New Strategies on Horizon

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Dept of Clinical Oncology
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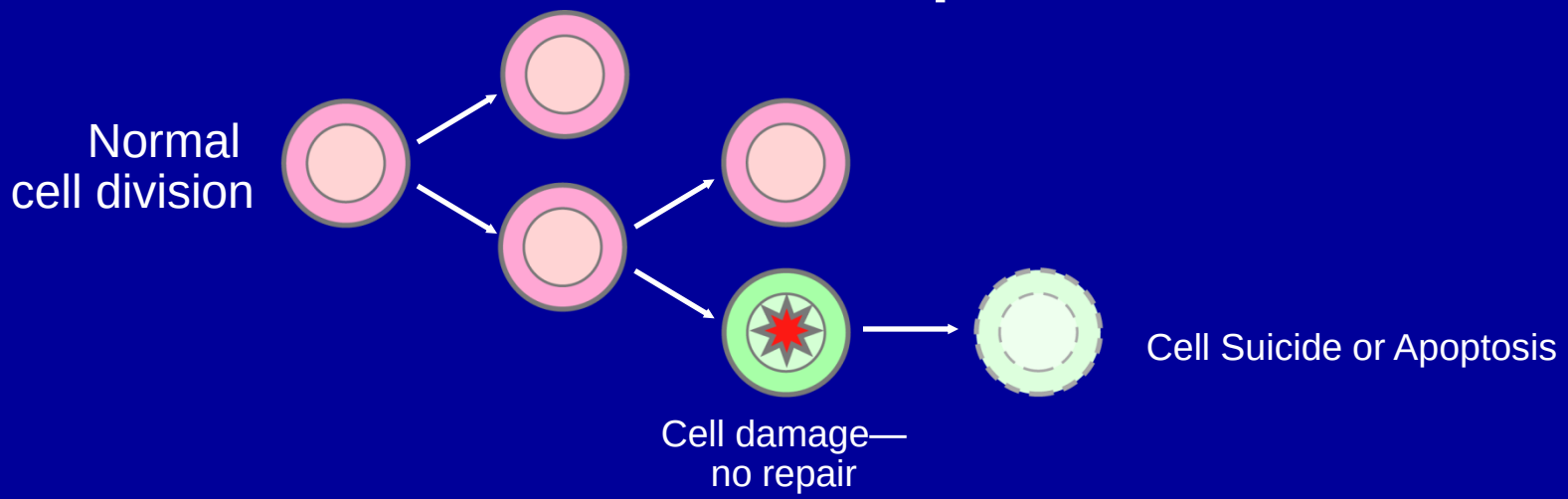
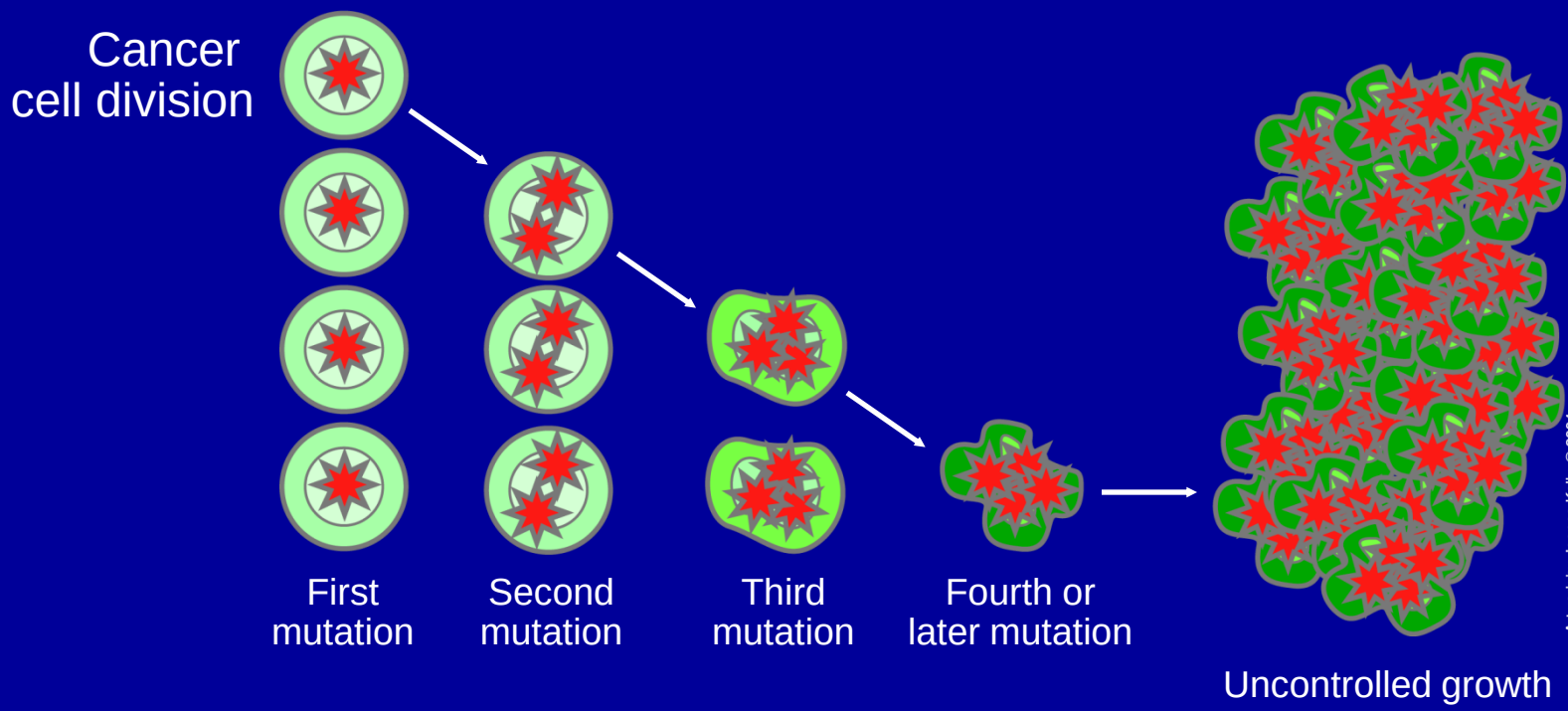
TKI is forever
Addressing the difference
A liquid profile
Man and his mice

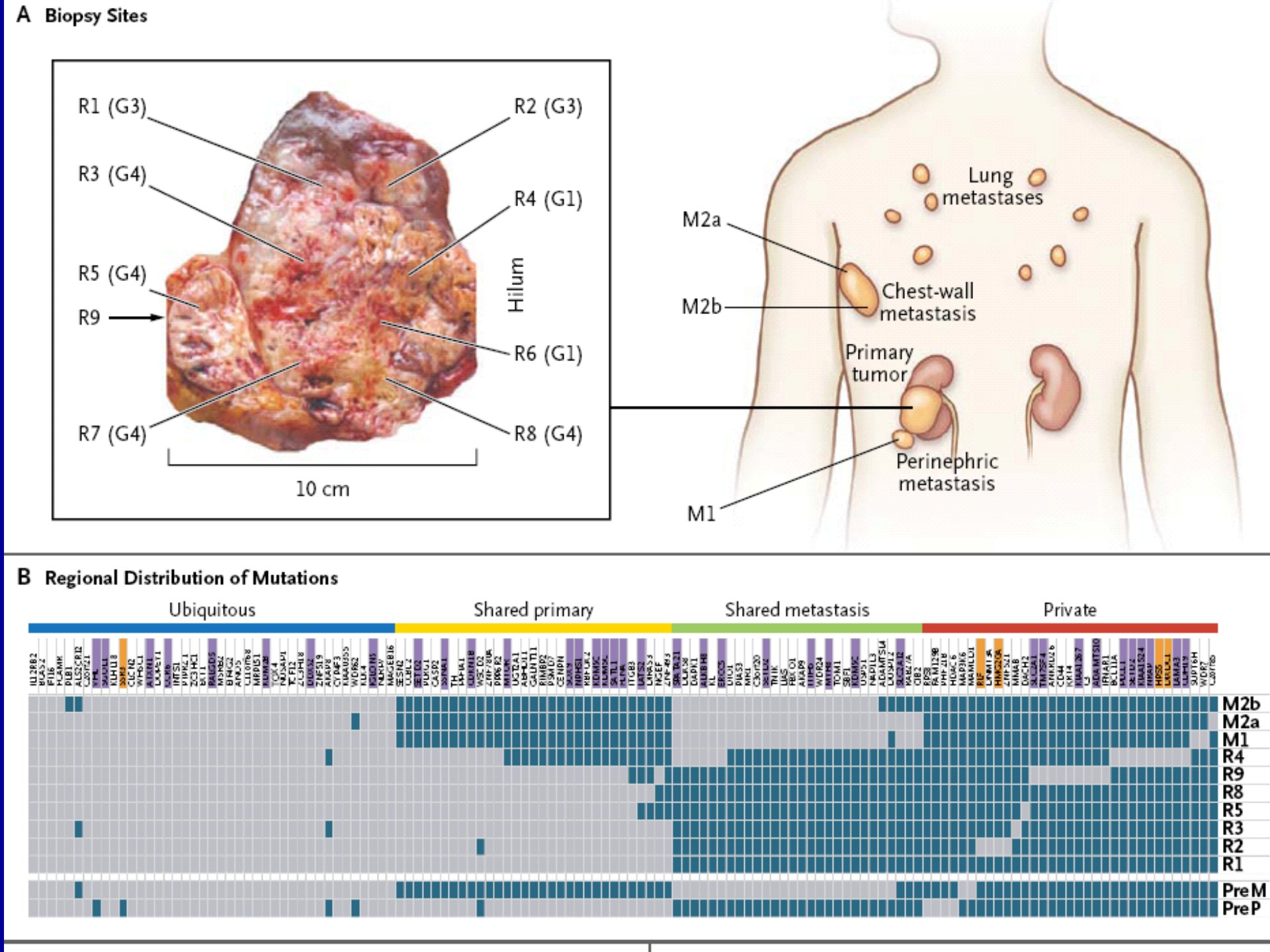
Strategy #1
Addressing the differences

Classic concept of cancer

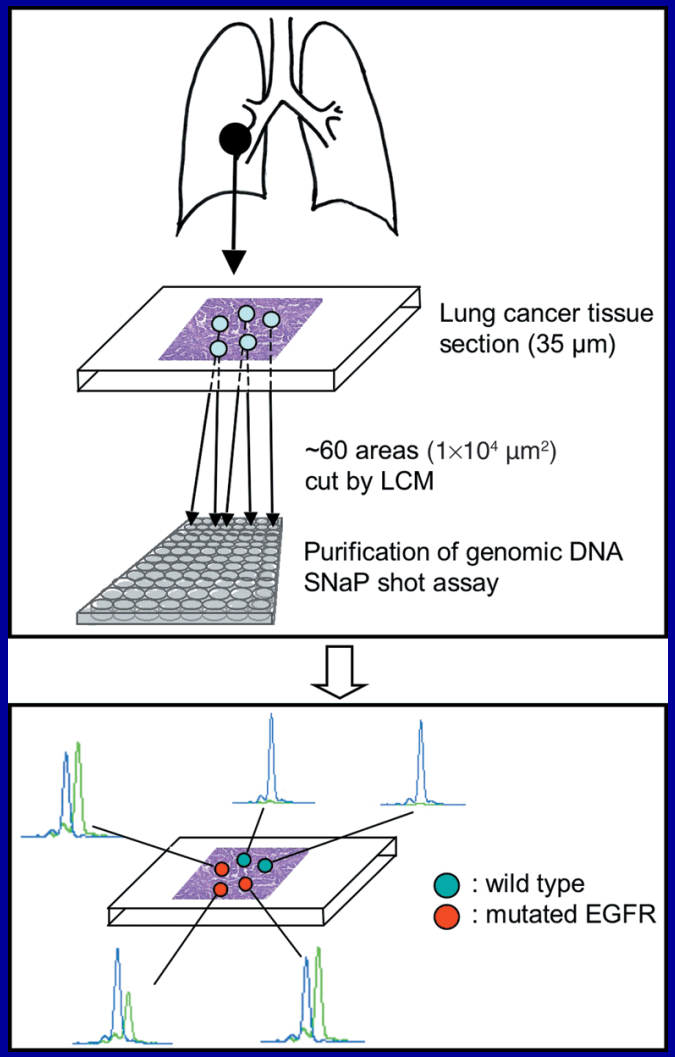




What if the cancer is not homogenous?


A Biopsy Sites
R1 (G3)
R2 (G3)
R3 (G4)
R4 (G1)
R5 (G4)
R9
R6 (G1)
R7 (G4)
R8 (G4)
Hilum
10 cm
Lung metastases
M2a
M2b
Chest-wall metastasis
Primary tumor
Perinephric metastasis
M1
B Regional Distribution of Mutations
Ubiquitous
Shared primary
Shared metastasis
Private
M2b
M2a
M1
R4
R9
R8
R5
R3
R2
R1
PreM
PreP

Early finding of intratumor heterogeneity in lung cancer



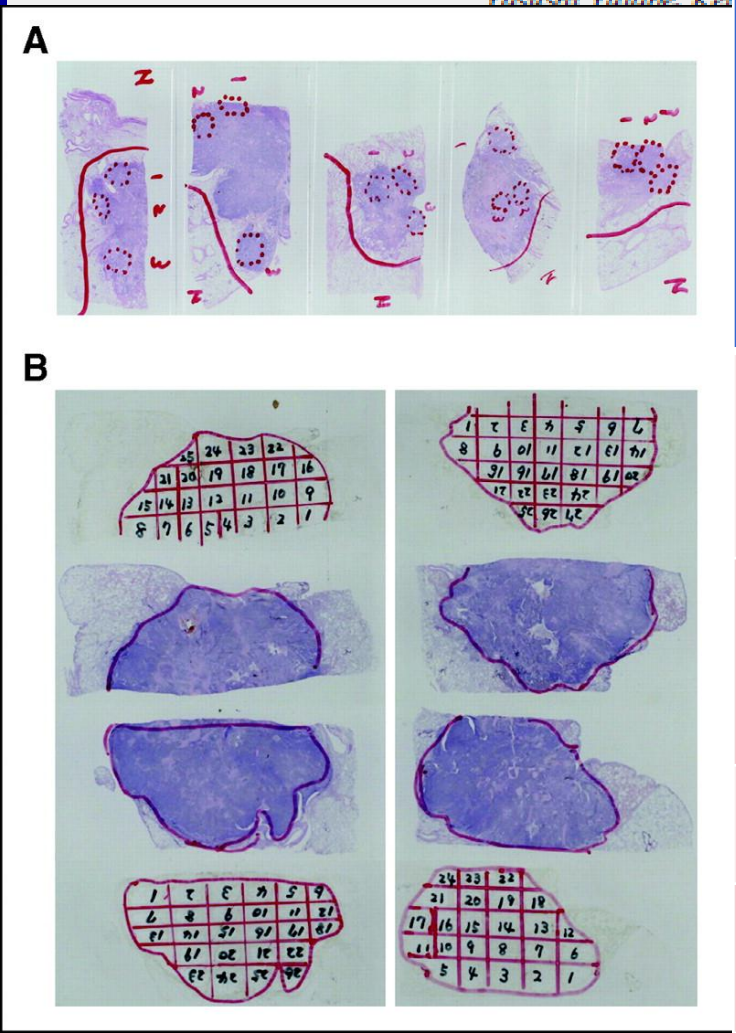
- **Twenty-one patients with recurrent EGFR mutation positive lung cancer**
- **Surgical specimens were retrieved from archive**
- **Using laser capture microdissection and analyzed 50–60 areas from each tissue**
- **Fifteen tissues consisted only of cells with EGFR mutations**
- **Six tissues contained both mutated and non-mutated cells.**

Taniguchi K, Cancer Sci, 2008 May;99(5):929-35

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Heterogeneous Distribution of *EGFR* Mutations Is Extremely Rare in Lung Adenocarcinoma

Yasushi Yatabe, Kei...

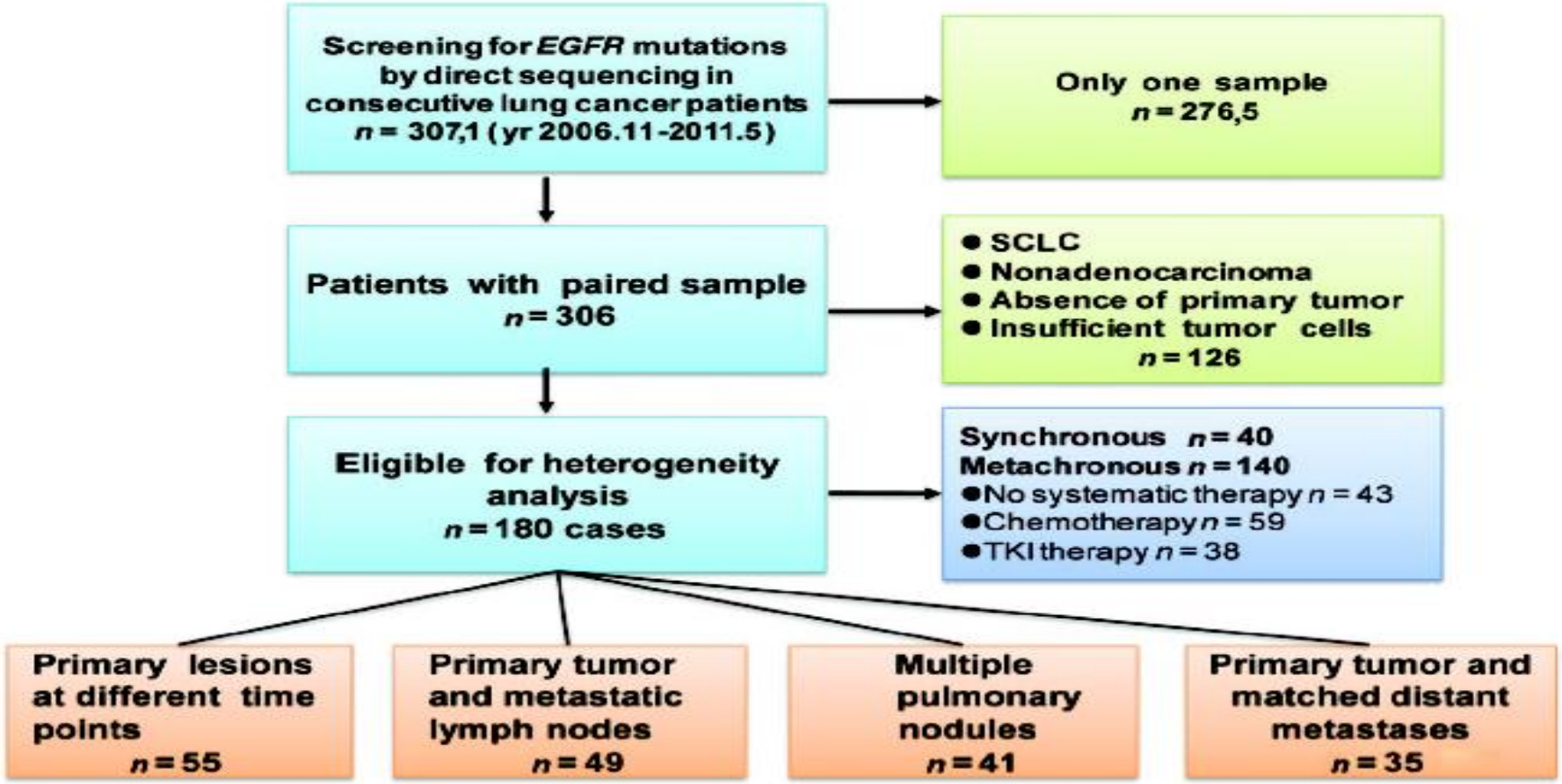


Primary	Lymph Nodes			
	Exon 19Del	Exon 20Ins	G719X	L858R
Exon 19Del	34	0	0	0
Exon 20Ins	0	5	0	0
G719X	0	0	3	0
L858R	0	0	0	35

The Oncologist®

EGFR Mutation Heterogeneity and the Mixed Response to EGFR Tyrosine Kinase Inhibitors of Lung Adenocarcinomas

ZHI-YONG CHEN,[a] WEN-ZHAO ZHONG,[a] XU-CHAO ZHANG,[a] JIAN SU,[a] XUE-NING YANG,[a] ZHI-HONG CHEN,[a] JIN-JI YANG,[a] QING ZHOU,[a] HONG-HONG YAN,[a] SHE-JUAN AN,[a] HUA-JUN CHEN,[a] BEN-YUAN JIANG,[a] TONY S. MOK,[b] YI-LONG WU[a]



	A: Primary lesions at different time points (*n*=55)	B: Primary tumor and metastatic lymph nodes (*n*=49)	C: Multiple pulmonary nodules (*n*=41)	D: Primary tumor and matched distant metastases (*n*=35)
Heterogeneity	5	5	10	5
Homogeneity	50	44	31	30

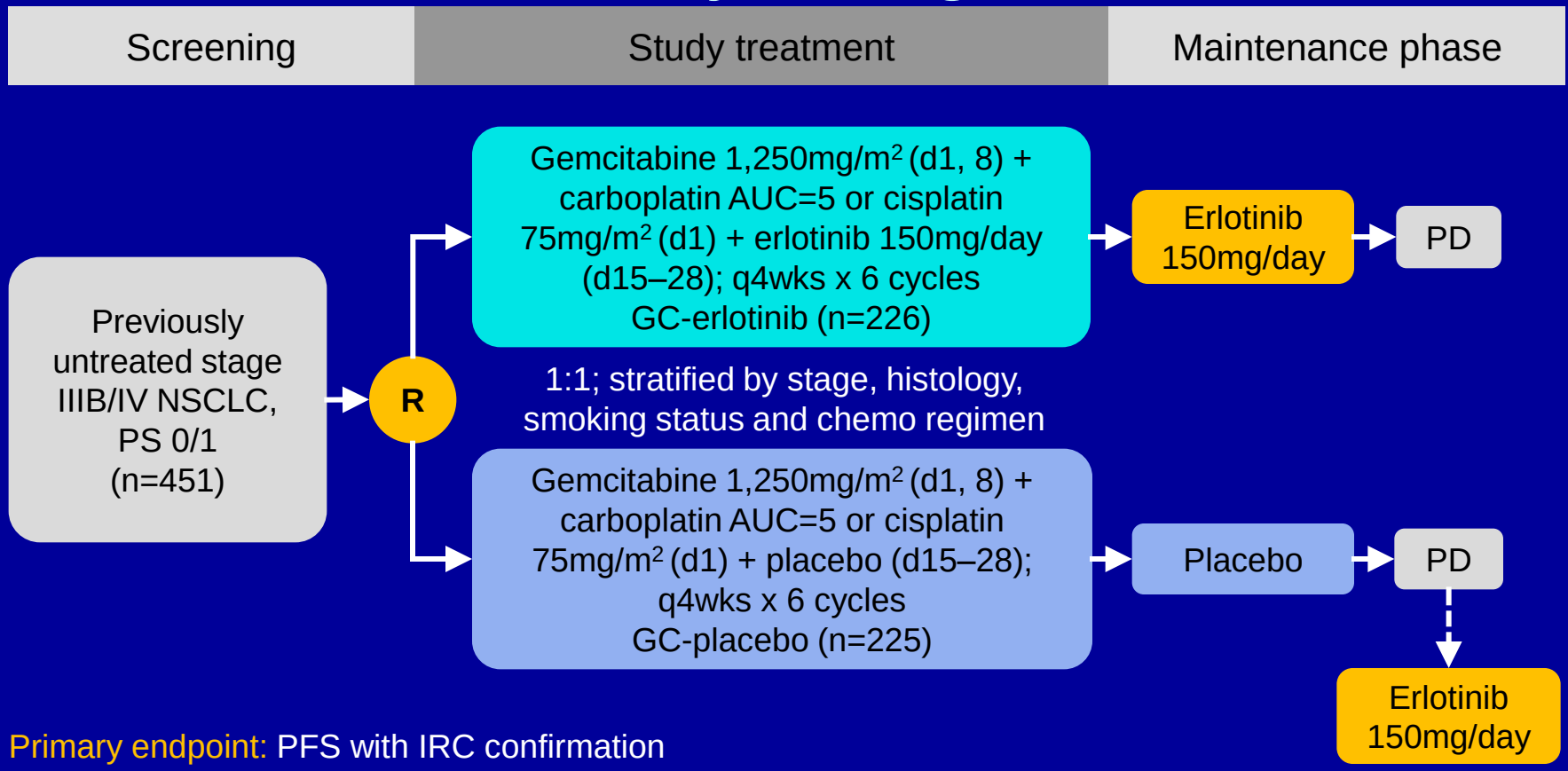
Comparison of *EGFR* mutation status between primary tumor and metastases

Author	No. of specimen	Detection technologya	Metastatic tumorb	Discordant rate (%)
Schimid	**96**	**Sequencing**	**Ly**	**6.3% (6/96)**
Kalikaki	**25**	**Sequencing**	**Ly**	**8.8% (7/80)**
Kalikaki	**25**	**Sequencing**	**Sk, Lu, TW, Br, AG, Li, Bo**	**28.0% (7/25)**
Matsumoto	**19**	**Sequencing**	**Br**	**0 (0/6)**
Park	**101**	**Sequencing Heteroduplex analysis**	**Ly**	**11.9% (12/101) 16.8% (17/101)**
Gomez-Roca	**49**	**IHC**	**-**	**32.7% (16/49)**
Badalian	**11**	**IHC**	**Bo**	**54.5% (6/11)**
Rao	**51**	**IHC**	**Ly**	**10.6% (5/47)**
Watzka	**39**	**IHC**	**-**	**30.8% (12/39)**
Italiano	**30**	**IHC/FISH**	**AG, Bo, Br, Lu, ST**	**33.3% (10/30) 26.9% (7/26)**
Bozzetti	**31**	**FISH**	**Li, Pl, Ab, Ri, Sk, Ly**	**32.1%（9/28）**
Daniele	**28**	**FISH**	**Br, AG**	**22.9% (8/35)**
Monaco	**40/366**	**FISH**	**Ly, Pl, Br, PF, Li, Bre**	**32.5% (11/34)**
Fang	**35**	**Taqman RT-PCR**	**Ly, Br**	**31.5% (11/35)**

Schmid K, Clin Cancer Res, 2009, 15(14): 4554-4560；Kalikaki A, Br J Cancer, 2008, 99(6): 923-929.；Matsumoto S，Int J Cancer, 2006, 119(6):1491-1494.
Park S, J Thorac Oncol, 2009, 4(7): 809-815.；Gomez-Roca C, J Thorac Oncol, 2009, 4(10): 1212-1220.；Badalian G, Pathol Oncol Res, 2007, 13(2): 99-104.
Rao C, J Exp Clin Cancer Res, 2010, 29: 7.；Watzka SB, Eur J Cardiothorac Surg, 2010, 38(1):34-37. Italiano A, Ann Oncol,2006, 17(6): 981-985.
Bozzetti C, J Thorac Oncol, 2008, 3(1): 18-22.；Daniele L, J Thorac Oncol, 2009, 4(6): 684-688. Monaco SE, Hum Pathol, 2010, 41(1): 94-102.

Strategy towards heterogeneity

FASTACT-2 (MO22201; CTONG0902) study design

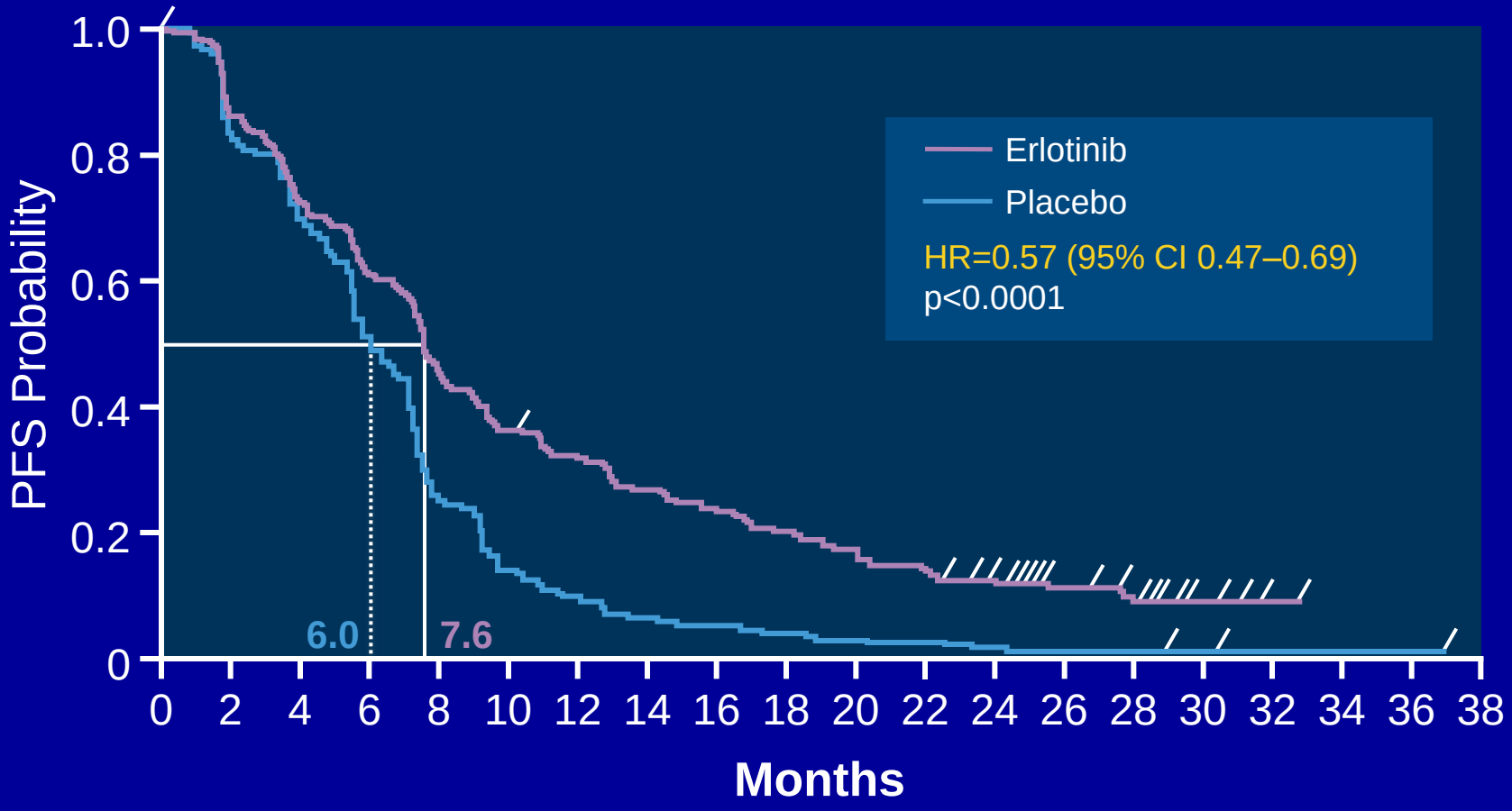


Primary endpoint: PFS with IRC confirmation

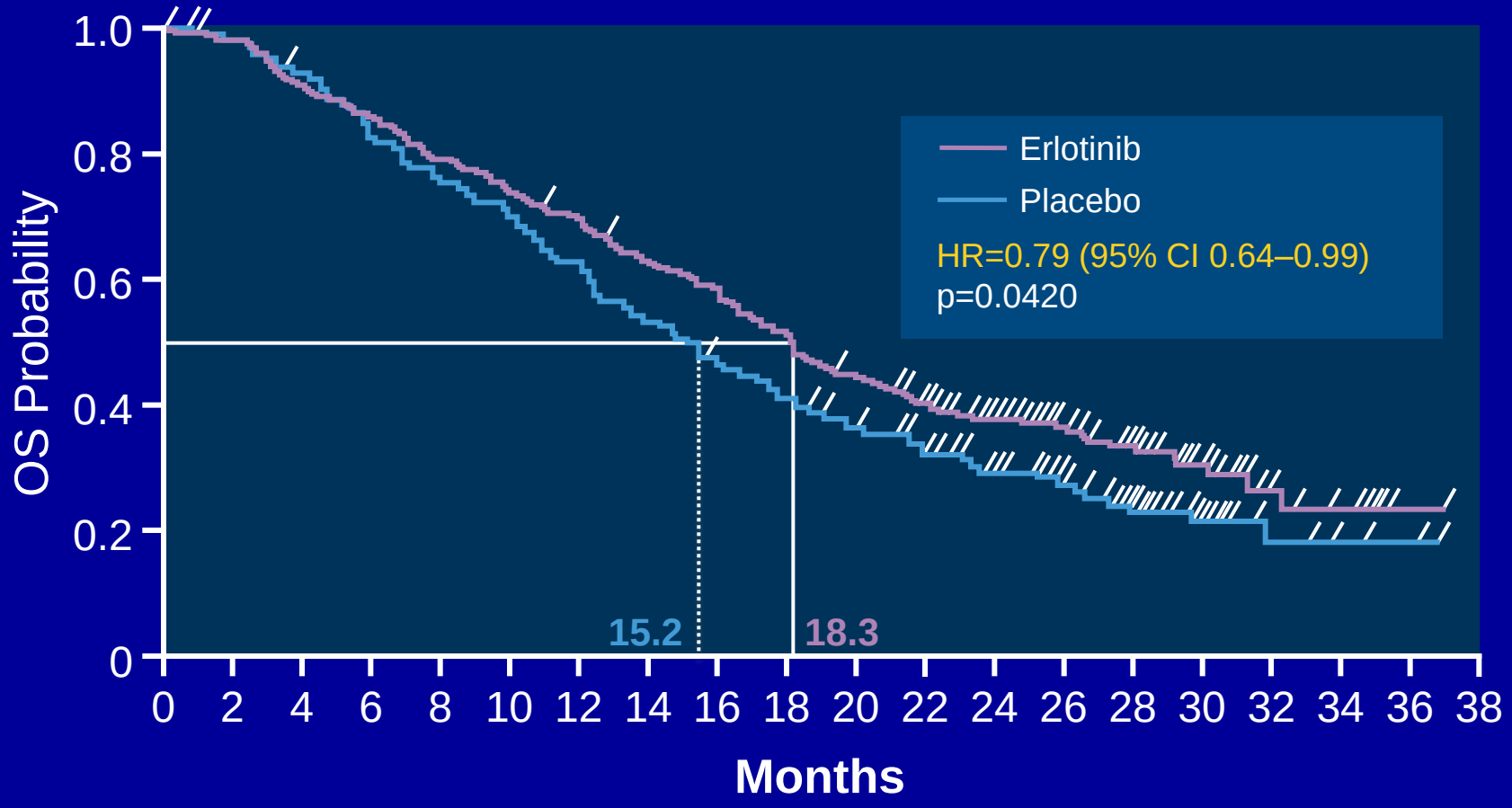
Secondary endpoints: subgroup analyses, OS in all patients and subgroups, ORR, duration of response, TTP, NPR at 16 weeks, safety, QoL

NSCLC = non-small cell lung cancer; PS = performance status; PD = disease progression; AUC = area under the curve; q4wks = every 4 weeks; IRC = independent review committee; OS = overall survival; ORR = objective response rate; TTP = time to progression; NPR = non-progression rate; QoL = quality of life

Updated primary endpoint: PFS in ITT population (27 Jun 2012)

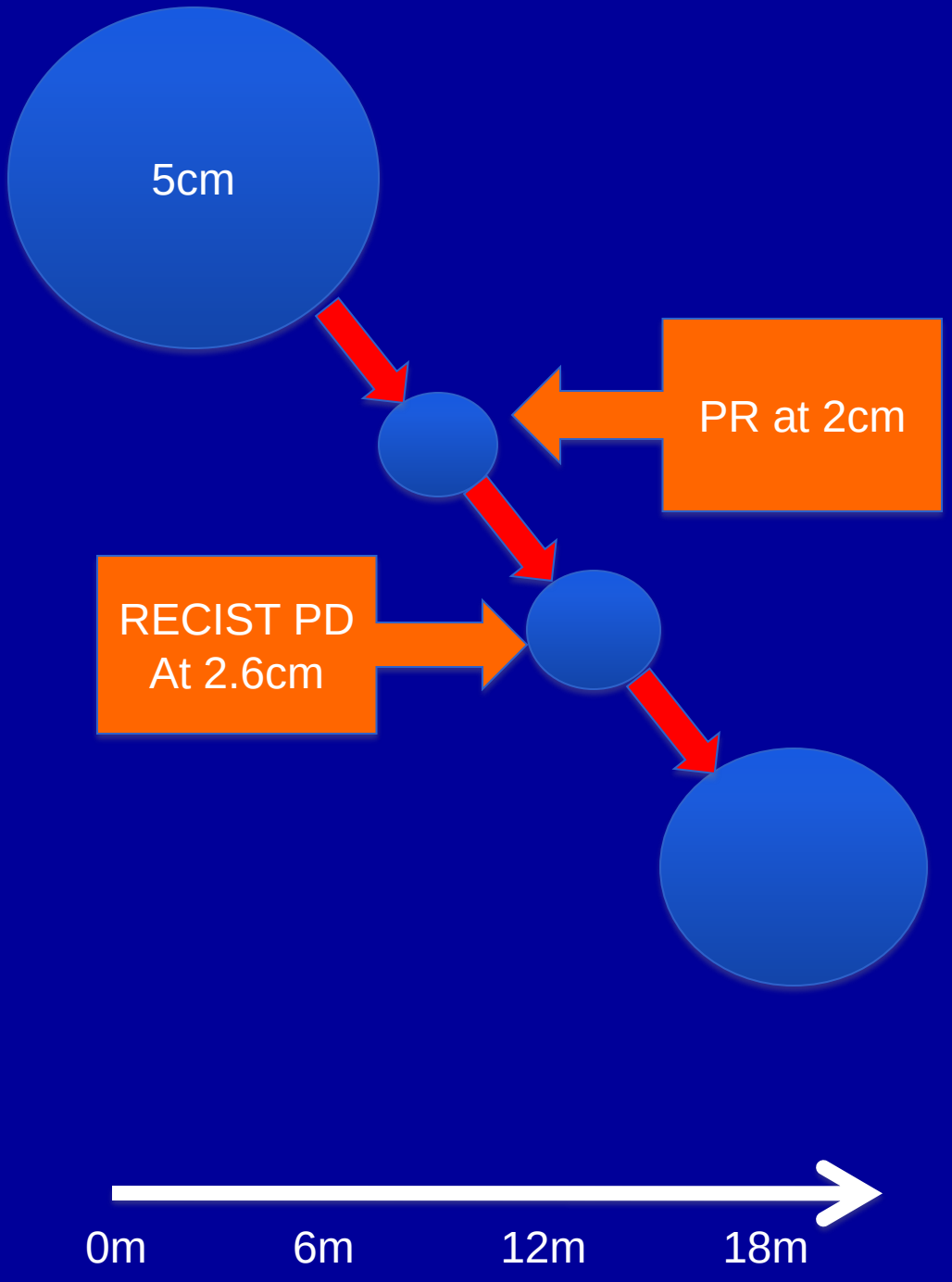


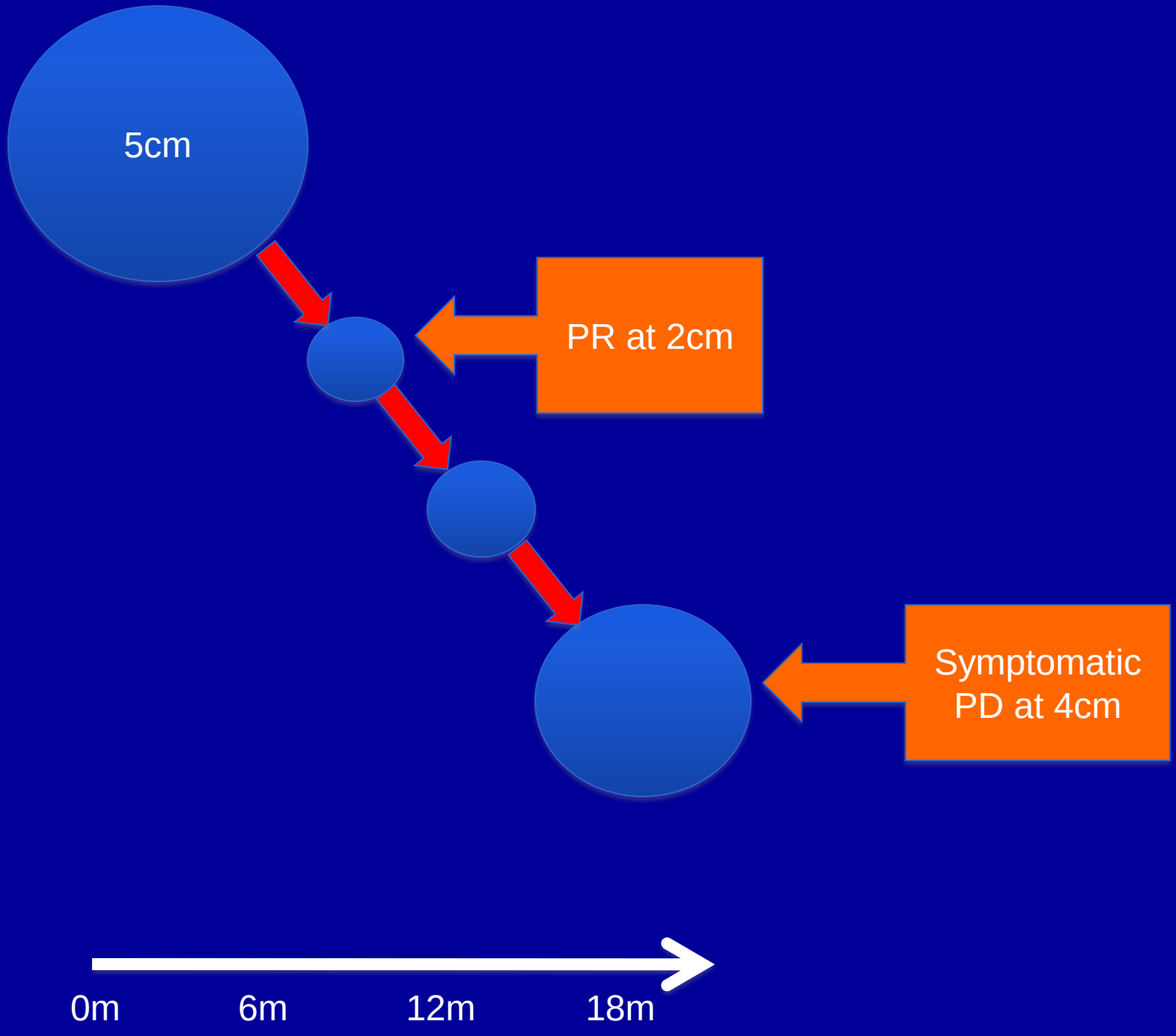
OS in ITT population (27 Jun 2012)

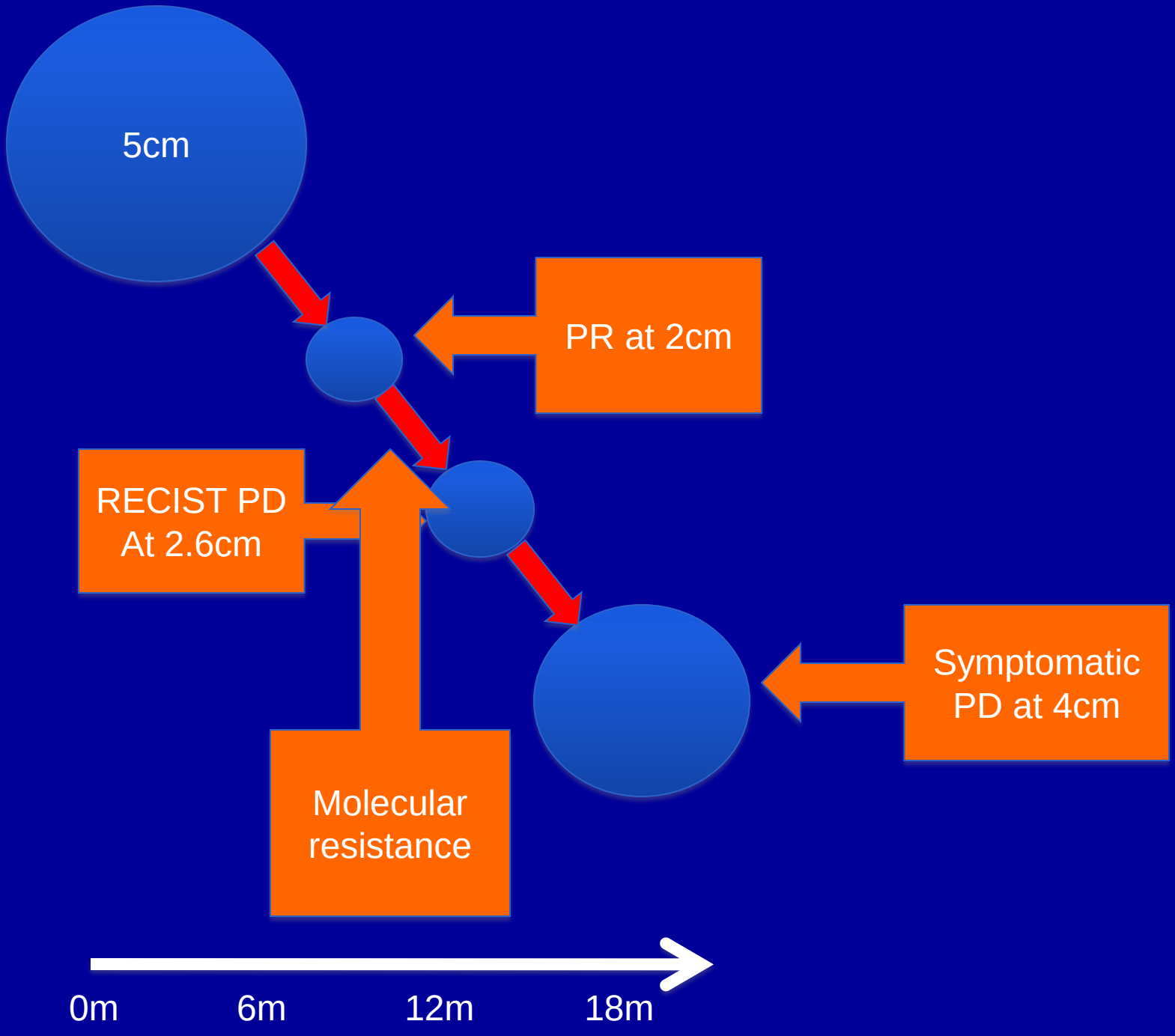


Strategy #2

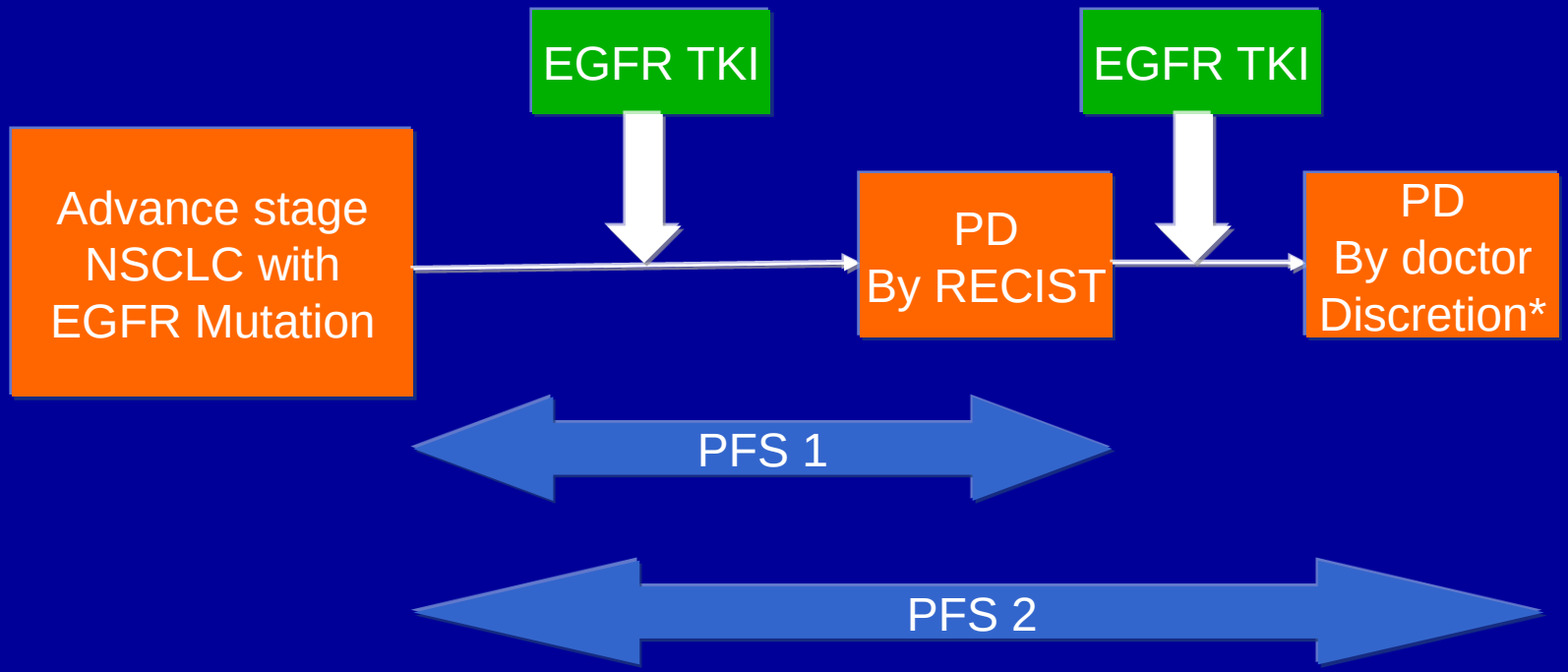
TKI is forever


5cm
PR at 2cm
RECIST PD
At 2.6cm
0m
6m
12m
18m


5cm
PR at 2cm
Symptomatic
PD at 4cm
0m
6m
12m
18m


5cm
PR at 2cm
RECIST PD
At 2.6cm
Symptomatic
PD at 4cm
Molecular
resistance
0m
6m
12m
18m

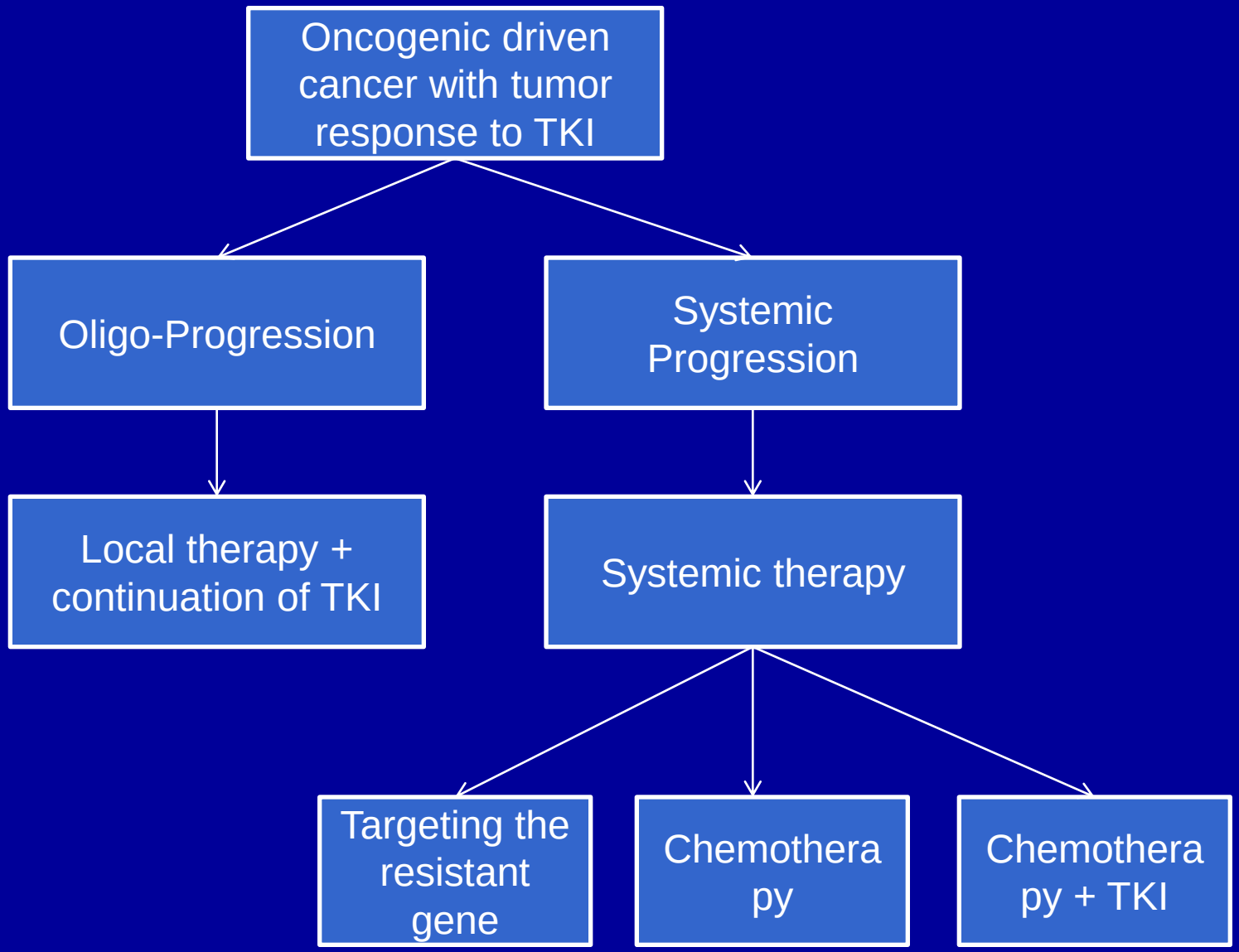
ASPIRATION: To optimize treatment duration



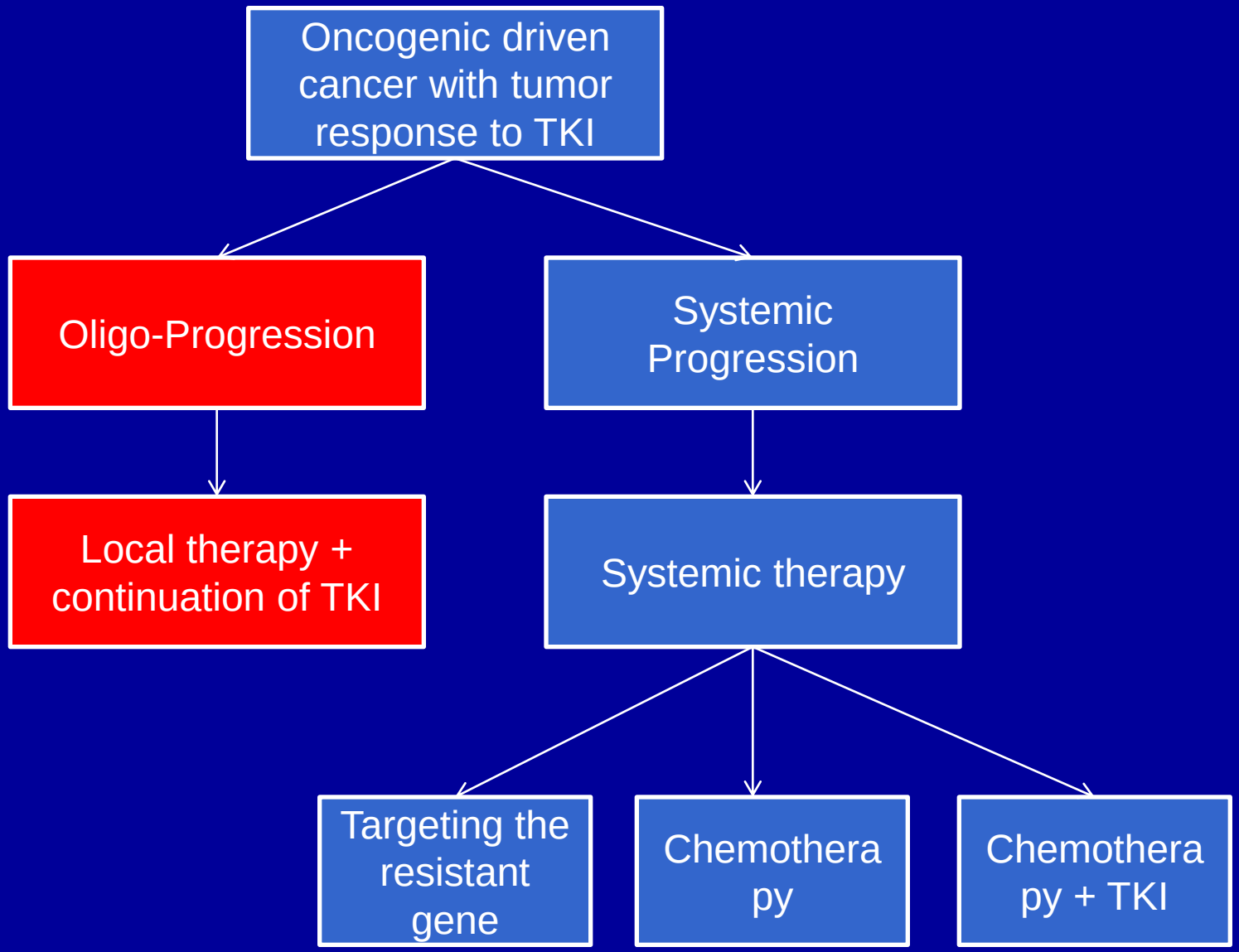
*Doctor Discretion: Symptomatic progression, multiple progression
Threat to major organ…etc

PI: K Park

TKI Resistance after ASCO 2012

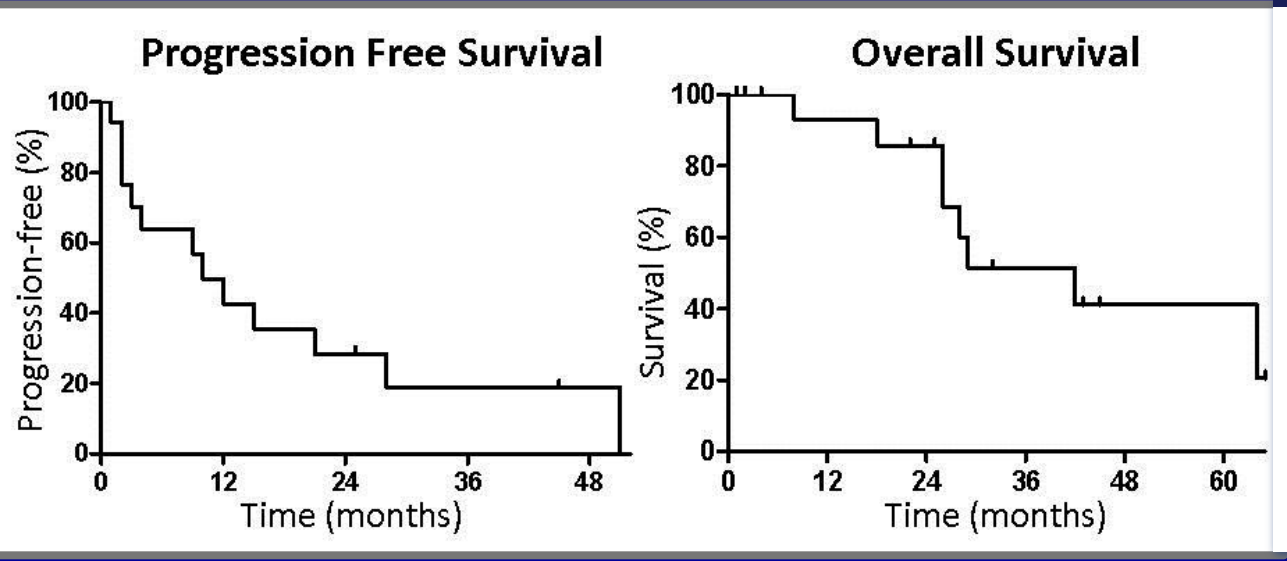


Treatment of TKI Resistance



Local Therapy in Acquired Resistance: MSKCC

- 18/184 pts/7+ yrs underwent local therapy for extracranial PD
  - CNS PD excluded
- From time of local therapy
  - Median TTP: 10 months
  - Median time to new systemic Rx: 22 months
  - Median OS: 41 months



Local Therapy Procedures	
Procedures Performed	18
Lung	15
Radiofrequency ablation	2
Radiation	2
Lobectomy	7
Wedge resection	1
Pneumonectomy	3
Lymph node- Radiation (mediastinum, supraclavicular lymph nodes)	1
Adrenals- Adrenalectomy	2

Yu, ASCO 2012, Abst#7527

Local treatment to oligo-progression plus continuation of TKI

- Colorado University collection of 65 patients with oncogenic driven cancer (EGFR mutation or ALK positive)
- All received EGFR TKI or Crizotinib
- PFS 1 defined as <4 sites of progression
  - Local ablative therapy offered to all sites of involvement and continue TKI
- PFS 2 defined as from time of local therapy to second progression

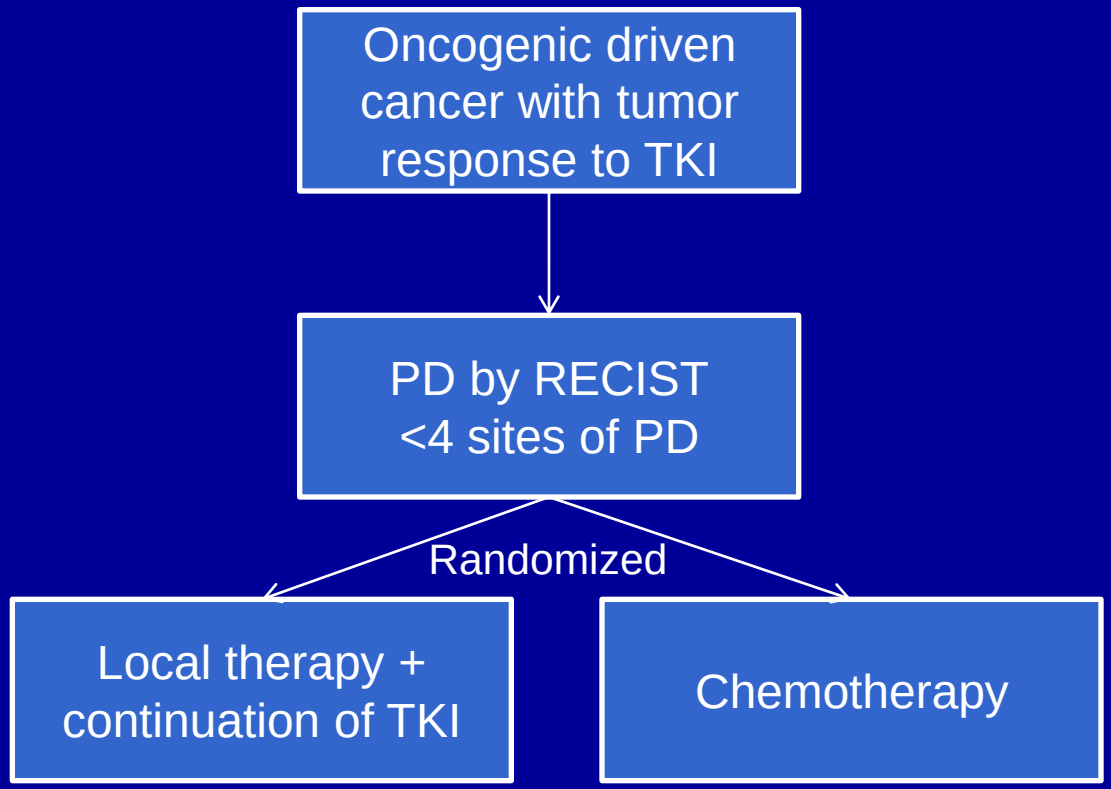
ASCO 2012 Abst 7526

PFS of patients treated with LAT and continuation of TKI therapy

Site of first progression	Number of patients	PFS1 (months)(95% CI)	PFS2 (months)(95% CI)	Site of 2nd progression	
CNS	10	10.9 7.3 – 18.3	**7.1** 1.7 – 11.3	2 (20%)	no prog
				3 (30%)	CNS
				5 (50%)	eCNS
eCNS†	15	9.0 6.5 – 13.8	**4.0** 2.7 -7.4	4 (27%)	no prog
				3 (20%)	CNS
				8 (53%)	eCNS
All patients	25	9.8 8.8 – 13.8	**6.2** 3.7 – 8.0	6 (24%)	no prog
				7 (28%)	CNS
				12 (48%)	eCNS

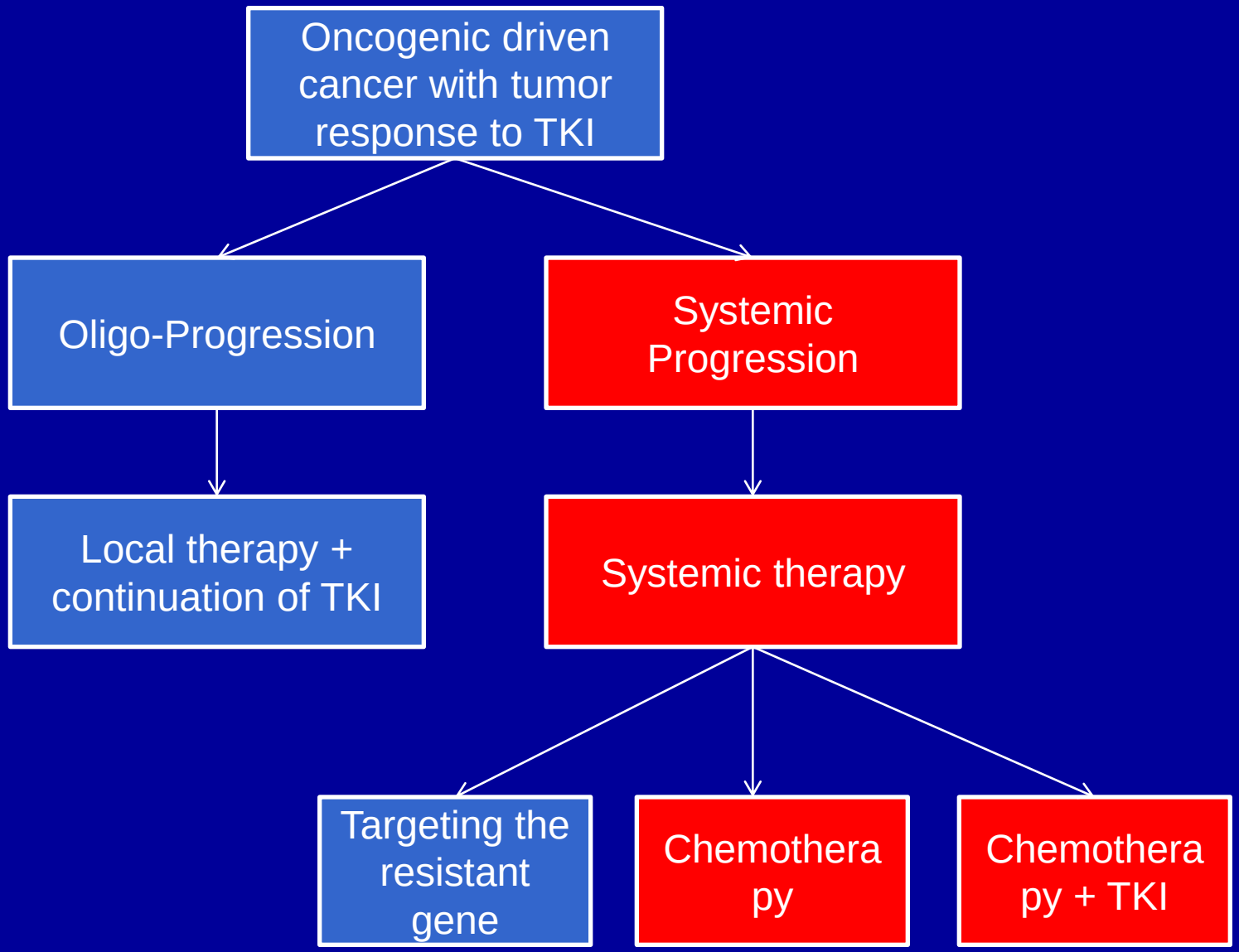
† Includes 3 patients who progressed systemically (eCNS) and simultaneously within the CNS

Future Prospective Study?



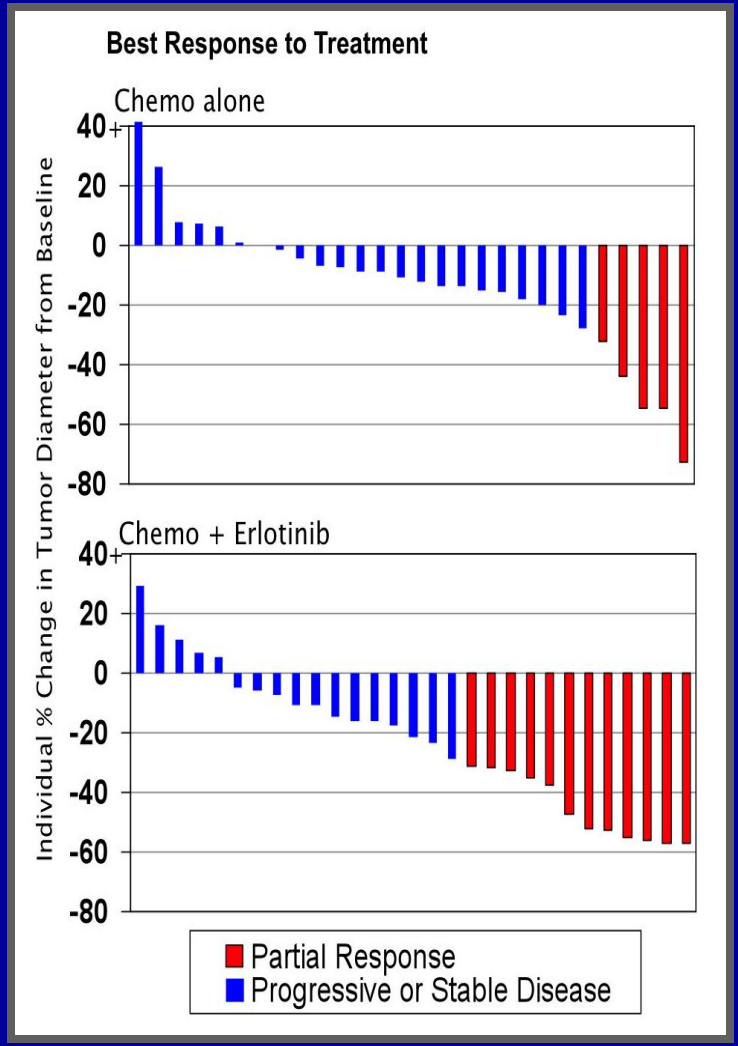
Primary endpoint: PFS
Secondary endpoint: OS, RR, QOL

Treatment of TKI Resistance



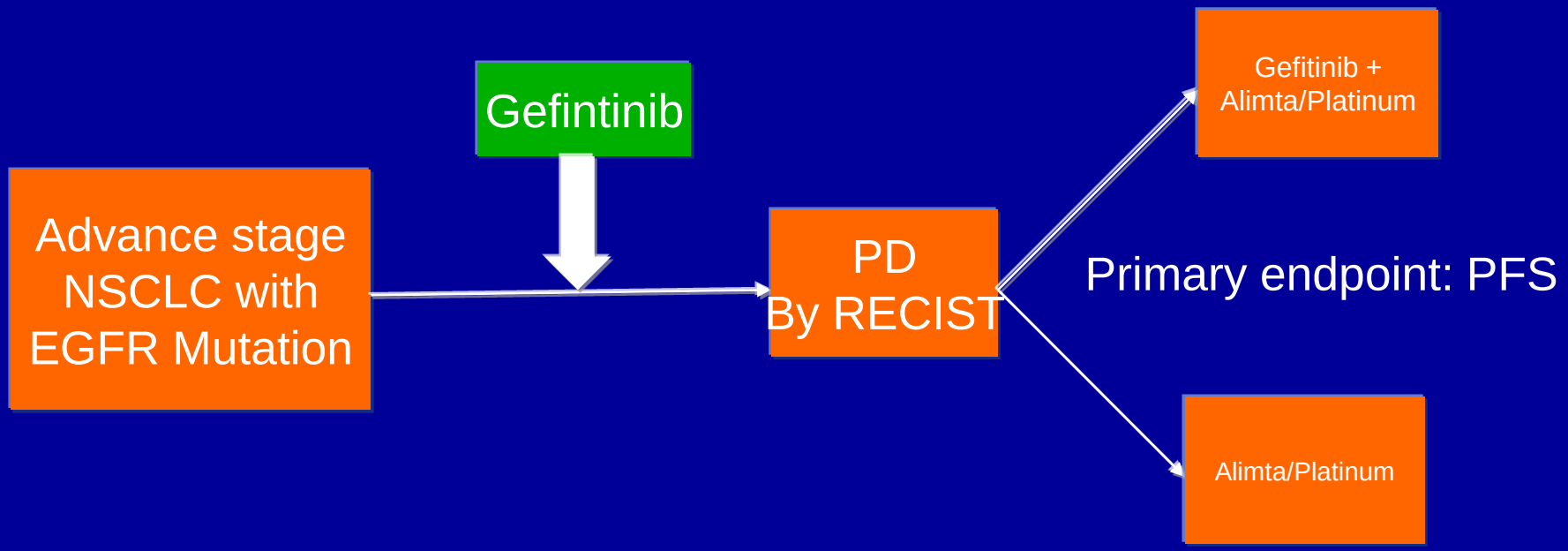
Chemo/Erlotinib vs. Chemo Alone at Progression after Acquired Resistance

- N = 78 retrospective review of outcomes
  - chemo alone (N = 44) or
  - chemo/erlotinib (N = 34)
- RR 18% (chemo) vs. 41% with chemo/erlotinib)
- No differences in PFS or OS between these two strategies



Goldberg, ASCO 2012, Abst#7524

IMPRESS: Chemotherapy with or with gefitinib at progression

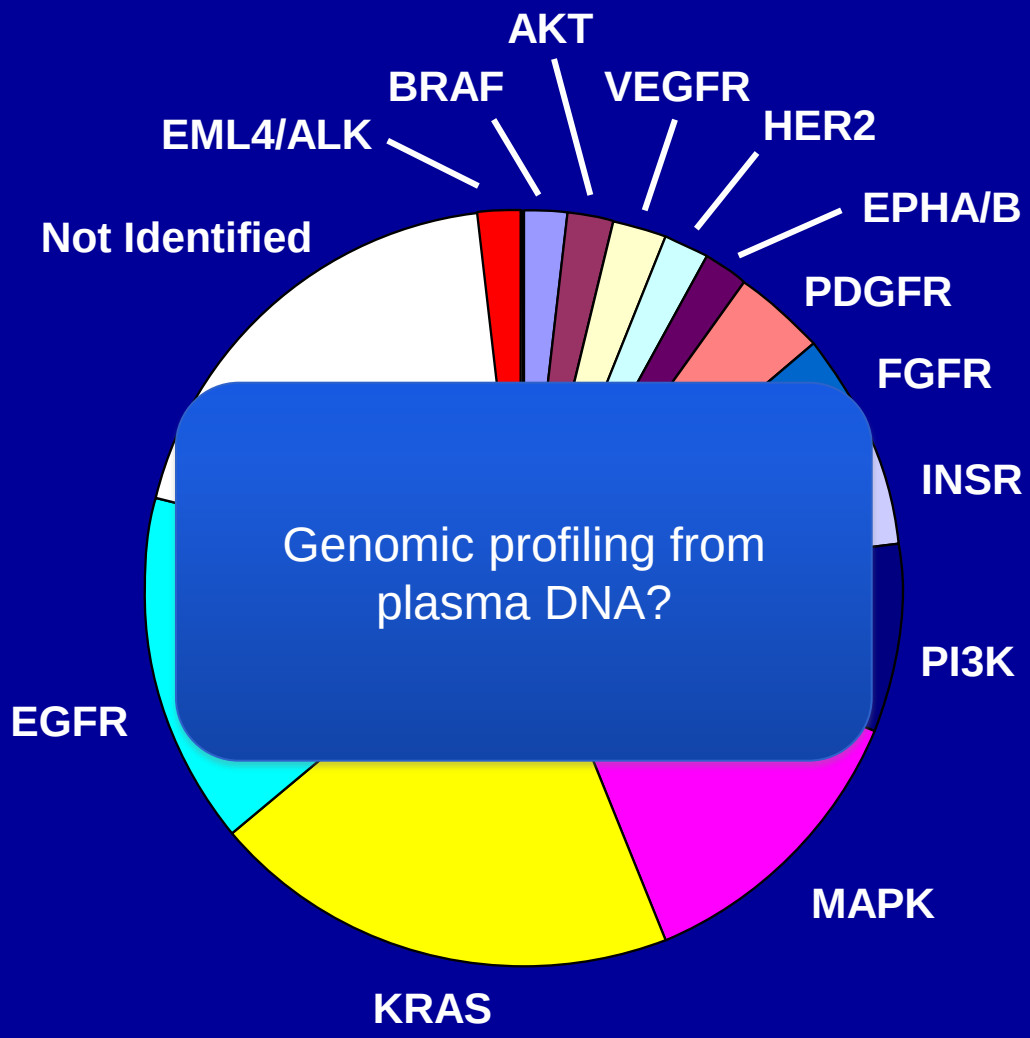


Co-PI: Soria J; Mok T

Strategy #3

A liquid profile

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



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

Characteristics of circulating DNA

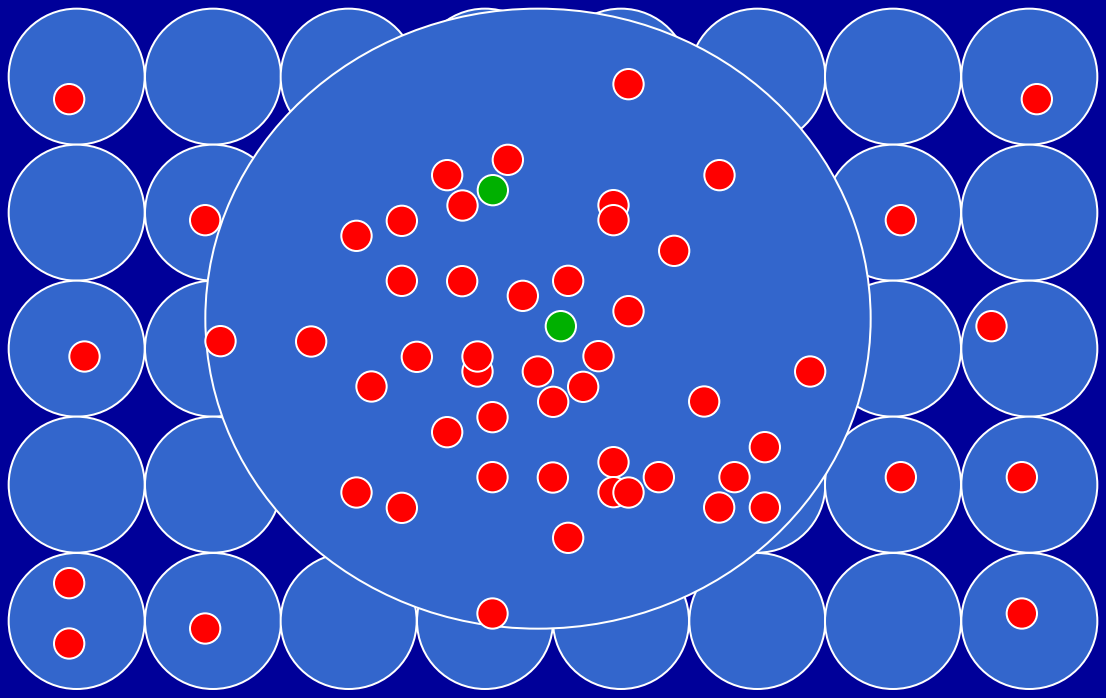
- Fragmented DNA at about 140 to 170 bp
- Only few thousands of amplifiable copies per ml of blood
- Circulating tumor DNA (ctDNA) may contain loci of mutated gene (driver or non-driver oncogene)
- Quantity of ctDNA could be related to tumor volume

Gormally et al Mutat Res 635: 105, 2007
Diehl et al Nat Med 14:985, 2008

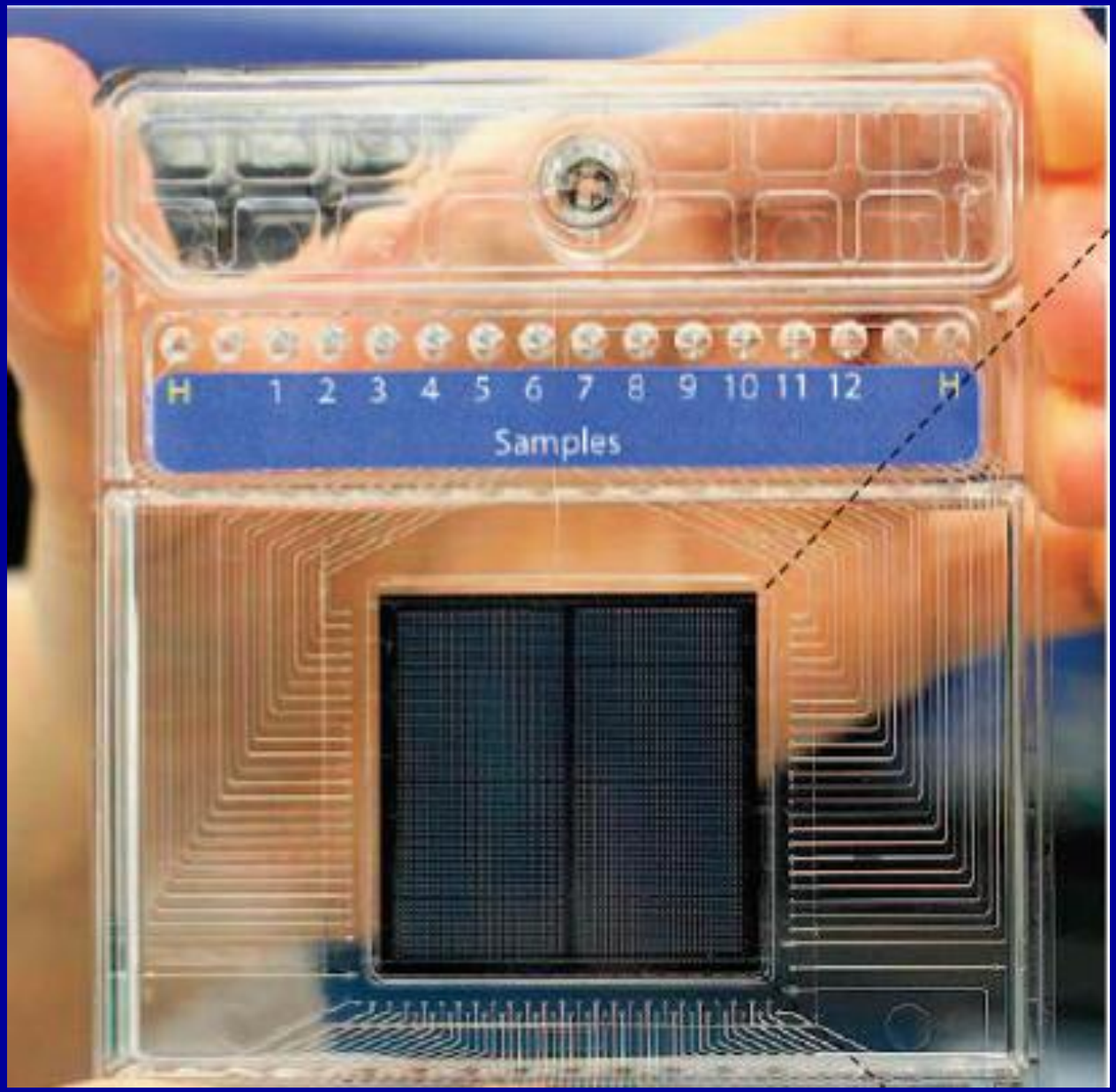
Detecting one mutated gene at a time

Mixture of molecules

Digital PCR

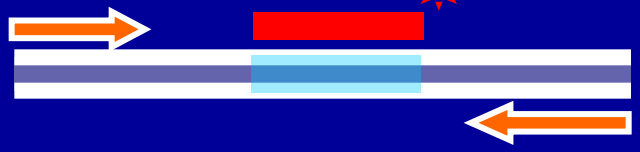


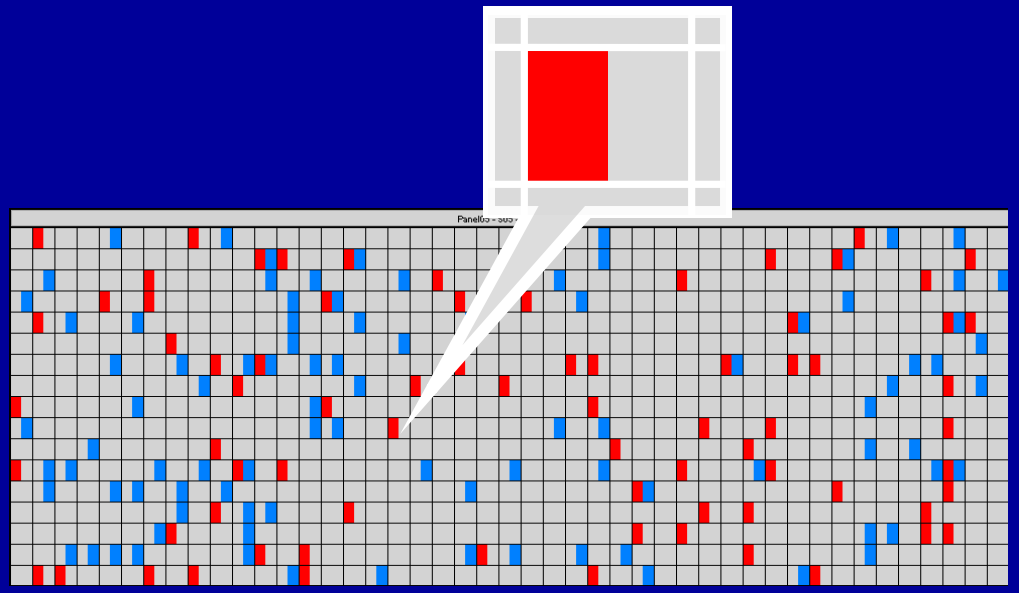
Microfluidics Digital PCR



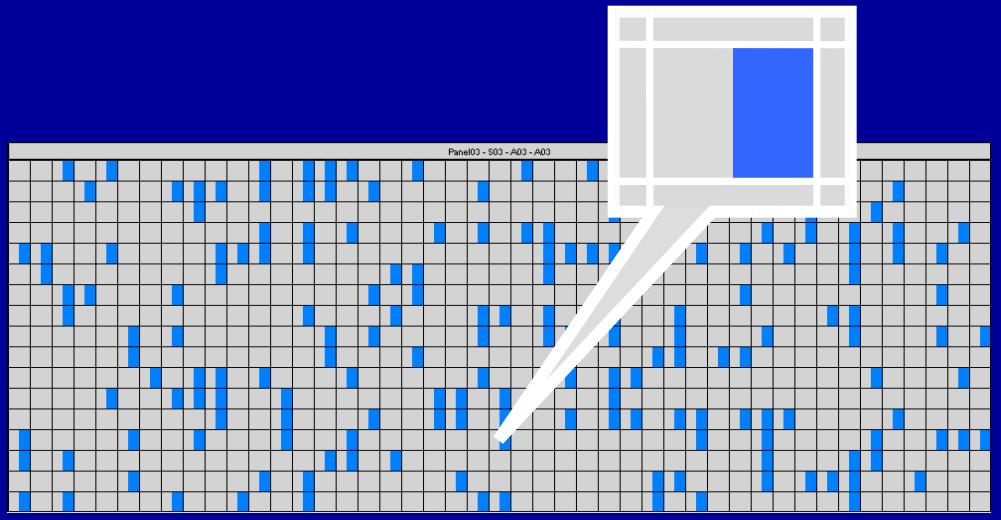
L858R mutation

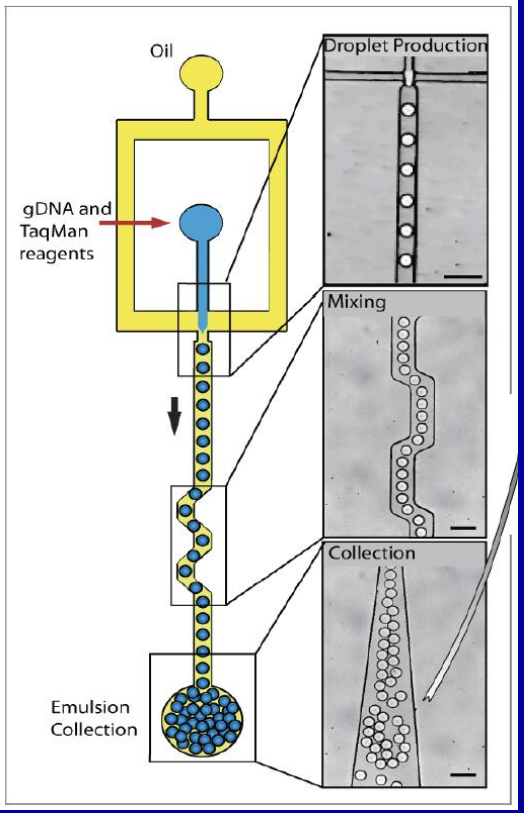
mutant-specific probe





wild-type-specific probe





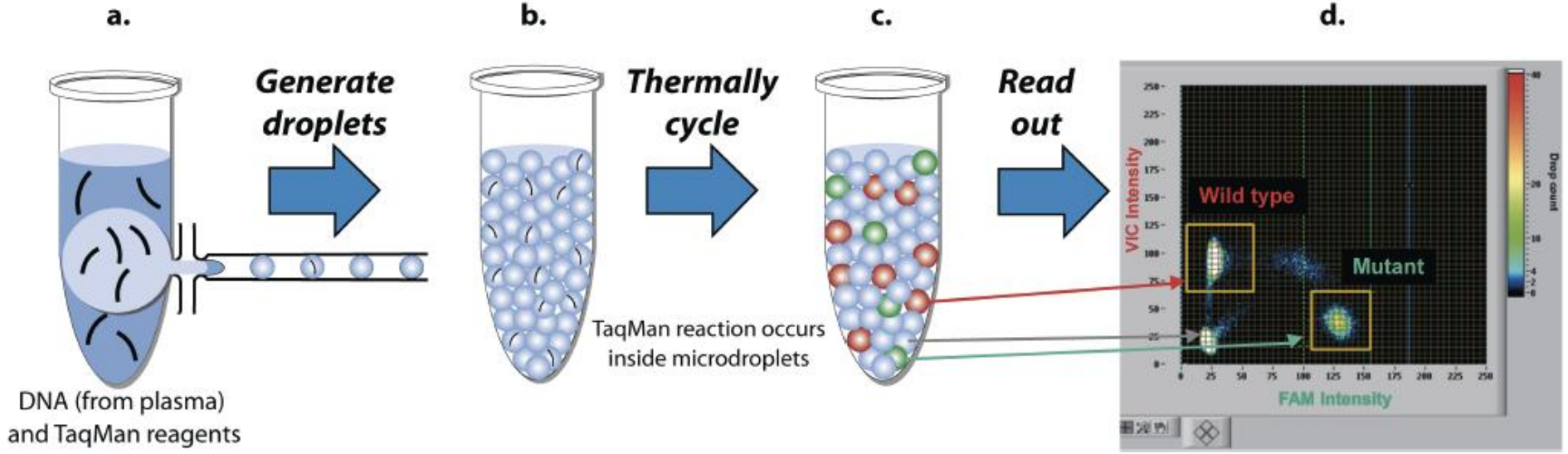
Droplet-based digital PCR

-Each micro-droplet contain a fragment of DNA

-TaqMan reaction take place within each droplet

-About 20,000 droplet per tube

Implied 20,000 single gene sequencing



Reconstruction of fetal chromosome from maternal plasma

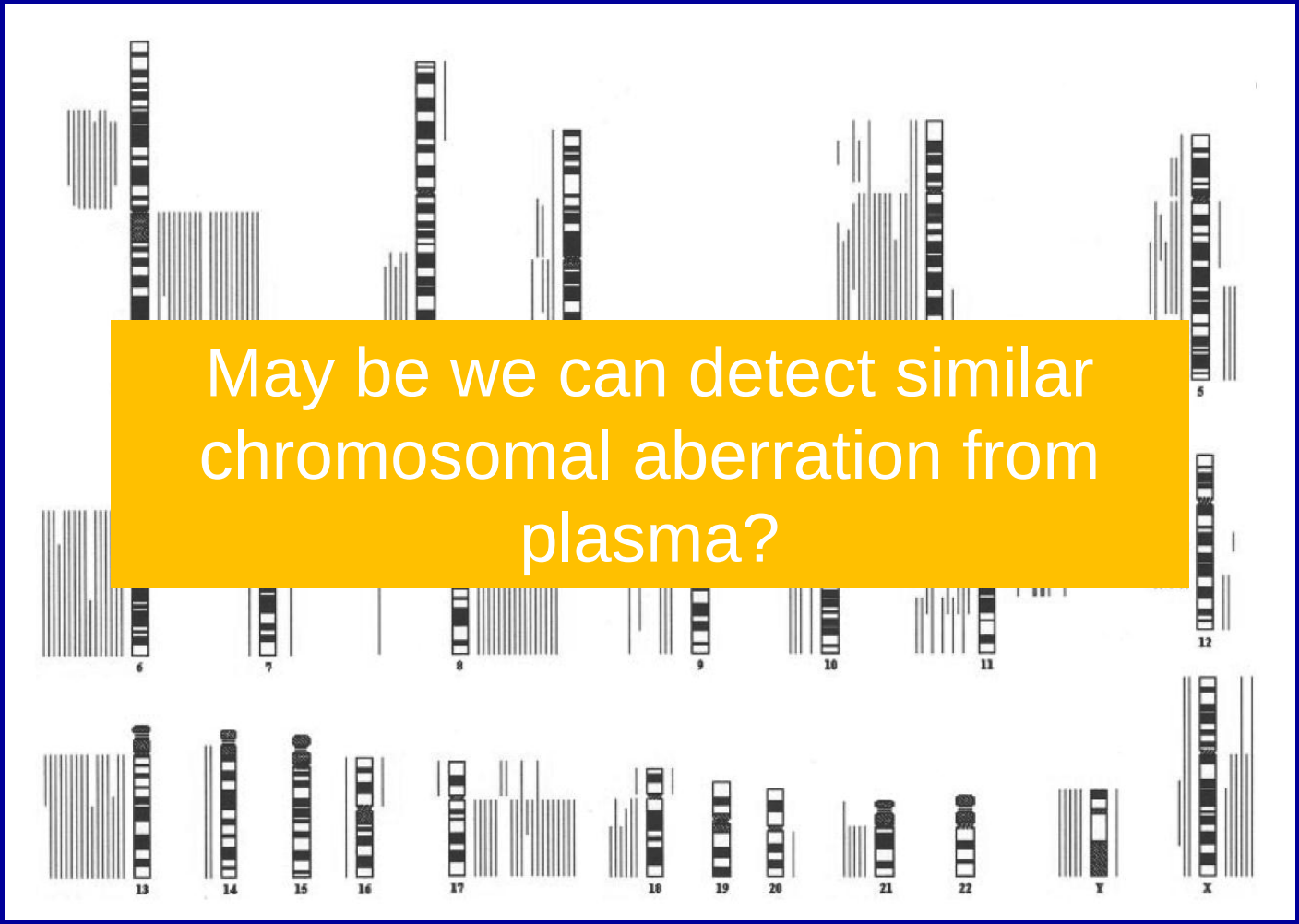
PNAS

Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma

Rossa W. K. Chiu[a,b], K. C. Allen Chan[a,b], Yuan Gao[c,d], Virginia Y. M. Lau[a,b], Wenli Zheng[a,b], Tak Y. Leung[e], Chris H. F. Foo[f], Bin Xie[c], Nancy B. Y. Tsui[a,b], Fiona M. F. Lun[a,b], Benny C. Y. Zee[f], Tze K. Lau[e], Charles R. Cantor[g,1], and Y. M. Dennis Lo[a,b,1]

20458–20463 | PNAS | December 23, 2008 | vol. 105 | no. 51

Common chromosomal aberrations in hepatocellular carcinoma (HCC)



Tornillo et al. J Pathol 2000

Detecting multiple mutated gene at a time

RESEARCH ARTICLE

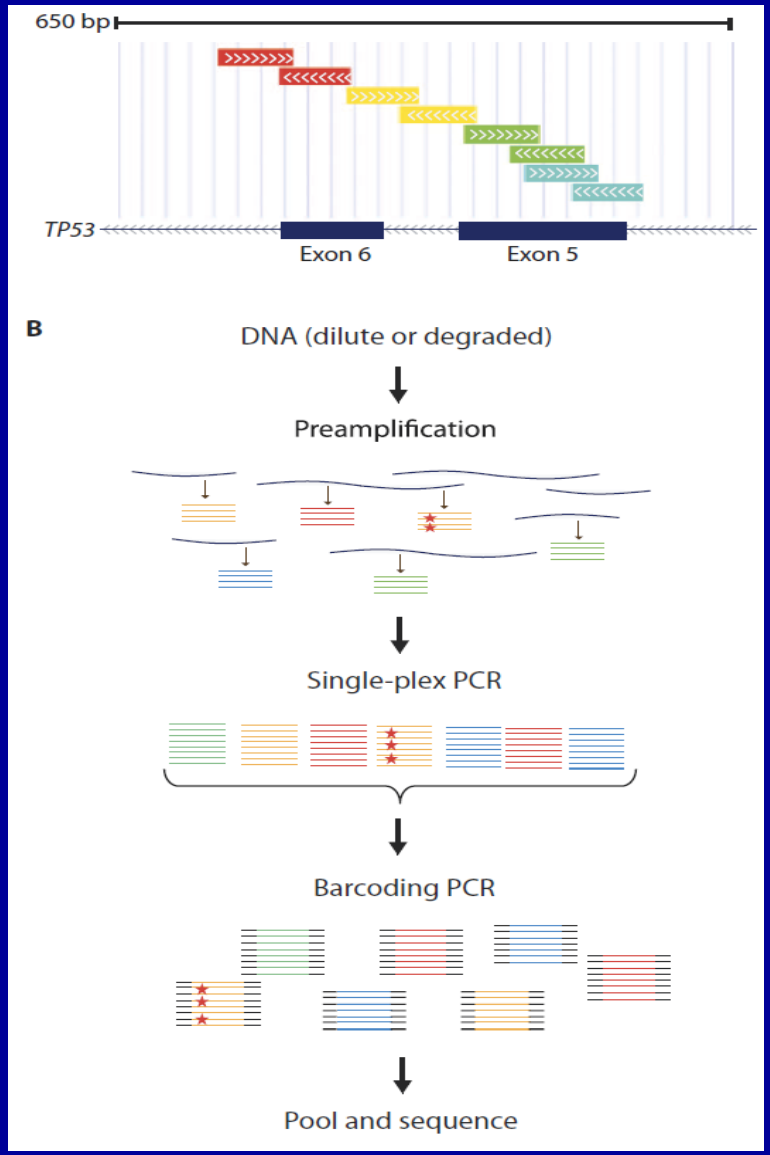
CANCER GENOMICS

Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA

Tim Forshew,[1*] Muhammed Murtaza,[1,2*] Christine Parkinson,[1,2,3*] Davina Gale,[1*] Dana W. Y. Tsui,[1*] Fiona Kaper,[4†] Sarah-Jane Dawson,[1,2,3] Anna M. Piskorz,[1,2] Mercedes Jimenez-Linan,[3,5] David Bentley,[6] James Hadfield,[1] Andrew P. May,[4] Carlos Caldas,[1,2,3,7] James D. Brenton,[1,2,3,7‡] Nitzan Rosenfeld[1,2‡]

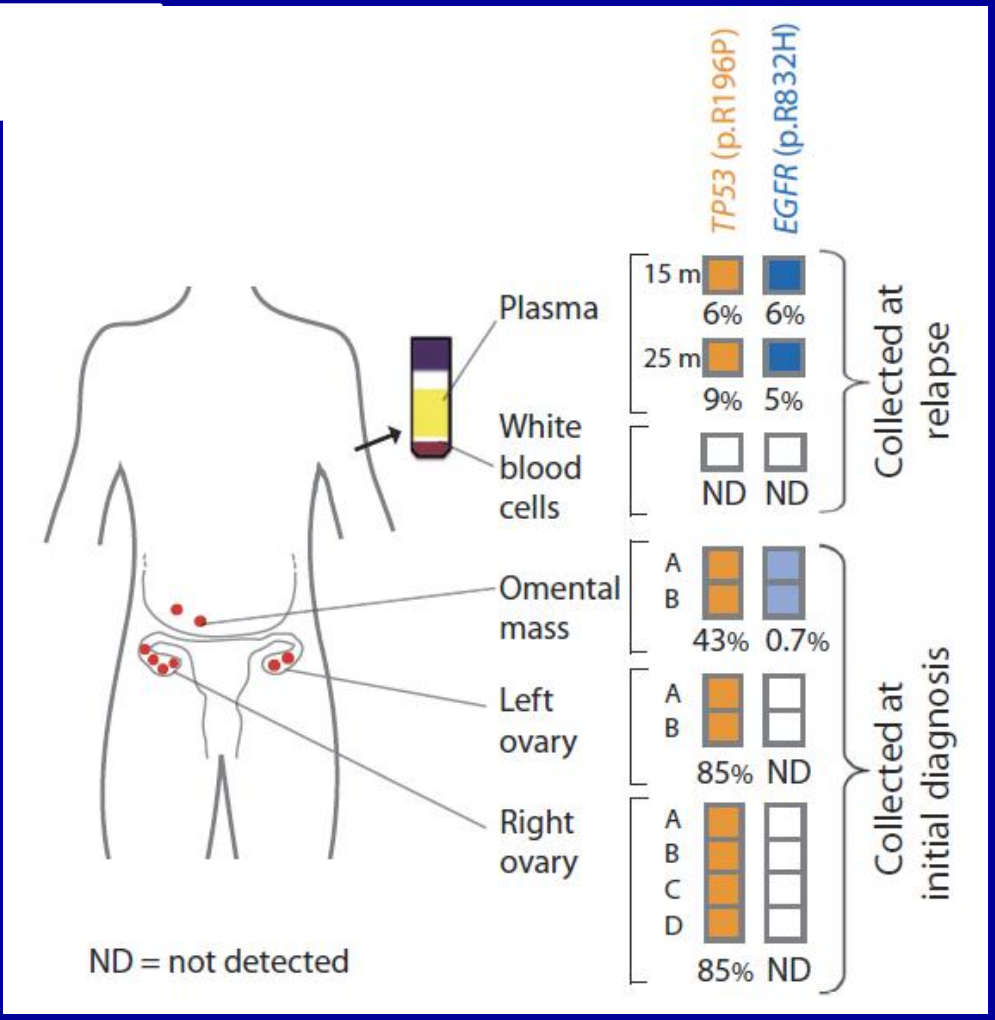
Forshew et al. Sci Transl Med 2012

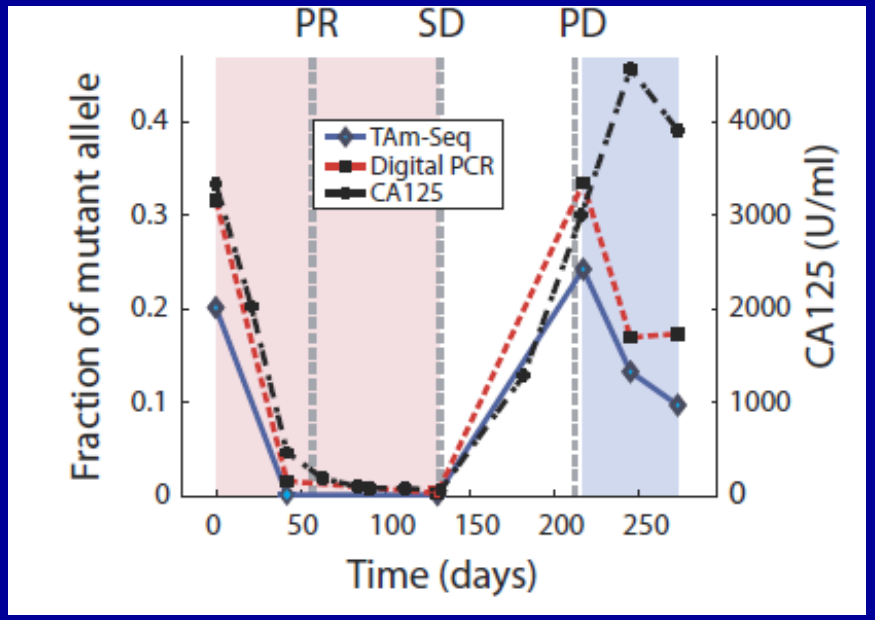
Tagged-amplicon deep sequencing (TAm-Seq)



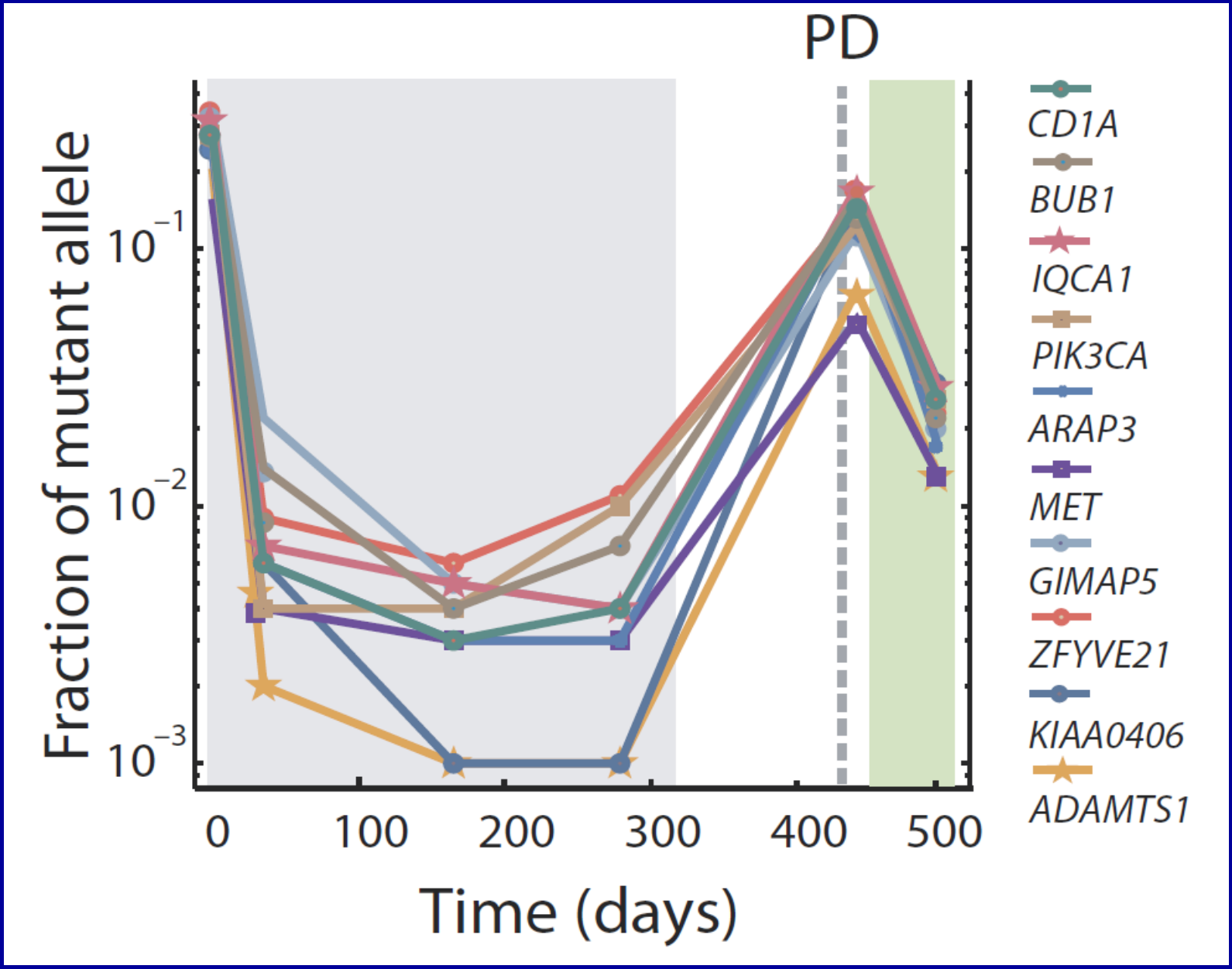
- 48 primer pairs
- ~6 kb genomic target
  - *TP53*
  - *PTEN*
  - *EGFR*
  - *BRAF*
  - *KRAS*
  - *PIK3CA*

Forshew et al. Sci Transl Med 2012


TP53 (p.R196P)
EGFR (p.R832H)
Plasma
White blood cells
Omental mass
Left ovary
Right ovary
15 m
25 m
6%
6%
9%
5%
ND
ND
A
B
43%
0.7%
A
B
85%
ND
A
B
C
D
85%
ND
Collected at relapse
Collected at initial diagnosis
ND = not detected


PR
SD
PD
TAm-Seq
Digital PCR
CA125
Fraction of mutant allele
CA125 (U/ml)
Time (days)
0
0.1
0.2
0.3
0.4
0
1000
2000
3000
4000
0
50
100
150
200
250

Breast cancer

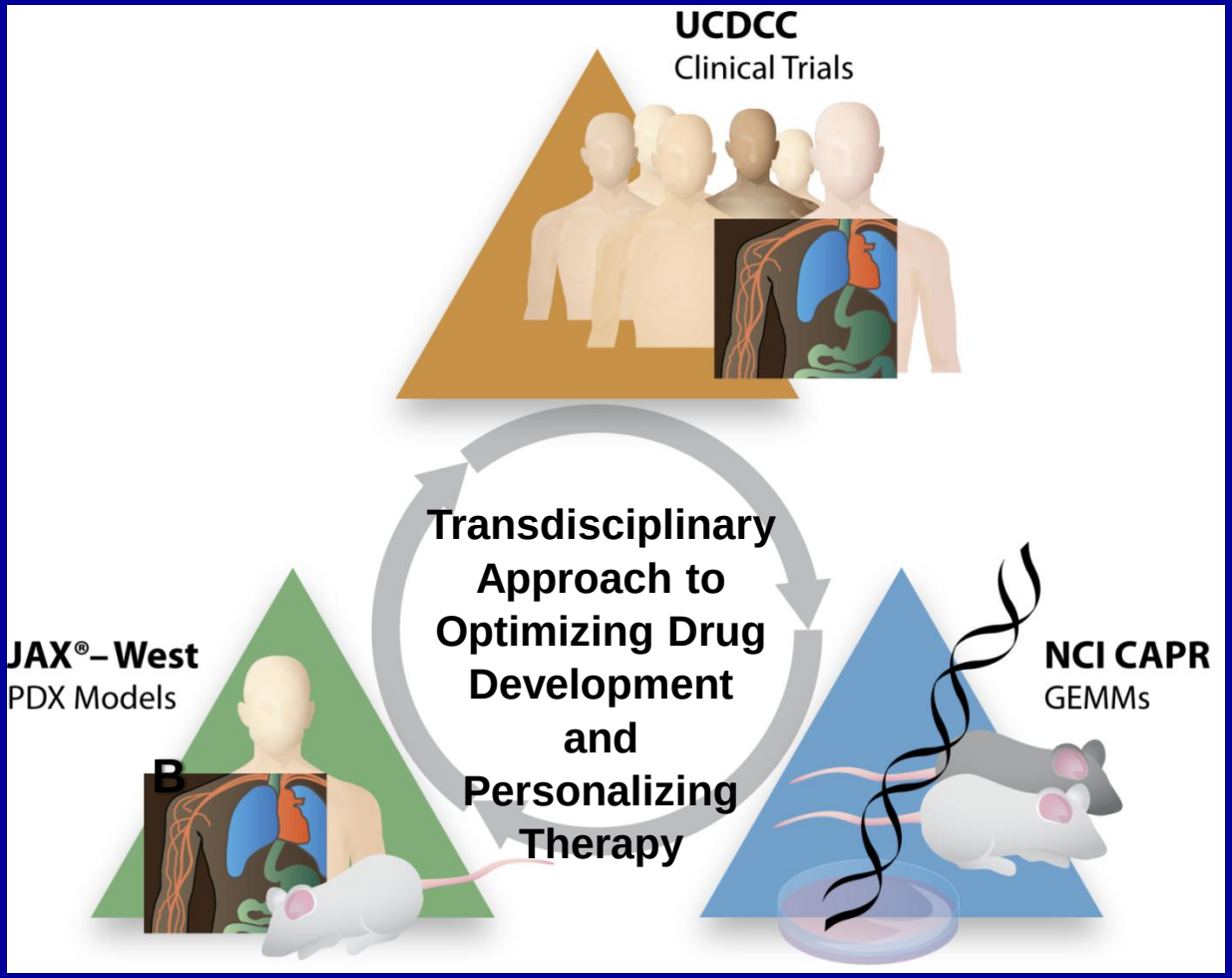


Strategy #4
Man and his mice

Patient Derived Xenograft (PDX)

*i*GXT Platform™

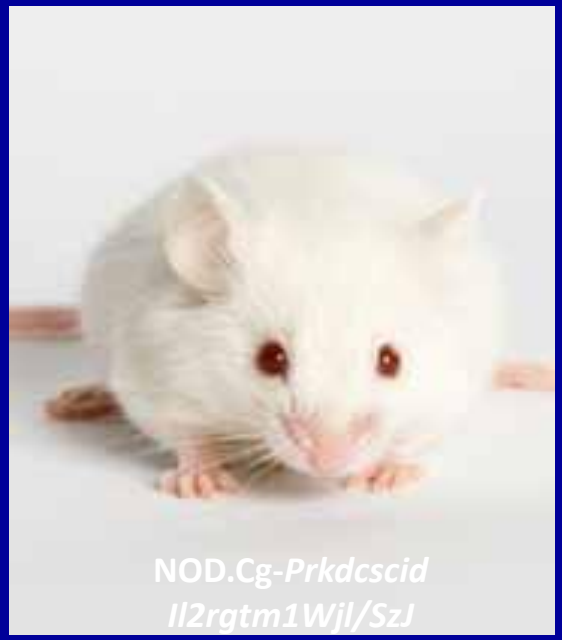
integrated GEMM • Patient-Derived Tumor Xenograft • Trials/Technology



CAPR: Center for Advanced Preclinical Research; JAX: Jackson Laboratories; UCDCC: UC Davis Comprehensive Cancer Center

Courtesy of Dr. David Gandara

JAX Lab NSG Mouse Model: PDX Host



NOD-scid Gamma Null (NSG) Mouse Model

- **Profoundly immune deficient**
  - **Lack mature T and B cells**
  - **No functional NK cells**
  - **Deficiencies in cytokine signaling**
  - **NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(CMV IL3,CSF2,KITLG)1Eav/MloySzJ availability**
- **High take rate of human tumors**
  - **2-3 mm^3 tumor fragments (optimal)**
  - **Cell pellets from pleural effusion, ascites**
  - **FNA from IR or EBUS**
- **Transportability**
  - **Over-night shipment of tumor specimens for implantation facilitates multi-institution collaboration**

Courtesy of Dr. David Gandara

UCD-JAX-WEST NSG Resource: Development Process from Patient → PDX host

Patient biopsy at UC Davis

to JAX

Patient tumor

P0

N=1-10

P1

N= 10-15

Freeze P1 fragments derived from P0 tumors for future use

Cryorecovery

P1 fragments

P1 or P2 tumors on drug study

Comparative Characterization of NSCLC Patient Tumors (PTs) & Patient-Derived Xenografts (PDXs) in NSG Mice (JAX-WEST & UC Davis)

Pretreatment Biopsy or Surgical Specimens (PTs) -Clinical Annotation

Transplantation of 2-3 mm^3 tumor fragments

JAX NSG PDXs

Director: Neal Goodwin

1. Histo-Morphologic Evaluation:
- FFPE
- IHC & Morphoproteomics

2. Clinically Applicable Molecular Biomarkers:
- Mutations: EGFR and K-RAS
- Fusion oncogene: EML4-ALK fusion variants
- mRNA levels of ERCC1, RRM1 and TS
- Genotyping for "driver" mutations

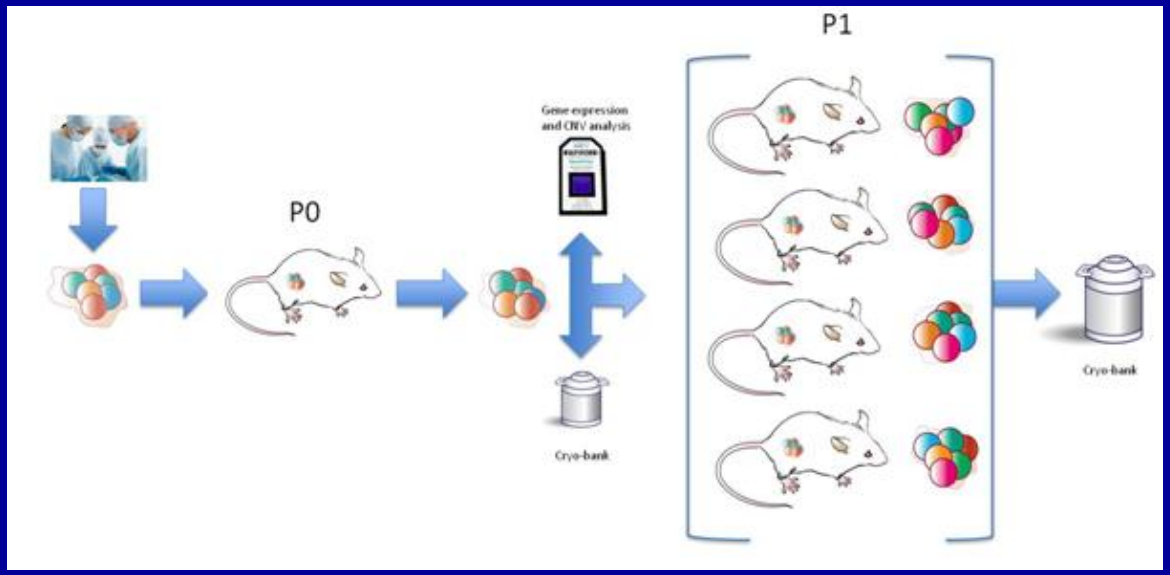
3. Genome-wide Molecular Profiling:
- CNV/SNP and gene expression arrays
- Next generation sequencing (Illumina)
- miRNA and epigenetic profiling

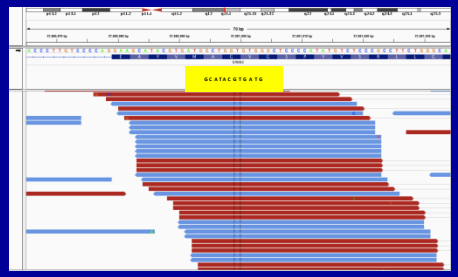
4. Targeted *in vivo* drug testing at JAX

5. Characterization of cancer stem cells in mediating resistance to therapy

From T. Li & P. Mack, UC Davis

Development of PDX Platform for Drug Testing in JAX-NSG Models: Selected EGFR-Mutation (MT+) Models





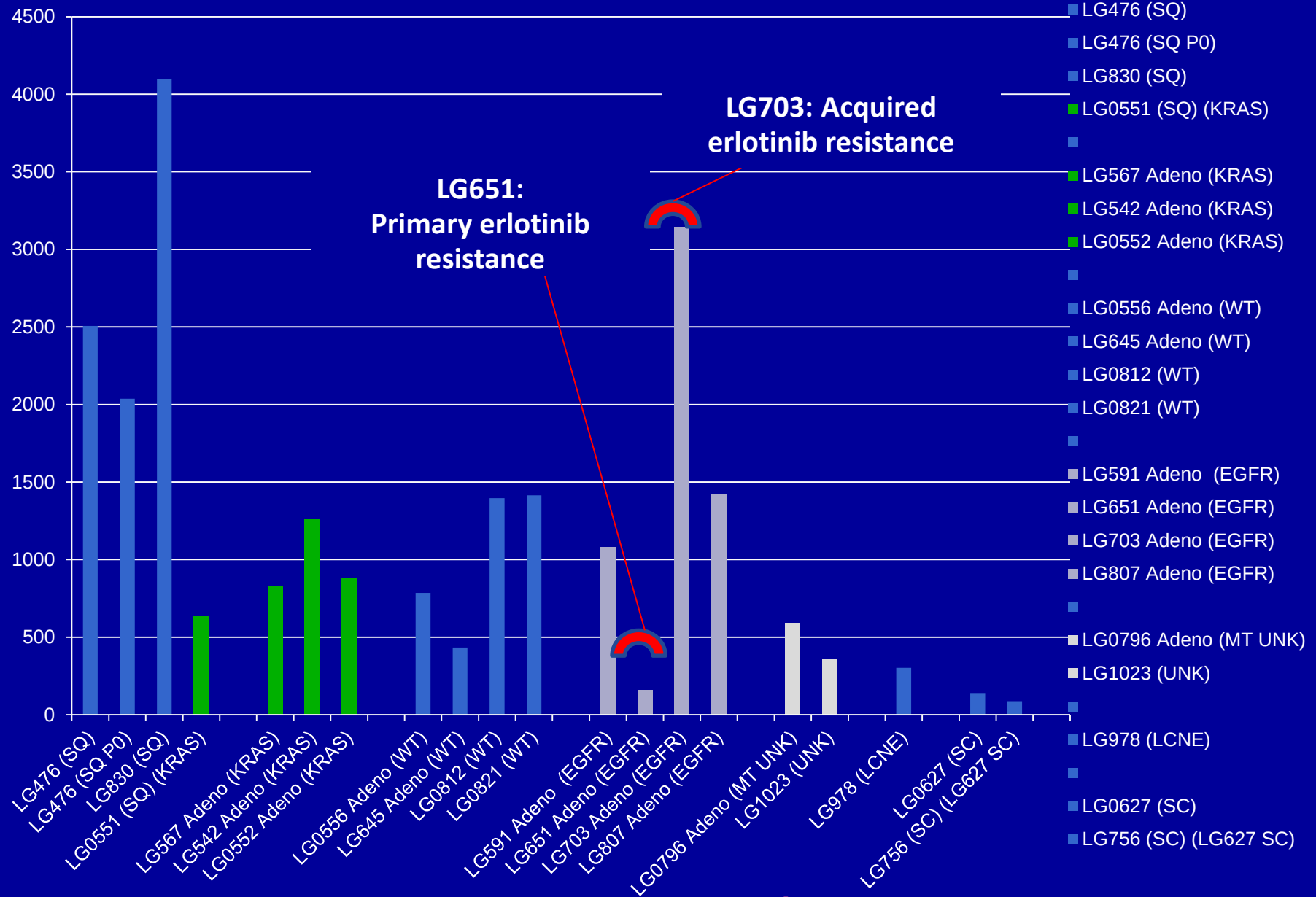
Molecular Characterization of Models

Selected Examples

EGFR activating MT+ Models (Examples)

LG628	LG631	LG703	LG651	LG807
EGFR aMT+ (E19del) Sensitive	EGFR aMT+ (L858R) Sensitive	EGFR aMT+ (L858R + MET) Acquired Resistance	EGFR aMT+ (L858R + T790M) De novo Resistance	EGFR aMT+ E19del+T790M Resistant
Regimens "A, B, C, D"	Regimens "A, B, C, D"	Regimens "A, B, C, D"	Regimens "A, B, C, D"	Regimens "A, B, C, D"

Examples: EGFR expression levels in Lung Cancer PDXs



Summary

- Addressing the difference
  - Tumor heterogeneity exists and may account to diversity in treatment outcome
- TKI is forever
  - Long term TKI for the concept of oncogenic addiction
- A liquid profile
  - Feasible to study genome profile from plasma DNA
- Man and his mice
  - In-vivo real time drug sensitivity testing method

Blind leading the blind