ASCP Annual Meeting
Las Vegas, October 22, 2011
Defining Best Practices for
KRAS Mutation in Colon Cancer
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Aschutz Medical Campus
University of Colorado
Colorado Molecular Correlates
*No conflicts to declare
MGM
AccessVegas.Com

Approach to Cancer Treatment before 2000

"Let's just start cutting and see what happens."

Personalized Medicine

- Personalized medicine is a medical model that tailors prevention and treatment to individual patient based largely on genomic and proteomic profiles
- Most emphasis in personalized medicine to date has been on somatic genetic changes in tumor cells to design cancer treatments
- Solid tumors (breast, lung, colon, skin cancers) as well as leukemias

Finding the right target

- Organ site
- Tumor type
- Focus on pathway
- Drug that works in a proportion of the treated population
- Mutation in the targeted gene or gene affecting the pathway

Predictive Biomarkers

- Important to distinguish predictive from diagnostic biomarkers
- Predictive marker status does not correlate well with tumor subtype
  - Must be measured directly
- Increasingly paired with therapeutic agents

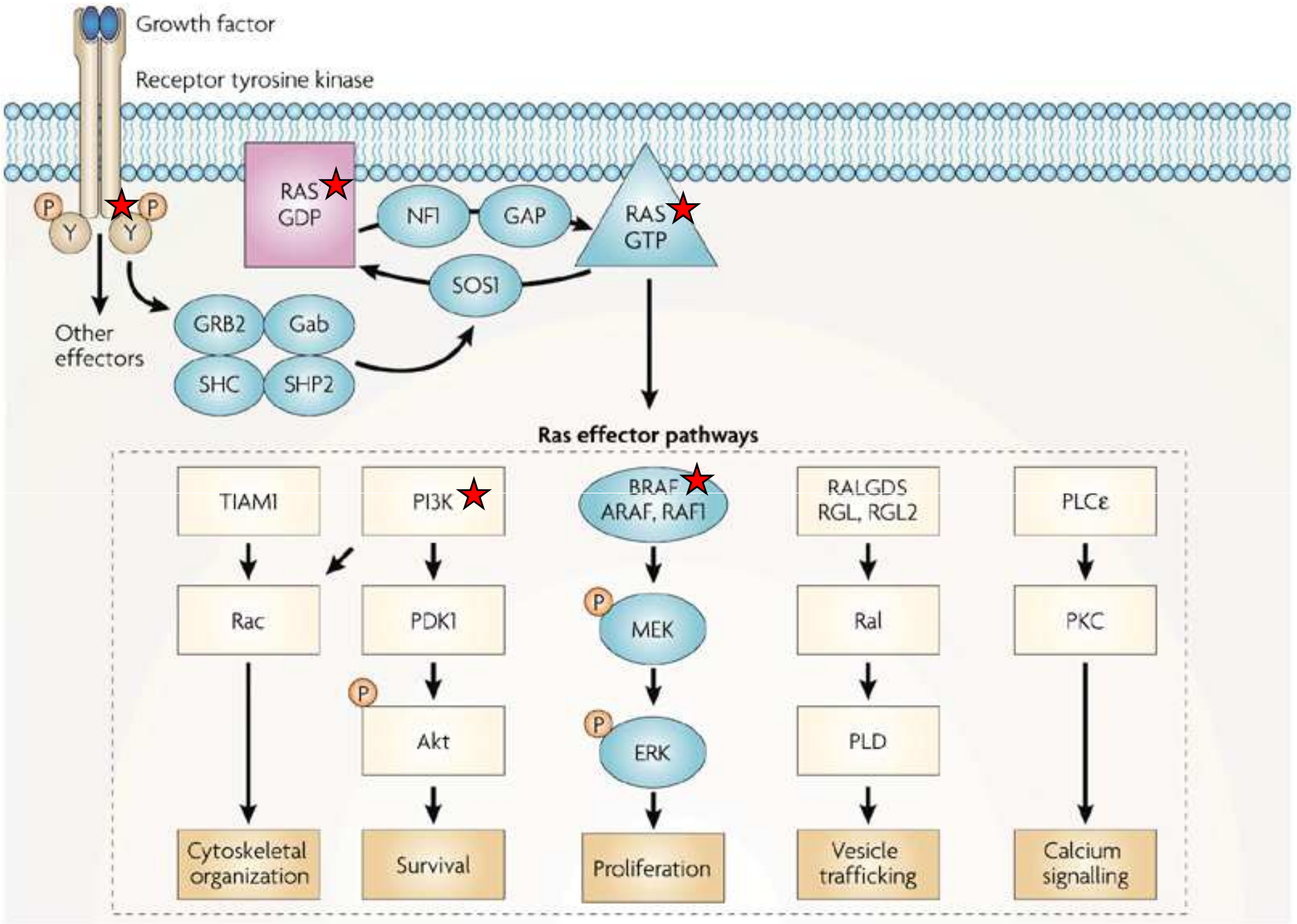
Drug	Tumor	Marker
Bevacizumab	Lung	Non-squamous histology
Pemetrexed	Lung	Non-squamous histology
Imatinib	GIST	Mutation, KIT
Small Mol. EGFR Inhibitors (Gefitinib, Erlotinib)	Lung	Mutation, EGFR
Anti BRAF	Melanoma, ?Colon	Mutation, *BRAF* (V600A)
MAGE vaccine	Lung	MAGE expression (RT-PCR/IHC)
Anti EGFR antibody (Cetuximab)	Colon	*KRAS* wild type
Anti IGF1R antibody	Lung	IHC IGF1R
Crizotinib (PF-02341066)	Lung	EML4-ALK translocation
Trastuzumab (Herceptin)	Breast, stomach	Overexpression/amplification

History of *KRAS**

- 1960's: Werner Kirsten, a pathologist at the University of Chicago isolated murine leukemia virus containing the *KRAS* oncogene.
- 1982: *v-KRAS* sequenced, published
- 1982-3: Identity of transforming oncogene with v-KRAS established
- 1984: EGFR found to stimulate KRAS activity
- 1984-7: KRAS mutation described in lung cancer, other epithelial tumors

Werner Kirsten

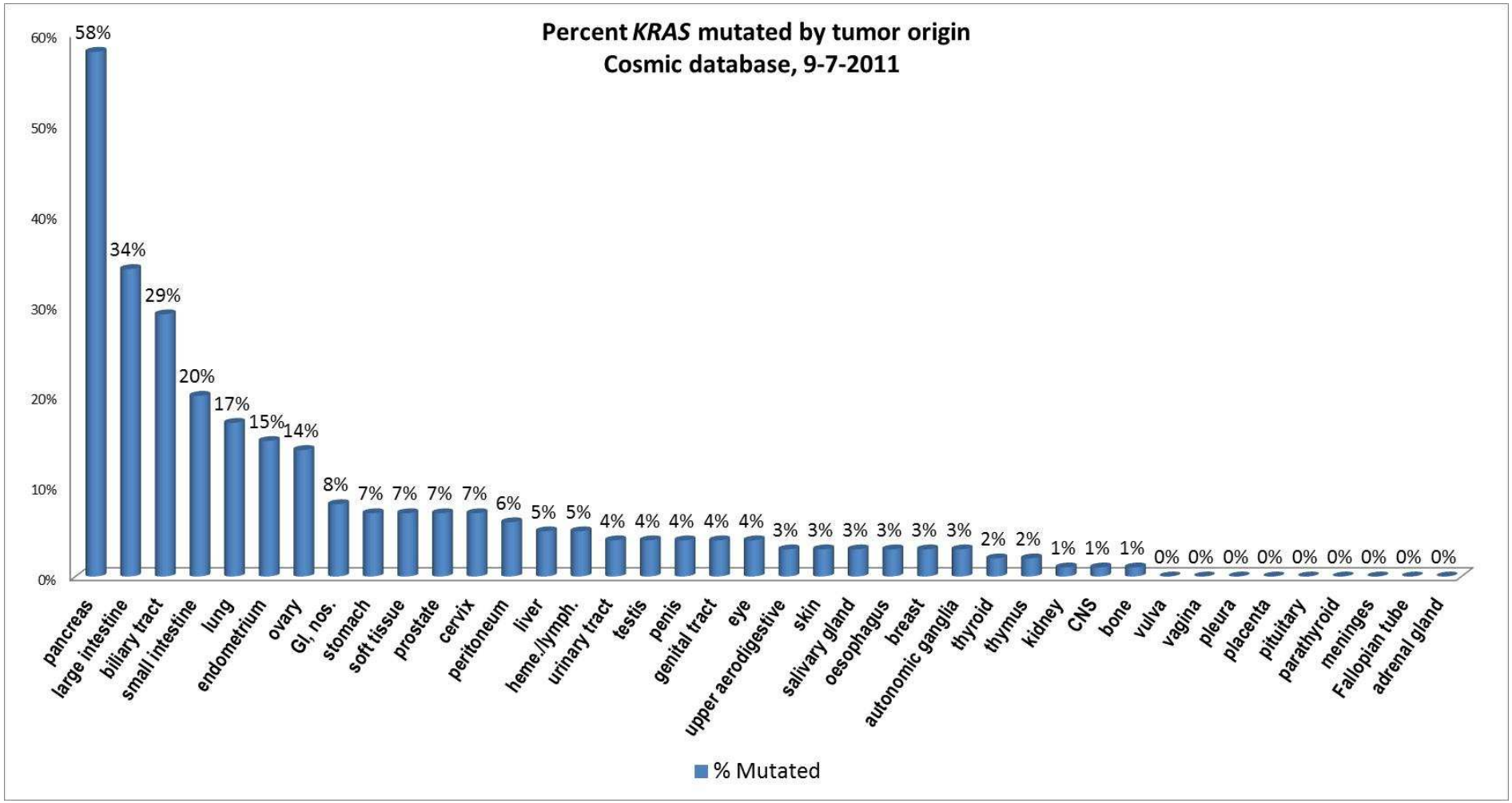
*Excellent review (Malumbres and Barbacid M Nat Rev Cancer 2003;3:459-65) listed in hand out



★ = mutations described

Nature Reviews | Cancer

Frequency of KRAS mutation in Human Tumors



KRAS as a Prognostic Marker (RASCAL)

Andreyev et al: *British Journal of Cancer* (2001) 85(5), 692–696

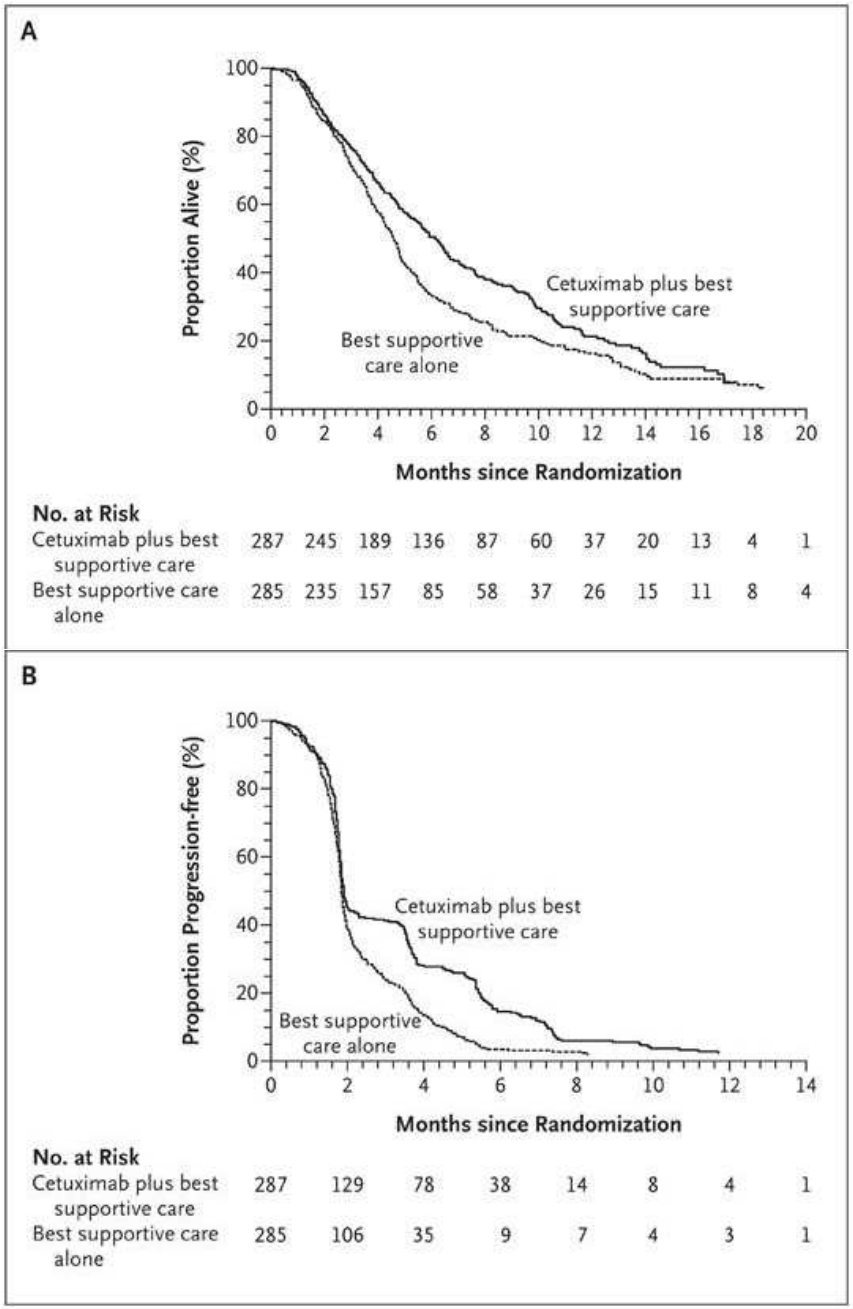
Table 5 2nd RASCAL study: results of the multivariate analysis when the effects of a valine mutation on the Dukes' B patients were analysed separately

	Total	Failure-free survival				Overall survival			
	n =	n = relapse or death	P-value	Hazard ratio	95% CIs	n = deaths	P-value	Hazard ratio	95% CIs
Dukes' stage B	1399	545				485			
Codon 12 mutations									
Valine	138	57	0.46	1.11	0.84–1.46	52	0.36	1.15	0.86–1.53
Aspartate	137	54	0.53	1.10	0.83–1.45	48	0.71	1.06	0.78–1.43
Cysteine	24	15	0.10	1.59	0.95–2.66	14	0.14	1.53	0.90–2.61
Serine	29	15	0.19	1.44	0.86–2.41	11	0.68	1.14	0.63–2.07
Alanine	28	10	0.55	1.22	0.65–2.28	8	0.66	1.18	0.58–2.37
Codon 13 mutations									
Aspartate	130	48	0.02	0.70	0.51–0.96	45	0.06	0.74	0.54–1.02
Mutation type									
Guanine to thymidine	168	74	0.08	1.25	0.98–1.59	69	0.11	1.24	0.96–1.60
Guanine to cytosine	44	16	0.30	1.30	0.80–2.12	15	0.25	1.37	0.82–2.30
Guanine to adenine	278	113	0.54	0.94	0.76–1.15	96	0.39	0.91	0.73–1.13

Table 6 The 2nd RASCAL study: results of the multivariate analysis, when the effects of a valine mutation on Dukes' C patients were analysed separately

	Total	Failure-free survival				Overall survival			
	n =	n = relapse or death	P-value	Hazard ratio	95% CIs	n = deaths	P-value	Hazard ratio	95% CIs
Dukes' stage C	1056	610	540						
Codon 12 mutations									
Valine	84	54	0.008		1.13–1.98	46	0.02	1.45	1.07–1.96
Aspartate	121	70	0.85	1.02	0.80–1.31	54	0.15	0.82	0.62–1.08
Cysteine	29	17	0.43	0.83	0.5 – 1.34	15	0.52	0.85	0.51–1.42
Serine	22	15	0.04	1.83	1.09–3.06	13	0.08	1.72	0.99–2.99
Alanine	20	12	0.82	1.07	0.60–1.90	11	0.72	1.12	0.61–2.03
Codon 13 mutations									
Aspartate	81	58	0.78	0.96	0.73–1.27	45	0.81	0.97	0.73–1.29
Mutation type									
Guanine to thymidine	119	70	0.10	1.24	0.97–1.58	63	0.13	1.23	0.95–1.60
Guanine to cytosine	28	16	0.79	1.07	0.66–1.73	16	0.62	1.14	0.69–1.87
Guanine to adenine	225	139	0.52	1.07	0.88–1.29	114	0.51	0.93	0.76–1.15

❖KRAS mutation subtype is marker for worse survivial



2007: Anti-EGFR antibody (Cetuximab) treatment improves PFS and OS in EGFR expressing colon carcinomas of 572 advanced **metastatic** tumors previously treated by conventional chemotherapy

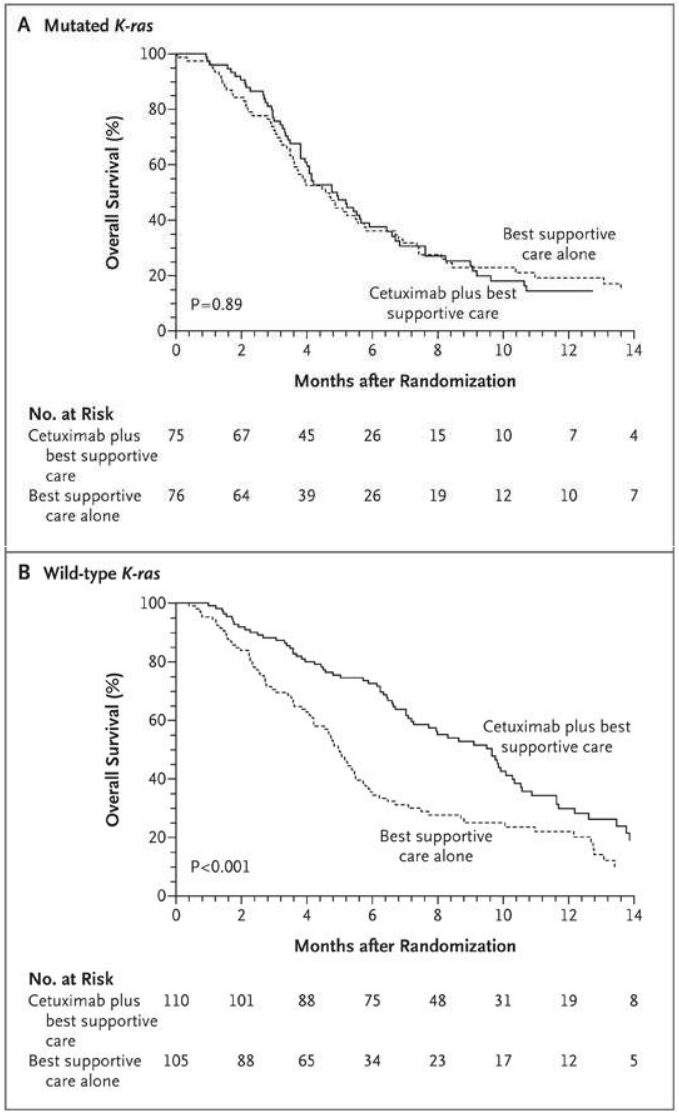
Kaplan-Meier Curves for Overall Survival (Panel A) and Progression-free Survival (Panel B)

Jonker DJ et al. N Engl J Med 2007;357:2040-2048

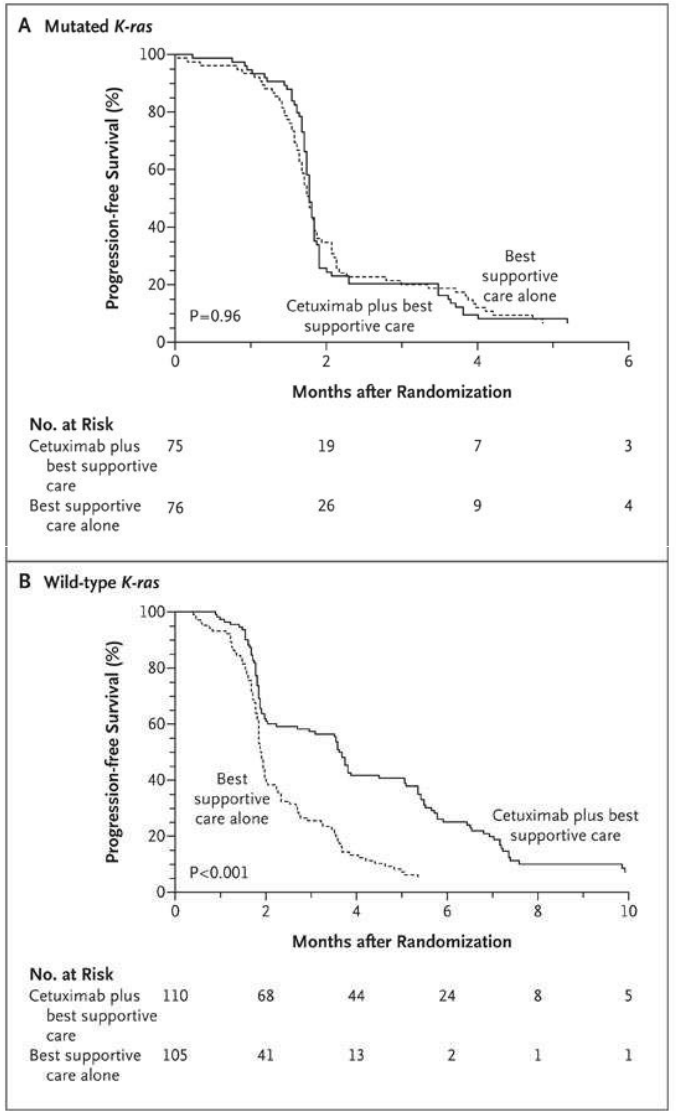
The NEW ENGLAND JOURNAL of MEDICINE

I wish I had held that stock a little longer

Kaplan-Meier Curves for Overall Survival According to Treatment



Kaplan-Meier Curves for Progression-free Survival According to Treatment



Karapetis CS et al. N Engl J Med 2008;359:1757-1765

The NEW ENGLAND JOURNAL of MEDICINE

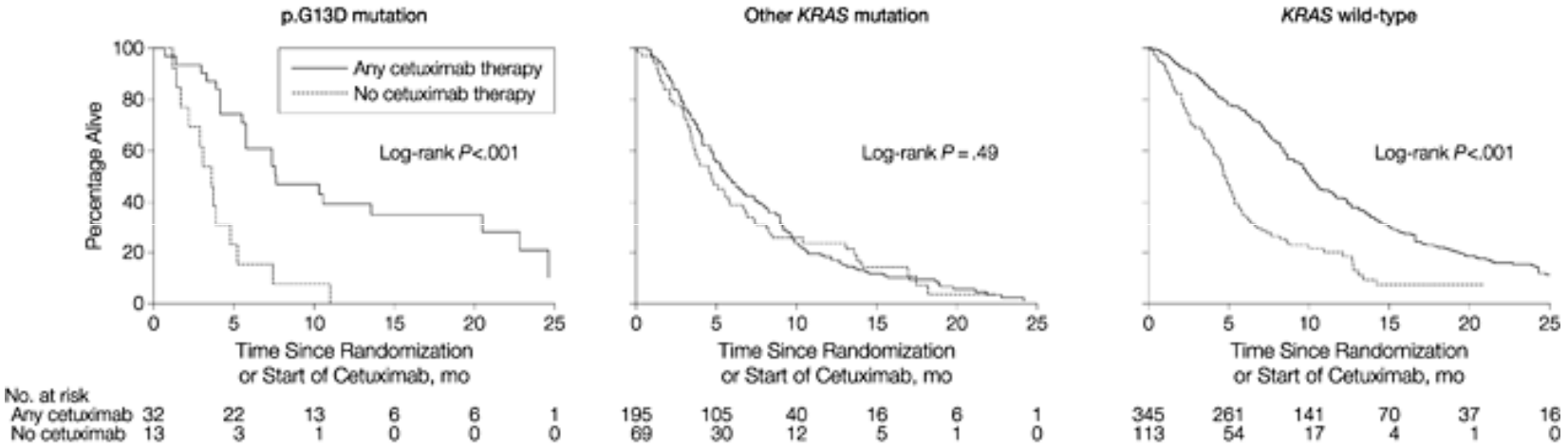
CRC Trials Incorporating KRAS testing

Study	Treatment	Pts	KRAS MT*	HR	KRAS WT*	HR	Reference
Amado	P versus BSC (3rd line)	427	7.4 wks	0.99	12.3 wks	0.45	JCO, 2008
Van Cutsem (CYRSTAL)	FOLFIRI + C (1st line)	540	7.6 mos	1.07	9.9 mos	0.68	NEJM, 2009
Bokemeyer (OPUS)	FOLFOX + C (1st line)	233	5.5 mos	1.83	7.7 mos	0.57	JCO, 2009
Karapetis	C versus BSC (3rd line)	572	1.8 mos	0.99	3.7 mos	0.40	NEJM, 2008

*PFS

How is KRAS testing done?
What factors affect testing?

Figure 1. Overall Survival: Predictive Analysis by KRAS Status for Patients Receiving Any Cetuximab-Based Therapy vs No Cetuximab



De Roock, W. et al. JAMA 2010;304:1812-1820

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Joint ASCO/CAP Statement on KRAS testing in CRC

American Society of Clinical Oncology Provisional Clinical Opinion: Testing for *KRAS Gene Mutations in Patients* With Metastatic Colorectal Carcinoma to Predict Response to Anti–Epidermal Growth Factor Receptor Monoclonal Antibody Therapy. Allegra et al, J Clin Oncol 2009;27:2091-6

Based on systematic reviews of the relevant literature, all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations in a CLIA-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients with metastatic colorectal carcinoma should not receive anti-EGFR antibody therapy as part of their treatment.

Questions Implicit in ASCO Statement

- Which patients should be tested and when should they be tested?
  - Mutual agreement between oncologist and laboratory when patients are considered for anti EGFR treatment
  - Reflex testing of metastatic CRC
- What samples are suitable for mutational testing?
  - "…*should be specifically chosen by a* ***pathologist*** *to include predominantly tumor cells without significant necrosis or inflammation."*

Multiple steps to detect mutations in clinical samples if you must

1. Obtain specimen
   - Surgical Resection
   - Biopsy
2. Preanalytical Processing
   - DNA Extraction
3. Testing platform
4. Data analysis

Factors Potentially Affecting Molecular Testing

1. Pre-analytical processing

- Type of fixation
  - Frozen tissue
  - Formalin breaks/unstacks and may bond covalently with DNA
    - PCR amplicons should be short
    - Sufficient DNA used in testing assay
- Selection of blocks for testing
  - Requires input of Pathologist
- Testing site (CLIA approval required)
- Sampling of tissue in paraffin blocks

Tissue Collection and Processing
Experience in CMOCO

- Although testing may be performed on fresh or frozen tissue, 3.7% buffered formalin is universal fixative that is now well accommodated in molecular platforms
- Surgical resection—usually no problem
- Biopsies (fine needle aspirates and cores)—may generate inadequate numbers of tumor cells
- Pitfalls
  - Excessive fixation: We recommend no more than 72 hours.
  - Inadequate fixation: Biopsy specimens should be fixed at least 6 hours. Resection specimens should be fixed overnight with a change in formalin to offset the effect of absorption of fixative by blood. Tissue should be sliced at 5 mm thickness during fixation.
  - Decalcification causes DNA deterioration and may be a cause amplification failure. Decalcification status should be kept in mind in interpretation of testing results.
  - Minimum cells per slide <100 (10 pg/cell x 100 cells = 1 ng/100 cells)

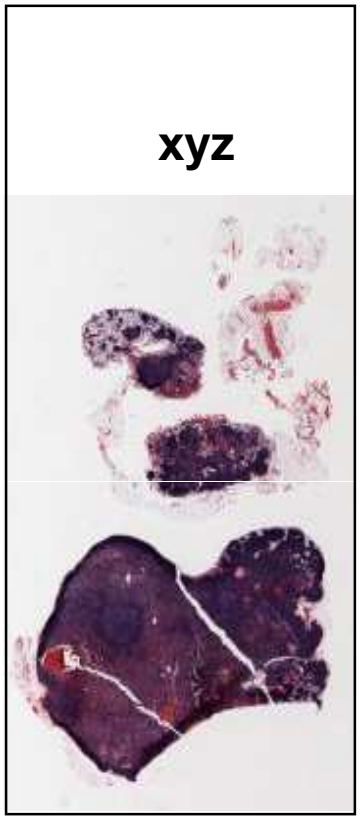
Documentation: A record of histological examination for specimen adequacy is retained by the testing laboratory, and includes:

- Time from removal to fixation (warm ischemia time, if available)
- Length of fixation time (if available)
- Presence of tumor
- Histological tumor type
- Percentage of viable tumor present in the specimen
- Final determination of specimen adequacy

Methods of Tumor Cell Enrichment

- Sections from face of trimmed block (scrolls): Easy but minimal but relative unselective
- Macrodissection: scrape from slide with microscopic assistance suitable for homogeneous tumors
- Microdissection: removal of cells from stained sections with assistance of dissecting microsope
- Laser capture: very selective but expensive and generally not needed
- Coring paraffin block: quick effective and generative excellent quality DNA

Methods of Tumor Cell Enrichment



Microdissection or scape of paraffin sections

1 mm tumor-enriched core
Beecher Instruments Tissue Microarrayer

Tissue must be adequately deparaffinized (overnight at room temperature)

Factors Potentially Affecting Molecular Testing

2. Properties of testing platforms

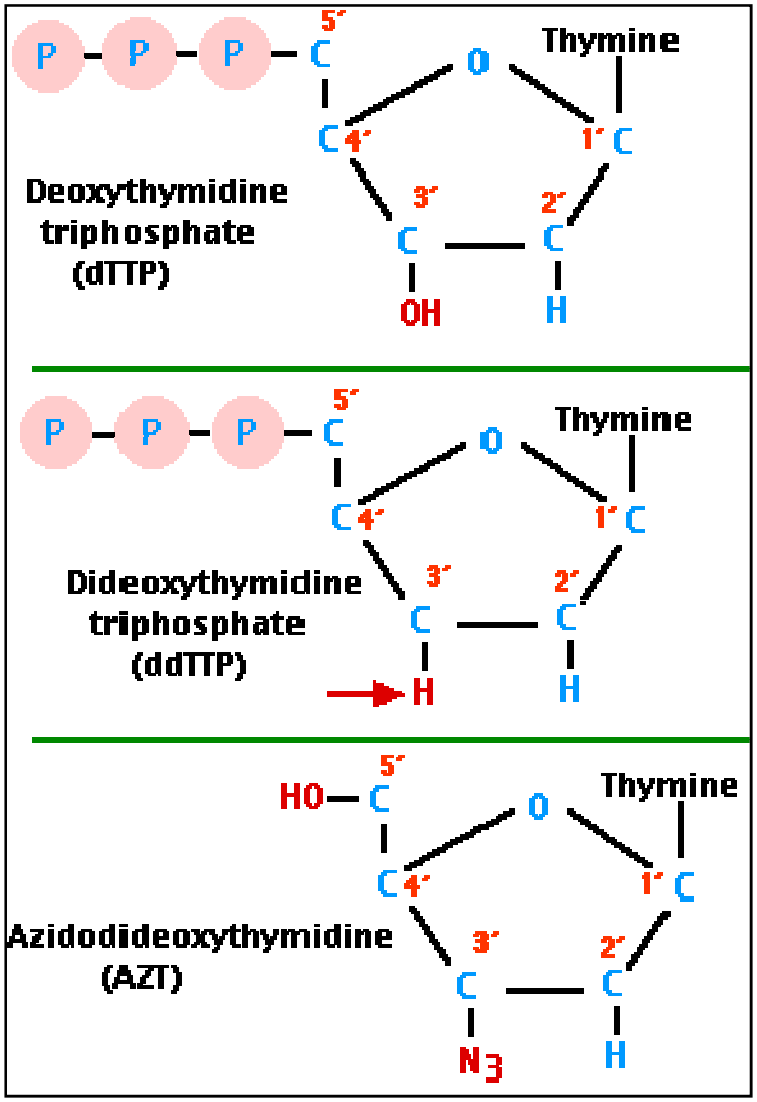
- Sensitivity
- Specificity
- Coverage of gene
- Turnaround time
- Simplicity
- Costs
  - Startup
  - Operating cost
- Clinical Validation

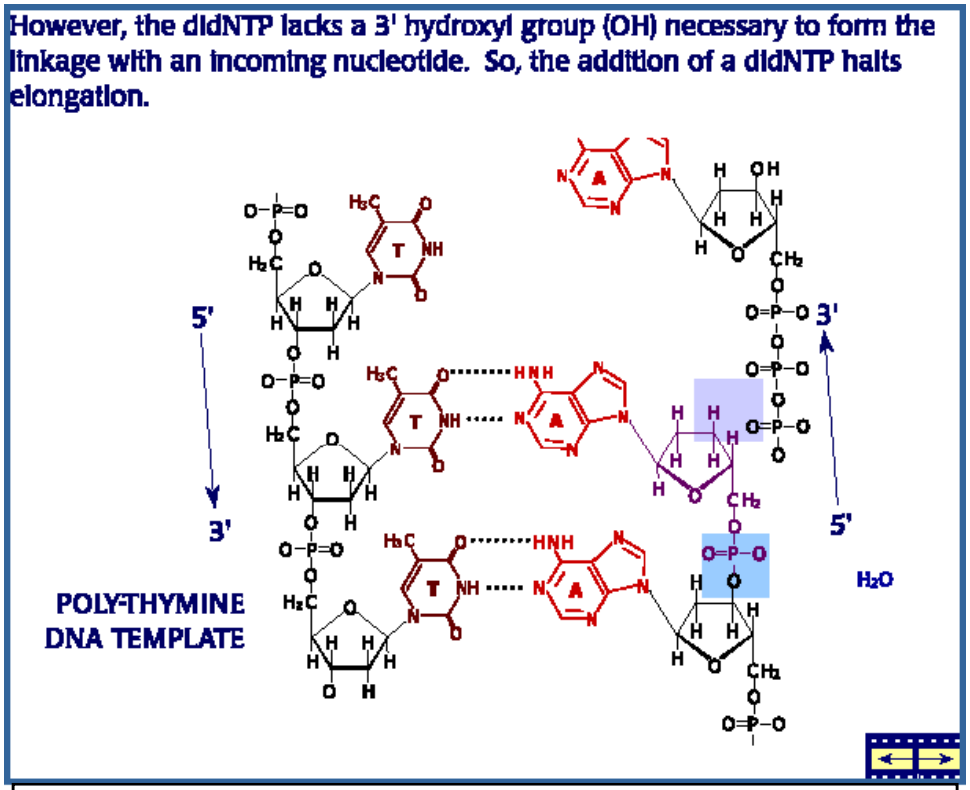
Recent *KRAS* Testing Methods

Method	Principle
PCR + Sanger sequencing (dideoxy chemistry)	Non-mutation-specific sequence comparison with normal sequence
High resolution melting (HRM) analysis (Roche Lightcycler)	Sequences with mutations hybridize at different, fixed temperatures
Bifunctional primer/probe (ARMS/Scorpion, DxS)	Mutation specific PCR/detection
Maldi-tof mass spectrometry array (Sequenom iPLEX)	Single base extension with mass bias nucleotide probes
Capillary electrophoresis, single base extension (SNaPshot, ABI)	Single base extension with florescent nucleotide probes
Pyrosequencing sequencing	PCR with phosphorescent probes

Sanger Sequencing

Dideoxy nucleotide inhibition of DNA synthesis due to nonreativity of 3' hydrogen





Animated version available at:
http://www.dnalc.org/ddnalc/resources/sangerseq.html

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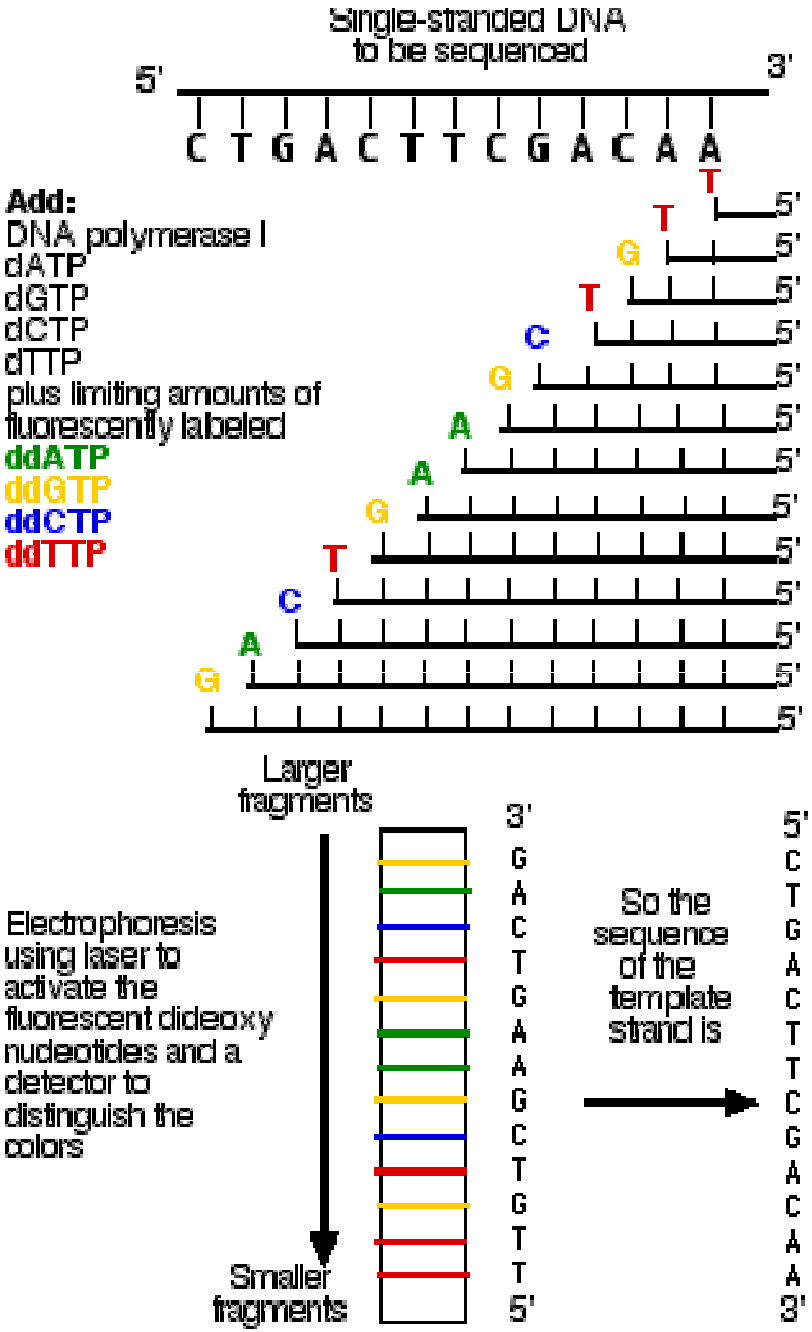

Many methods now available for detection of sequences derived from the dideoxy-reaction including:

- **Radioautography (P-32)**
- **Gel chromatography**
- **Capillary Electrophoresis**
- **MALDI-TOF (Sequenom)**

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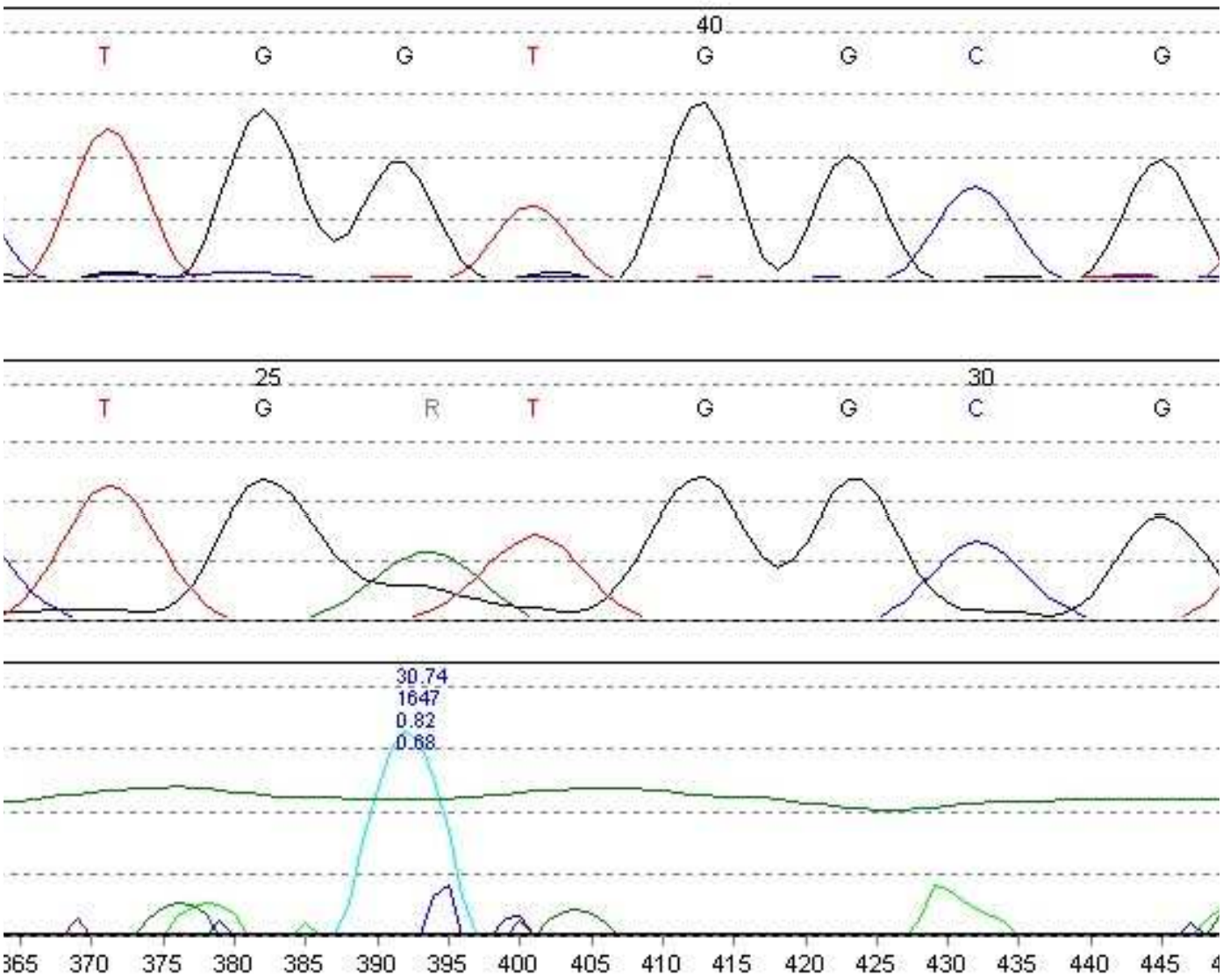
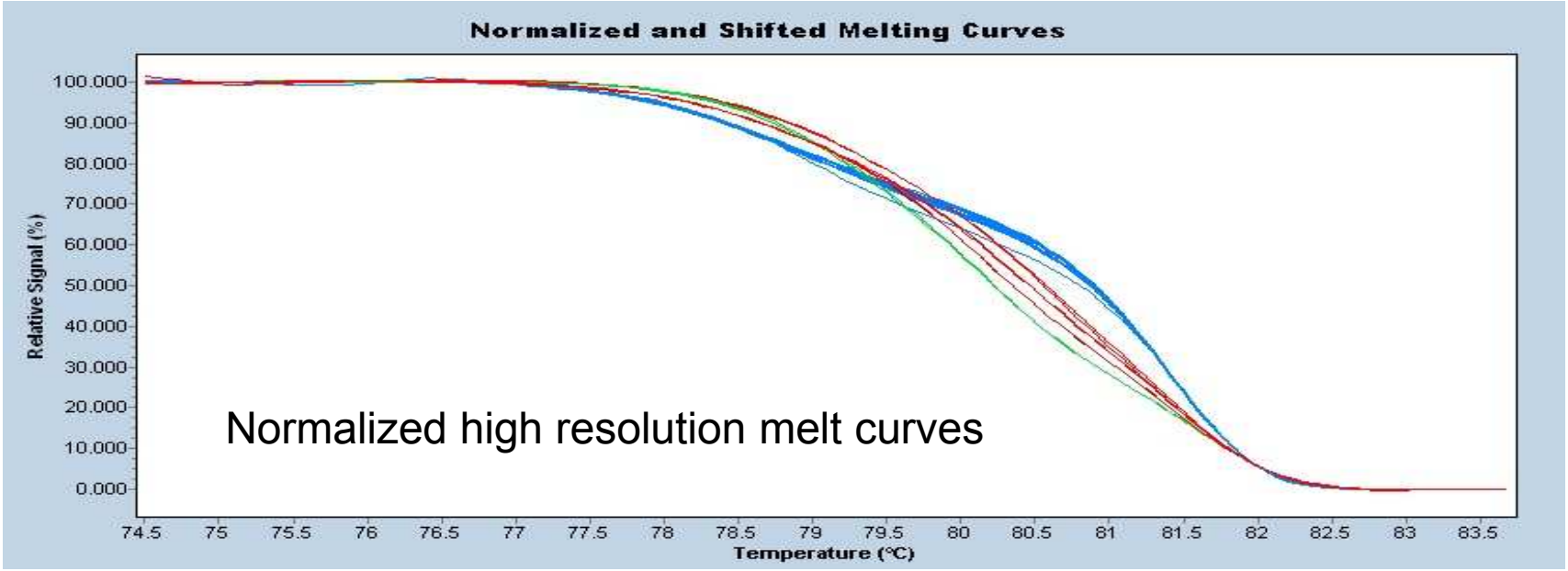
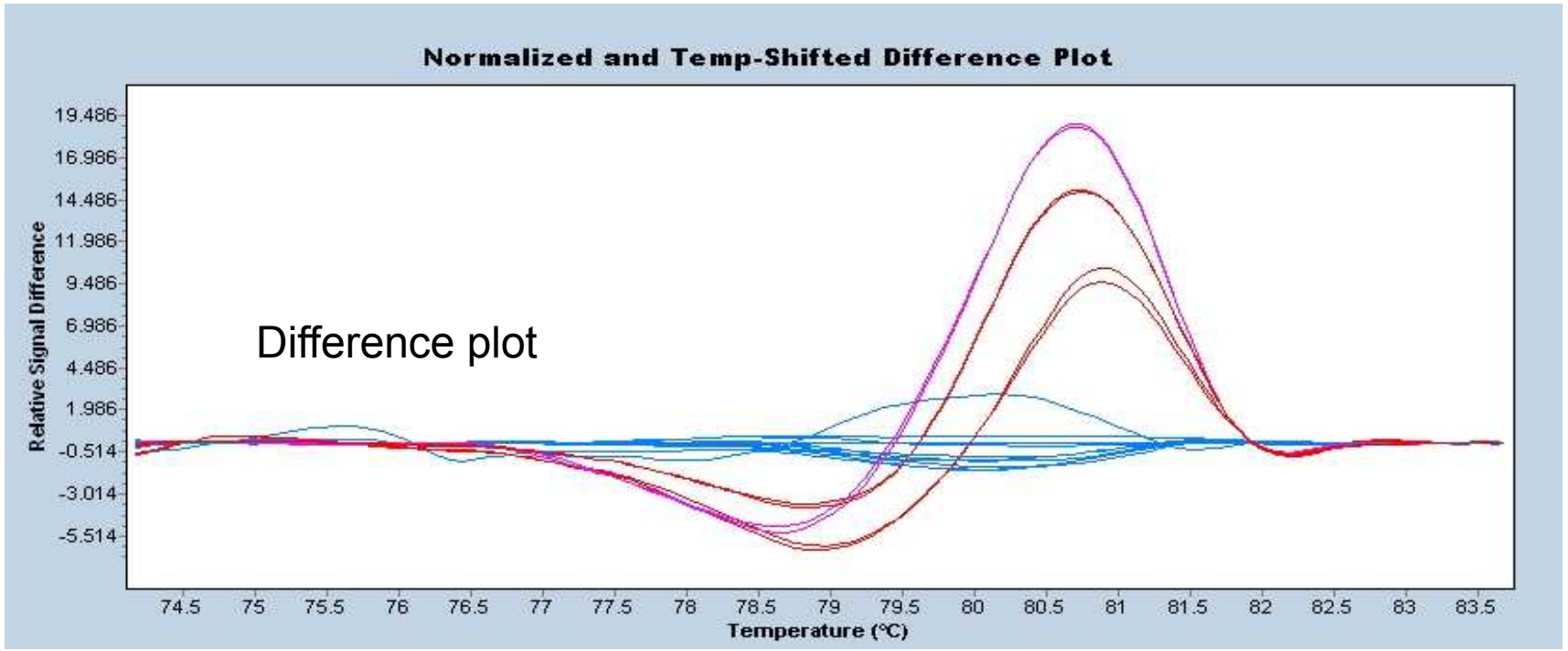


Mutation Surveyor readout for *KRAS* codon 12 mutation (GGT→GAT, G12D)

A.

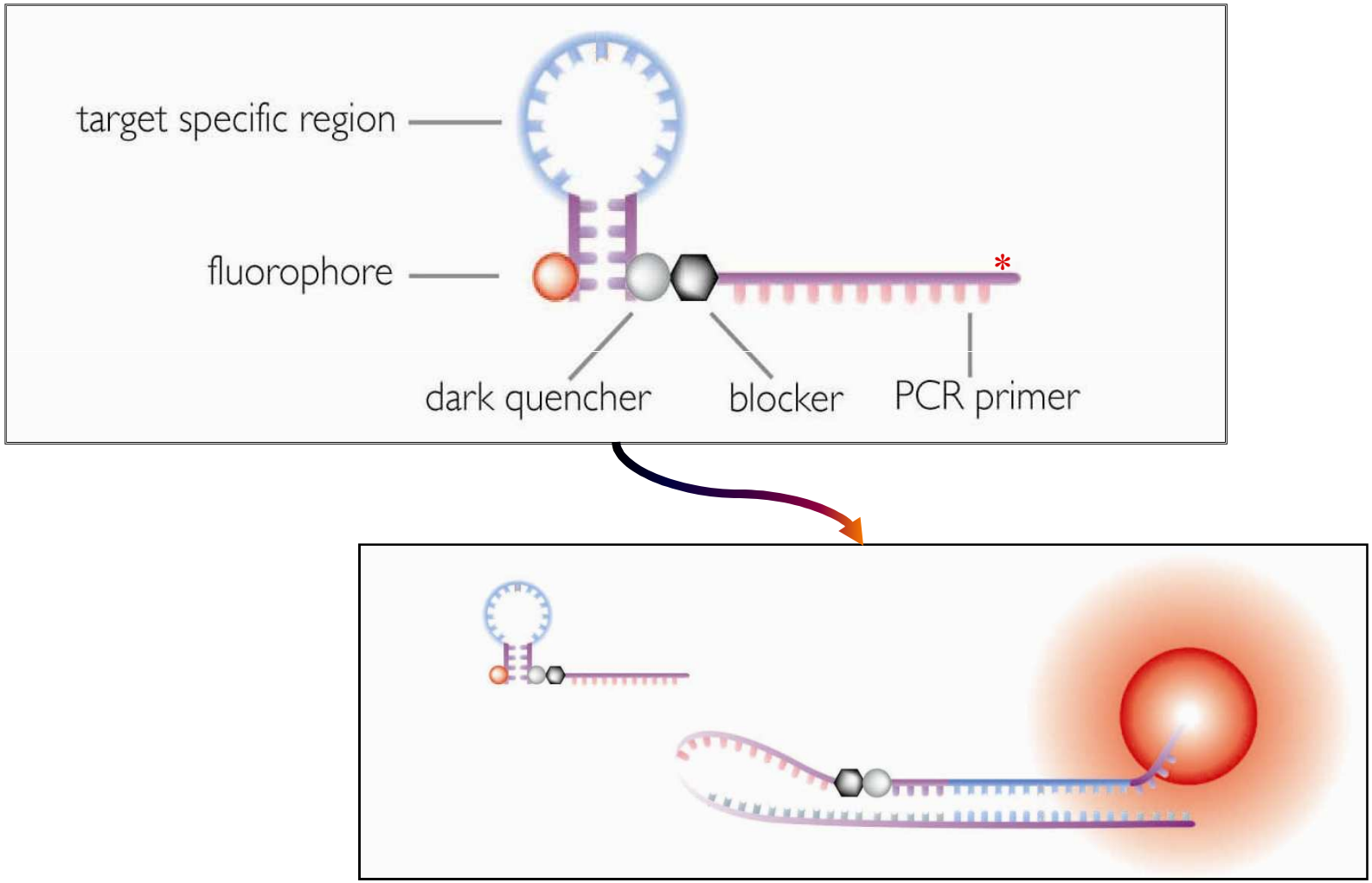

Normalized and Shifted Melting Curves
Relative Signal (%)
100.000
90.000
80.000
70.000
60.000
50.000
40.000
30.000
20.000
10.000
0.000
74.5
75
75.5
76
76.5
77
77.5
78
78.5
79
79.5
80
80.5
81
81.5
82
82.5
83
83.5
Temperature (°C)
Normalized high resolution melt curves

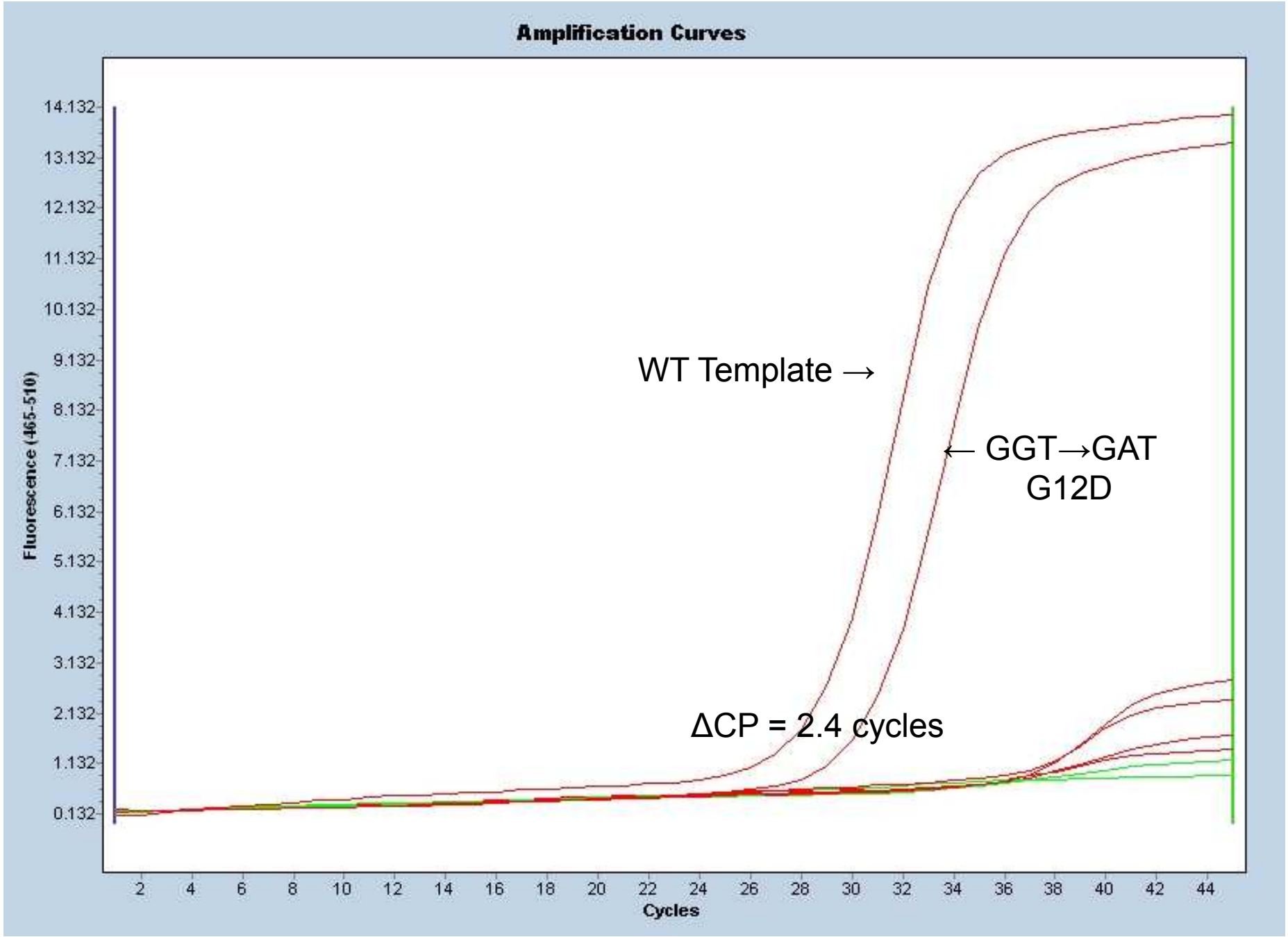
B.


Normalized and Temp-Shifted Difference Plot
Relative Signal Difference
19.486
16.986
14.486
11.986
9.486
6.986
4.486
1.986
-0.514
-3.014
-5.514
74.5
75
75.5
76
76.5
77
77.5
78
78.5
79
79.5
80
80.5
81
81.5
82
82.5
83
83.5
Temperature (°C)
Difference plot

Activation of Scorpions Probes During PCR

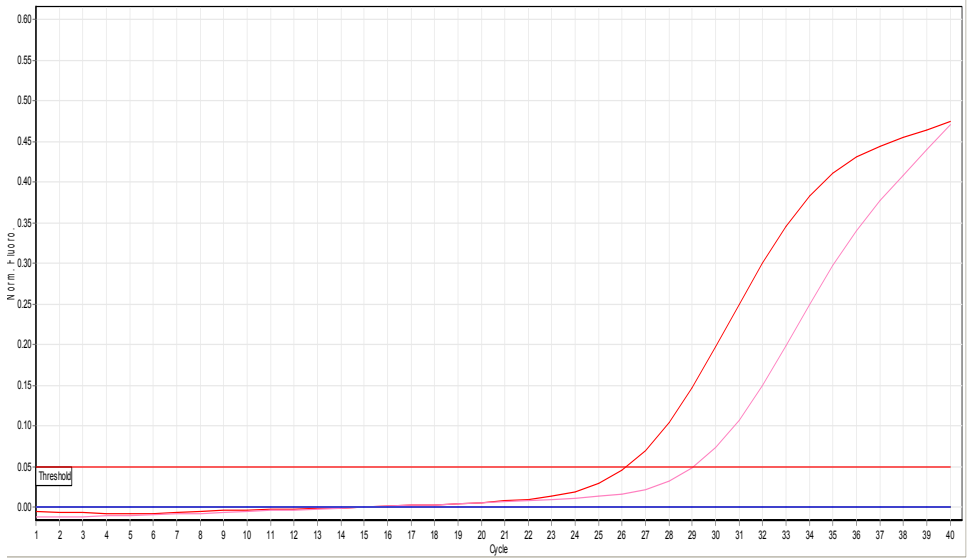
(depicted on DxS website)




Amplification Curves
Fluorescence (465-510)
14.132
13.132
12.132
11.132
10.132
9.132
8.132
7.132
6.132
5.132
4.132
3.132
2.132
1.132
0.132
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
Cycles
WT Template →
← GGT→GAT
G12D
ΔCP = 2.4 cycles

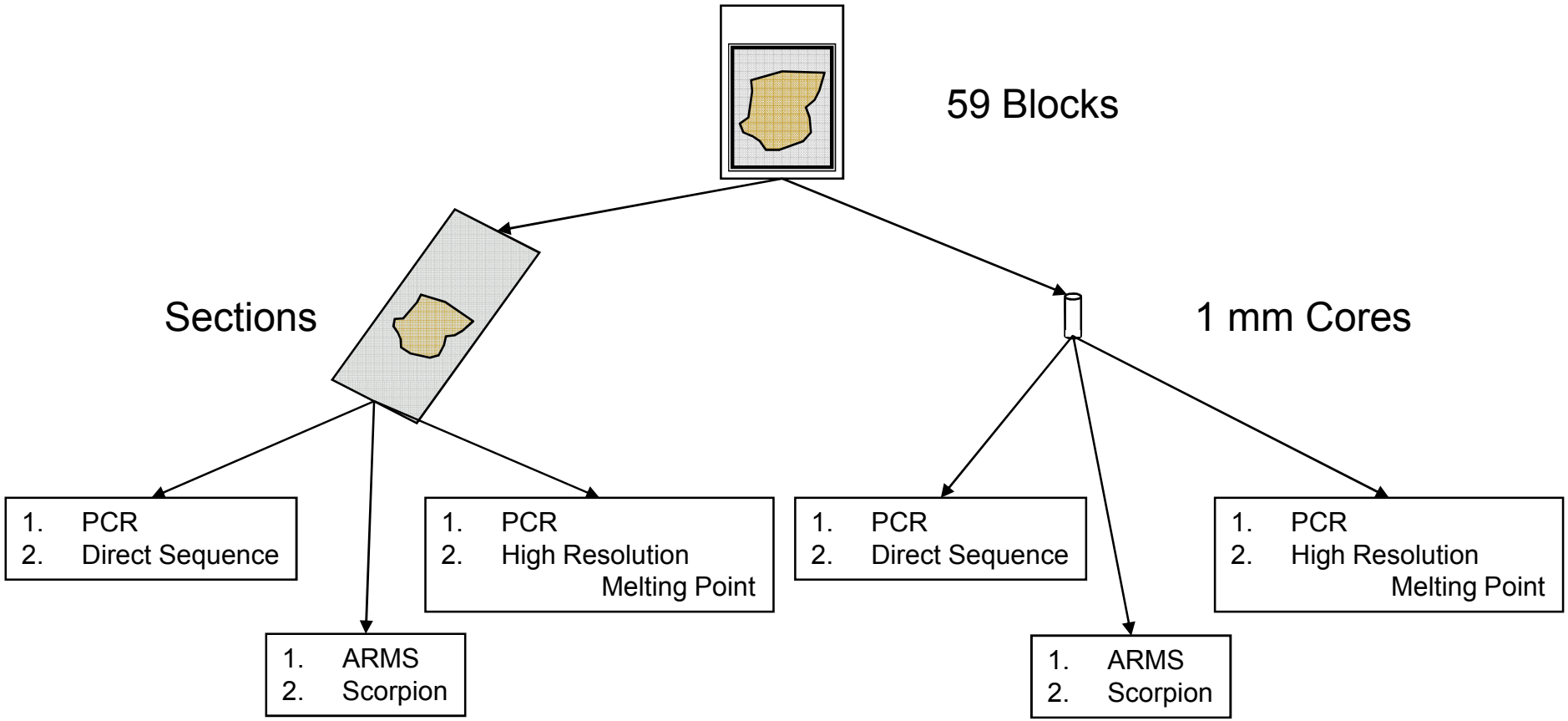
Qiagen RGQ
Preliminary Experience

		AA subst.	CT	ΔCT	
25	CUD000001158	WT	26.2		
26	CUD000001158	12ALA			WT
27	CUD000001158	12ASP			WT
28	CUD000001158	12ARG	29.06	2.86	M
29	CUD000001158	12CYS			WT
30	CUD000001158	12SER			WT
31	CUD000001158	12VAL			WT
32	CUD000001158	13ASP			WT



- DxS recent acquired by Qiagen
- ARMS/Scorpions technology now marketed through the Rotogene Q platform (RGQ)
- qPCR occurs in heated centrifuge chamber equipped with fluorescence detector.
- Paired with Qiagility sample prep station
- KRAS results similar to those obtained using Roche Lightcycler platform
- Initial testing suggests that nothing lost in moving KRAS to new RGQ platform

Which testing platform is best?
Schema for *Ki-ras* Testing Study



708 data points (total)

Figure 1

Cross Comparison
Sampling and Testing Methods

	ARMS/S		HRM		Direct Seq	
	Mutant	**%**	**Mutant**	**%**	**Mutant**	**%**
Sections	24	41%	31	53%	19	32%
Cores	27	46%	33	56%	23	39%

- 6 specimens had abnormal HRM curves but were not confirmed by ARMS/S or direct sequencing—false positive
- 4 ARMS/S-positive/Direct Seq-negative cases were cloned and resequenced DNA had concentration of 1/12 to 1/24 mut/wild type

Single Gene Platforms Employed in CRC Trials Assessing *KRAS*

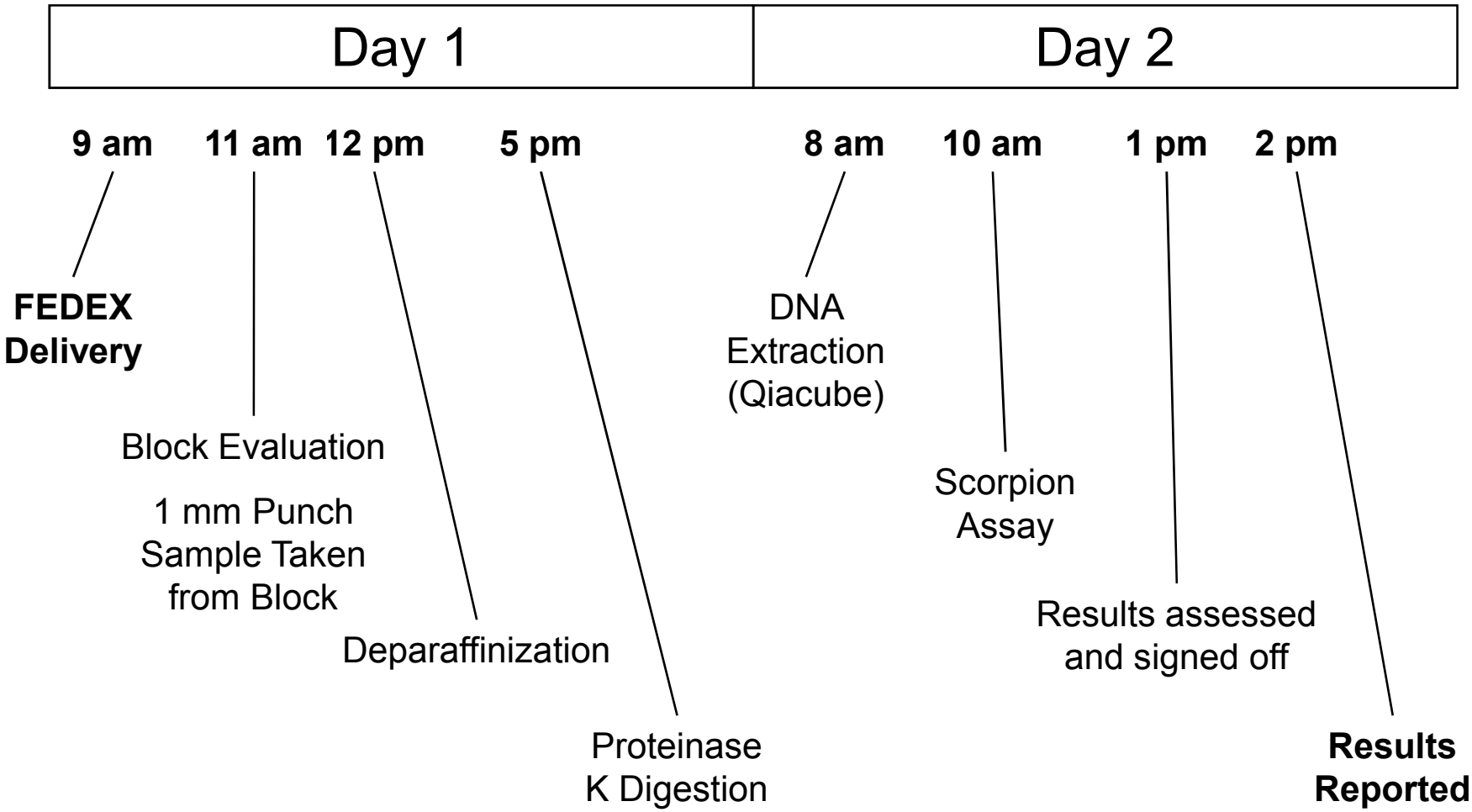
Study	Method	% mutant	p value PFS benefit
Amado	ARMS/S	43%	0.0001
Van Cutsem	HRM + clamp*	36%	0.02
Bokemeyer	HRM + clamp	42%	0.016
Karapetis	Sanger	42%	0.001

*Results confirmed by ARMS/S

Similar percentages are mutation positive regardless of testing methodology

- Small differences in sensitivity and specificity may not be reflected in these trials
- None of the platforms detect codon 61 or other pathway mutations

Performance of ARMS/Scorpions under field conditions C80405*



*A Phase III Trial of Irinotecan/5-FU/Leucovorin or Oxaliplatin/5-FU/Leucovorin with Bevacizumab, or Cetuximab (C225), or with the Combination of Bevacizumab and Cetuximab for Patients with Untreated Metastatic Adenocarcinoma of the Colon or Rectum

Performance of ARMS/Scorpions*
CALGB/Intergroup 80405 (N=2151)

AA Substitution	Codon	Mutant (N)	%
12ALA	GGT→GCT	40	1.9%
12ASP	GGT→GAT	261	12.1%
12ARG	GGT→CGT	17	0.8%
12CYS	GGT→TGT	87	4.0%
12SER	GGT→AGT	51	2.4%
12VAL	GGT→GTT	210	9.8%
13ASP	GGC→GAC	171	7.9%
Totals		837	39.0%

*Assay misses 12PHE (0.2%), 12ILE (0.1%), 13CYS (0.3%), 13ARG (0.1%), codon 61 (8%), codon 146 (0.1%)

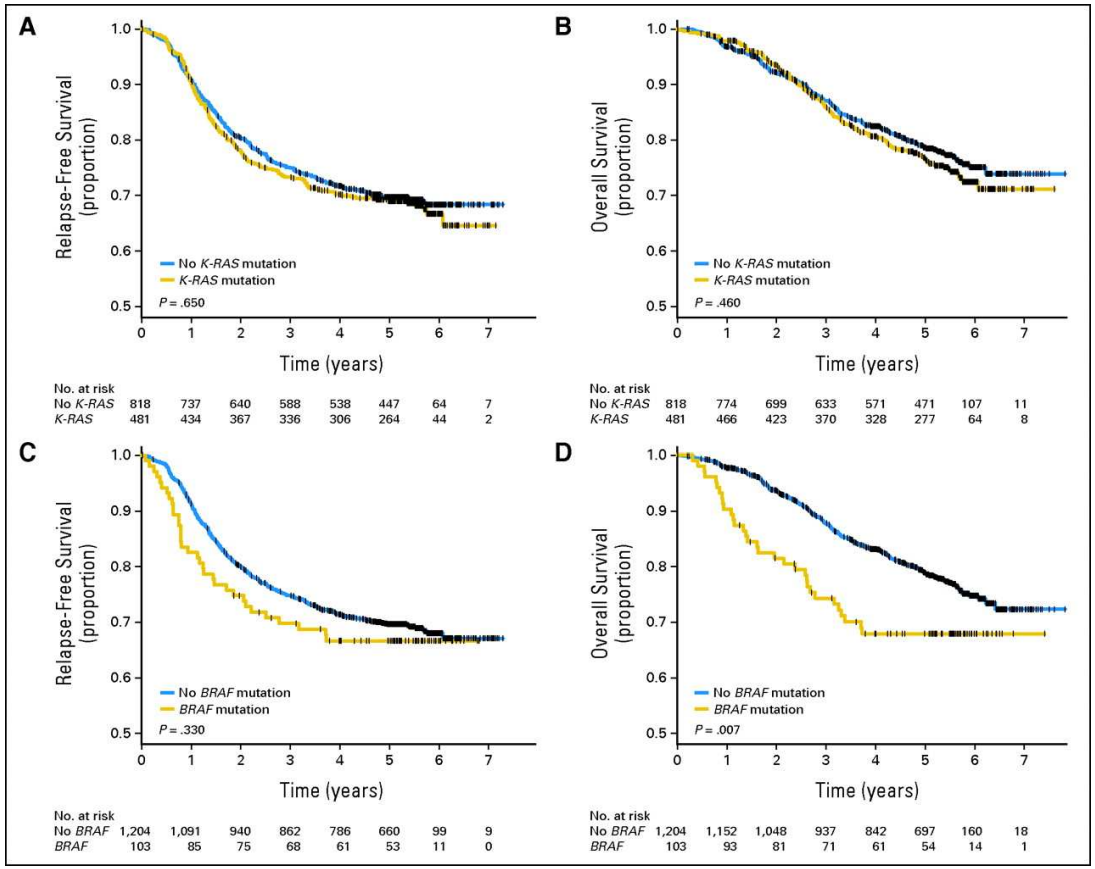
Comparative ratings of KRAS testing methods

Testing Method	*Analytical Sensitivity*	*Specificity*	*Coverage of gene*	*Turnaround time*	*Simplicity*	*Startup Costs*	*Cost per exon*	*Clinical Validation*
Sanger Seq	+	+++	+++	-	-	High	Mod	+
HRM	++	-	+	+++	+++	Mod	Low	-
HRM + Clamp	+++	++?	+	+++	++	Mod	Mod	+
ARMS/Scorpion	+++	+++	+	+++	++	Mod	High	+
iPLEX mass spectrometry	++	+++	+	+++	+	High	Low	-
Pyrosequencing	++	+++	+++	++	+	Mod	Mod	-
KRAS Scorecard								

Conclusions--Methods

- Several methods have similar but not identical sensitivities
- Method must be validated and performance characteristics defined before application to clinical material (CLIA certification required)
- Single mutation testing likely to give way to high throughput, multimutation platforms in near future
- Additional mutation and copy number data are likely to refine therapeutic targeting and provide an improved biological basis for treatment of CRC

Fig 1. Relapse-free survival (RFS) and overall survival (OS) curves estimated by the method of Kaplan-Meier for stage II and III colorectal cancer comparing cases respectively with and without mutations in KRAS (A: RFS; B: OS) and BRAF (C: RFS; D: OS)



- Stage II and III tumors
- BRAF a druggable target
- Early detection of mutation important

Roth, A. D. et al. J Clin Oncol; 28:466-474 2010

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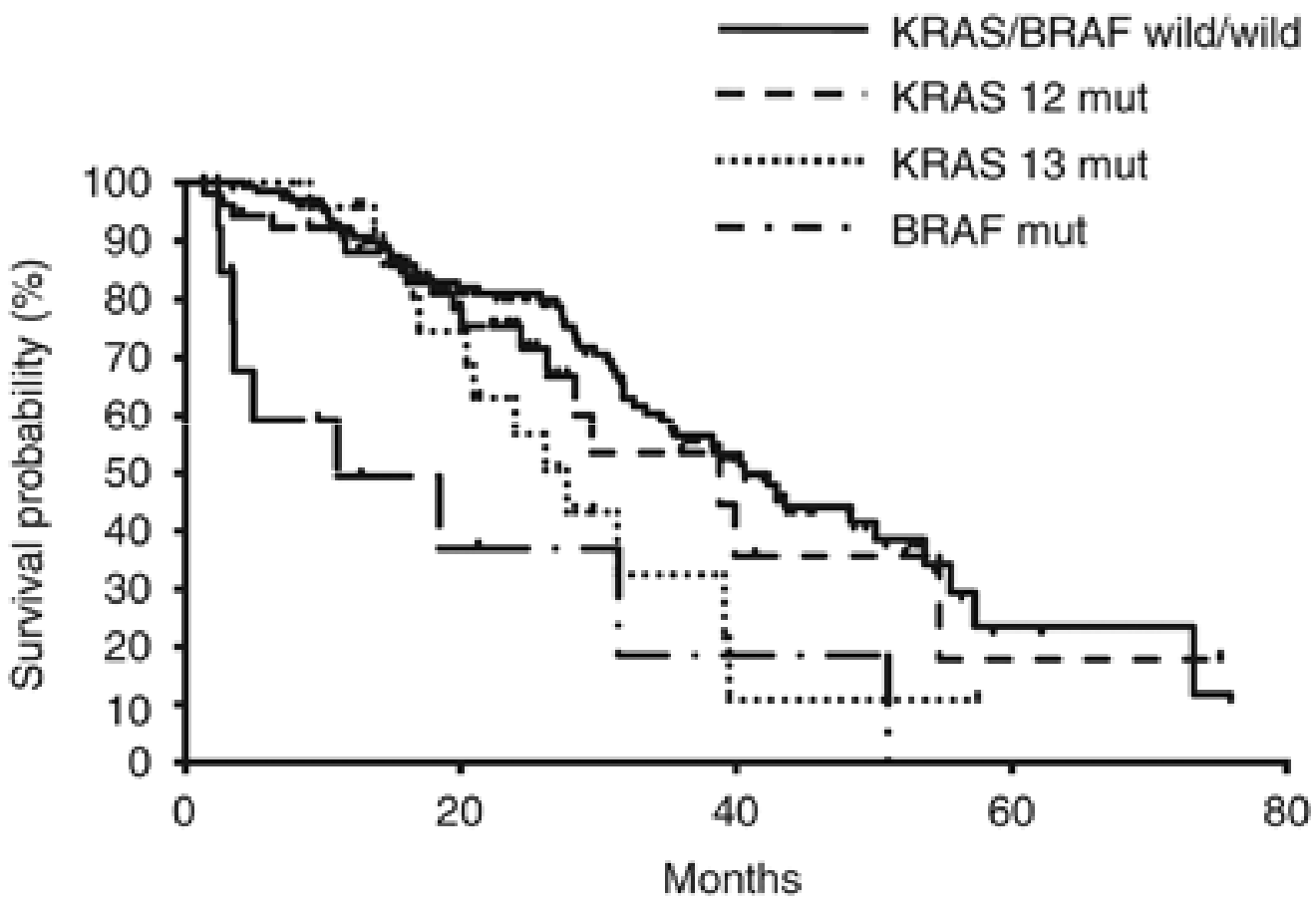
BRAF as a Prognostic Marker

T Yokota *et al*, *British Journal of Cancer* (2011) **104**, 856–862.



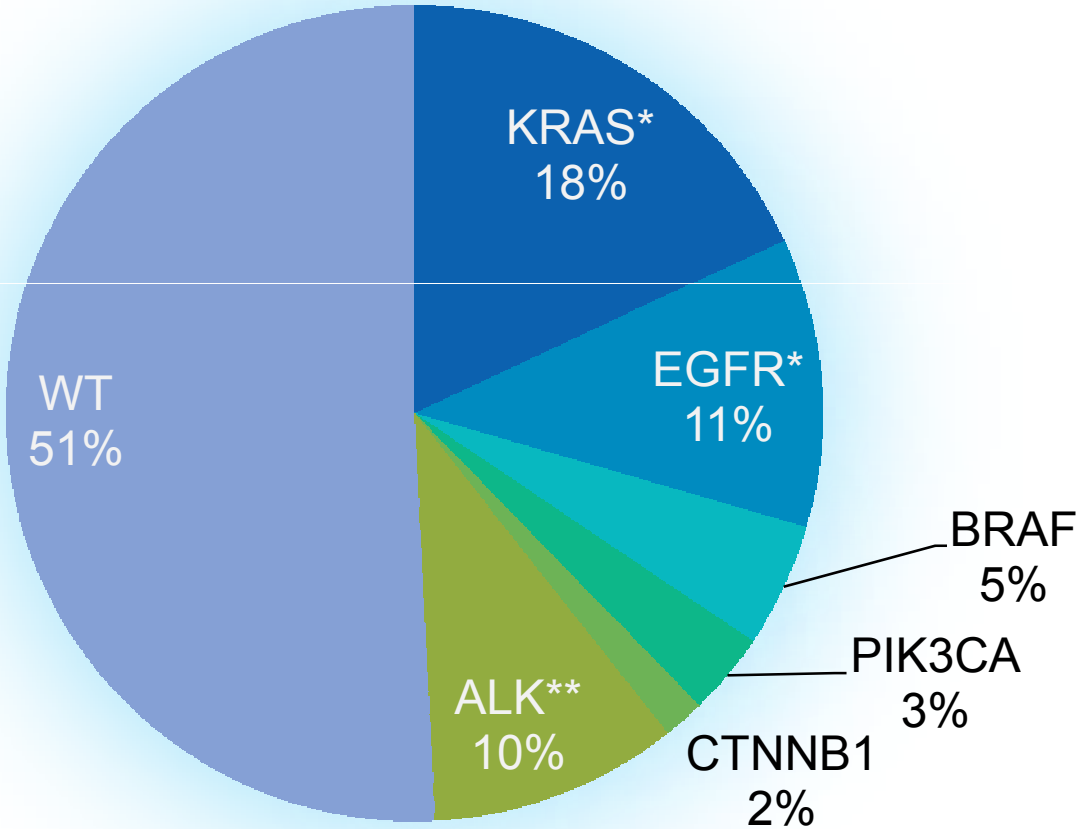
Kaplan–Meier plot showing overall survival in metastatic and recurrent colon cancer patients according to KRAS and BRAF V600E mutational status (n=229). mut, mutated.

Next Gen used for Lung Adenocarcinomas

- 1,013 non-synonymous somatic mutations
- 163 of the 188 tumors
  - 915 point mutations
  - 12 dinucleotide mutations (mutations affecting two consecutive bases on same allele)
  - 29 insertions
  - 57 deletions

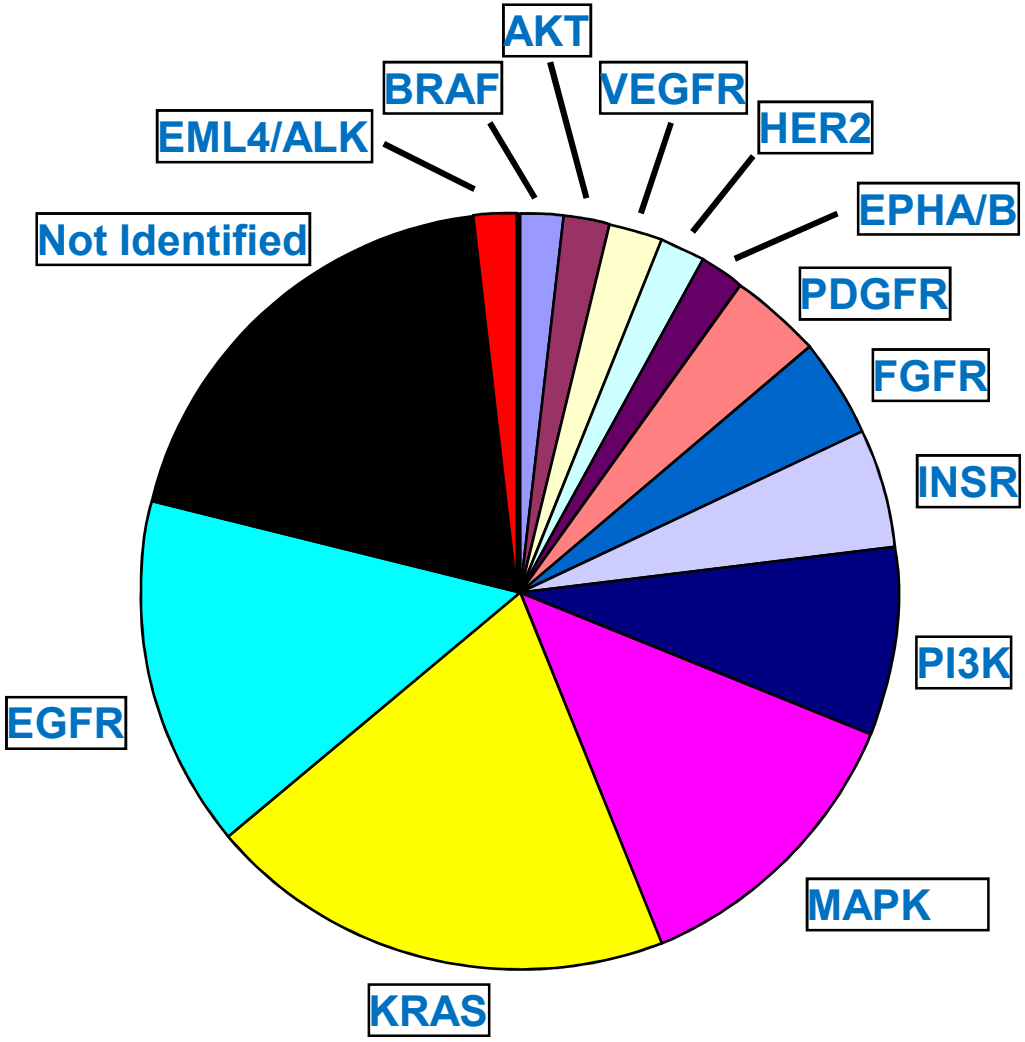
Molecular Binning of Cancer

Mutations in Lung Adenocarcinoma
SNaPshot Platform, N=61
Lung Cancer Mutation Consortium
Colorado Laboratory



**TP53* mutation, one case
***SNaPshot -/*ALK*+

Somatic Mutations Known Oncogenes in Lung Adenocarcinoma N=188 Tumors and 623 Genes



Modified from L Ding *et al. Nature* 455, 1069-1075 (2008)

Dealing with the Lung Cancer Genome
Lung Cancer Mutation Consortium

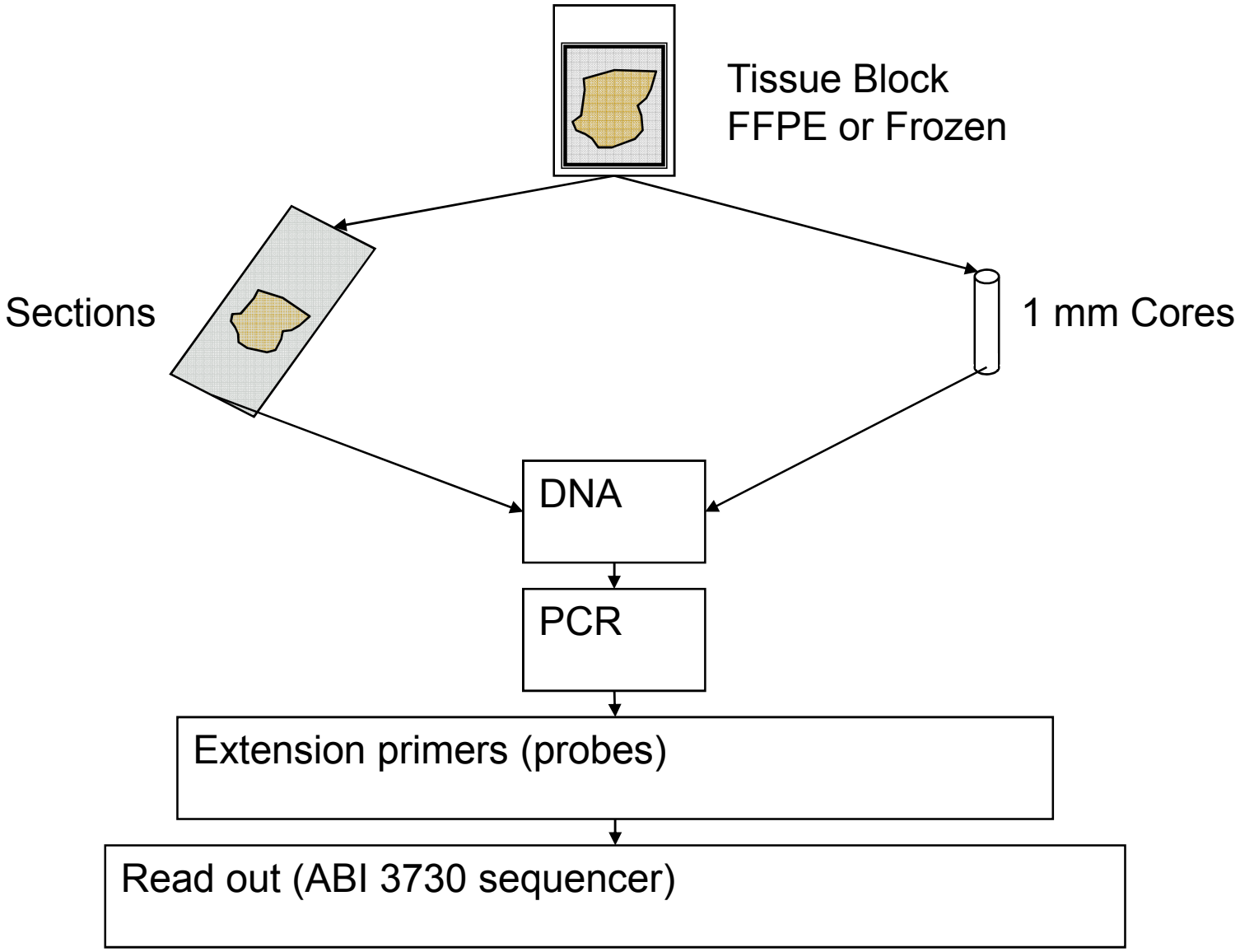
Aims

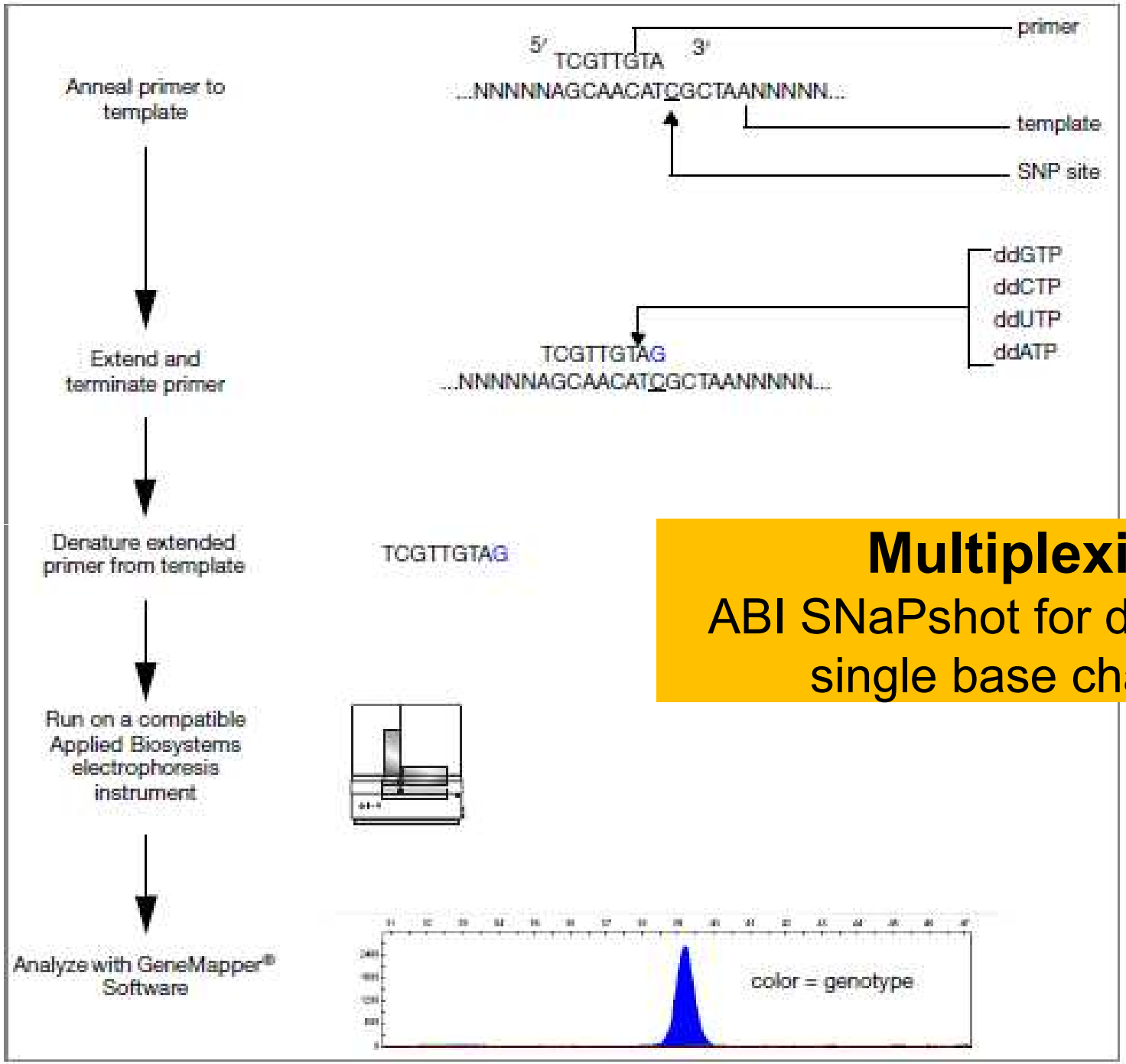
- Establish the LCMC with institutions experienced in lung cancer clinical trials and molecular testing with an administrative core, a clinical operations core, a web-based clinical trial network, a pathology core and protocol, CLIA and LCMC certified mutation analysis cores, and validated protocols for testing multiple mutations in multiple oncogenes;
- Develop and implement a clinical trial in 1000 patients with advanced lung adenocarcinoma that includes clinical information linked with tissue collection and mutation analysis;
- Analyze the tumor tissue from the 1000 lung adenocarcinoma patients on the trial for mutations in multiple oncogenes in CLIA and LCMC approved labs;
- Describe the clinical features and prognosis associated with specific oncogene mutations and the link between each oncogene and their signal pathways.
- Develop an annotated bank with lung adenocarcinoma specimens from lung cancer tissue, PBMC, serum, plasma, and urine from the 1000 patients for future research testing and,
- Provide the scientific basis and feasibility for future mutation specific therapeutic trials with novel agents aimed at these mutated targets using the identified cases for enrollment.

Consortium Members

Institution	PI	NCI Cancer Center	NCI Lung Spore
Mass General	Jeff Engelman	x	x
DFCI	Bruce Johnson	x	x
MSKCC	Mark Kris	x	
Pittsburgh	Jill Siegfried	x	x
Vanderbilt	William Pao	x	x
Colorado	Paul Bunn	x	x
Johns Hopkins	Stephen Baylin	x	x
HL Moffitt	Gerold Bepler	x	x
UTSouthwestern	Joan Schiller		x
MD Anderson	Ignacio Wistuba	x	x
UCLA	Steven Dubinett	x	x
Emory	Fadlo Khuri	x(new)	pending
NCI Intramural	Guiseppe Giaccone	N/A	N/A

SNaPshot work flow

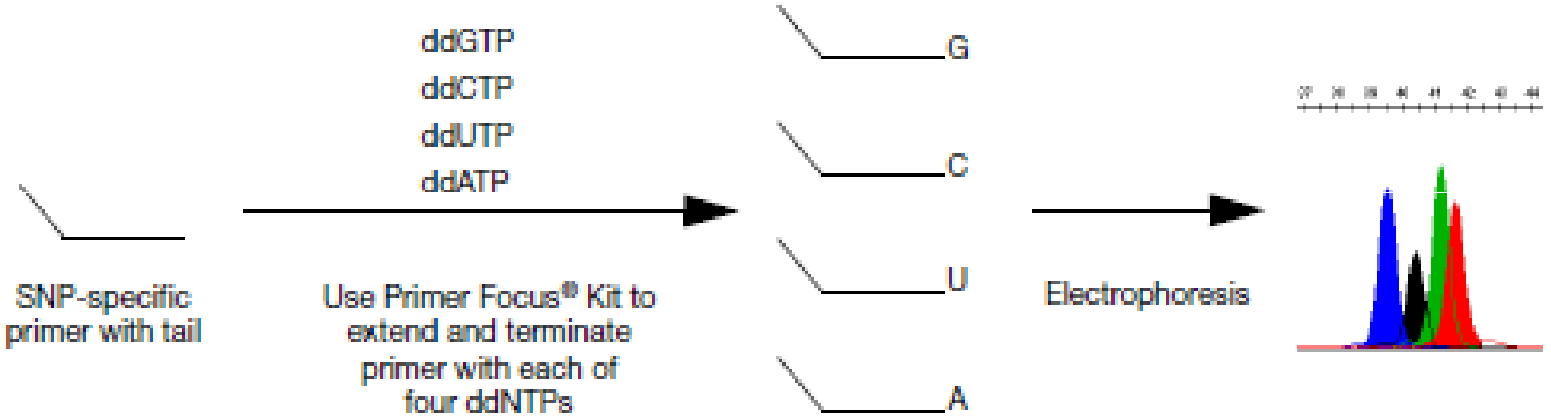




Multiplexing

ABI SNaPshot for detection of single base changes

Verifying Primers



GO Gene Panel
APC
AKT1
BRAF
CTNNB1
EGFR
FLT3
JAK2
KIT
KRAS
MAP2K1 (MEK1)
NOTCH1
NRAS
PIK3CA
PTEN
TP53

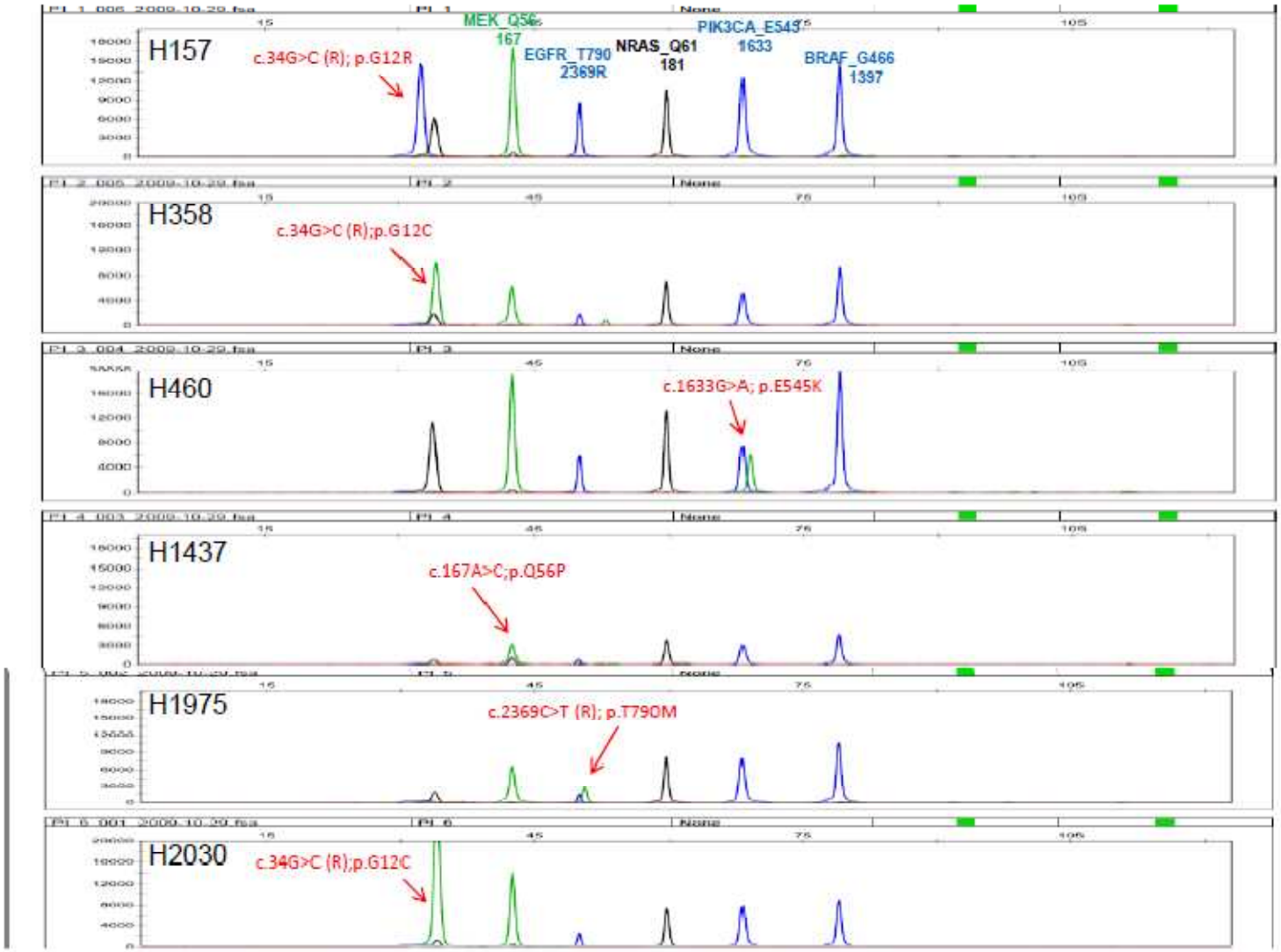
Representative Primer Sets*

Gene	Exon	Mutated Amino Acid	Allele / SNaPshot Extension Primer
BRAF	15	L597?	BRAF1798_extF
BRAF	15	V600?	BRAF1799_extF
CTNNB1	3	G34?	CTNNB1_101_extF
CTNNB1	3	T41?	CTNNB1_109_extF
CTNNB1	3	T41?	CTNNB1_110_extF
CTNNB1	3	S45?	CTNNB1_121_extR
CTNNB1	3	S45?	CTNNB1_122_extR
CTNNB1	3	D32?	CTNNB1_94_extF
CTNNB1	3	S33?	CTNNB1_95_extR
CTNNB1	3	G34?	CTNNB1_98_extF
EGFR	18	G719?	EGFR2155_extF
EGFR	19	Exon 19 deletions	EGFR2235_2249del_extF
EGFR	19	Exon 19 deletions	EGFR2235_2249del_extR
EGFR	19	Exon 19 deletions	EGFR2236_2249del_extF
EGFR	19	Exon 19 deletions	EGFR2236_2249del_extR
EGFR	20	Exon 20 insertions	EGFR2369_extR
KRAS	2	G12?	KRAS34_extR
KRAS	2	G12?	KRAS35_extF
KRAS	2	G13?	KRAS37_extF
KRAS	2	G13?	KRAS38_extF
NRAS	3	Q61?	NRAS181_extF
NRAS	3	Q61?	NRAS182_extF
NRAS	3	Q61?	NRAS183_extR
PIK3CA	10	E542?	PIK3CA1624_extR
PIK3CA	10	E545?	PIK3CA1633_extF
PIK3CA	10	E545?	PIK3CA1636_extF
PIK3CA	10	E545?	PIK3CA1637_extF

- Over 69 primer sets for >200 mutations in 15 genes require standardization
- Run in sets (panels) of 5-6 primer
- Heavy pipetting burden requires robotics

Panel Validation (Courtesy Wm. Pao Vanderbilt)

Panel I

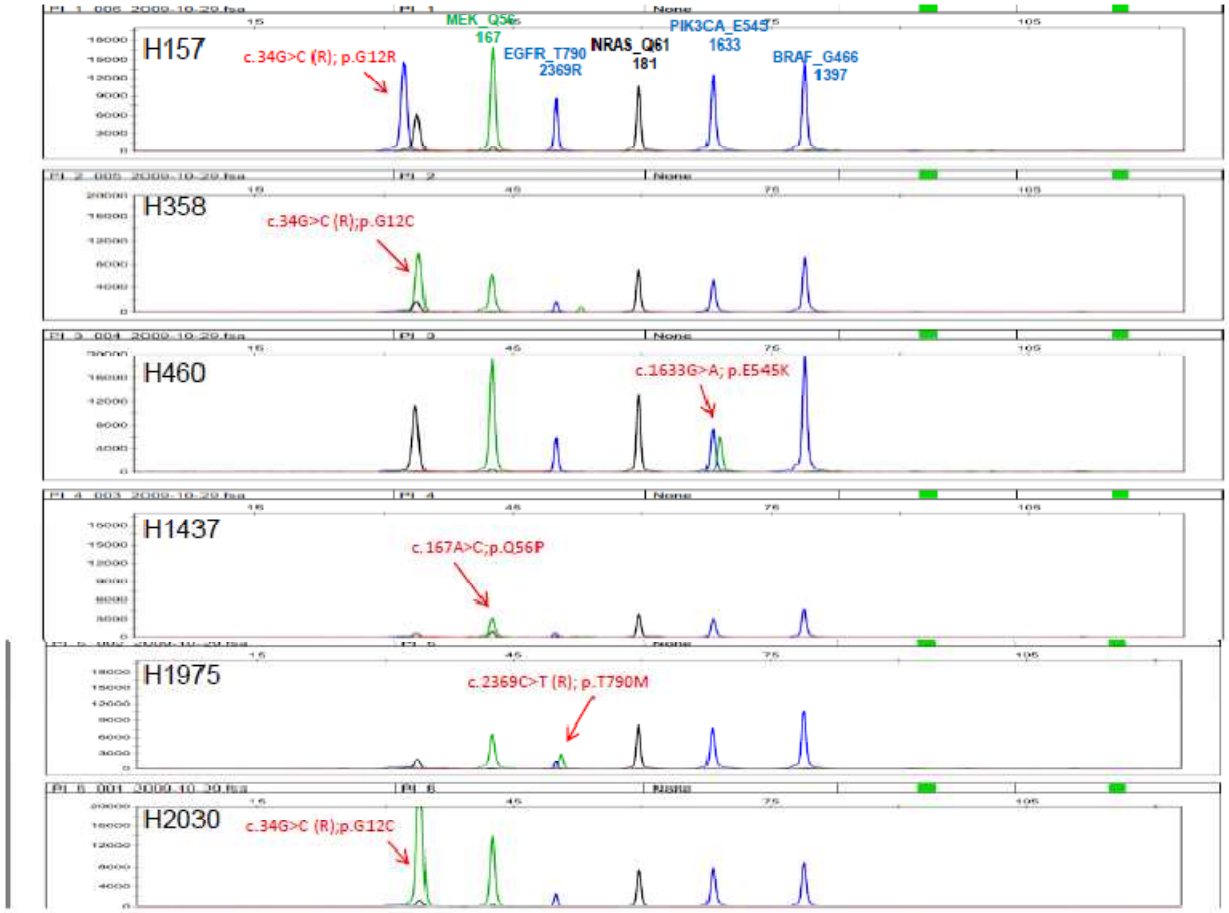


Now cutting edge of mutational testing of solid tumors (but probably transitional)

- Multiplex testing—15-20 gene
  - SNaPshot (combined with FISH)
  - Sequenome
- Chemistry same for different platforms
  - Single base extension with labeled nucleotides
- Permits binning of tumors into therapeutic groups

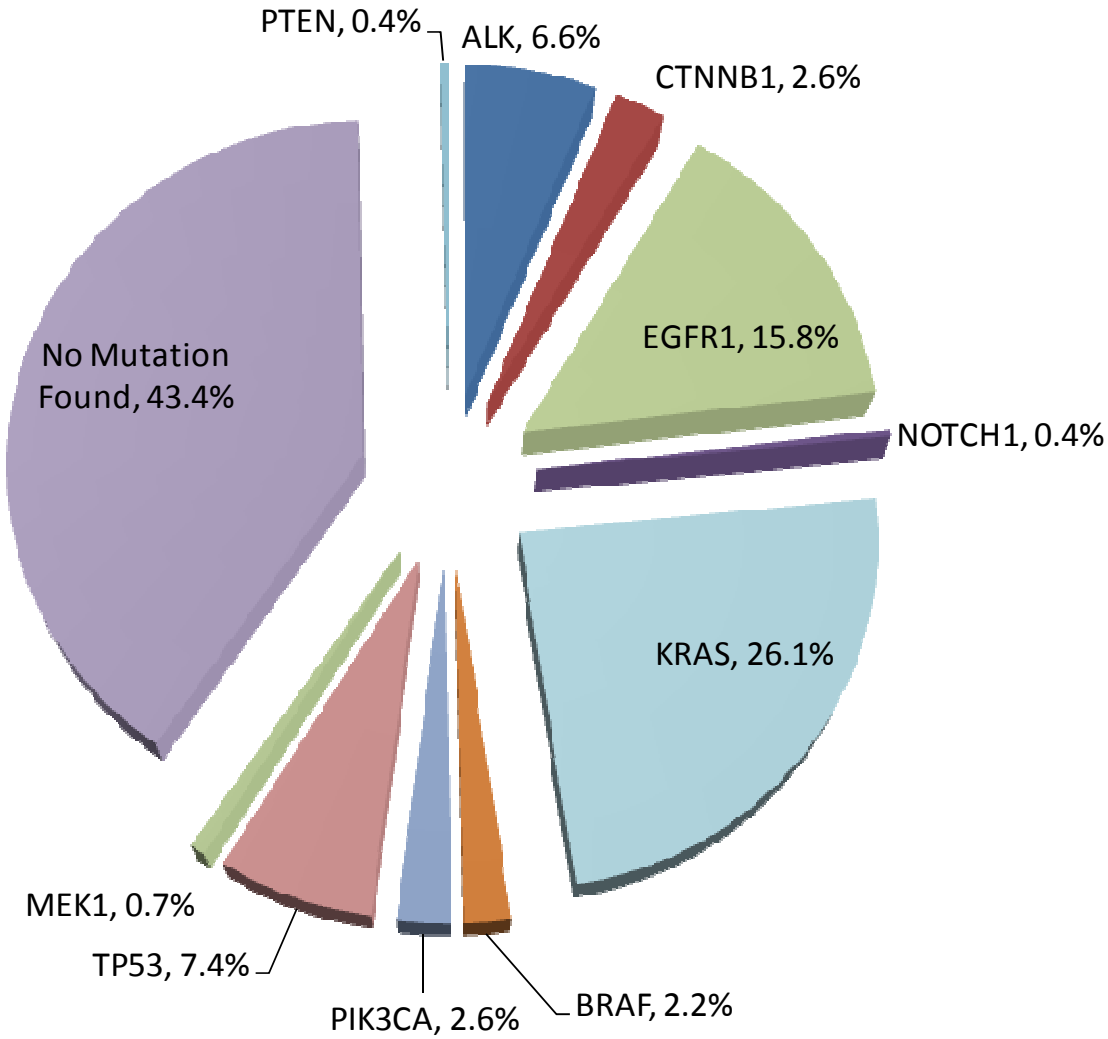
GO Gene Panel
APC
AKT1
BRAF
CTNNB1
EGFR
FLT3
JAK2
KIT
KRAS
MAP2K1 (MEK1)
NOTCH1
NRAS
PIK3CA
PTEN
TP53

Panel I



Molecular Binning of Cancer

Mutations in Lung Adenocarcinoma
SNaPshot Platform, N=272
Lung Cancer Mutation Consortium/Colorado Laboratory



Sequenome:
Comparison of results and added mutation info

Validation set of 32 colon carcinomas

Gene	KRAS				cMET	PIK3CA	EGFR	BRAF
Method	DxS	HRM	Dseq	Sqom	Sqom	Sqom	Sqom	Sqom
C001	G13D	MT	G13D	G13D	WT	E545K	WT	WT
C002	WT	WT	WT	WT	WT	WT	WT	WT
C003	G12C	MT	WT	G12C	T992I	WT	WT	V600E
C004	WT	WT	WT	WT	WT	WT	WT	WT
C005	G13D	MT	WT	G13D	WT	WT	WT	WT
C006	WT	WT	WT	WT	WT	WT	WT	V600E
C007	G12D	MT	G12D	G12D	WT	WT	WT	WT
C008	WT	WT	WT	WT	WT	WT	WT	V600E
C009	G12C*	?	WT	WT	WT	WT	WT	WT
C010	WT	MT	WT	WT	WT	E545K	WT	WT
C011	G12V	MT	G12V	G12V	T992I	WT	WT	WT
C012	WT	WT	WT	WT	WT	WT	WT	WT
C013	G12C	MT	G12C	G12C	WT	WT	A750P and	WT
C014	WT	WT	WT	WT	WT	WT	WT	V600E
C015	G12V	MT	G12V	G12V	WT	WT	WT	WT
C016	WT	WT	WT	WT	WT	WT	WT	WT
C017	G12V	MT	G12V	G12V	WT	WT	WT	WT
C018	WT	WT	WT	WT	WT	WT	WT	WT
C019	G12V	MT	G12V	G12V	WT	WT	WT	WT
C020	G12C	MT	G12C	G12C	R970C	WT	WT	WT
C021	G12D	MT	G12D	G12D	WT	E545K	WT	WT
C022	WT	WT	WT	WT	WT	WT	WT	WT
C023	G12D	MT	G12D	G12D	WT	WT	WT	WT
C024	WT	WT	WT	WT	WT	WT	WT	WT
C025	G13D	MT	G13D	G13D	WT	WT	WT	WT
C026	WT	WT	WT	WT	WT	WT	WT	WT
C027	WT	?	WT	WT	WT	WT	WT	WT
C028	G12V	MT	G12V	G12V	WT	WT	WT	WT
C029	WT	WT	WT	WT	WT	WT	WT	WT
C030	G12V	MT	G12V	G12V	WT	WT	WT	WT
C031	WT	?	WT	WT	WT	WT	WT	WT
C032	WT	WT	WT	WT	WT	WT	WT	WT

***Cloned sequences positive**

Future Platforms

- Array-base analysis
  - Hundreds of SNPs (mutations) assessed simultaneously
  - Maybe limited by DNA amounts in small samples (FNAs)
- Deep sequencing
  - Data handling limiting
  - FFPE limiting

Current KRAS Best Practices, 2011

- Patients: Metastatic colorectal carcinoma
- Initiation: Clinical service/reflex
- Starting Material: Well fixed paraffin block
- Sampling method: Core, microdissection
- Testing platform: ARMS/scorpions, Sanger, Pyro, multigene, depending on local capability
- Validation: Cell lines, clinical samples processed in standard way
- FDA approved kits in development
- Pathologist interpretation