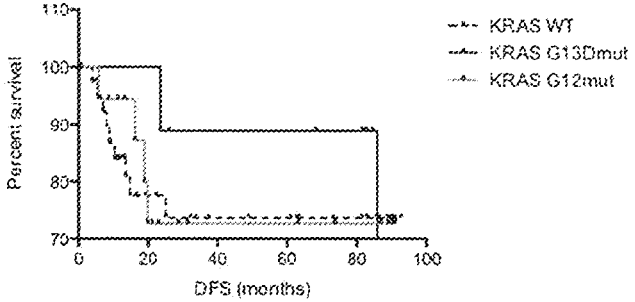
p (Log rank): 0.03 (*)
p (Log rank test for trend): 0.03 (*)



(pPI3K/pHER3) ratio < 2/10

Better Survival

p (Log rank): 0.90
p (Log rank test for trend): 0.84



(pPI3K/pHER3) ratio > 2/10

Worse Survival

FIG. 9

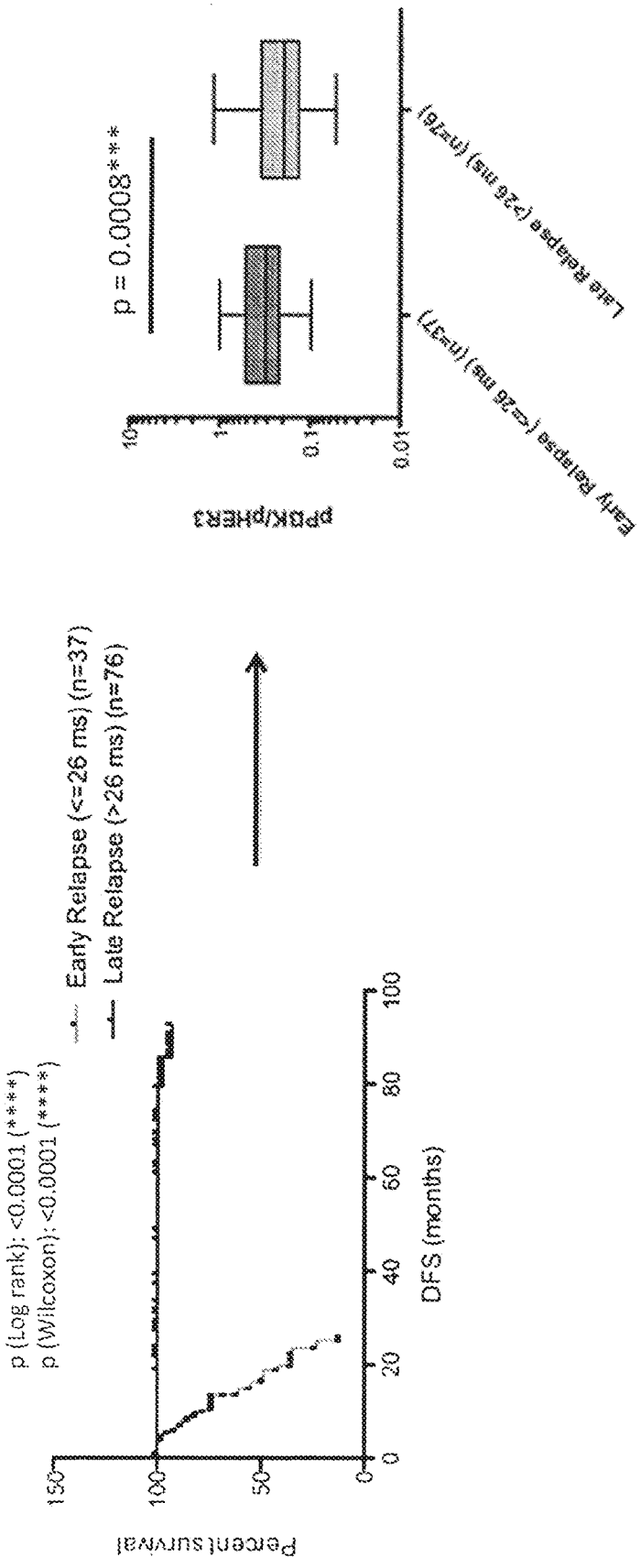

p (Log rank): <0.0001 (****)
p (Wilcoxon): <0.0001 (****)
Early Relapse (<=26 ms) (n=37)
Late Relapse (>26 ms) (n=76)
Percent survival
DFS (months)
p = 0.0008***
pPDK/pHER3
Early Relapse (<=26 ms) (n=37)
Late Relapse (>26 ms) (n=76)

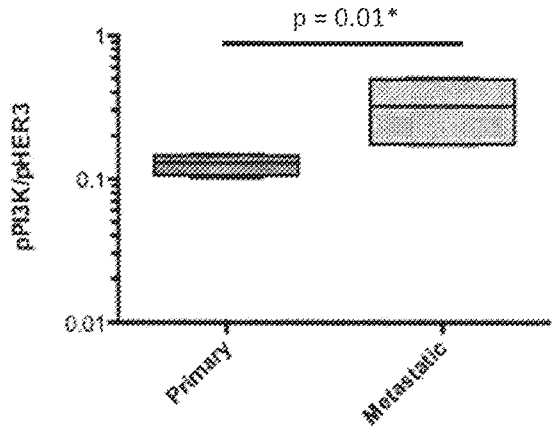
FIG. 10

CEER data available on 10 pairs of matched primary and metastatic CRCs

- 4 pairs of primary/mets
- All primaries except 1 stage 4
- 3 samples KRAS-WT, 1 sample KRAS-G12mut
- 2/4 primaries received chemotherapy, data unavailable for one patient

- 6 pairs of primary/mets
- All primaries stage 4
- 4 samples KRAS-WT, 2 samples KRAS-G12mut. 1 KRAS-WT sample has a BRAF mutation
- All Primaries received chemotherapy

(pPI3K/pHER3) ratio < 2/10



(pPI3K/pHER3) ratio > 2/10

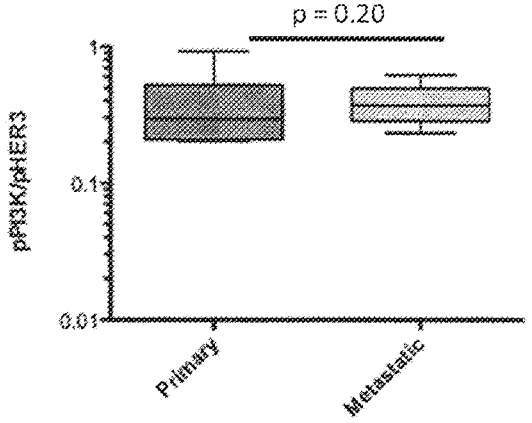


FIG. 11

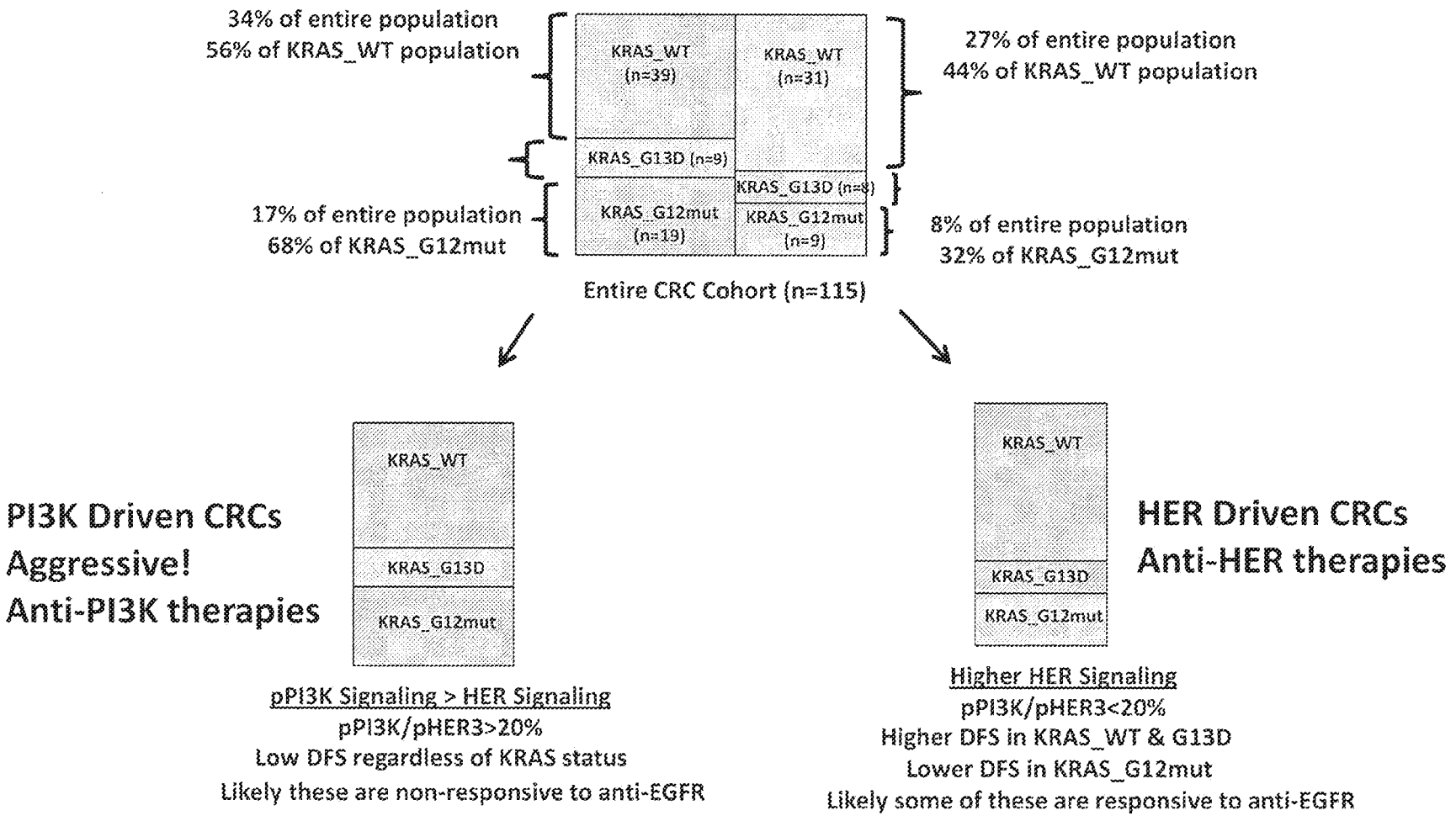

34% of entire population
56% of KRAS_WT population
KRAS_WT (n=39)
KRAS_WT (n=31)
27% of entire population
44% of KRAS_WT population
KRAS_G13D (n=9)
KRAS_G13D (n=9)
17% of entire population
68% of KRAS_G12mut
KRAS_G12mut (n=19)
KRAS_G12mut (n=9)
8% of entire population
32% of KRAS_G12mut
Entire CRC Cohort (n=115)
PI3K Driven CRCs
Aggressive!
Anti-PI3K therapies
KRAS_WT
KRAS_G13D
KRAS_G12mut
pPI3K Signaling > HER Signaling
pPI3K/pHER3>20%
Low DFS regardless of KRAS status
Likely these are non-responsive to anti-EGFR
KRAS_WT
KRAS_G13D
KRAS_G12mut
HER Driven CRCs
Anti-HER therapies
Higher HER Signaling
pPI3K/pHER3<20%
Higher DFS in KRAS_WT & G13D
Lower DFS in KRAS_G12mut
Likely some of these are responsive to anti-EGFR

FIG. 12

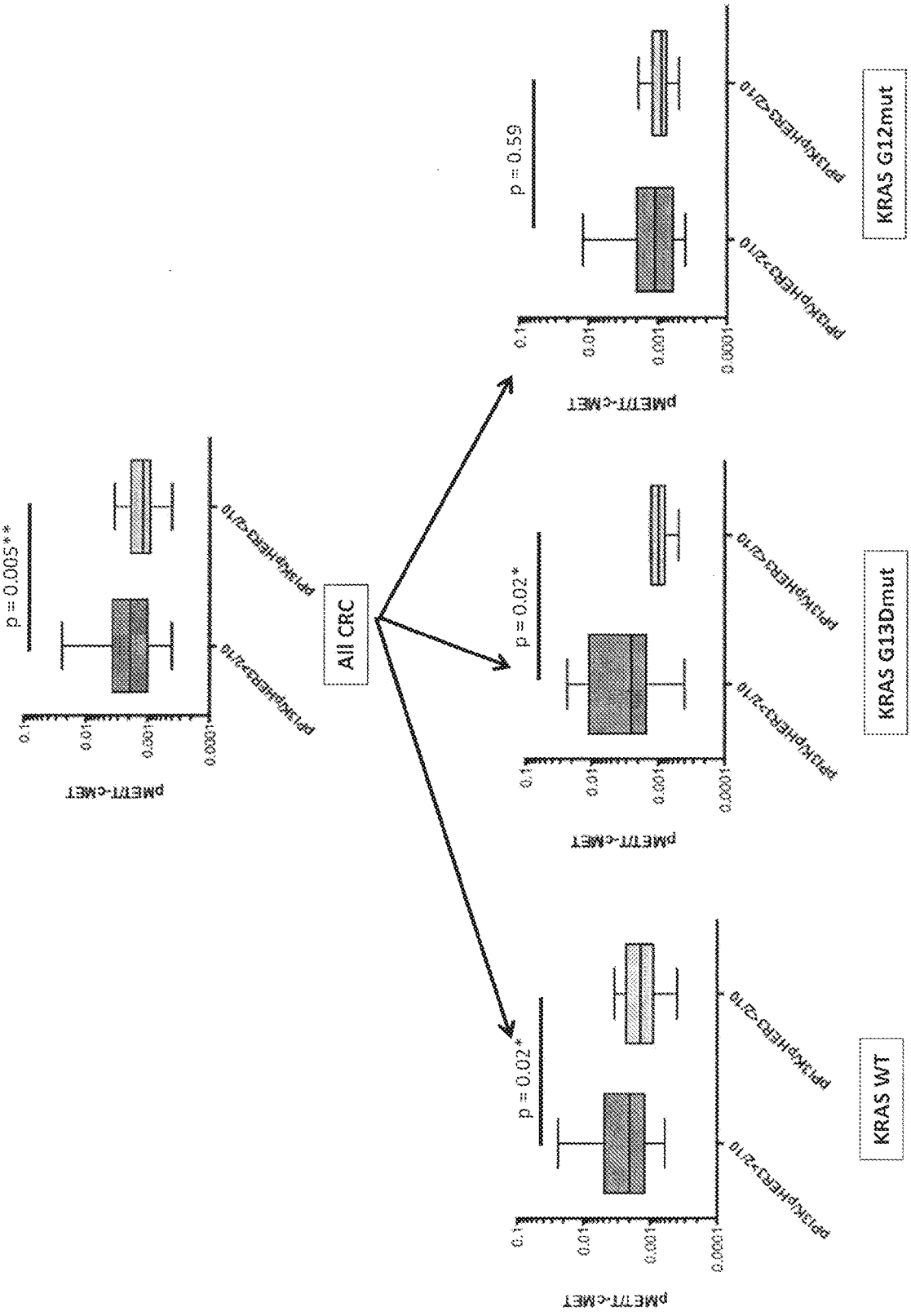

All CRC
p = 0.005**
pMET/T-cMET
pPI3Kp/pHER3-2/1/0
pPI3Kp/pEGFR-2/1/0
p = 0.02*
KRAS G13Dmut
p = 0.59
KRAS G12mut
p = 0.02*
KRAS WT

FIG. 13

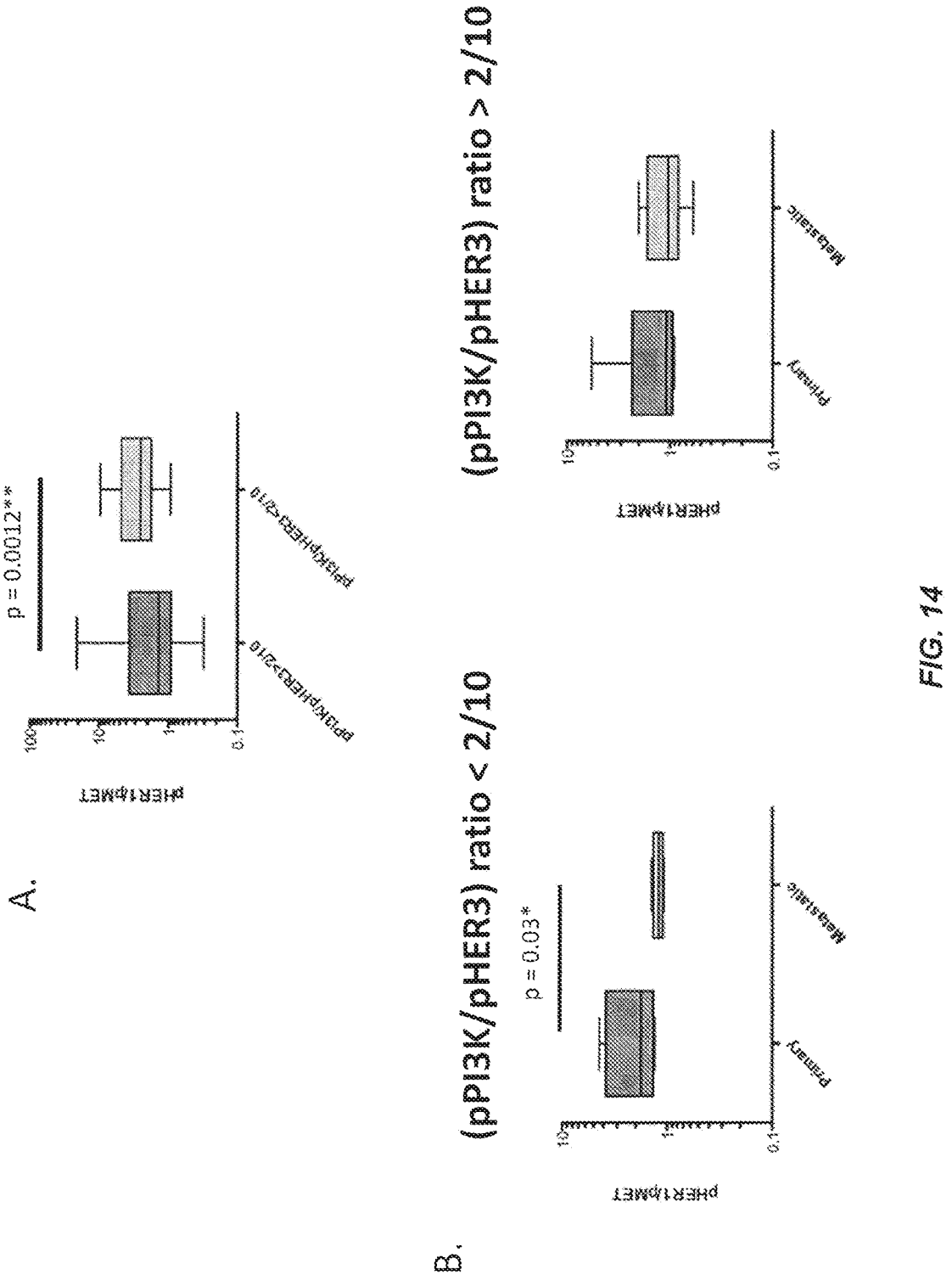

A.
p = 0.0012**
pHER1/pMET
100
10
1
0.1
pPI3K/pHER2>2/10
pPI3K/pHER2<2/10
B.
(pPI3K/pHER3) ratio < 2/10
p = 0.03*
pHER1/pMET
10
1
0.1
Primary
Metastatic
(pPI3K/pHER3) ratio > 2/10
pHER1/pMET
10
1
0.1
Primary
Metastatic

FIG. 14

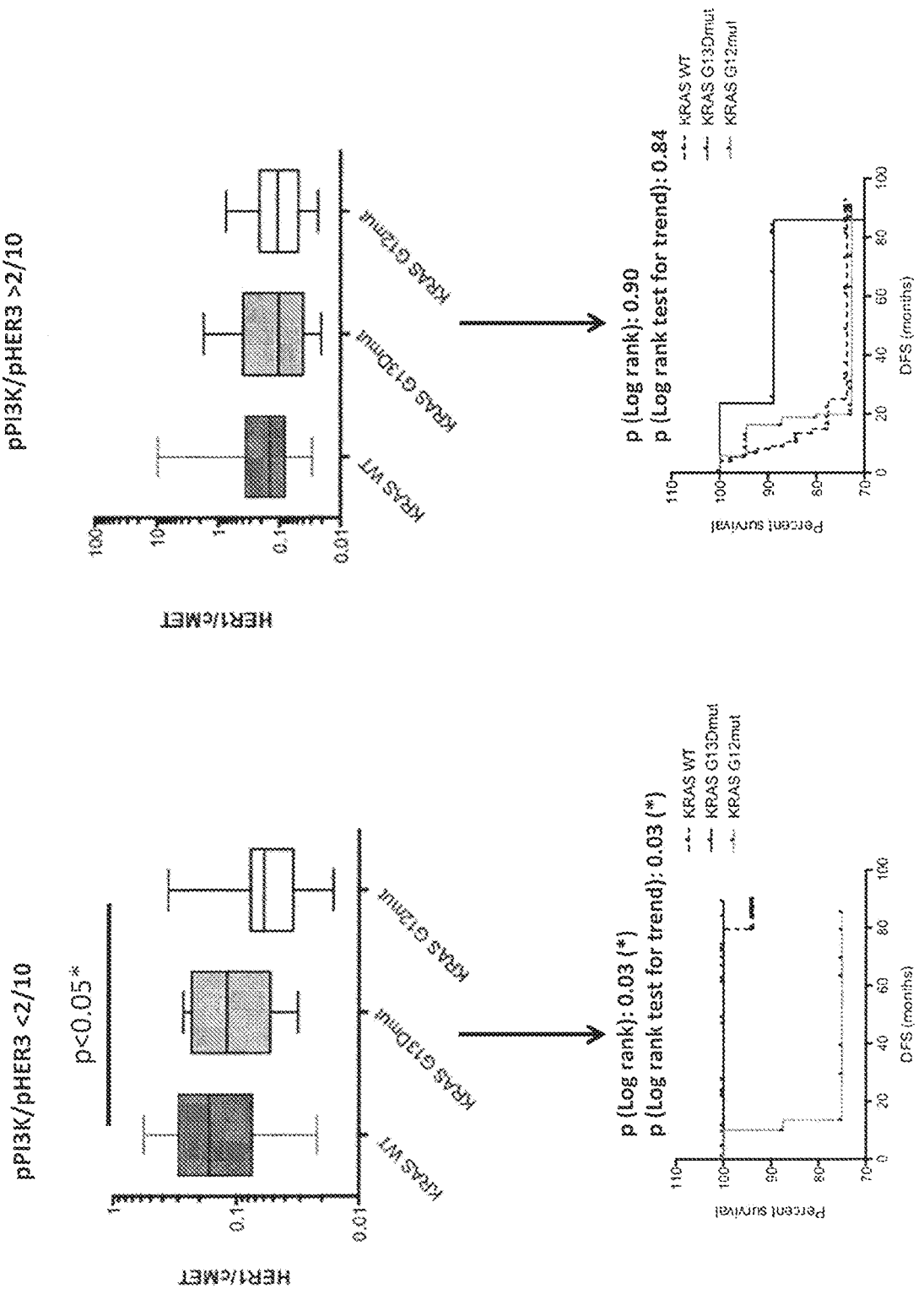

pPI3K/pHER3 >2/10
HER1/cMET
100
10
1
0.1
0.01
KRAS WT
KRAS G13Dmut
KRAS G12mut
p (Log rank): 0.90
p (Log rank test for trend): 0.84
KRAS WT
KRAS G13Dmut
KRAS G12mut
Percent survival
110
100
90
80
70
0
20
40
60
80
100
DFS (months)
pPI3K/pHER3 <2/10
p<0.05*
HER1/cMET
1
0.1
0.01
KRAS WT
KRAS G13Dmut
KRAS G12mut
p (Log rank): 0.03 (*)
p (Log rank test for trend): 0.03 (*)
KRAS WT
KRAS G13Dmut
KRAS G12mut
Percent survival
110
100
90
80
70
0
20
40
60
80
100
DFS (months)

FIG. 15

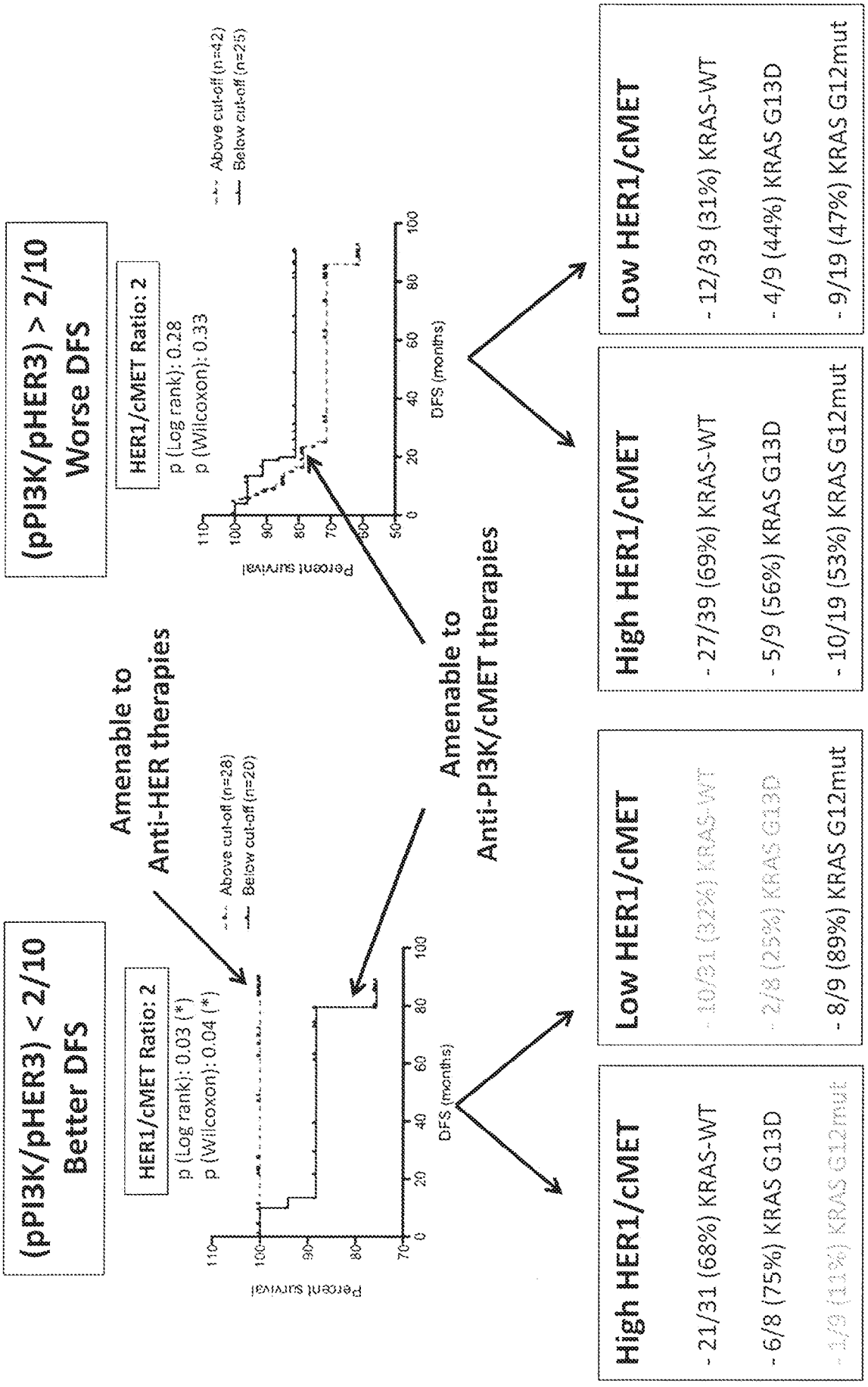

(pPI3K/pHER3) < 2/10
Better DFS
Amenable to
Anti-HER therapies
HER1/cMET Ratio: 2
p (Log rank): 0.03 (*)
p (Wilcoxon): 0.04 (*)
Above cut-off (n=28)
Below cut-off (n=20)
Percent survival
DFS (months)
High HER1/cMET
- 21/31 (68%) KRAS-WT
- 6/8 (75%) KRAS G13D
- 2/9 (11%) KRAS G12mut
Low HER1/cMET
- 10/31 (32%) KRAS-WT
- 2/8 (25%) KRAS G13D
- 8/9 (89%) KRAS G12mut
(pPI3K/pHER3) > 2/10
Worse DFS
Amenable to
Anti-PI3K/cMET therapies
HER1/cMET Ratio: 2
p (Log rank): 0.28
p (Wilcoxon): 0.33
Above cut-off (n=42)
Below cut-off (n=25)
Percent survival
DFS (months)
High HER1/cMET
- 27/39 (69%) KRAS-WT
- 5/9 (56%) KRAS G13D
- 10/19 (53%) KRAS G12mut
Low HER1/cMET
- 12/39 (31%) KRAS-WT
- 4/9 (44%) KRAS G13D
- 9/19 (47%) KRAS G12mut

FIG. 16

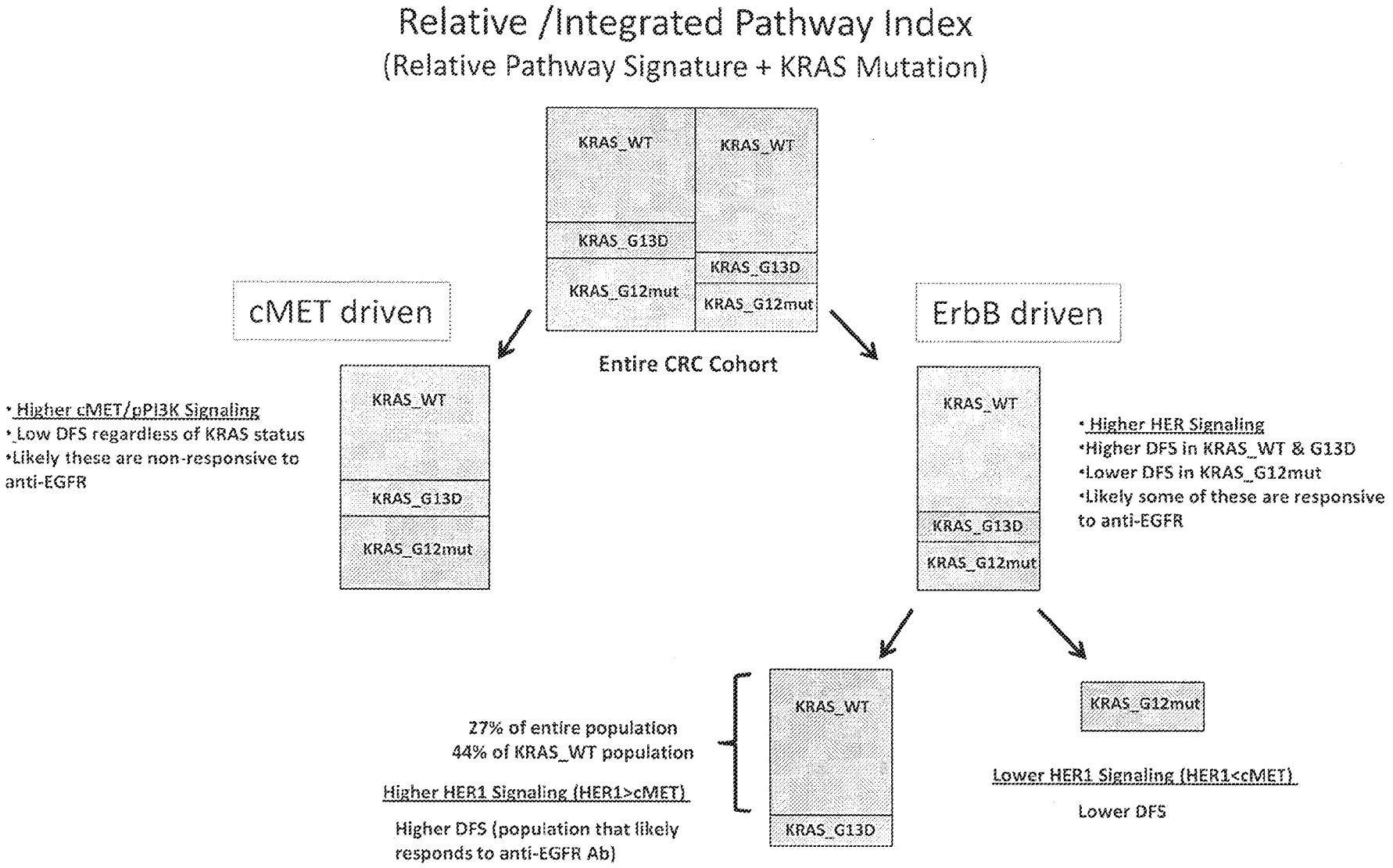

Relative /Integrated Pathway Index
(Relative Pathway Signature + KRAS Mutation)
KRAS_WT
KRAS_WT
KRAS_G13D
KRAS_G13D
KRAS_G12mut
KRAS_G12mut
Entire CRC Cohort
cMET driven
ErbB driven
• Higher cMET/pPI3K Signaling
• Low DFS regardless of KRAS status
•Likely these are non-responsive to anti-EGFR
KRAS_WT
KRAS_G13D
KRAS_G12mut
KRAS_WT
KRAS_G13D
KRAS_G12mut
• Higher HER Signaling
•Higher DFS in KRAS_WT & G13D
•Lower DFS in KRAS_G12mut
•Likely some of these are responsive to anti-EGFR
27% of entire population
44% of KRAS_WT population
Higher HER1 Signaling (HER1>cMET)
Higher DFS (population that likely responds to anti-EGFR Ab)
KRAS_WT
KRAS_G13D
KRAS_G12mut
Lower HER1 Signaling (HER1<cMET)
Lower DFS

FIG. 17

pPI3K/pHER1, pPI3K/pHER2, pPI3K/pHER3
All correlate negatively and significantly with relapse in CRC (Higher the marker, quicker is the relapse)

	pPI3K	pPI3K/pHER1	pPI3K/pHER2	pPI3K/pHER3
Number of XY Pairs	113	113	113	113
Pearson r	-0.24	-0.23	-0.25	-0.29
95% confidence interval	-0.41 to -0.06	-0.40 to -0.05	-0.42 to -0.07	-0.45 to -0.12
P value (one-tailed)	0.0054	0.0069	0.00	0.00
P value summary	**	**	**	***
R square	0.06	0.05	0.06	0.09

pPI3K/pHER1, pPI3K/pHER2, pPI3K/pHER3
All correlate negatively and significantly with disease free survival (DFS) in CRC

Parameter	pPI3K/p-HER3	pPI3K/pHER1	pPI3K/pHER2
Number of XY Pairs	113	113	113
Pearson r	-0.17	-0.19	-0.17
95% confidence interval	-0.3464 to 0.01253	-0.3654 to -0.00928	-0.3415 to 0.01800
P value (one-tailed)	0.034	0.0199	0.0382
P value summary	*	*	*
Is the correlation significant? (alpha=0.05)	Yes	Yes	Yes
R square	0.030	0.038	0.028

FIG. 18

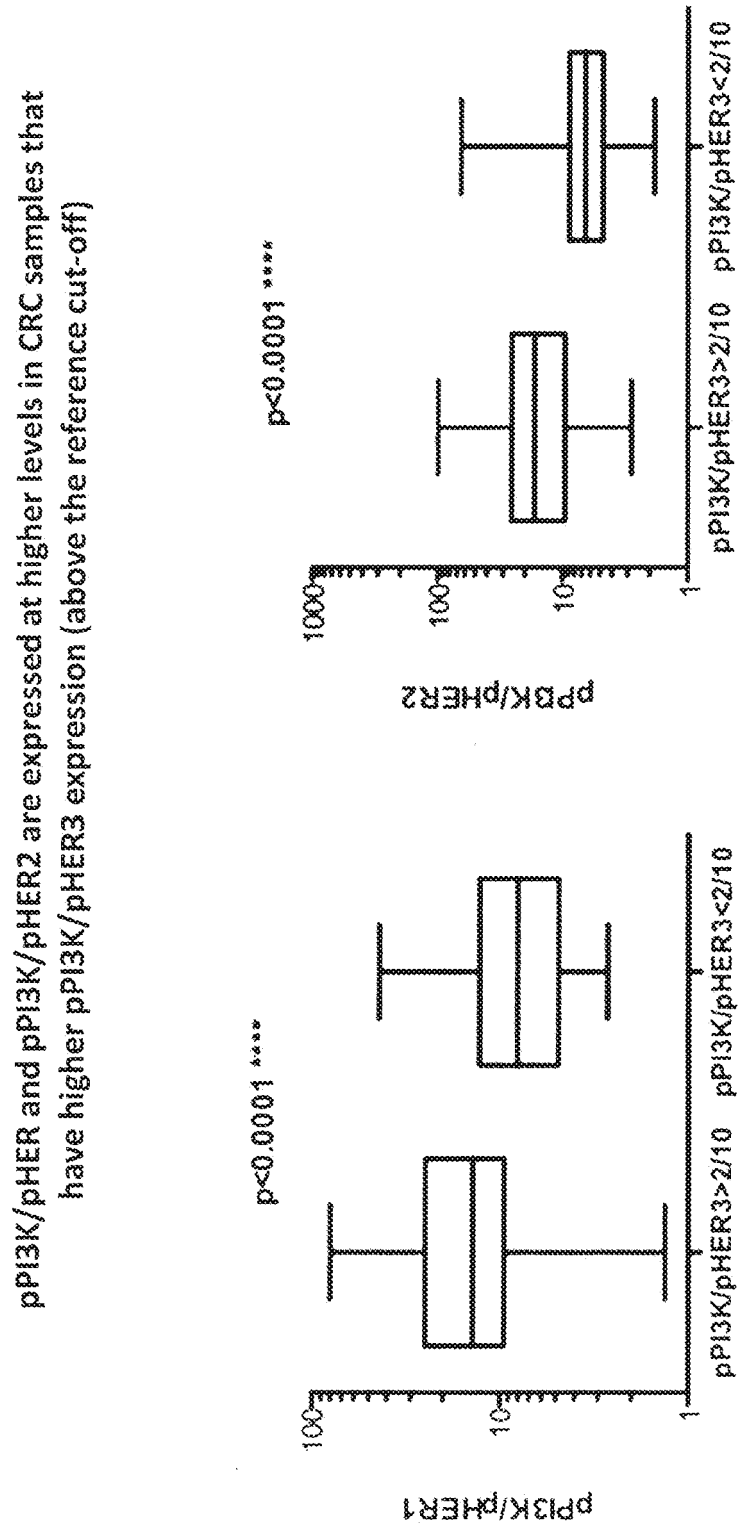

pPI3K/pHER and pPI3K/pHER2 are expressed at higher levels in CRC samples that have higher pPI3K/pHER3 expression (above the reference cut-off)
p<0.0001 ****
pPI3K/pHER1
100
10
1
pPI3K/pHER3>2/10
pPI3K/pHER3<2/10
p<0.0001 ****
pPI3K/pHER2
1000
100
10
1
pPI3K/pHER3>2/10
pPI3K/pHER3<2/10

FIG. 19