



Diplôme Universitaire
de Carcinologie Thoracique Intégrée :
**Module 1 – Base de la
cancérologie thoracique**

- **Biologie moléculaire :
techniques en routine
et en recherche**
- **ADN circulant**

Ludovic Lacroix

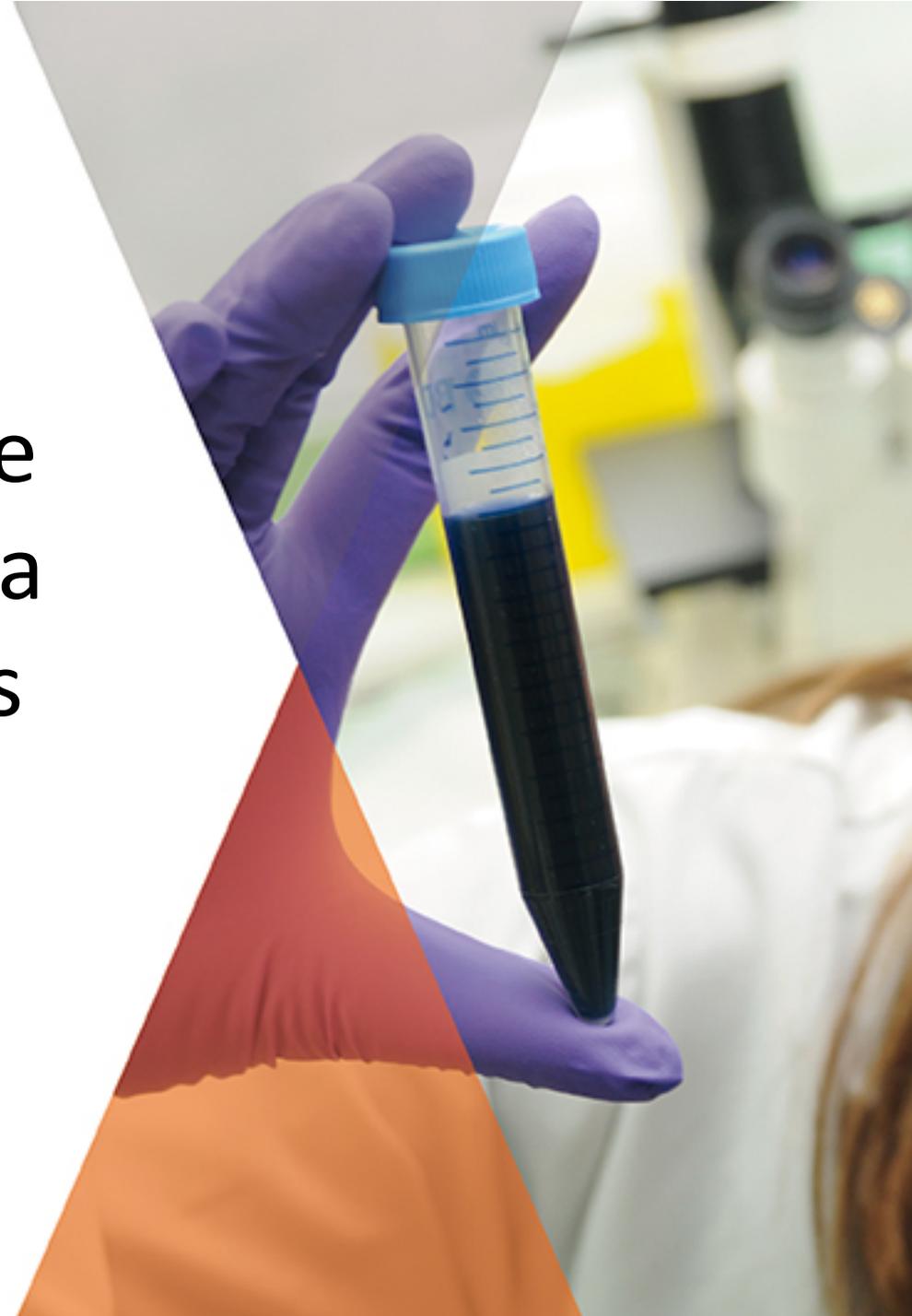
janvier 2017



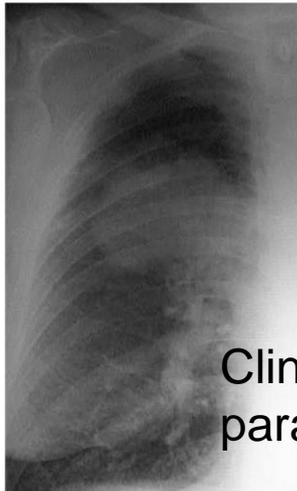
Thèmes abordés

- **Place de la biologie moléculaire dans la caractérisation des tumeurs bronchiques**
- **Techniques de base et organisation d'un laboratoire de Biologie Moléculaire**
 - > Pré-analytique
 - > Analytique
 - > Programme Inca
- **Les techniques de nouvelle generation**
 - > La cytogénétique haut débit : CGHa/SNPa
 - > Les approches multiplex haut débit : le NGS
- **Biopsie liquides : l'ADN libre circulant**
- **Conclusion**

Place de la biologie
moléculaire dans la
caractérisation des
tumeurs
bronchiques

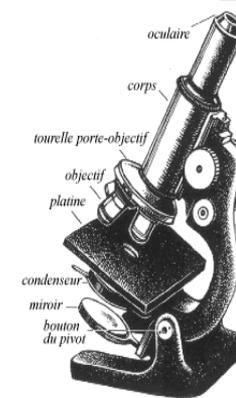


Un nouvelle conception du la caractérisation de la tumeur

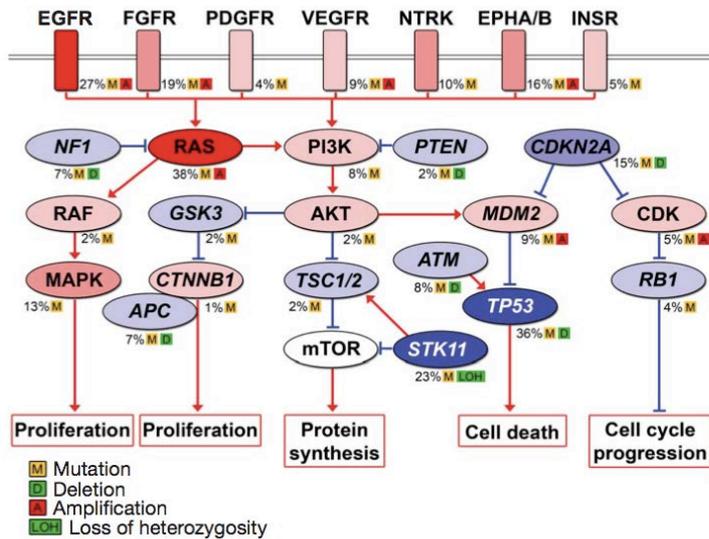


Clinical parameters

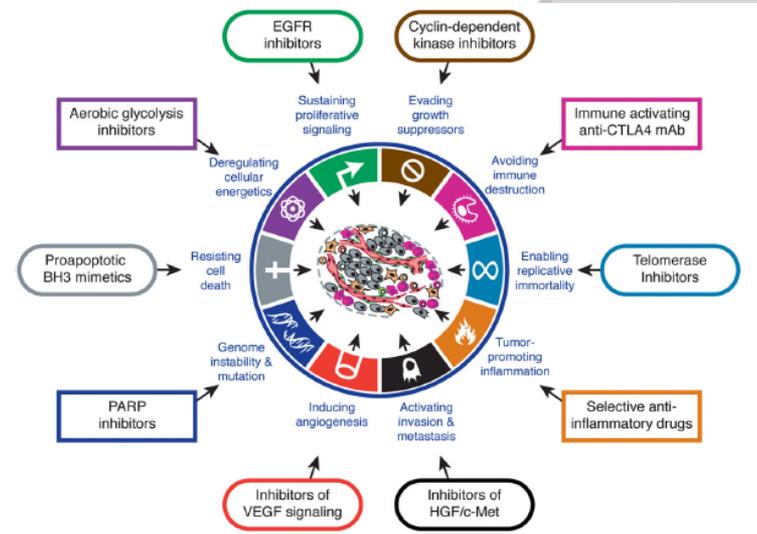
Anatomo
Histological
parameters



Histological
microscopic
parameters



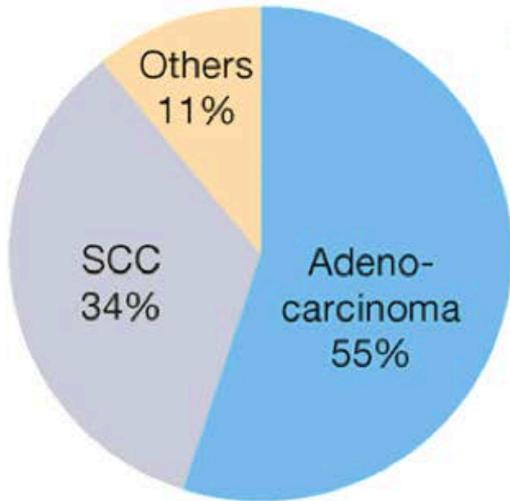
Ding et al. Nature 2008



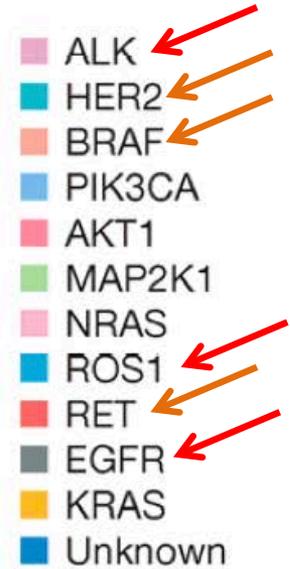
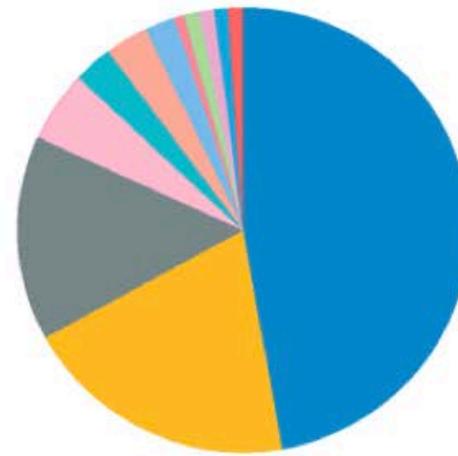
Hanahan, Weinberg Cell 2011



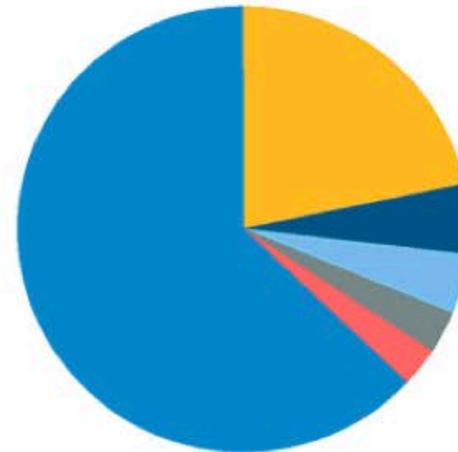
Histology-based subtyping

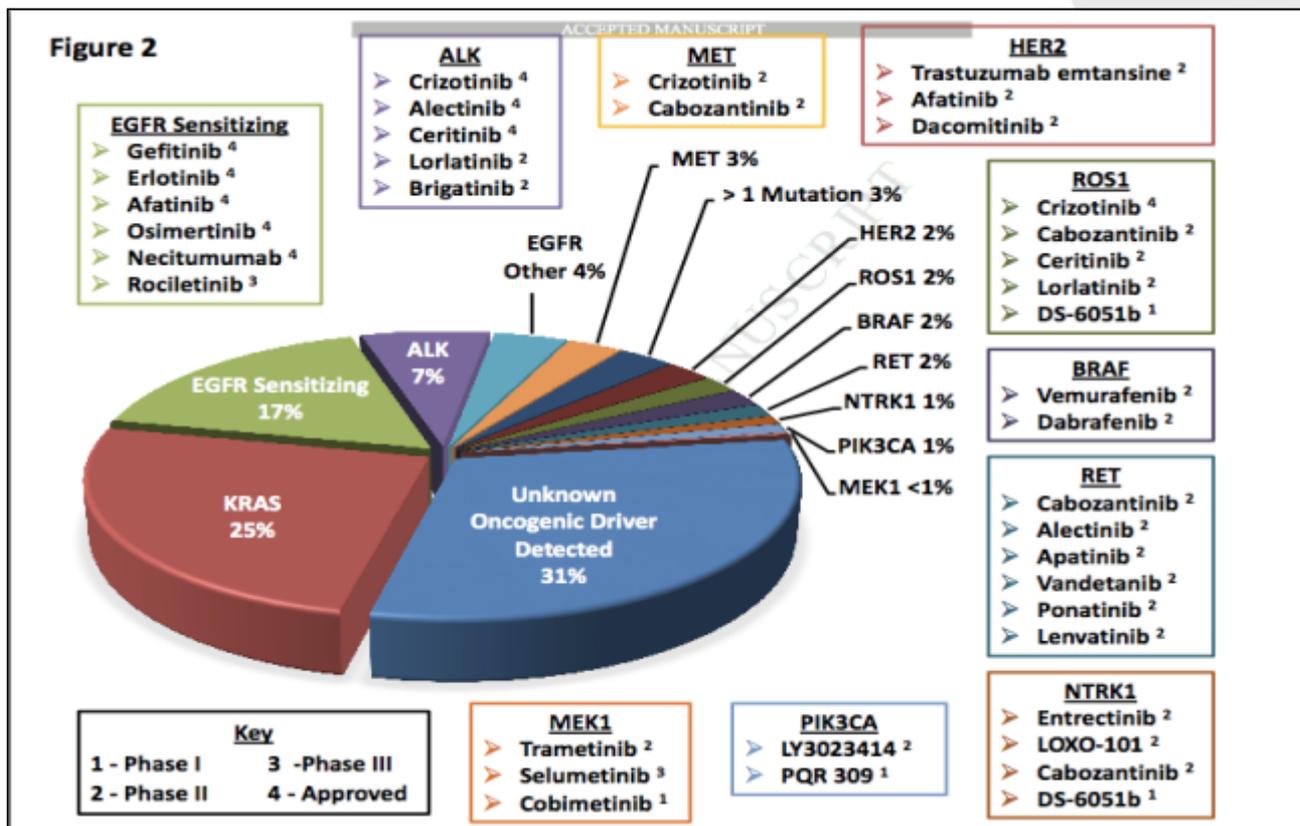


Adenocarcinoma



SCC





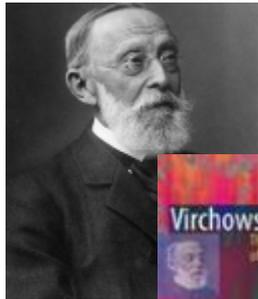
Tsao – J Thorac Oncol 2016

Biomarker Need for standard drug :

EGFR mutations (KRAS mutation?)
 ALK / ROS 1 fusions

BRAF/MET/MEK1/HER2 mutations
 RET fusion ; NTRK1 fusion
 MET/ HER2 Amplification

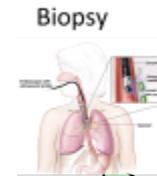
→ Changement de paradigme



Une tumeur
un organe
Un prélèvement diagnostic

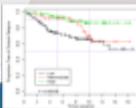
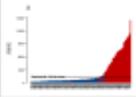
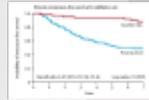
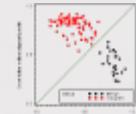
Biomarqueurs Moléculaire

“One size fits all”



Precision Medicine

High throughput
Technologies
GE / CGH / NGS



Diagnosis

Prognosis

New Target

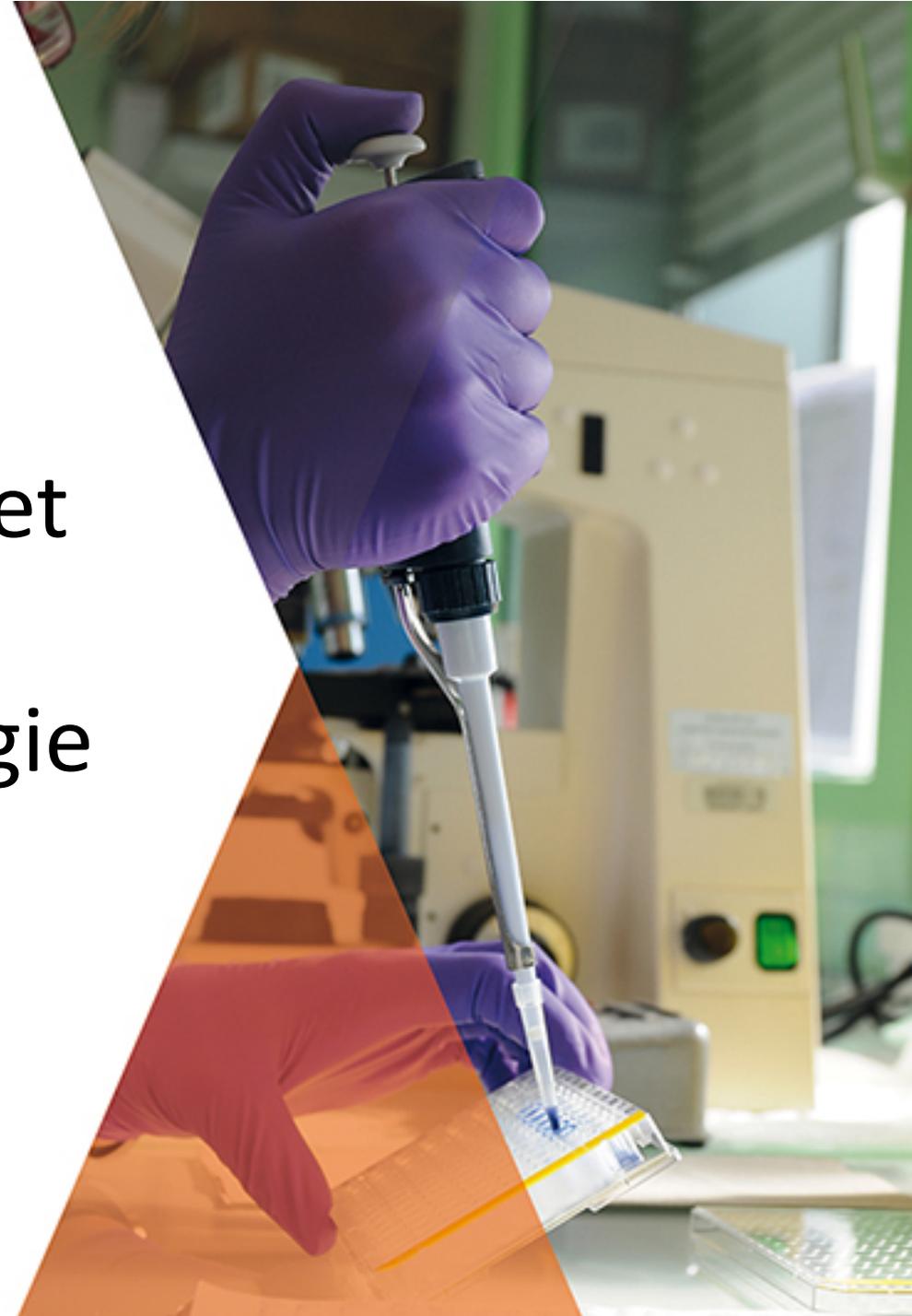
Conventional
treatment



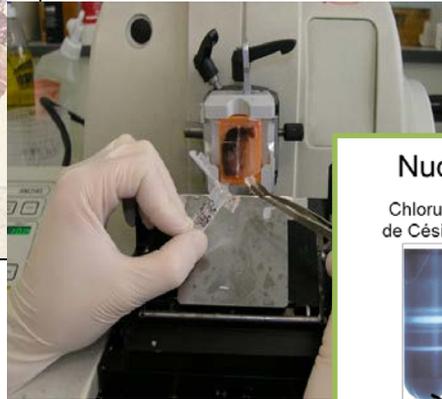
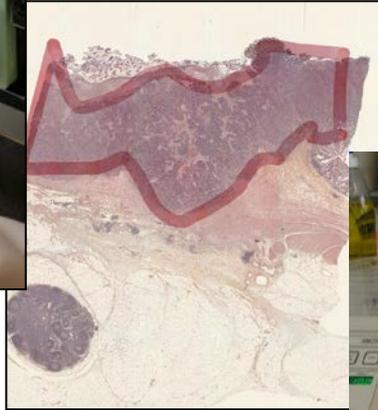
Théranostique=>Méd. Stratifiée=>Méd. personnalisée=>Méd. de Précision

- Le concept de « *Precision Medicine* » change le paradigme basé sur la **classification** essentiellement **histologique** des cancers vers un paradigme intégrant une **classification moléculaire** des cancers.

Techniques de base et organisation d'un laboratoire de Biologie Moléculaire



Continuum avec l'anatomopathologie



Nucleic Acid Extraction :

Chlorure de Césium	Phénol-Chloroforme	Matrice de Sil
<p>ADN mitochondrial ADN nucléaire ARN</p>	<p>ARN/ADN (pH) phase aqueuse</p>	

1 Cell = 6pg DNA ; 1g = 10⁶-10⁹ ;

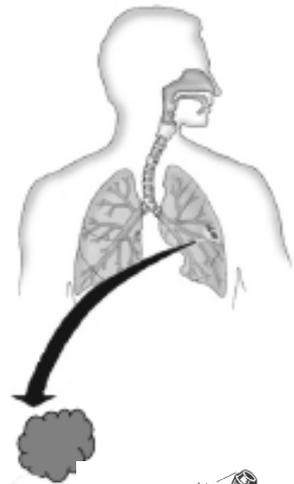
➔ Storage +4°C/-20°C/-80°C,



MUTATION ?

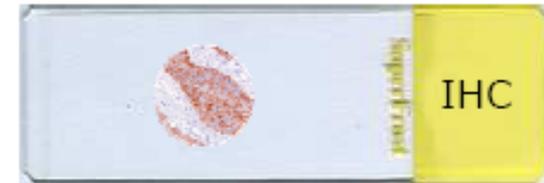
→ Change of paradigm and limitation in real Life...

Diagnostic fresh or paraffin embedded specimens collected and/or frozen



Cut and stain slides

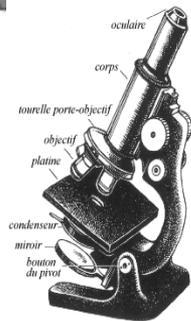
Tumor tissue marked by pathologist



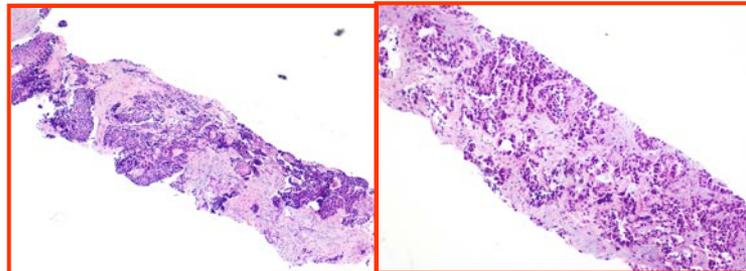
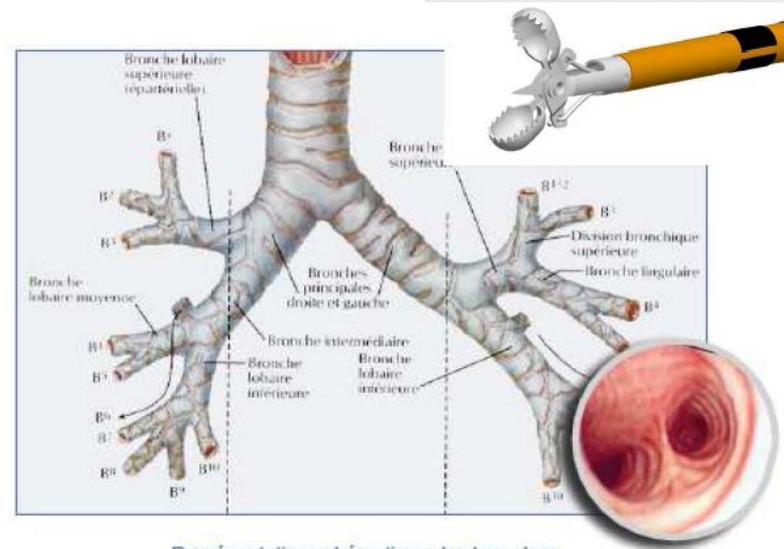
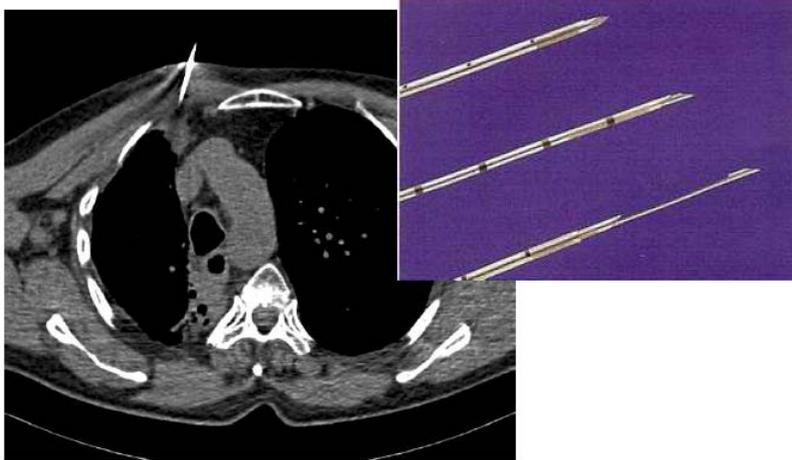
Quality of nucleic acid

ISOLATE TUMOR DNA
PCR Amplify

- EGFR
- PI3KCA
- K-ras ...

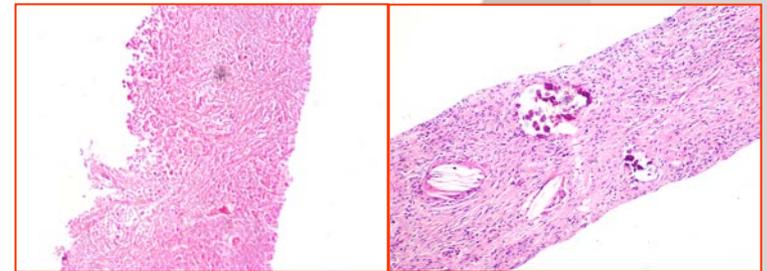


Limitation in real Life...



Squamous Cell Ca Adenocarcinoma

Adequate Biopsies

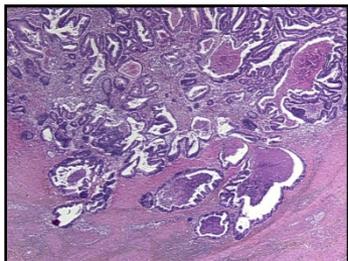


Necrosis

Fibrosis

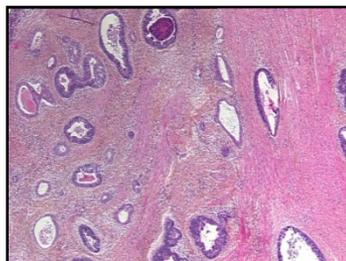
Inadequate Biopsies

Statut *KRAS*: impact cellularité !



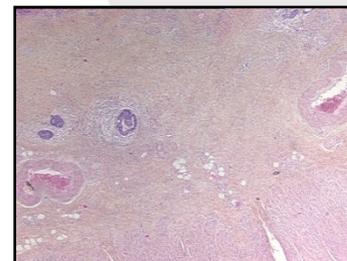
> 50%

Si 1 allèle muté sans amplification
=> 25% d'ADN muté



> 20 < 50%

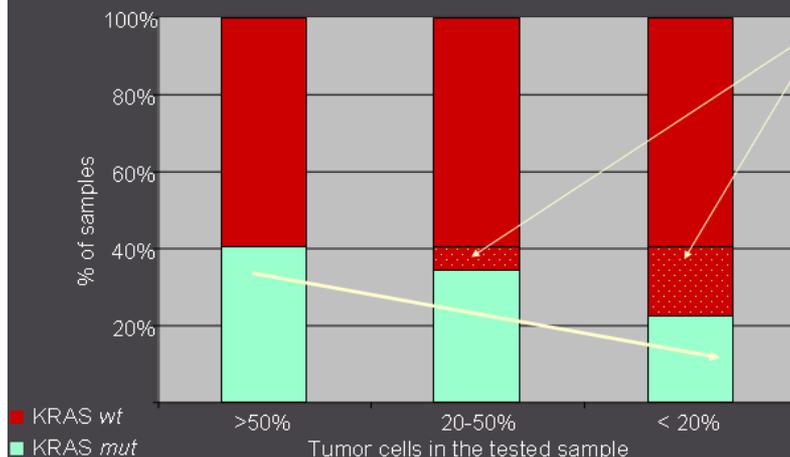
Si 1 allèle muté sans amplification
=> 10 à 25% d'ADN muté



< 20%

Si 1 allèle muté sans amplification
=> Moins de 10 % d'ADN muté

RESULTS (IV): MUTATION RATE & TUMOR CELLULARITY



False negative cases ?

Groups	Pearson Chi2 test
[>50%] vs [20-50% and <20%]	$p = 0.056$
[<20%] vs [20-50% and >50%]	$p = 0.044$
[<20%] vs [20-50%] vs [>50%]	$p = 0.061$

→ *KRAS* mutations rate decreased with the ratio of tumor cells/non tumor cells

→ Major risk of false negative in tumor presenting a low amount of tumor cells

→ *Biopsie dédiée à la pathologie moléculaire si traitement néoadjuvant*

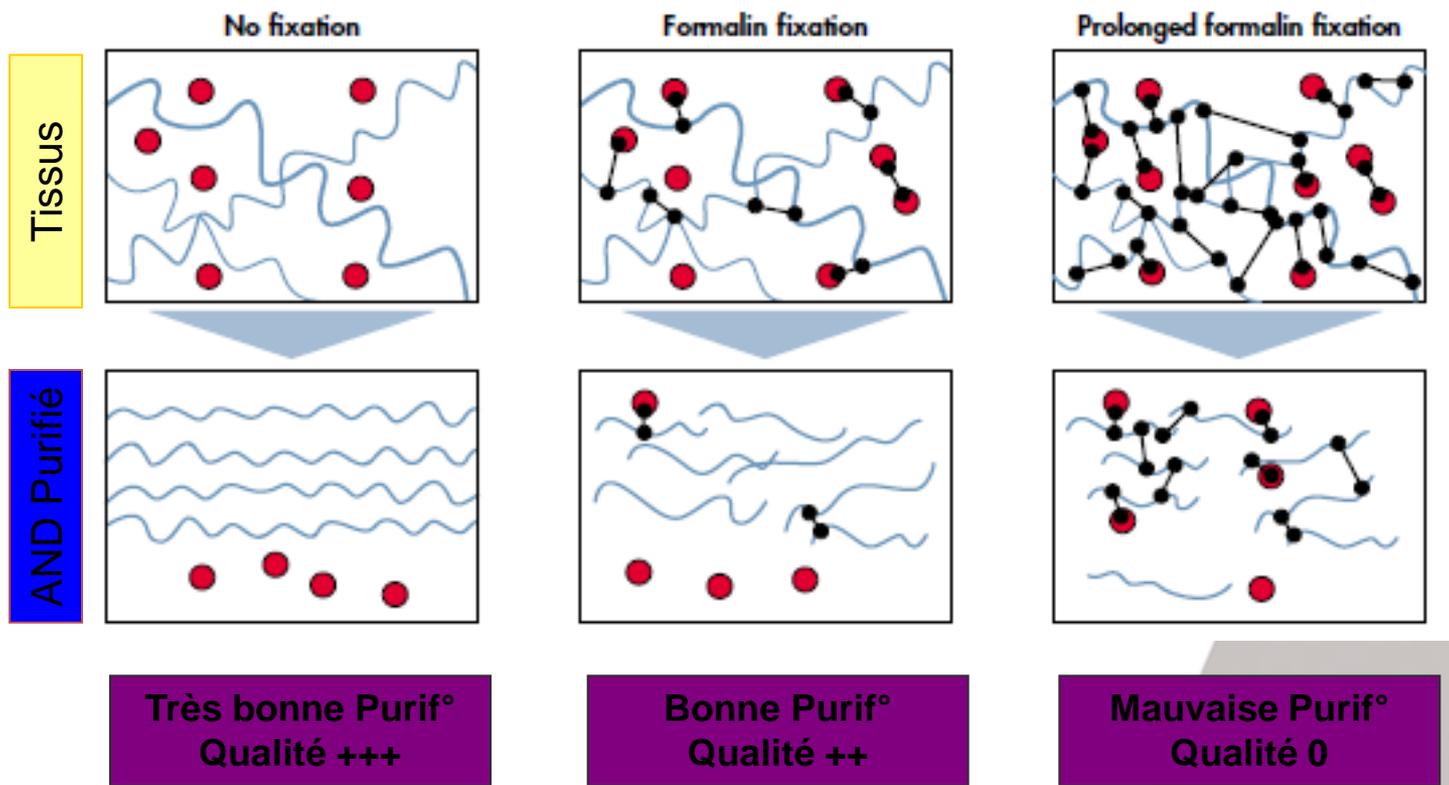
Tissus fixés : Facteurs liés à la qualité de l'ADN



Taille du tissu
Fixateur (type/Vol./conc.)
Temps

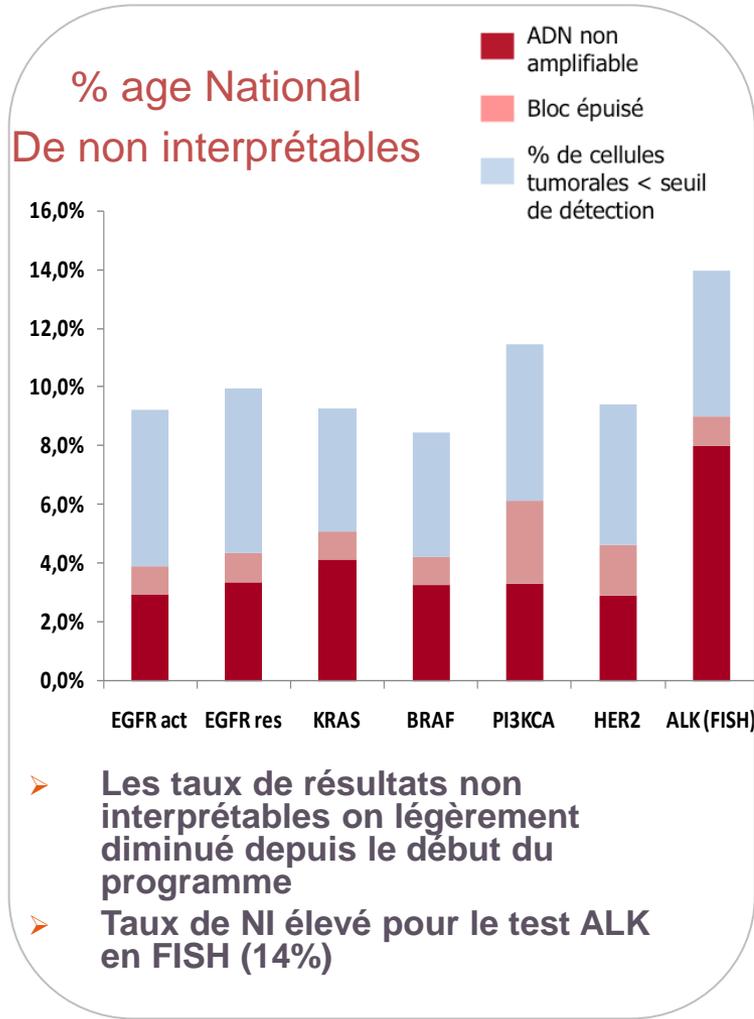
Température
Durée

Méthode
Equipement / Techniques d'analyses

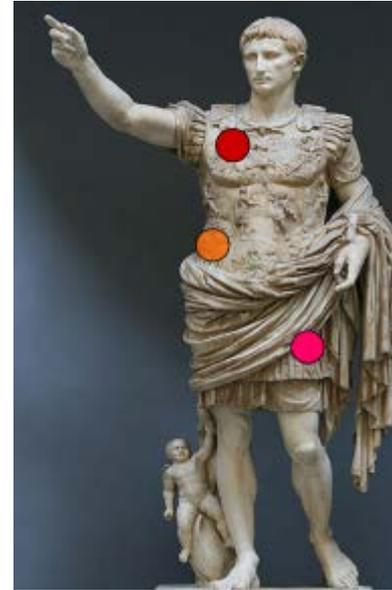


Biomarqueurs moléculaires : Limitations

Résultats non-interprétables



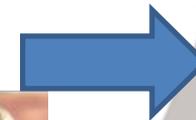
Le bon prélèvement ?



Primitif ou
Métastases
(synchrones ou
métachrones)

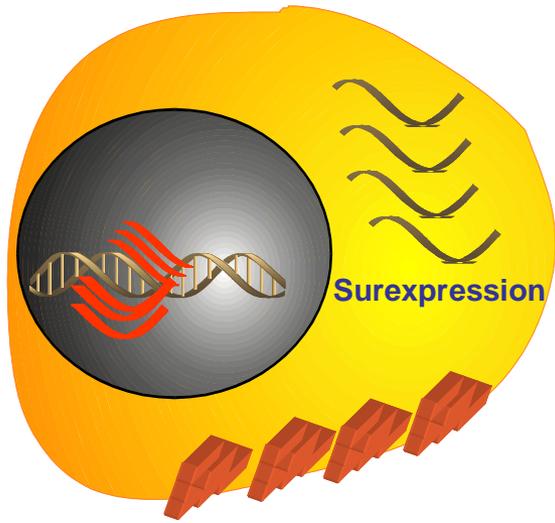
Au diagnostic

Après rechutes aux
traitements



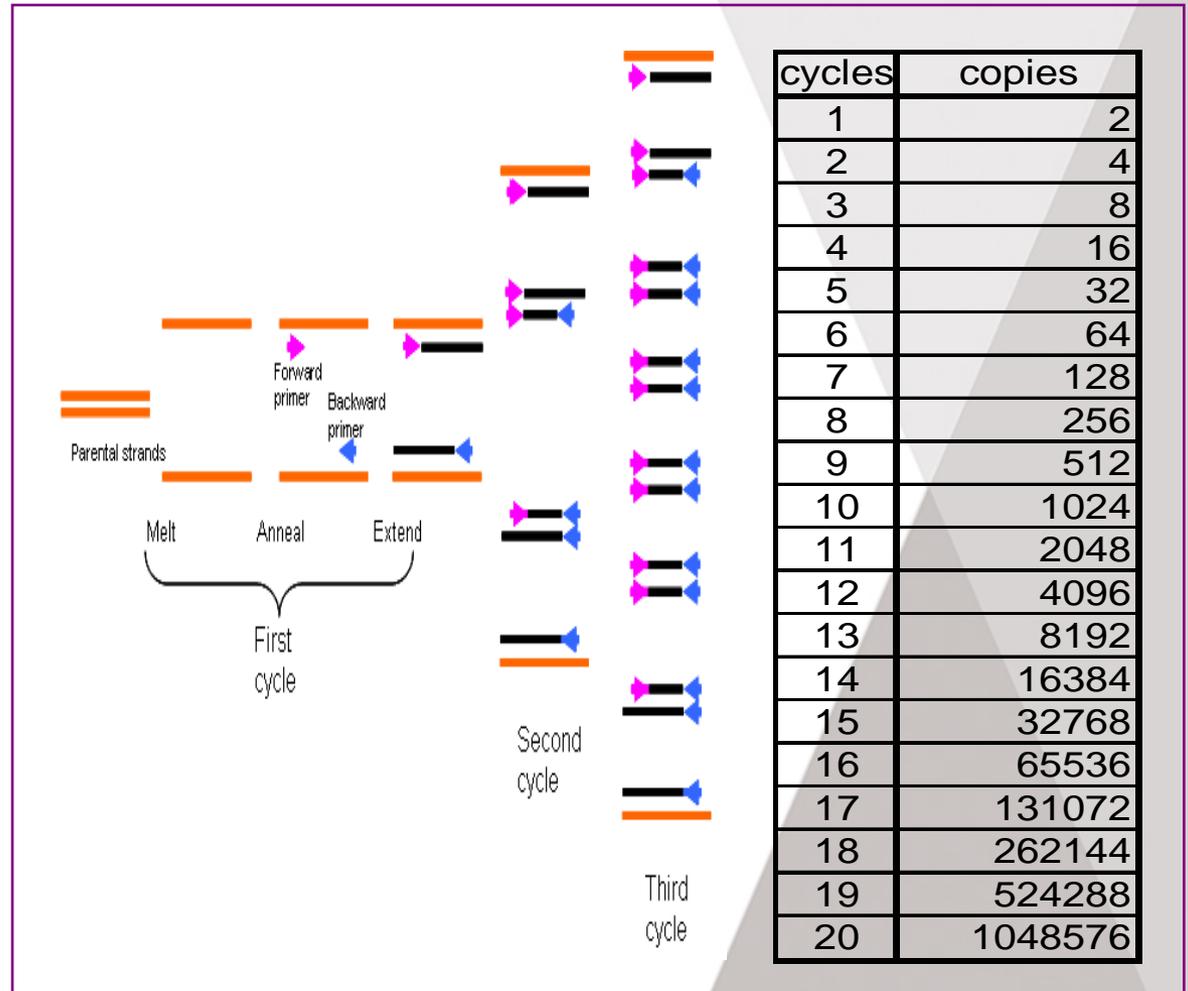
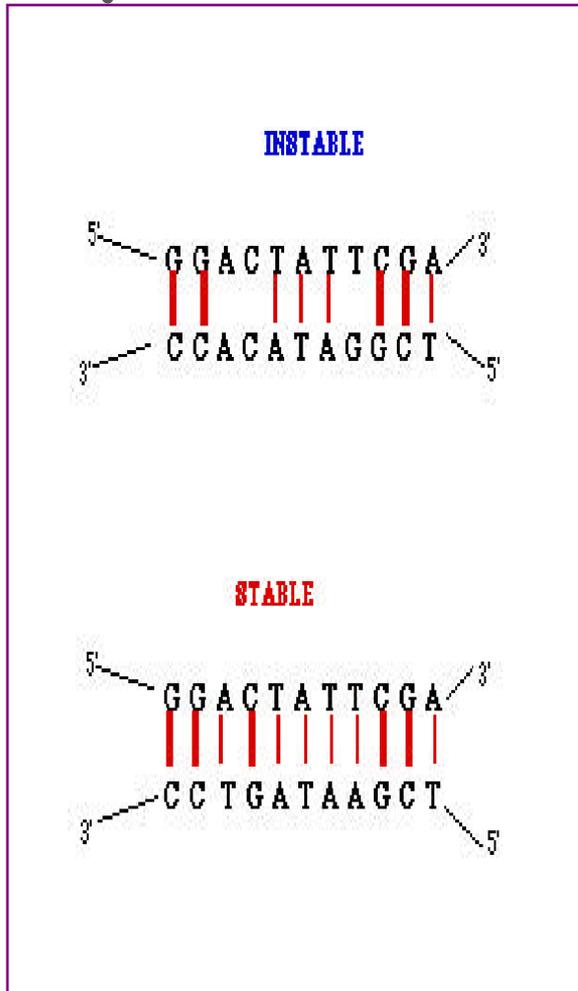
Pré-analytique

- **Importance de la qualité de l'échantillon biologique collecté**
- **Contrôle de la cible à analyser (Histologie)**
- **La conservation est elle compatible avec la méthode appliquée?**
- **L'ADN se conserve bien (l'ARN est très "fragile")^o**



Phase analytique

Hybridation & PCR, l'outil universel



- association de deux séquences complémentaires d'acide(s) nucléiques(s).

- très spécifique : permet la détection de faibles quantités d'A.N.

- Amplification à spécifique « l'infinie »

Techniques utilisées

- **Technique de détection des mutations ponctuelles**
- **Analyse de régions mutées = hotspot**
- **Techniques**

> Allèles spécifiques / ciblées = recherche d'un unique hotspot

☺ Sensibilité

☹ Faux négatif sur des mutations rares

> De criblage = information sur toute une région

☺ Détection de toutes les mutations d'une région

☹ Faux négatif si cellules tumorales à un taux trop faible

- **Techniques**

- Simplex = recherche de l'altération d'un unique marqueur voire d'une unique mutation

- Multiplex = recherche en parallèle de plusieurs mutations sur plusieurs marqueurs

A>C



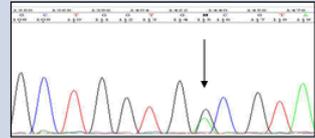
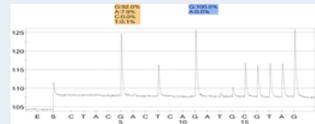
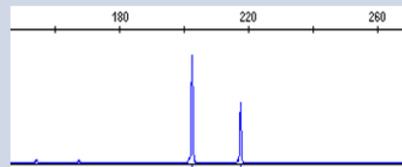
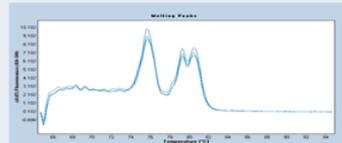
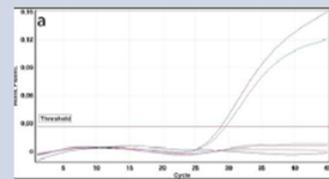
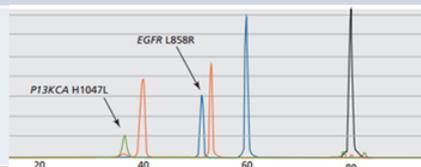
```
ACGTGCTATGCCTAGC
TGCACGATACGGATCG
```

```
ACGTGCTATGCCTAGC
TGCACGATACGGATCG
```

Neal I. Lindemane, Philip T. Cagle, Mary Beth Beasley, Dhananjay Arun Chitale, Sanja Dacic, Giuseppe Giaccone, Robert Brian Jenkins, David J. Kwiatkowski, Juan-Sebastian Saldivar, Jeremy Squire, Erik Thunnissen, Marc Ladanyi Accepted 12 February 2013. published online 08 April 2013.

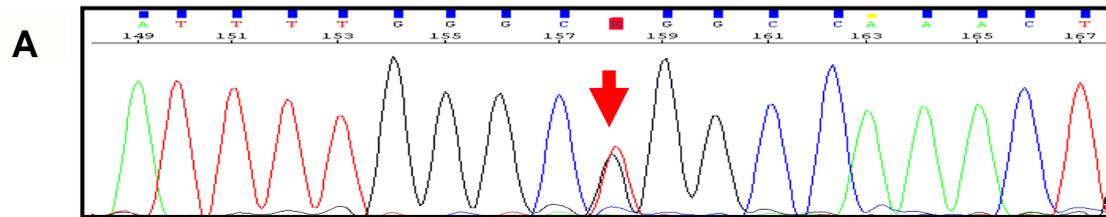
Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology

Diversité technologique biologie moléculaire

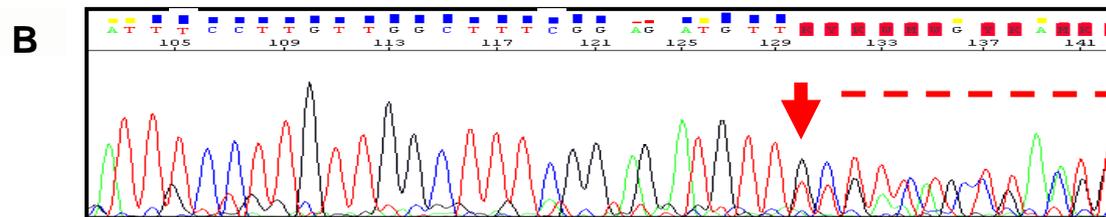
Technique	Limite de détection	Principe	Etapes	
Séquençage Sanger	10-20%	Criblage	PCR Séquence	
Pyroséquençage	5-10%	Criblage/mutations ciblées	PCR Séquence	
Analyse de fragments	5-10%	Criblage uniquement délétion ou duplication	PCR Migration	
HRM	5-10%	Criblage	PCR/HRM	
Allele spécifique Taqman ARM Scorpion	1-5%	Mutations ciblées	PCR	
Snap Shot	5-10%	Mutations ciblées	PCR Migration	

La méthode « Gold standard » : le séquençage direct

- ❖ Lecture directe de la séquence après amplification par PCR de la zone cible (e.g EGFR exons 19-21)



Mutation L858R



**Délétion de 15bp del745-
750**

• Pro. :

- robust & universal technic.
- easy to develop.
- Not limited to known mutation

• Cons:

- PCR >150bp , not quantitative
- time consuming (2 days),
- Sensitivity (30%-50% Cell.Tum)

Évolution technologique vers le NGS

Cible	Enrichissement	Nombre de bases	Technologie	Application FFPE
Génome entier	Non	3,4 billion bases (Gb)	Illumina HiSeq	Oui
Exome	Capture	1-2% du génome 50Mb 26,000 gènes	Illumina HiSeq NexSeq MiSeq S5	Limité
Ciblé (target)	Capture PCR	1 à 190 gènes...	Illumina MiSeq PGM	Oui +++

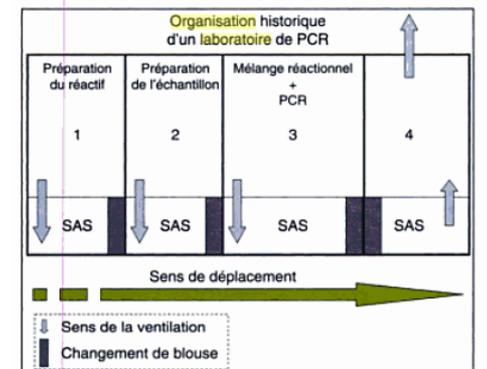
D'après Mardis Annu. Rev. Anal. Chem. 2013. 6:287–303

Organisation du laboratoire

Doit être conforme aux bonnes pratiques des laboratoires :

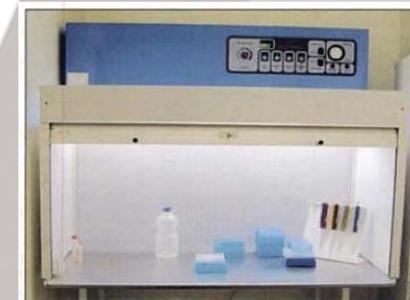
- Norme ISO15189 – Famille « génétique somatique »

- > SMQ,
- > Organisation des locaux,
 - Eviter les contaminations / FP
 - Séparation pré-PCR et post-PCR
 - Hottes spécifiques
 - Utilisation de Cônes à Filtre...



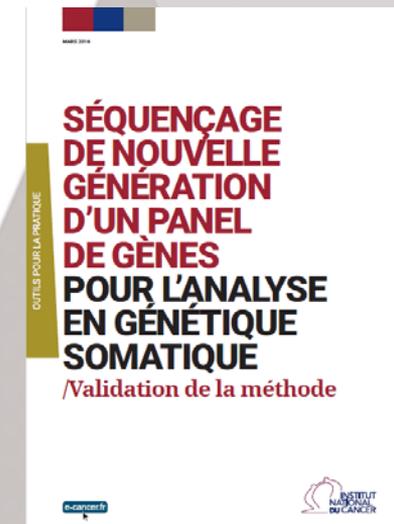
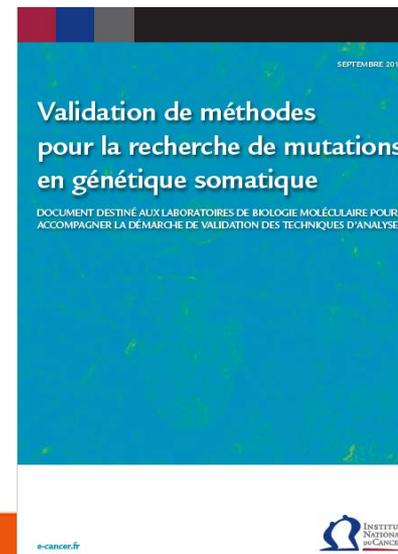
- > Validation de méthode, Procédures et modes opératoires normalisés (MON), Fiches techniques,
 - Eviter les résultats faussement négatifs
 - Validation(sensibilité/spécificité reproductibilité/répétabilité)
 - Contrôles Positifs (et négatifs...), CQE
 - Stabilités dans les réactifs/concentrations...

- > Métrologie
- > Traçabilité,
- > Indices « Qualité »



Choix de la méthode

- **Compatibilité avec la paraffine**
- **Sensibilité/Spécificité/Reproductibilité**
- **Délai/temps d'analyse**
- **Equipements du laboratoire**
- **Qualité / Normes ISO 15189**
- **Coûts**
- **Recommandation**



Le compte rendu (recommandations 2010)

- Éléments demandés à la prescription
 - > nom, num bloc, **diagnostic, histologie, %age de Cell.Tum...**
 - > Identifiant unique de l'échantillon par le laboratoire ;
 - > Date d'arrivée du prélèvement dans la plateforme ;
 - > Nom du pathologiste ayant qualifié le prélèvement ;
- Date du compte rendu ;
 - > Identifiant unique sur chaque page ; nb total de pages
 - > **Méthode(s) utilisée(s)** avec indication de la (des) **sensibilité(s) analytique(s)** ; liste des **mutations cherchées** ;
 - > En cas de Mutation identifiée et notée (à l'exception des délétions de l'exon 19 de l'*EGFR*) selon la **nomenclature internationale** (e.g p.Glu12Asp et c.35G>A).
- Commentaires sur les résultats
 - > Statut **WT ou Muté**
 - > **Précision sur le type mutation**
 - > **Prédiction de l'impact thérapeutique**
- Nom du biologiste/pathologiste moléculaire.

transmis au maximum 7 à 10 jours ouvrables après réception du prélèvement par la plateforme.

(délai de 2 à 3 semaines à compter de la date de la prescription)

=> Nécessite prescription avec information patient/maladie/objectif recherché ?

Annotation officielle selon HGVS

~~KRAS G12S~~

Transition de G en A dans l'exon 2.
Substitution faux-sens.
Gly à la position 12 change en Ser.

Selon les recommandations de l'HGVS (version 2.0), cette variation doit être libellée:

Niveau ADN (cDNA):

NM_033360.3:c.34G>A

Niveau ADN (génomique):

Chr12(GRCh37):g.25398285C>T

Niveau protéique:

p.Gly12Ser

Et non pas G12 ou G12S....

~~EGFR del Exon19~~

Délétion (15 pbs) dans l'exon 19.
Cette délétion entraîne la perte de 5 résidus.

Nomenclature HGVS v2.0

Niveau cDNA:

NM_005228.3:c.2235_2249del

Niveau gDNA:

Chr7(GRCh37):g.55242465_55242479del

Niveau protéique:

p.Glu746_Ala750del

Annotation officielle selon HGVS

Substitution

c.123A>G sur le cDNA, A, en position 123, est remplacé par G
p.Pro252Arg (P252R) sur la protéine, proline (P) remplacée par arginine (R)

Délétion

c.546delT délétion de T en 546
c.586_591del six bases délétées
p.Phe 508del délétion de phenylalanine (F) en 508

Duplication

c.546dupT duplication de T en 546
c.586_591dup duplication du segment 586 -> 591
p.Gly4_Gln6dup duplication du segment à partir de glycine (G) en 4 jusqu' à glutamine (Q) en 6

Insertion

c.546_547insT insertion de T entre 546 et 547
c.1086_1087insGCGTGA insertion de GCGTGA
p.Lys2_Leu3insGlnSer insertion de glutamine sérine entre lysine (K) en 2 et leucine (L) en 3

Inversion

c.546_2031inv segment 546 -> 2031 inversé

Décalage cadre de lecture

p.Arg83SerfsX15 arginine (R) est le premier acide aminé changé, c' est en position 83, cela donne une sérine (S) à la place, la taille du segment en aval est de 15, codon stop (X) inclu

Croissance d'une « nouvelle discipline »

Organisation des Tests moléculaires en France. (marqueurs biologie moléculaire + cytogénétique + histo.)

Lancement en **2006** des « plateformes de génétique moléculaire des cancers » supportées par l'INCa,

- Laboratoire Hospitalier
(CHR, CHU, CLCC+qq privé)
- Fond DGOS via l'INCa
MIGAC/MERRI - annuel
mise en place du système RIHN (2016)
- Recommandations
- Support pour la mise en place
de l'AQ – ISO 151689 – CQE
- Support pour le dvpt de
biomarqueurs innovants et de
nouvelles technologies



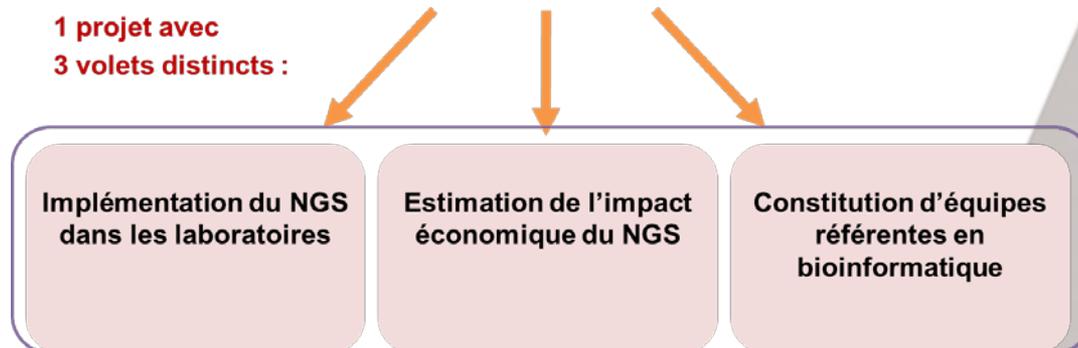
www.e-cancer.fr

(rubrique « les Soins »)

Programmes spécifiques

- ❖ Le programme « **Biomarqueurs émergents** » depuis 2009
- ❖ Le projet **BIOMARQUEURS France**
- ❖ Lancement du Contrôle Qualité National (Gen&Tiss et BRAF)
- ❖ Le programme **AcSé**
(screening des cibles du Crizotinib et du Vémurafenib) depuis 2013
- ❖ Le programme **NGS** lancé en 2013-2014

1 projet avec
3 volets distincts :



Suivi de 44 tests dans 23 pathologies

✓ 89 000 tests déterminants pour l'accès à une thérapie ciblée ont été réalisés en 2013 pour 65 000 patients.

Tableau 1. Nombre de recherches de marqueurs prédictifs de la réponse à une thérapie ciblée en 2013

Pathologie	Biomarqueur	Nombre de tests	
Cancer gastrique	Amplification d'HER2	8 924	
			Cancer du sein
Cancer colorectal métastatique	Amplification d'HER2	709	
	Cancer colorectal	Mutations de KRAS	19 347
		Mutations de NRAS	3 330
GIST (Gastro-Intestinal Stromal Tumor)	Mutations de KIT	1 105	
	Mutations de PDGFRA	1 005	
	Mutations d'EGFR	23 336	
Cancer du poumon	Translocation d'ALK	18 861	
	Mélanome	Mutation de BRAF V600	5 026
	Leucémies	Détection de BCR-ABL	6 750
		Mutations d'ABL	861
TOTAL		89 254	

Pathologie	Marqueurs de suivi	Indication
Mélanome	Mutations de BRAF Mutations de KIT*	Prescription de vemurafenib ou de dabrafenib
Glioblastome	Méthylation de MGMT	Sensibilité au temozolomide
Leucémie myéloïde chronique (LMC) / Leucémie aiguë lymphoblastique (LAL)	Translocation de BCR-ABL au diagnostic Détection de BCR-ABL pour le suivi de la maladie résiduelle Mutation d'ABL	Prescription d'imatinib ou de nilotinib en 1 ^{re} ligne de traitement. Résistance à l'imatinib: prescription de dasatinib, de bosutinib ou de ponatinib en 2 ^e ou 3 ^e ligne.
Neuroblastome	amplification de MYCN	traitement
LAL-LAM	Quantification d'anomalies chromosomiques Quantification WT1	Suivi de la maladie
	Quantification du réarrangement des gènes du TCR ou des Ig Clonalité B/T	Suivi de la maladie résiduelle

RIHN :
37 actes liste RIHN
+ 21 liste compl.

Nombre de NSCLC patients testés (2013)

Tableau 11. Activité 2013 dans le cancer du poumon

Marqueur	Nombre de patients	% d'altérations moléculaires	% de non interprétables
Mutations <i>EGFR</i>	23 336	10,0 %	8,0 %
Translocation <i>ALK</i>	18 861	3,5 %	13,4 %
Mutations <i>KRAS</i>	22 958	27,0 %	7,9 %
Mutations <i>BRAF</i>	20 100	2,0 %	8,9 %
Mutations <i>HER2</i>	17 843	0,7 %	10,1 %
Mutations <i>PI3KCA</i>	17 375	2,4 %	10,4 %

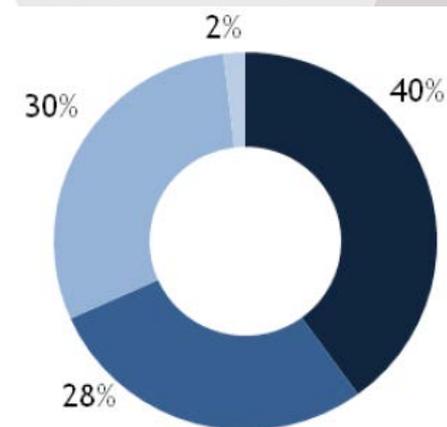
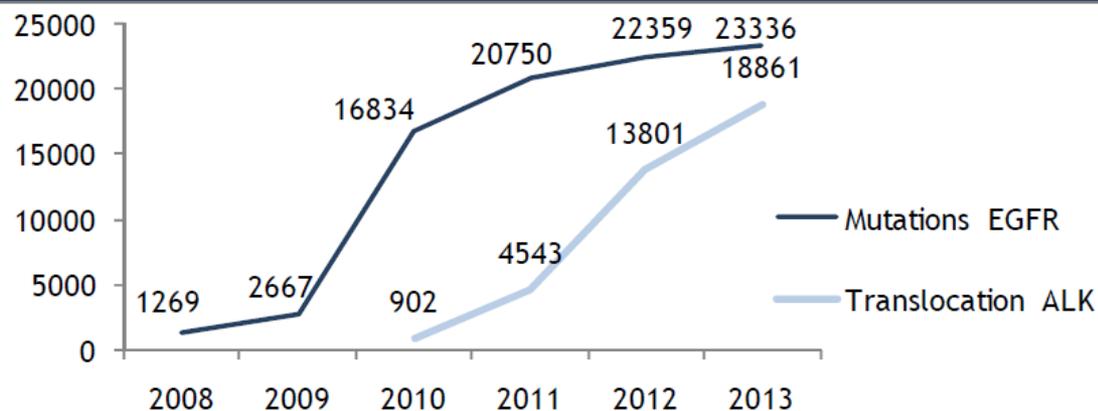
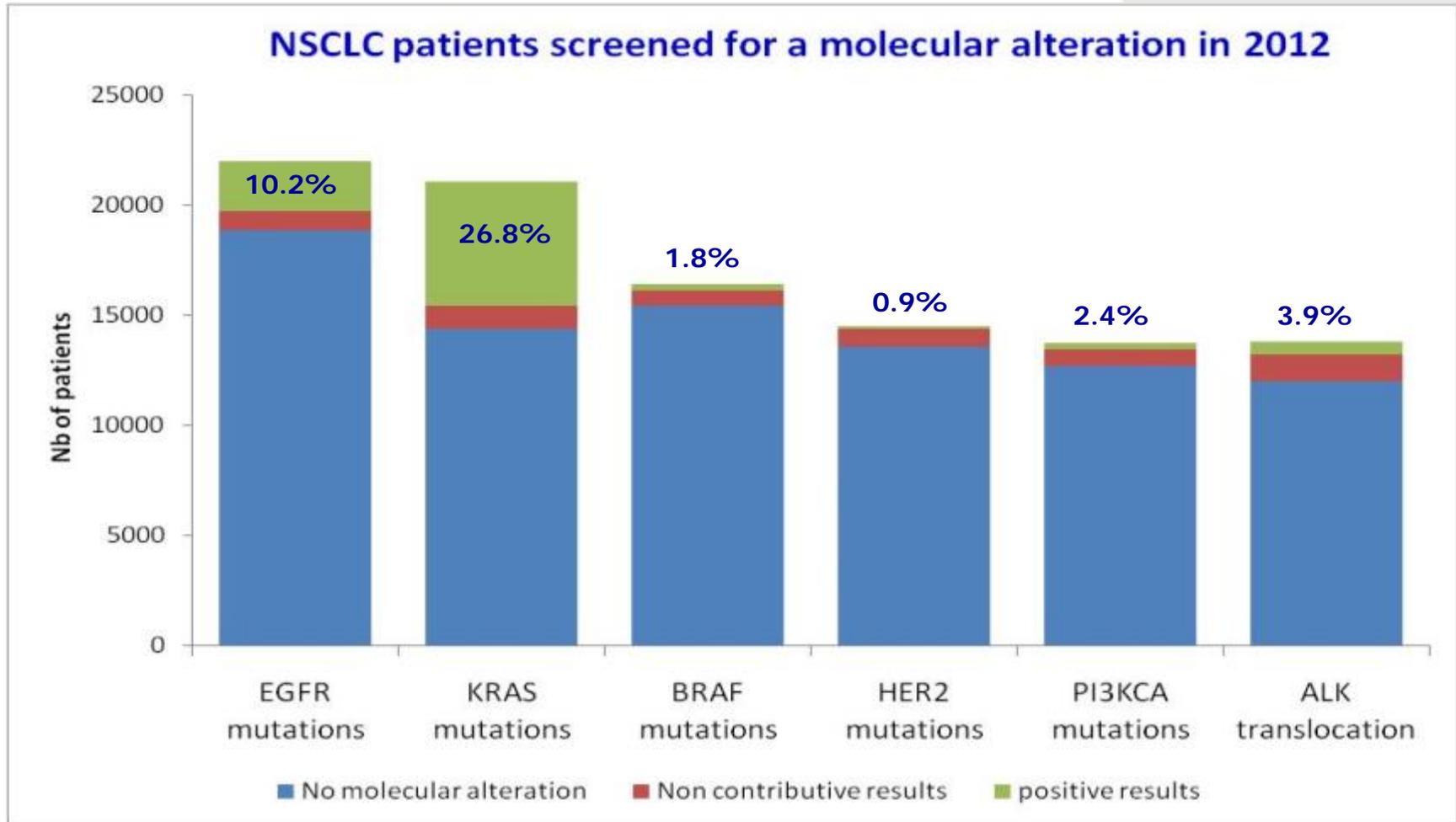


Figure 17. Évolution de l'activité



- Patients pris en charge dans un établissement de la plateforme
- Patients pris en charge dans un CH hors plateforme
- Patients pris en charge dans un établissement privé
- Prescription provenant d'une autre plateforme

Cancer du poumon (N=22 359)

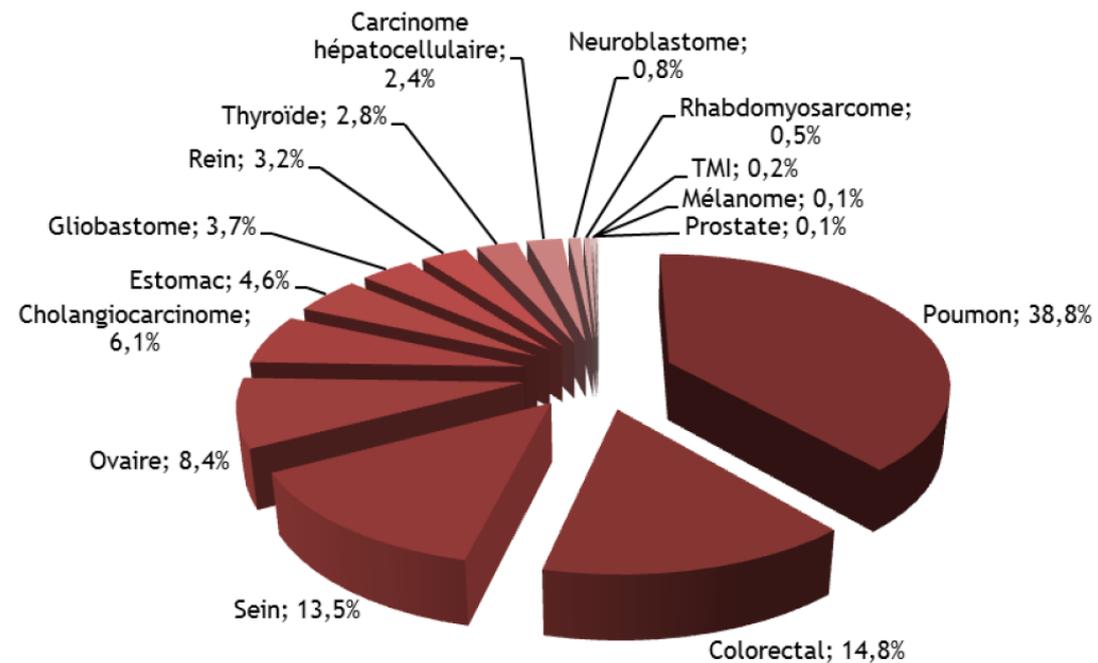


Programme Acsé (2013)

Type de cancer	Transloc ALK	Amp ALK	Amp MET	Transloc ROS	Mut ALK	Mut MET
Cancer colorectal	x		x			x
CBNPC	AMM	x	x	x		
Cancer du sein	x	x				
Cholangiocarcinome				x		
Cancer de l'ovaire			x			
Cancer du rein	x	x				x
Cancer du foie			x			x*
Cancer de la thyroïde	x				x	x
Glioblastome			x			
Neuroblastome		x			x	
Cancer gastrique			x			
Tumeur myofibroblastique inflammatoire (TMI)	x					
Rhabdomyosarcome		x				
Lymphomes anaplasiques à grandes cellules	x					

* seulement pour les cancers du foie pédiatriques

Figure 5. Répartition des tests par localisation tumorale



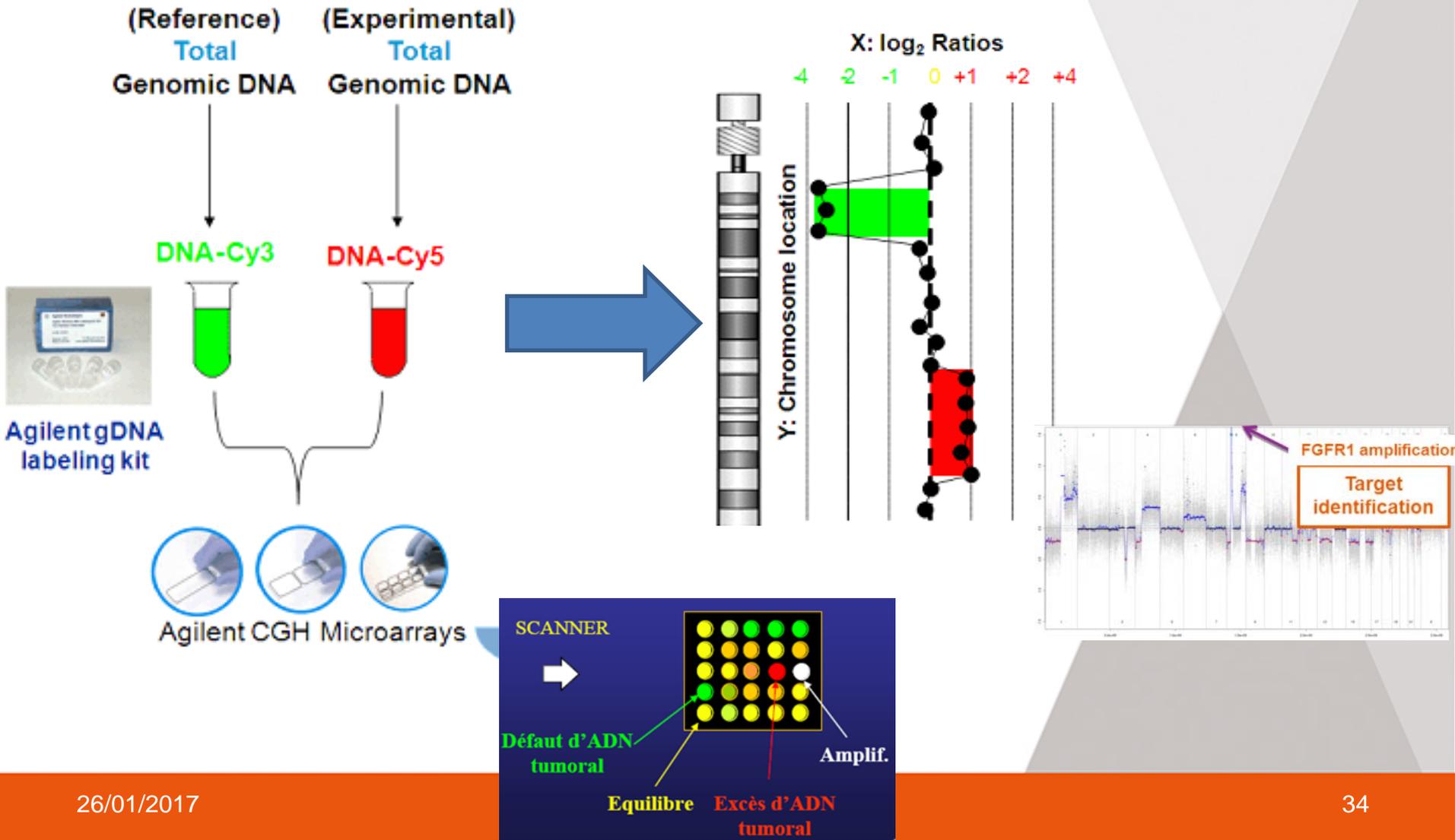
Les approches
Émergentes,

Analyse CGH
(Comparative Genomic Hybridization)

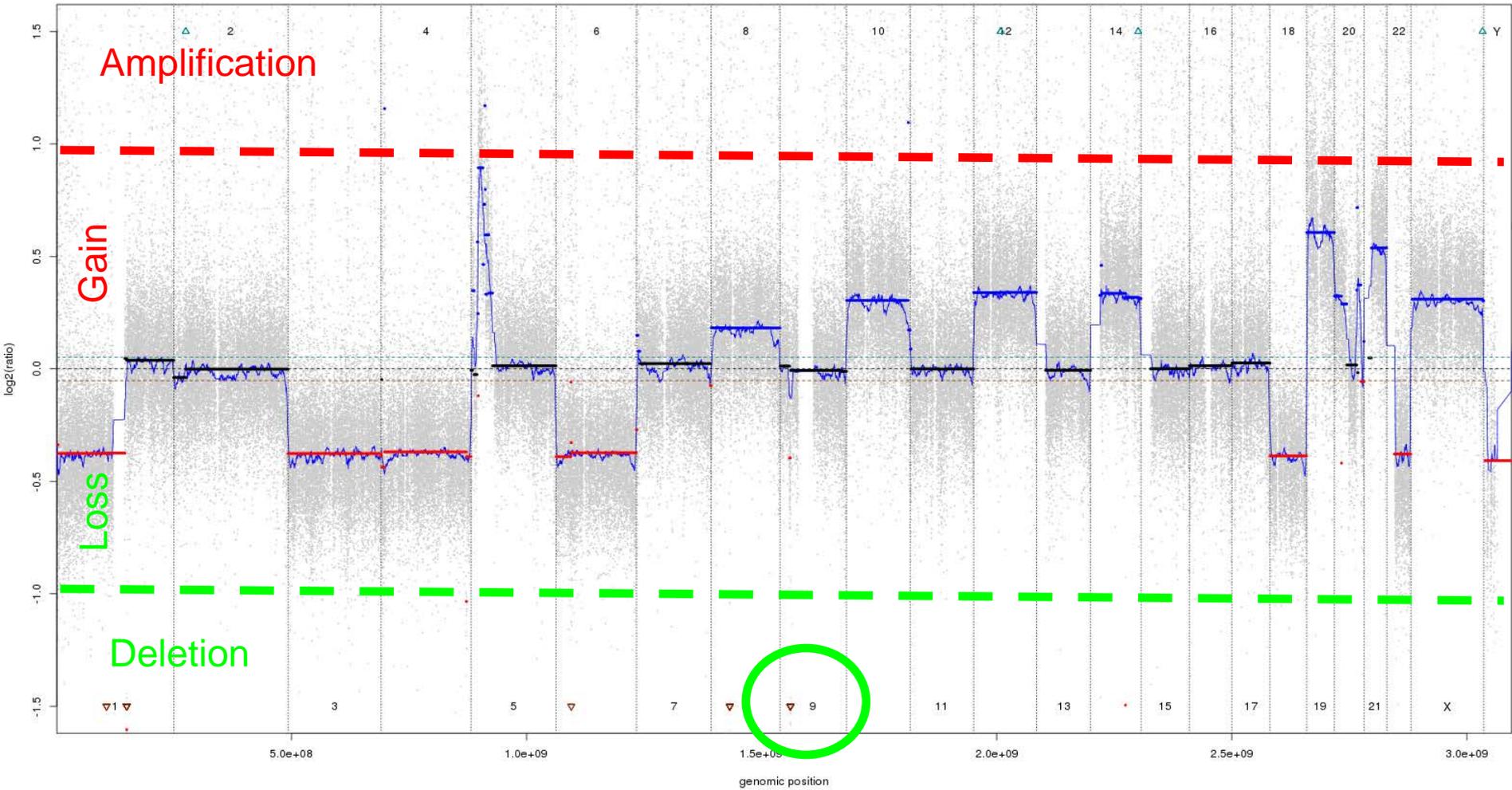


→ Comparative Genomic Hybridization array

CGH-array 180K -Whole Genome Coverage (Agilent Technologies)



C04 28425_1_3 final, centered profile | MAD = 0.2078 | SSAD = 111.4

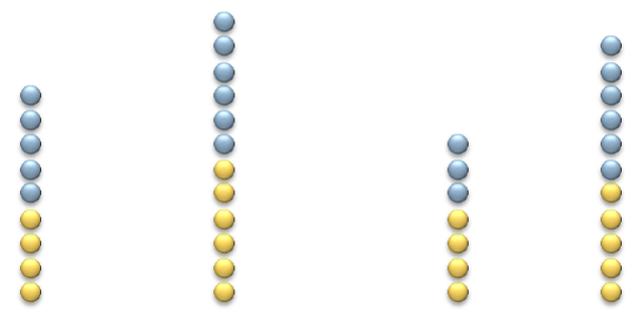


cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)

SNParrays CNV + Allele Freq.

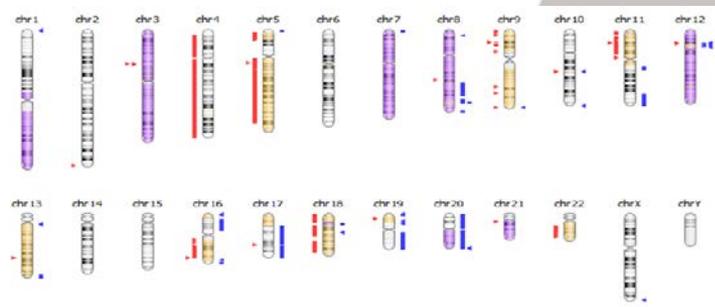
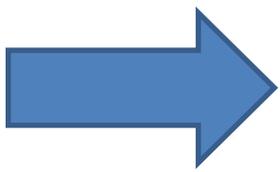
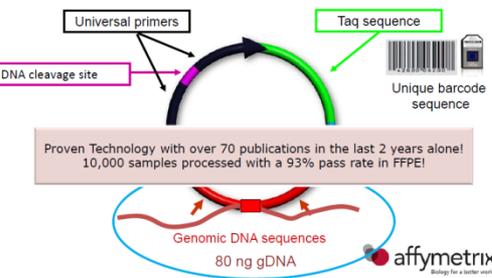
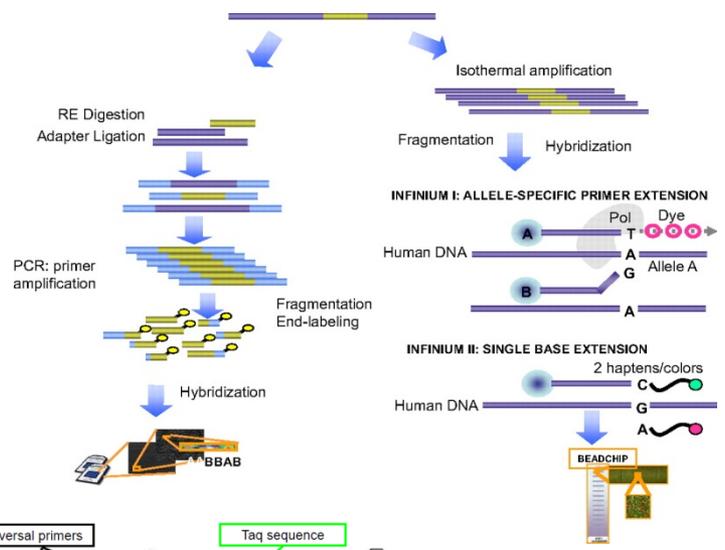
BAF

0.44 0.5 0.57 0.45



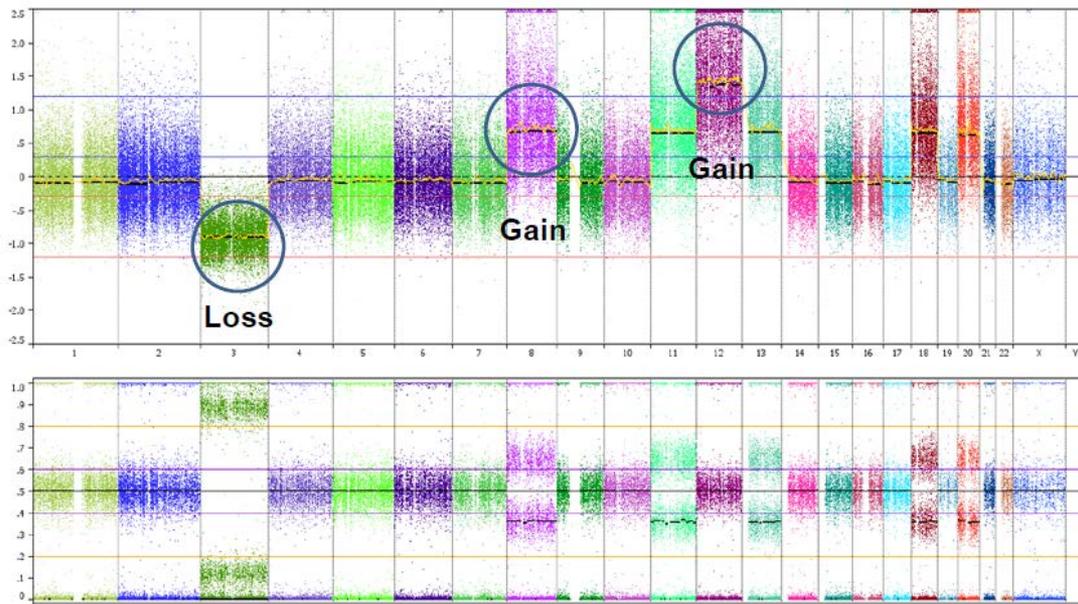
acGatgacgtcaAatgctagcgcagGcacacaATac
acCatgacgtcaTatgctagcgcagCcacacaAAc

B allele frequency = $B / (A+B)$

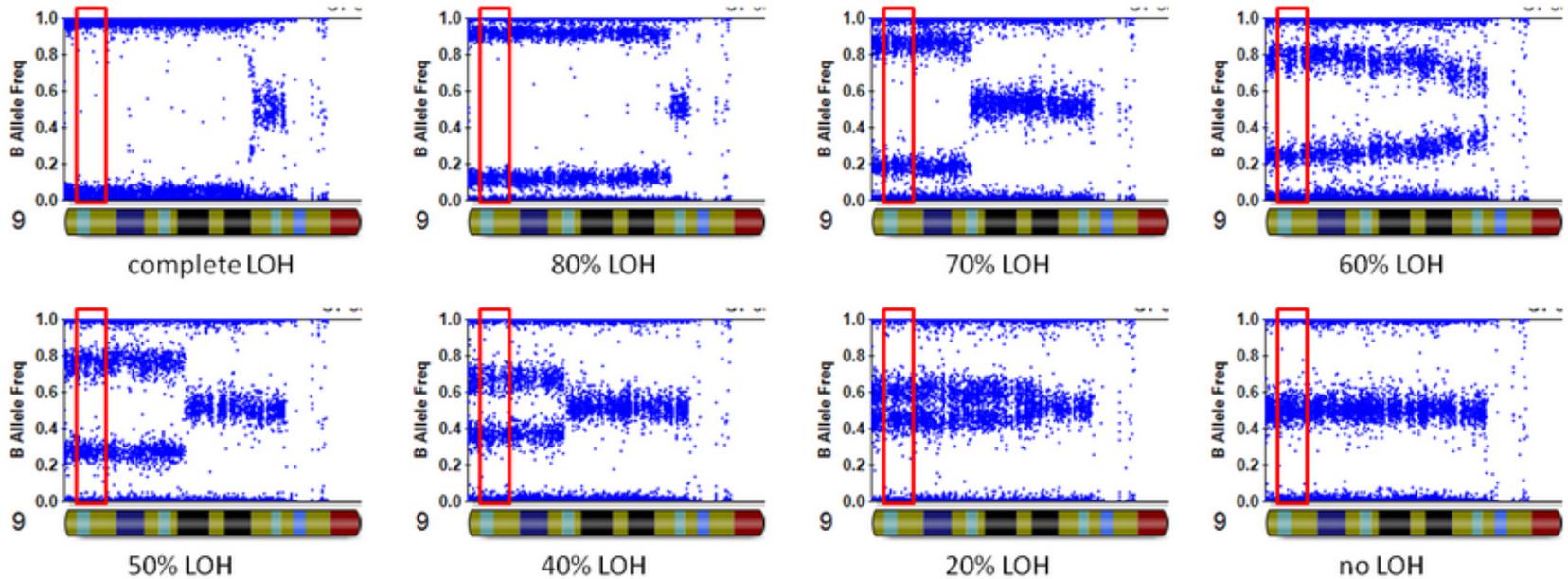
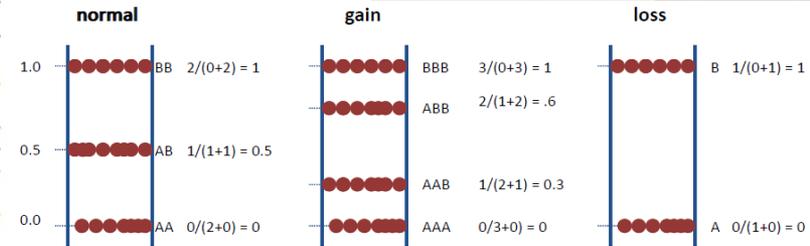


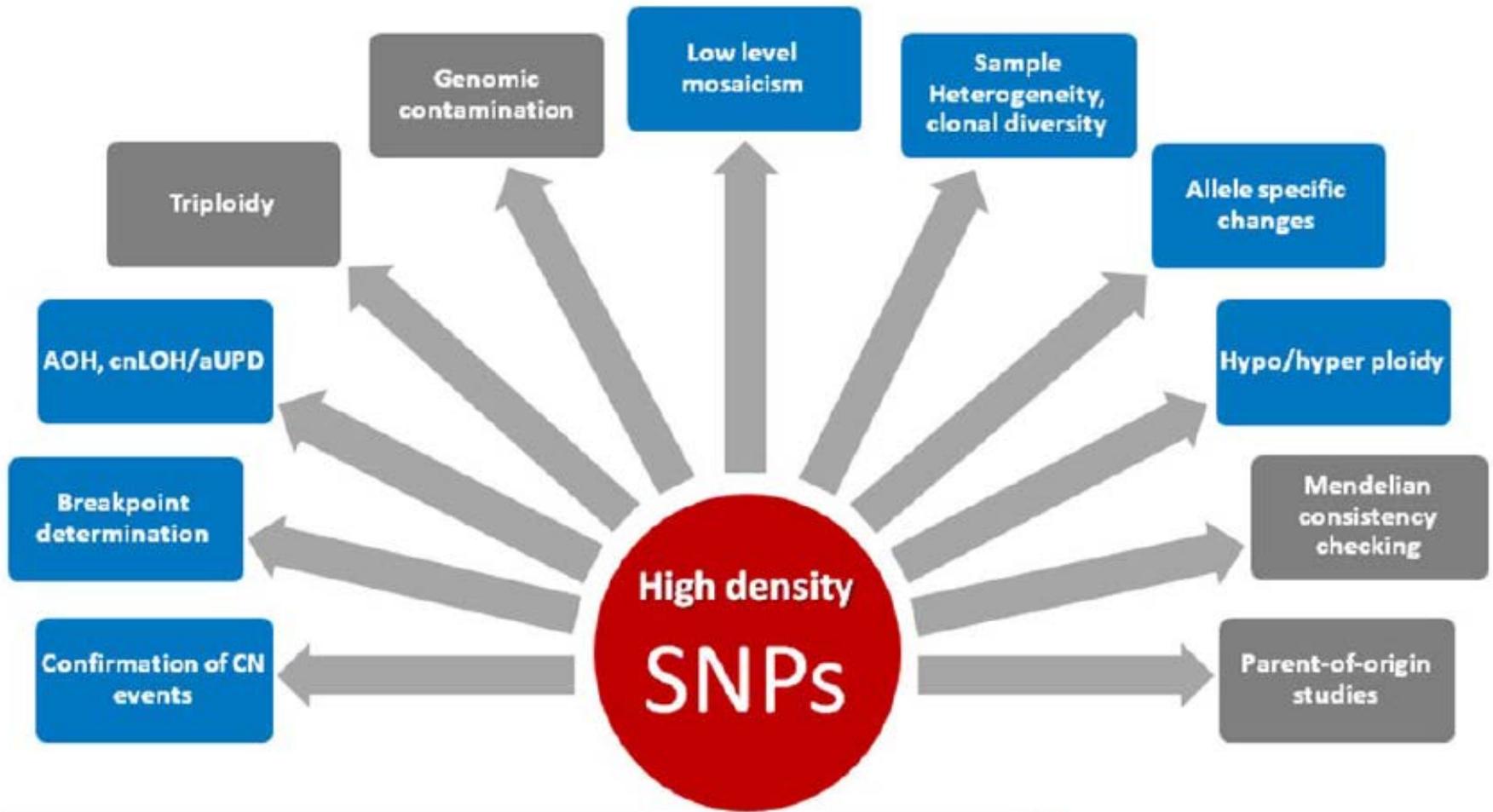
CN:
• blue = CN gain
• Red = CN loss

Allelic events:
• Gold = LOH
• Purple = Allelic imbalance



$$B \text{ allele frequency} = B / (A+B)$$





High-density SNPs allow for all of these performance attributes to be measured at gene-level resolution.



- **Analyse pangénomique des anomalies chromosomiques de nombre : perte, délétion, gain, amplification (mais pas pour les translocations...)**
- **Limité pour la Paraffine et 30% de cellules tumorales**
- **Quelques application de « Routine »**
- **Selon les méthodes approches ou pas des LOH (intérêt en association es mutations de gènes supprimeur de tumeur)**

Les approches
Émergentes,

Techniques NGS

(Séquençage de Nouvelle Génération)



→ From discovery to technology explosion

- 1868: Discovery of DNA
- 1953: Watson and Crick propose double helix structure
- 1977: Sanger sequencing
- 1985: PCR
- 2000: Working draft human genome announced (Sanger method)
- 2005: 454 sequencer launch
 - (pyrosequencing)
- 2006: Genome Analyzer launched
 - (Solexa sequencing)
- 2007: SOLiD launched
 - (ligation sequencing)
- 2009: Whole human genome no longer merits Nature/Science paper
- 2013 : “third-gen” systems on the horizon

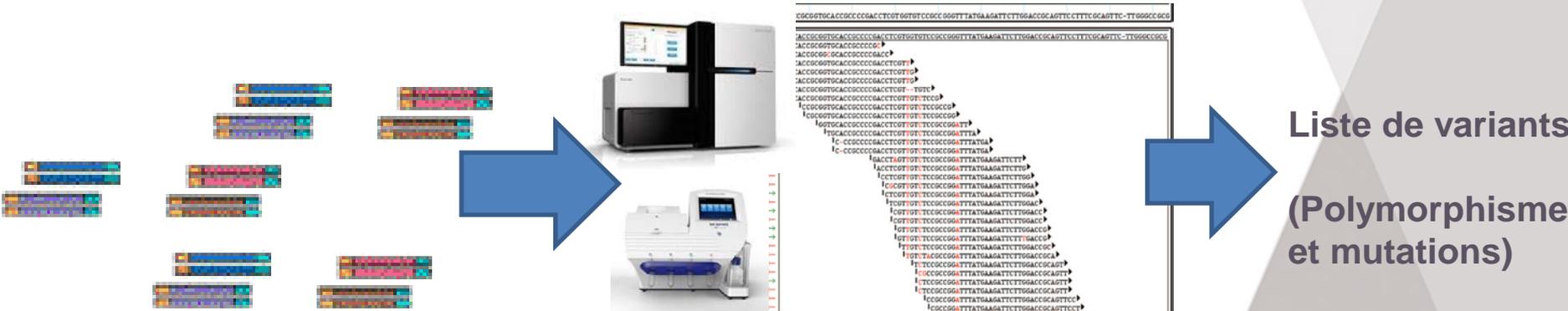
	Efficiency (bp/person/year)
	1
\$ human	15
Genome	150
\$3 billion	1,500
	15,000
	25,000
	50,000
\$2-3 million	200,000
	50,000,000
\$250k	
\$50k	
\$20k	100,000,000,000
?<\$5k?	



Next Generation Sequencing - NGS

2005 première étude NGS, en 2016 = outil de base

Principe: chaque copie d'ADN sélectionnée (ou non) est clonée puis séquencée séparément et analysé informatiquement pas rapport aux séquences de référence.



4 étapes:

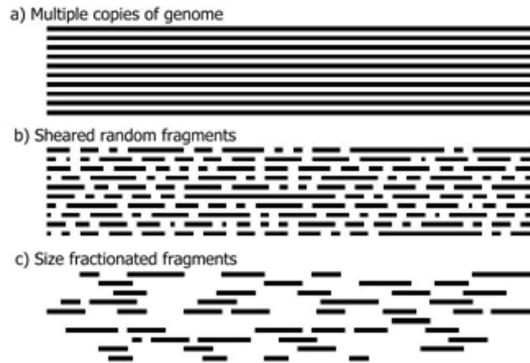
- 1-Préparation de "library"
- 2-Sequencage
- 3-Bioinformatique
- 4-Compte rendu clinique

Liste de variants
(Polymorphisme
et mutations)

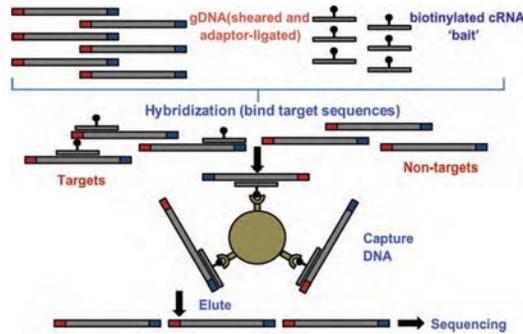
Préparation des *Librar*



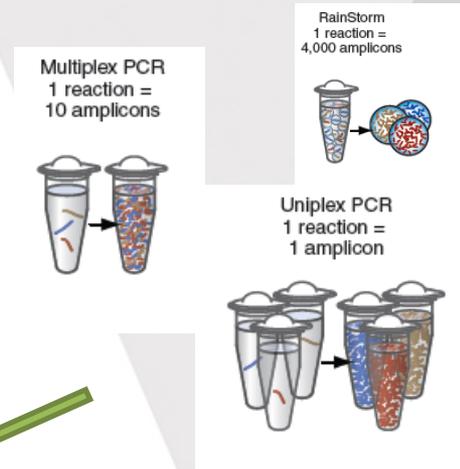
Shot Gun



Capture Based

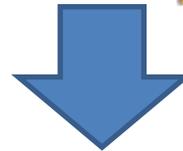


PCR Based



Analyse Génome entier

- 3 jours
- >1µg ADN (3µg)
- Quantitatif
- Congélation +
- Application génomes entier / RNA seq



Analyse Exome ou région cible large

- Prep. 3 jours
- ~1µg ADN
- Quantitatif
- Congélation+ (FFPE?)
- Application exome entier ou grande zone cible / RNAseq



Analyse région cible

- Prep. 1 jours
- ~100 ng ADN
- Peu quantitatif
- FFPE ok
- Application zone cible

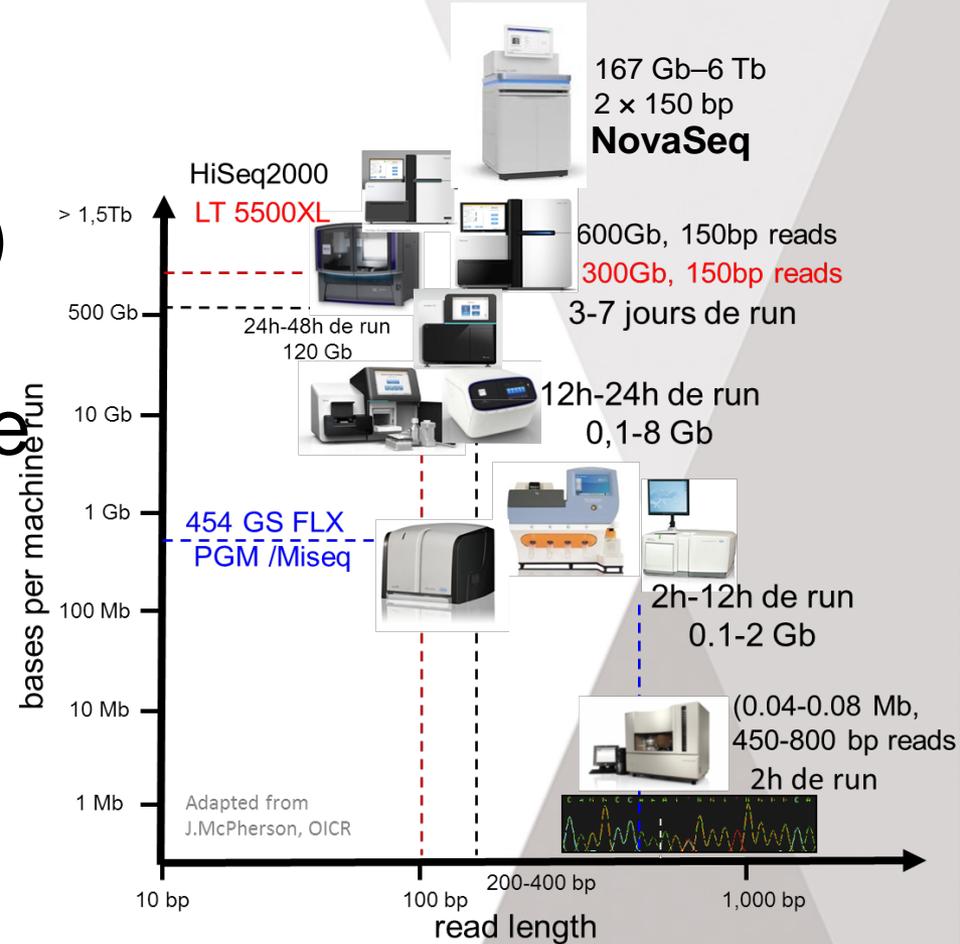
Les types de séquenceur- NGS

- Capacité :

$$\text{Nb de clone (read)} \times \text{longueur de lecture}$$



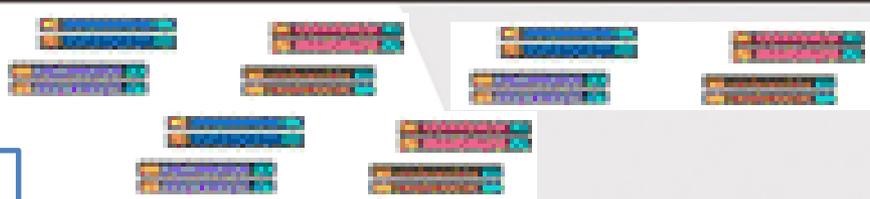
- Type de séquenceur
 - High Capacity NGS
 - 200-600Gb-1To-6To
 - BenchTop NGS
 - 0.1-8Gb



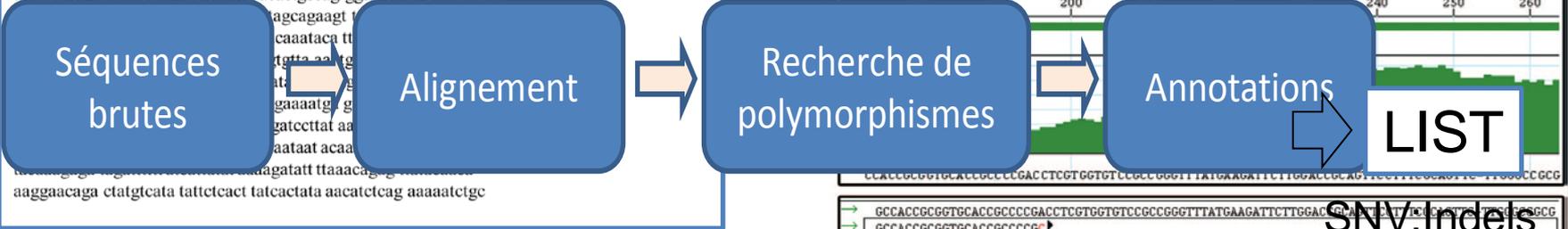
Adapted from J.McPherson, OICR

Human genome (3Gb)
 Human exome (50Mb)
 Target genes (0,2Mb)

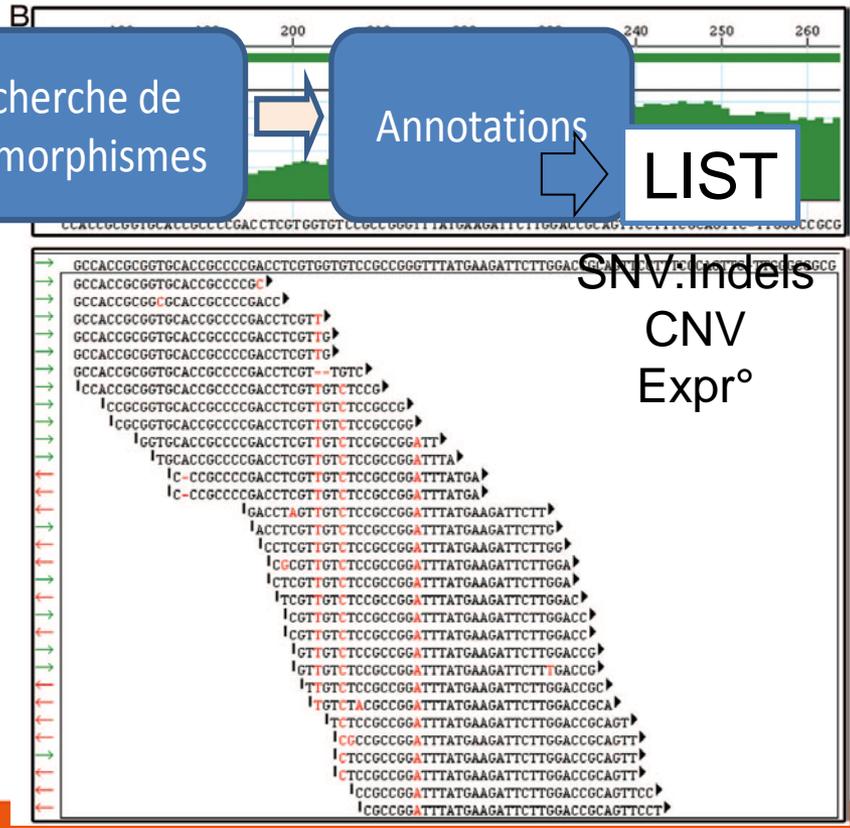
NGS data



```
>ggatcctcac atgagtcag tatataattg taacagaata aaaaatcaat tatgtattca
agtgtctagt gtcttaagag gttcacattt ttactaacct gattatcaca aaaatacttc
gagttacttt tcaftataat tctgactac acatgaagag actgacacgt aggtgcctta
cttaggtagg ttaagtaatt tatccaaaac cacacaatgt agaacctaaag ctgattcgcc
>catagaaaca caataatgfgg tataaatgag acagagggat ttctctcctt cctatgctgt
cagatgaata ctgagataga atatttagtt catctatcac acattaaacg ggactttaca
ttctgtctg ttgaagattt ggggtgfgggg ataactcaag gtatcatatc caaggatgg
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gtatatgtaa aatccacatt aagcttcttt ctgcatgcat tggcaatggt tatgaataat
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tacctattca ccaaaattta ttaaatgect aactgtatc agatatacaa ttatctggcg
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cccataattt gtgtaggttg aatgagctta tagaaaaatg cagtatattt atcagaagtc
tttaggtctca tgaaaaagaa tegtcaactg acactgcccag ggactcatat gtaagaata
agcagaagt t
caaataca tt
gttta aa tg
at
gaaaatg g
gattccttat aa
aataat acaa
adagattt taaacag
aaggaacaga ctatgcata tattctact taccactata aacatctcag aaaaatctgc
```



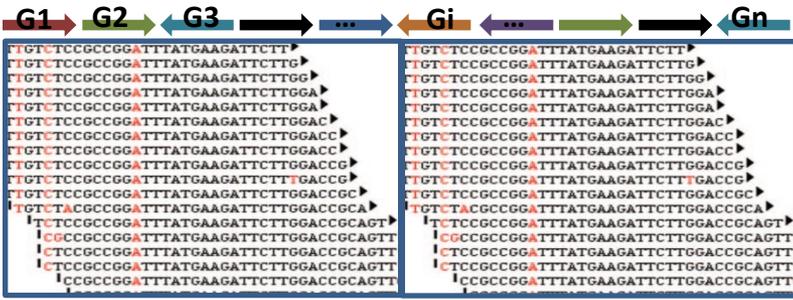
- Le NGS permet de produire des séquences correspondantes à chaque clone (Read)



Capacité du NGS

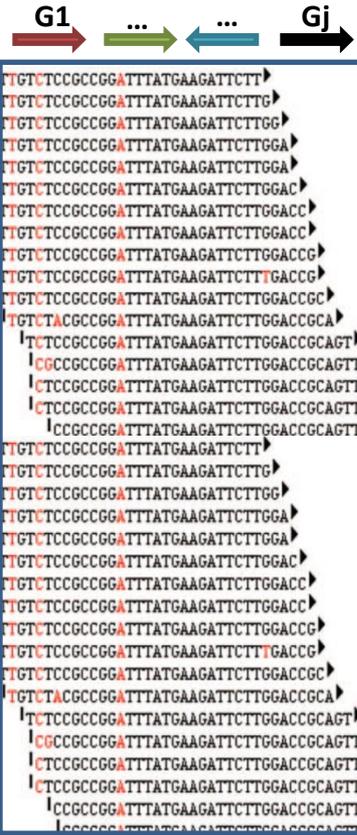
1 échantillon

peu de lectures sur beaucoup de positions

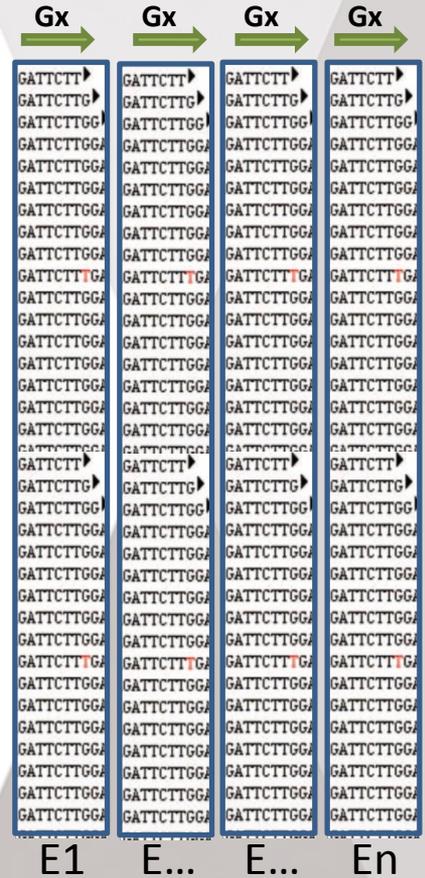


1 échantillon

beaucoup de lectures sur peu de positions



Multiplexage
Plusieurs échantillons



Profondeur
Ou X

- Human genome (3Gb)
 - 30X => 90Gb (8 jrs max – 6/run)
- Human exome (50Mb)
 - 100X => 5Gb (3 jrs max – 24/run)

- Target genes (0,2Mb)
 - 500X => 1Gb (<1jr) 24/48 run)

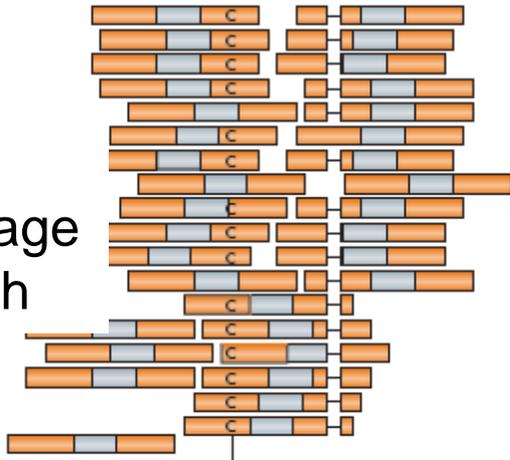
Reference sequence
Chr 1

A



nX

Coverage
depth



Point mutation

Indel

Detect° 50% variants 30X

Detect° 10% variants 100-500X

Detect° 1% variants >1000X

⇒ Exome 30x ⇒ 1,5 Gb / 100x ⇒ 5Gb

⇒ Genome 30x ⇒ 90 Gb / 100x ⇒ 300Gb

Visualisation de toutes les séquences en parallèle



Figure : Visualisation des couvertures des exons 18 et 19 du gène *EGFR*

Sensibilité

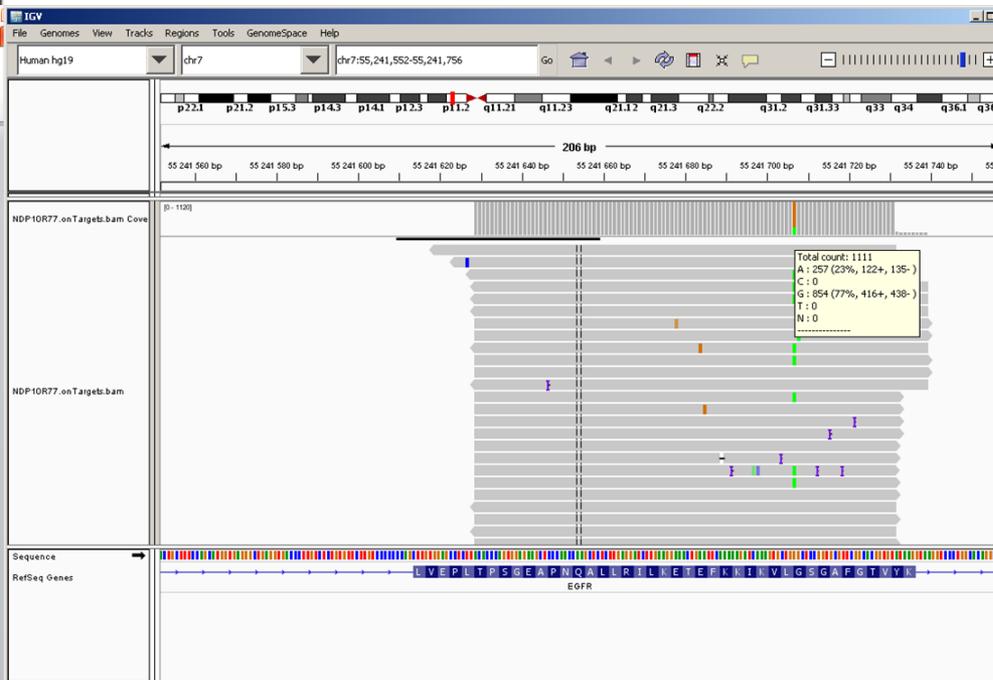
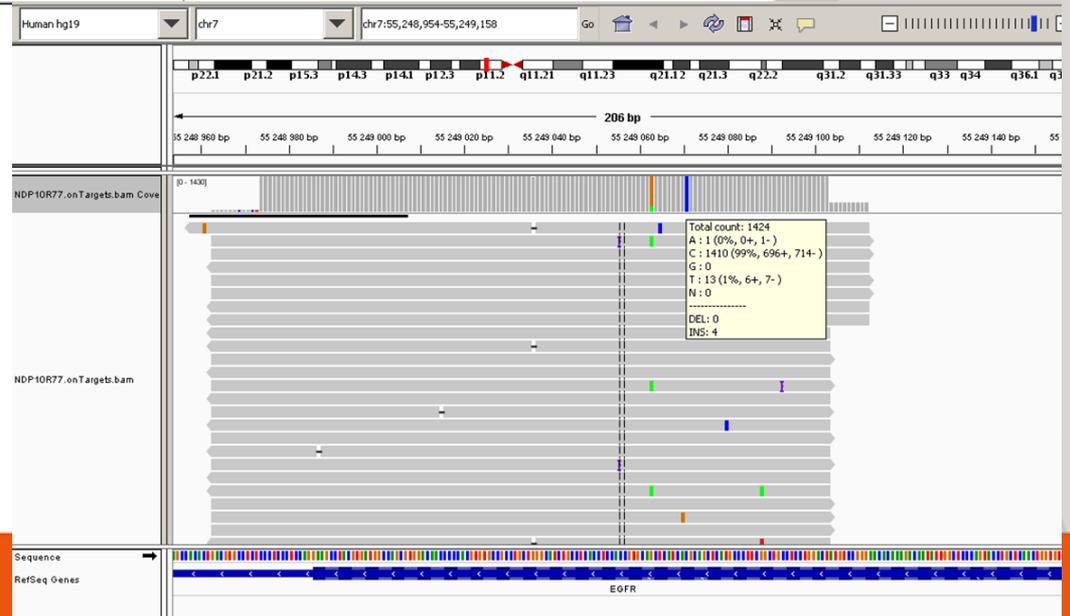


Figure : visualisation sous Alamut du contrôle interne : mutations *EGFR* : c.2155G>A, p.Gly719Ser (exon 18)



applications et exemples

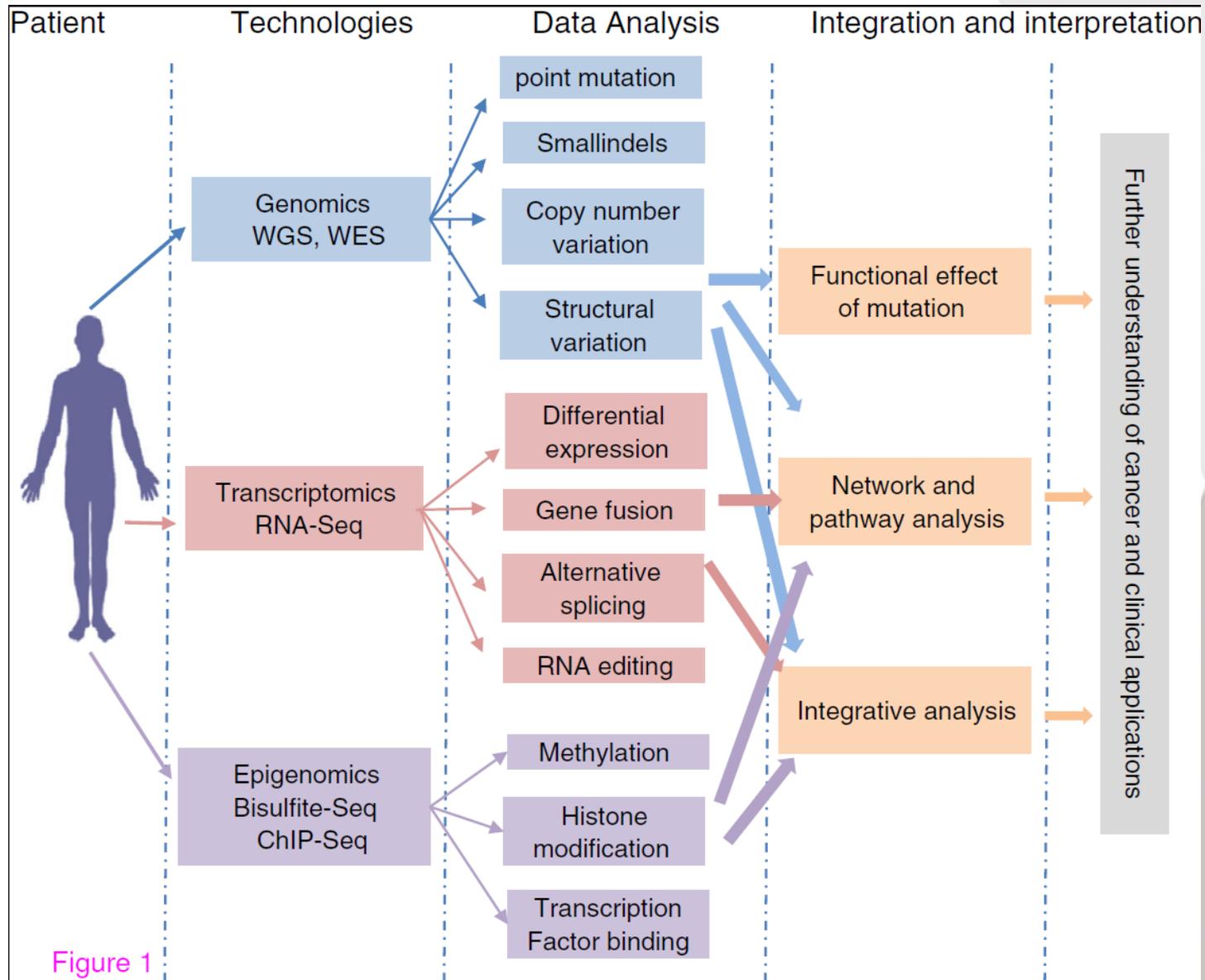
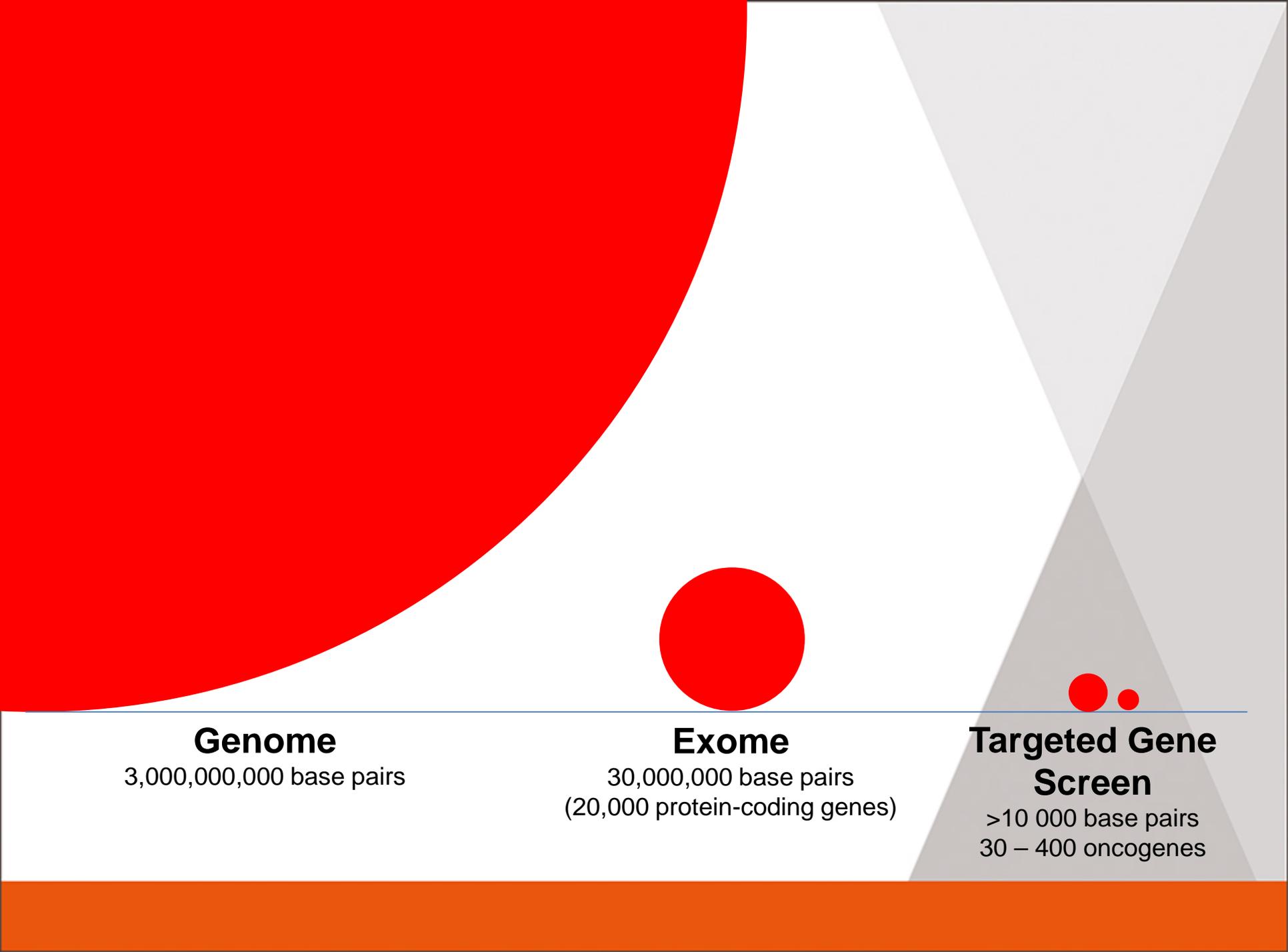


Figure 1



Genome

3,000,000,000 base pairs

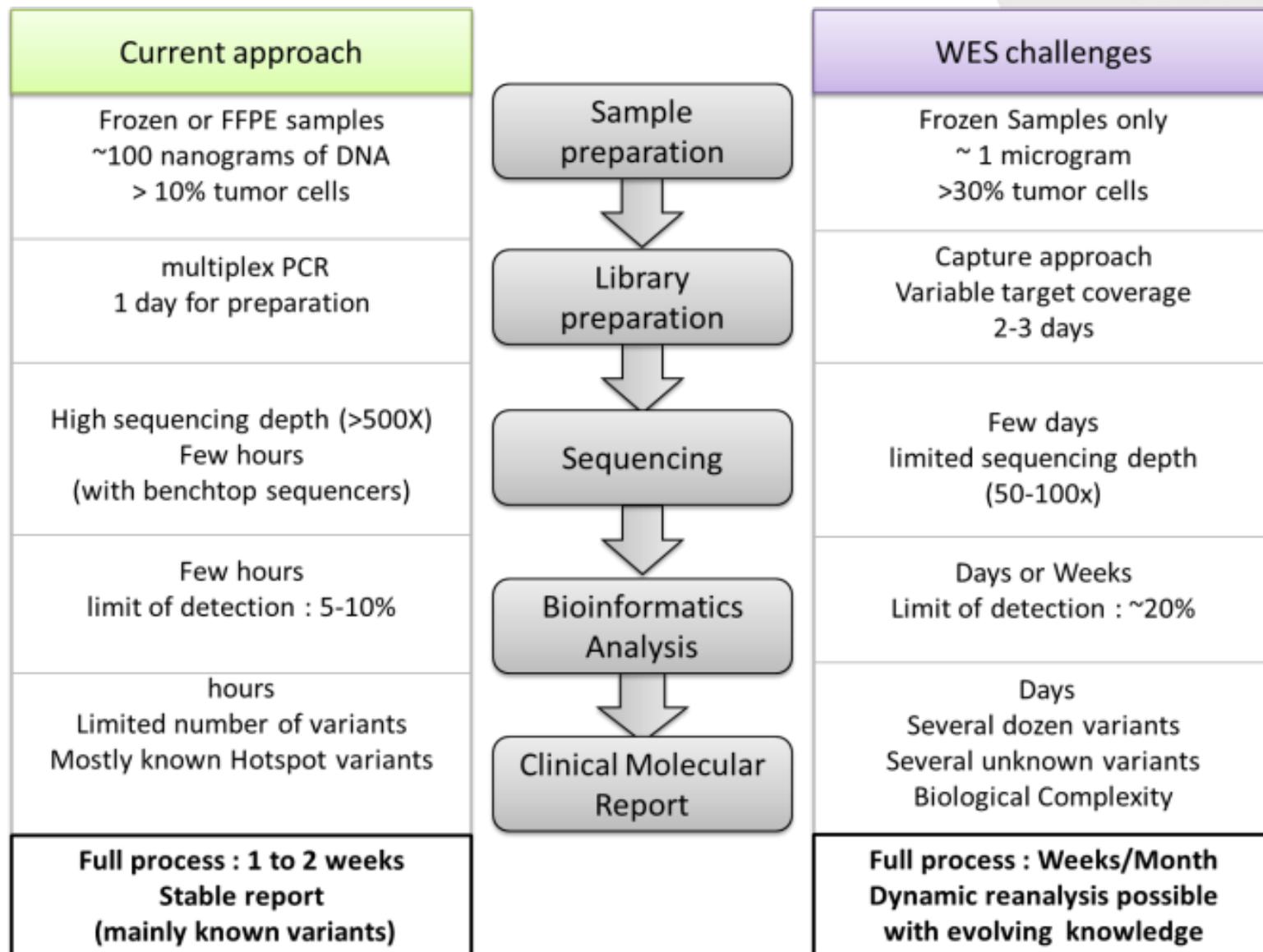
Exome

30,000,000 base pairs
(20,000 protein-coding genes)

Targeted Gene Screen

>10 000 base pairs
30 – 400 oncogenes

Challenges to launch exomic analysis with clinical setting

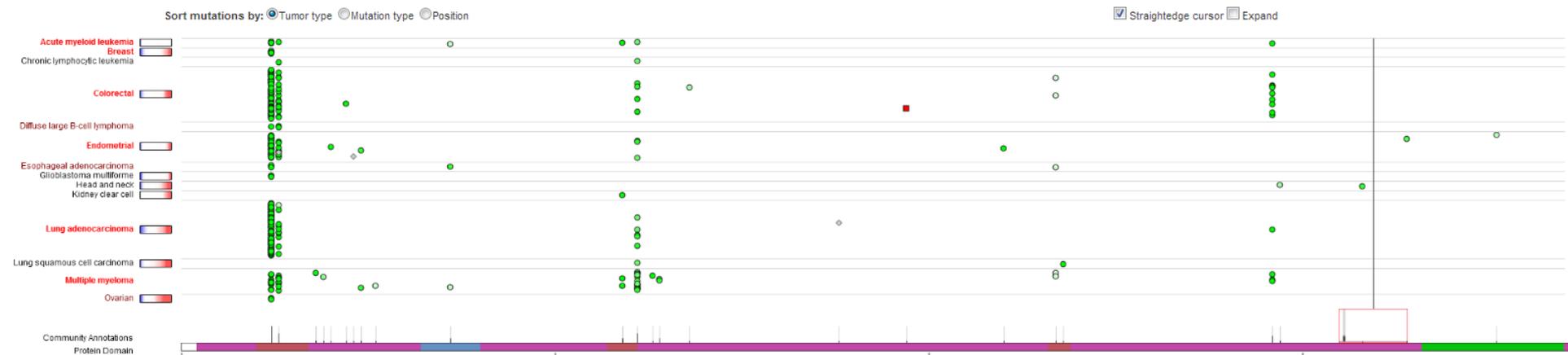


Interprétation des variants

Community Annotation of KRAS

Q Add / Edit KRAS: Annot

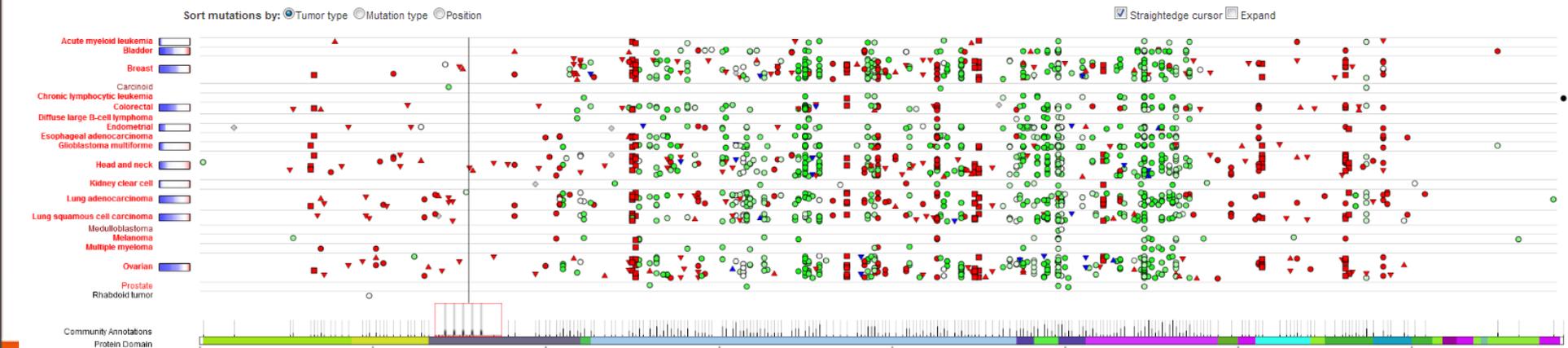
No community annotations yet for KRAS.



Community Annotation of TP53

Q Add / Edit TP53: Annot

No community annotations yet for TP53.



Interprétation des variants

Community Annotation of ERBB2

Q Add / Edit ERBB2: Annotations

No community annotations yet for ERBB2.

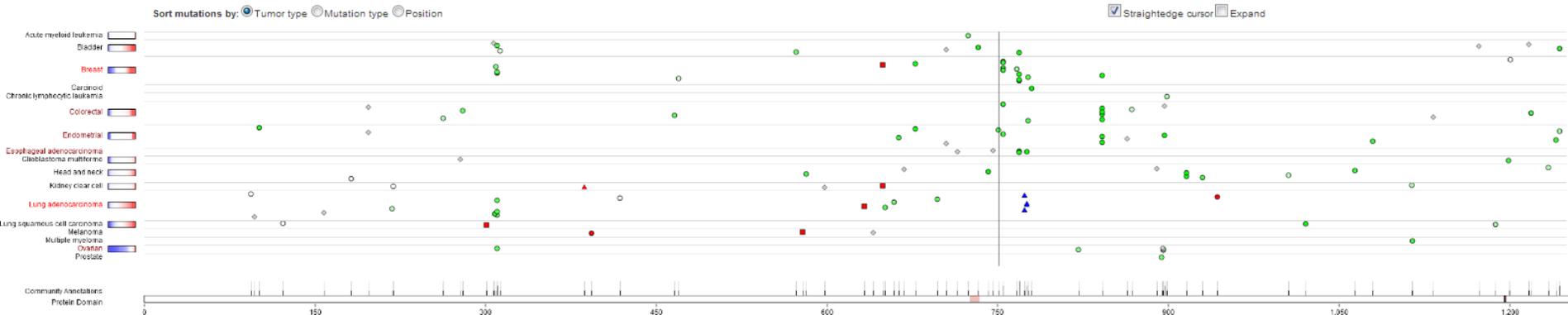


Figure notes

- "Mouse over" a mutation to see details.
- Missense green saturation indicates evolutionary conservation of the mutated positions.
- Red hashes in protein strip are splice sites.
- Blue-white-red bars are log₂ copy ratio distributions (-1 to +1) from Zack et al. (2013).

- Synonymous mutation
- Missense mutation
- ▼ In-frame insertion
- ▲ In-frame deletion
- Splice-site mutation
- Nonsense mutation
- ▼ Frameshift insertion
- ▲ Frameshift deletion

Community Annotation of PIK3CA

Q Add / Edit PIK3CA: Annotations

No community annotations yet for PIK3CA.

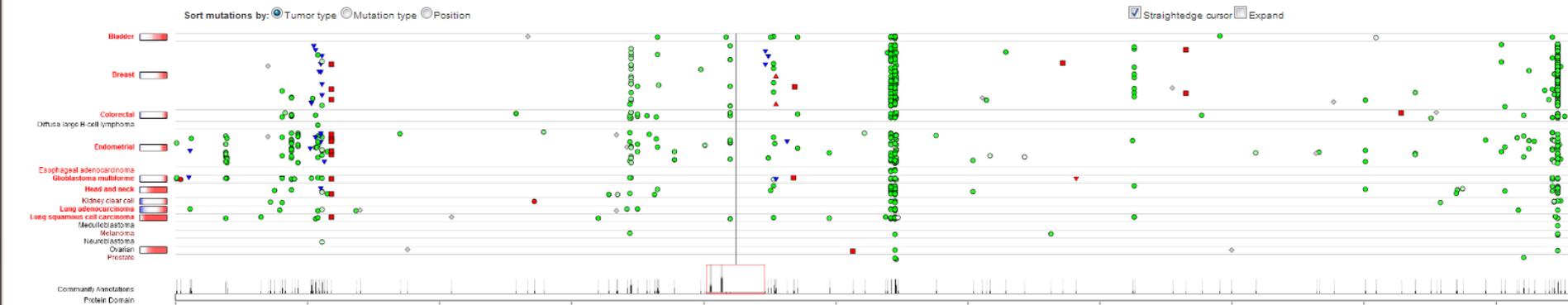


Figure notes

- "Mouse over" a mutation to see details.
- Missense green saturation indicates evolutionary conservation of the mutated positions.
- Red hashes in protein strip are splice sites.
- Blue-white-red bars are log₂ copy ratio distributions (-1 to +1) from Zack et al. (2013).

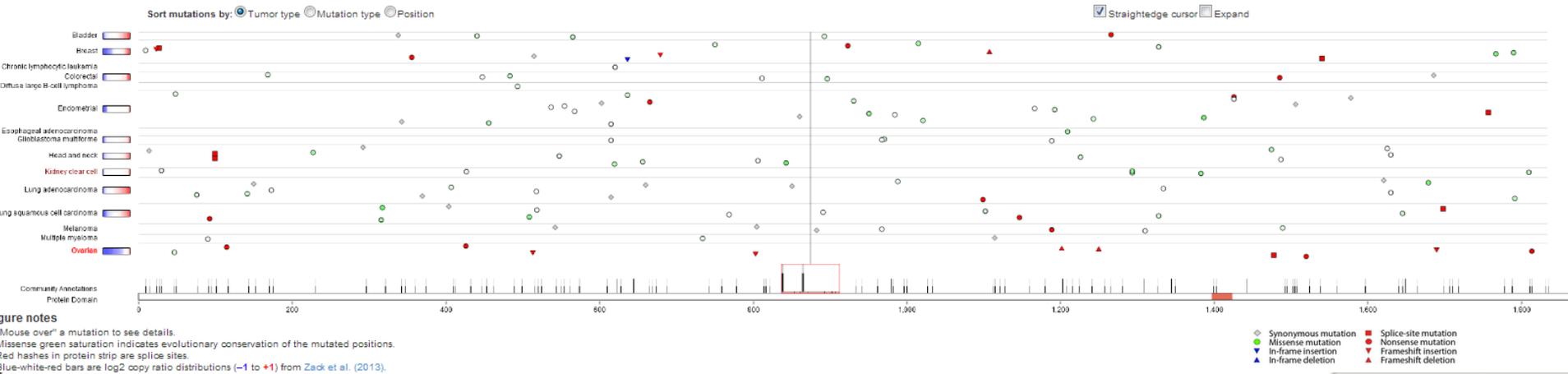
- Synonymous mutation
- Missense mutation
- ▼ In-frame insertion
- ▲ In-frame deletion
- Splice-site mutation
- Nonsense mutation
- ▼ Frameshift insertion
- ▲ Frameshift deletion

Interprétation des variants

Community Annotation of BRCA1

Q Add / Edit BRCA1: Annotations

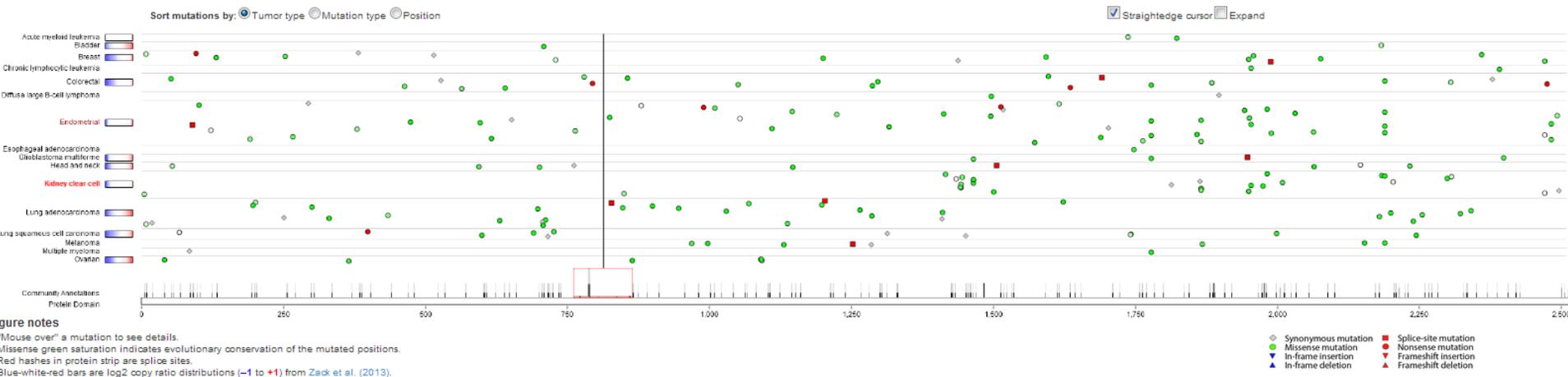
Community annotations yet for BRCA1.



Community Annotation of MTOR

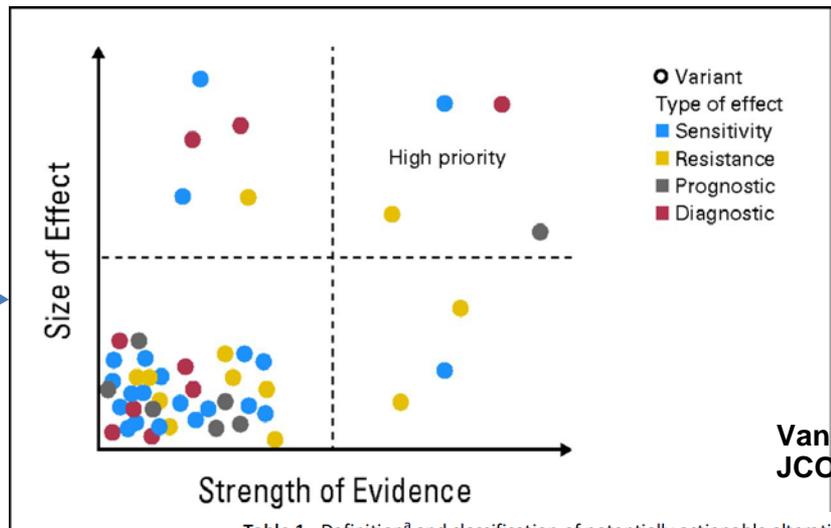
Q Add / Edit MTOR: Annotations

Community annotations yet for MTOR.



Spectrum of variants (not gene):

- Actionable
- Potentially actionable (clinical Research)
- Pathogenic non actionable (cancer gene variant)
- Variant of unknown effect
- Variant probably none pathogenic
- Frequent Polymorphisms (1%, 0,1%)
- ...



Van Allen E M et al.
JCO 2013;31:1825-1833

Table 1. Definition^a and classification of potentially actionable alterations

Category	Classification
Category 1	Gene variant predicts sensitivity to approved therapy in a particular malignancy.
Category 2	Gene variant predicts sensitivity for an approved therapy in any malignancy, but data for efficacy is lacking in that tumor type.
Category 3	Gene variant is an eligibility criterion for a clinical trial, or there is published evidence of clinical efficacy with an investigational agent.
Category 4	Gene variant with only preclinical support for use of an investigational therapy.

^aPotentially actionable alteration is defined as genetic alteration that is predicted to confer sensitivity to either approved targeted agent or experimental targeted agent in clinical trials.

Reporting: items discussed...

- Does the molecular profiling report communicated to the clinician need to contain ?:
 - > Mutation specific aspects:
 - Inclusion of all mutations identified or only significant mutations...
 - Processes involved (eg. Expert panel, Bioinfo, functional validation...)
 - Mutation nomenclature (chromosome position v amino acid change...)
 - > Clinical significance aspects:
 - Inclusion of literature-based information (clinical significance and frequency)
 - evaluation of level of evidence of clinical data; Inclusion of preclinical data
 - Listing of clinical trials involving novel agents targeting identified mutations
 - > Legal aspects:
 - Disclaimer, research use only.
 - Germline Aspect ?
 - Laboratory CAP/CLIA/ISO-accreditation/certification
 - > Process specific aspects:
 - Listing all mutations for which analysis occurred
 - Listing of analysis failures
 - Description of platforms and methods used
 - Details of platform sensitivity and specificity
 - > Report presentation
 - Executive summary / conclusion / clinical recommendation ...

applications et exemples

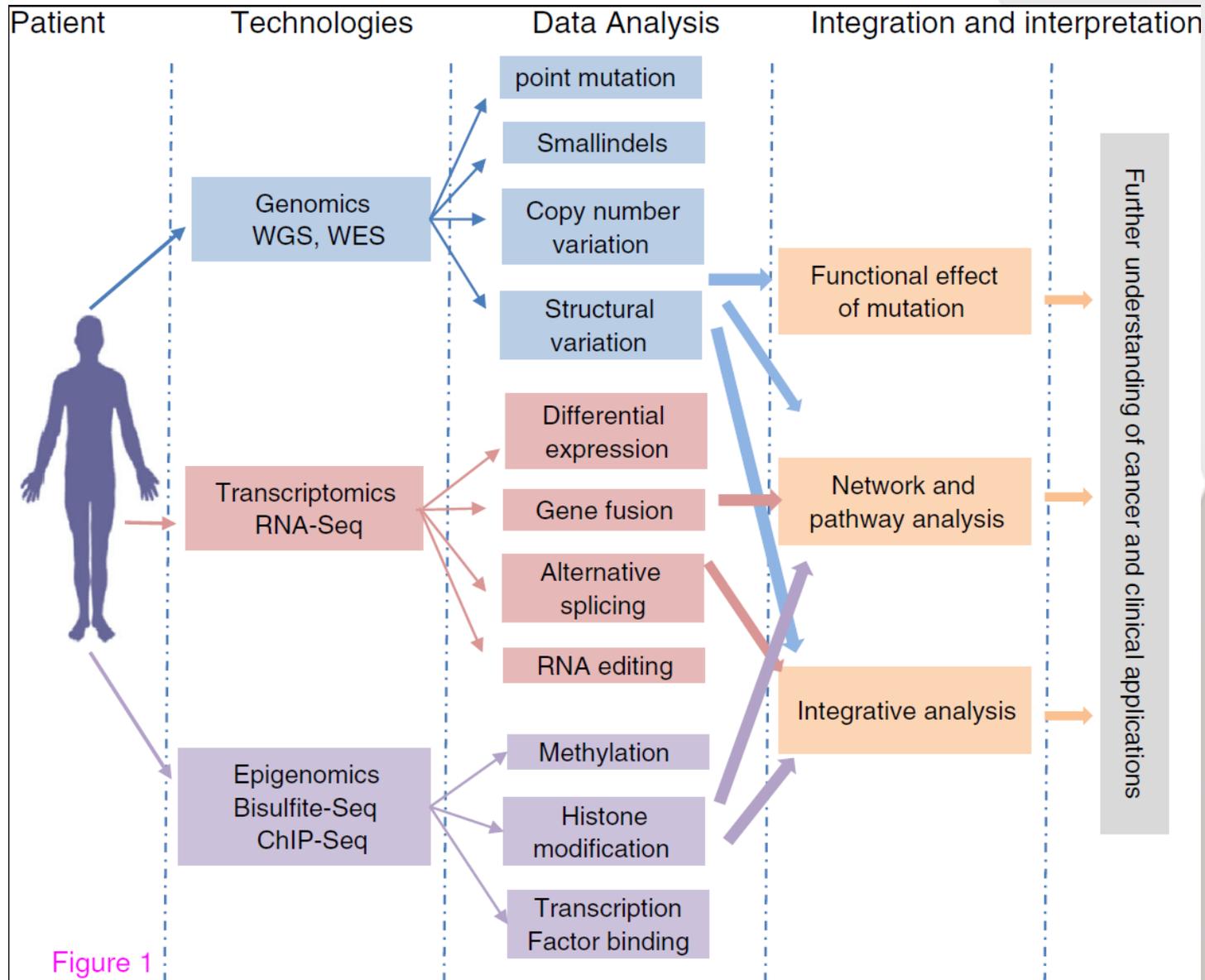


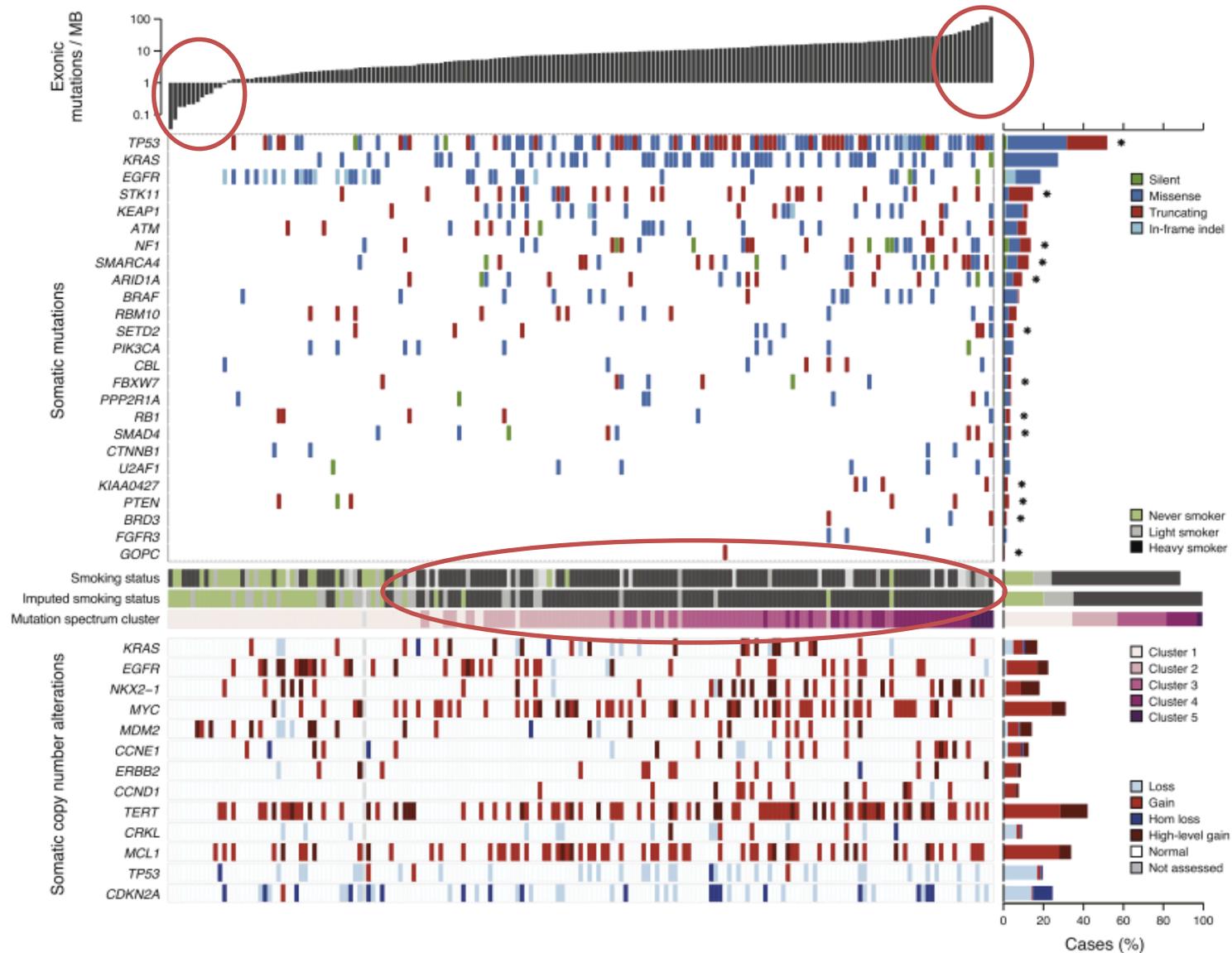
Figure 1

Mapping the Hallmarks of Lung Adenocarcinoma with Massively Parallel Sequencing

Cell

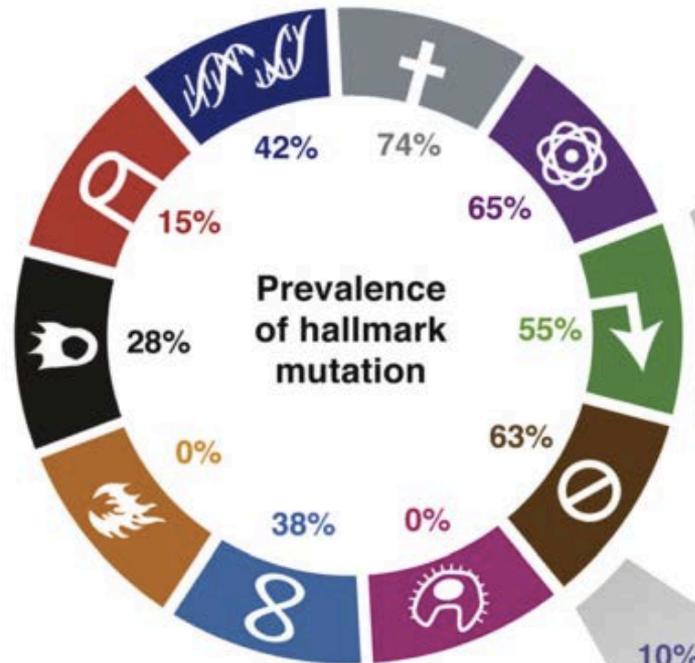
Imielinski et Al. Cell 2012

Cell 150, 1107–1120, September 14, 2012

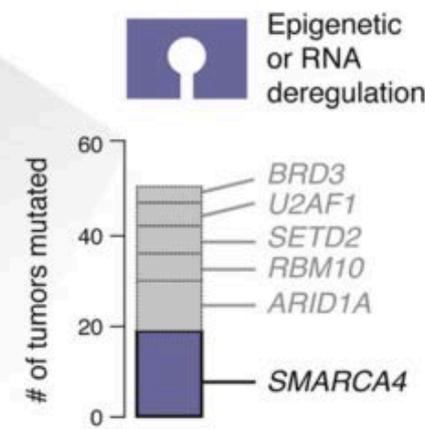
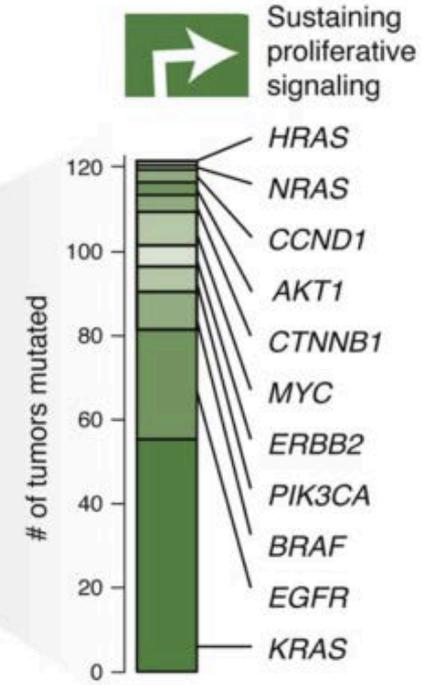


Mapping the Hallmarks of Lung Adenocarcinoma with Massively Parallel Sequencing

Cell



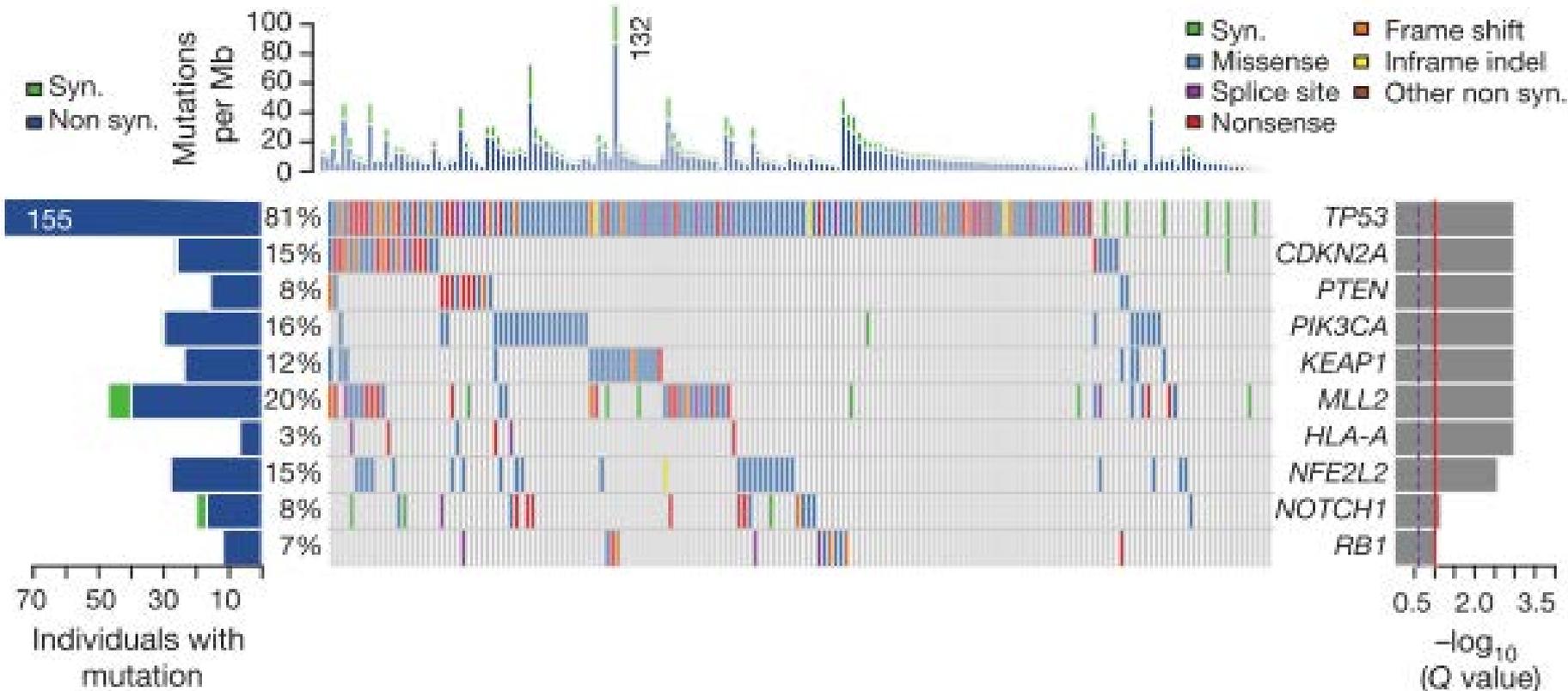
- | | | |
|------------------------------------|--|---------------------------------|
| Resisting cell death | | Classic hallmarks |
| Sustaining proliferative signaling | | |
| Evading growth suppressors | | |
| Enabling replicative immortality | | |
| Activating invasion and metastasis | | Emerging hallmarks |
| Inducing angiogenesis | | |
| Deregulating cellular energetics | | Enabling characteristics |
| Avoiding immune destruction | | |
| Genome instability and mutation | | |
| Tumor-promoting inflammation | | |



Comprehensive genomic characterization of squamous cell lung cancers

The Cancer Genome Atlas Research Network*

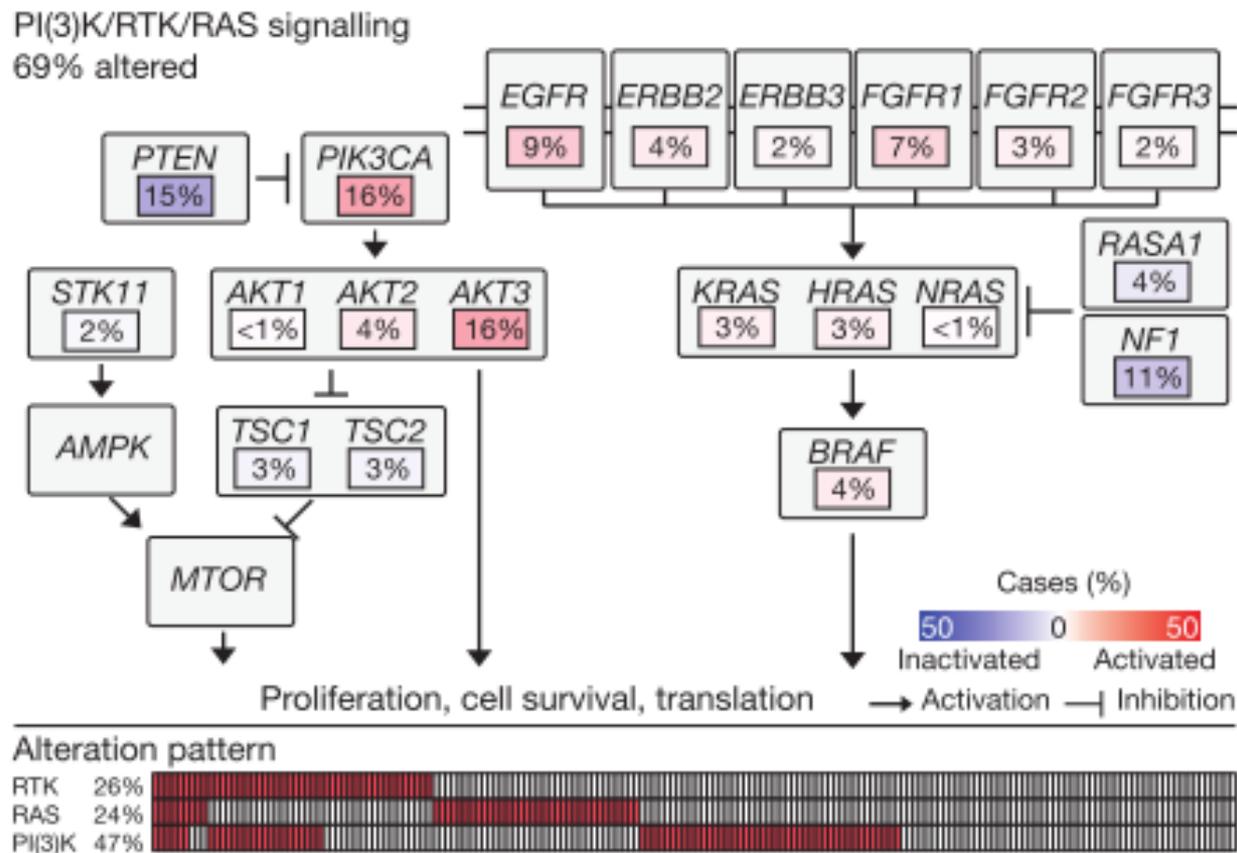
178 SCC
 Exon sequencing
 WGS
 RNA seq
 mRNA expression
 Promoter methylation



Comprehensive genomic characterization of squamous cell lung cancers

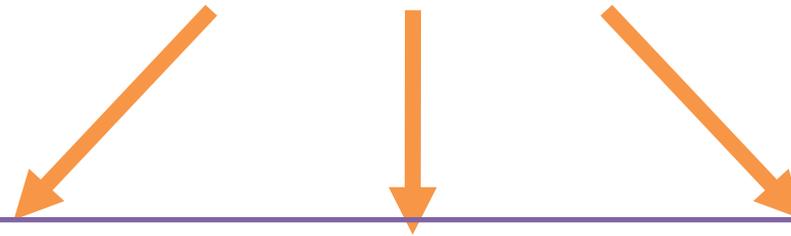
The Cancer Genome Atlas Research Network*

Many actionable abnormalities



➤ Préparer l'implémentation nationale du NGS à visée diagnostique dans les laboratoires d'oncogénétique constitutionnelle et les plateformes de génétique moléculaire, en mettant en place une phase pilote sur un nombre limité de laboratoires.

**1 projet avec
3 volets distincts :**

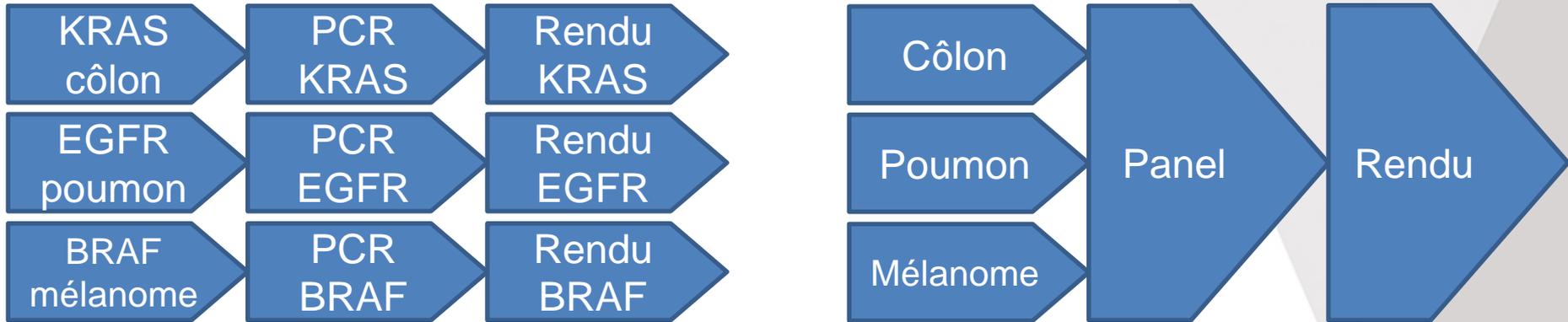


**Implémentation du NGS
dans les laboratoires**

**Estimation de l'impact
économique du NGS**

**Constitution d'équipes
référentes en
bioinformatique**

Changement d'organisation



Implications

- Des économies d'échelle, mais une complexité d'analyse
- Importance de la qualité du matériel extrait - problème des non contributifs
- Limite de détection $\leq 1\%$ vs. 20% pour le séquençage direct
- Validation du panel et de la bioinformatique associée – **biais de filtre**
- Intégration des résultats dans un système dédié
- Limitation actuelle – **PGM 318 < 30 échantillons – 1 semaine**

Retained approach for molecular screening in standard car at Gustave Roussy

Sentosa Next Generation Sequencing NGS automation for the IVD routine laboratory



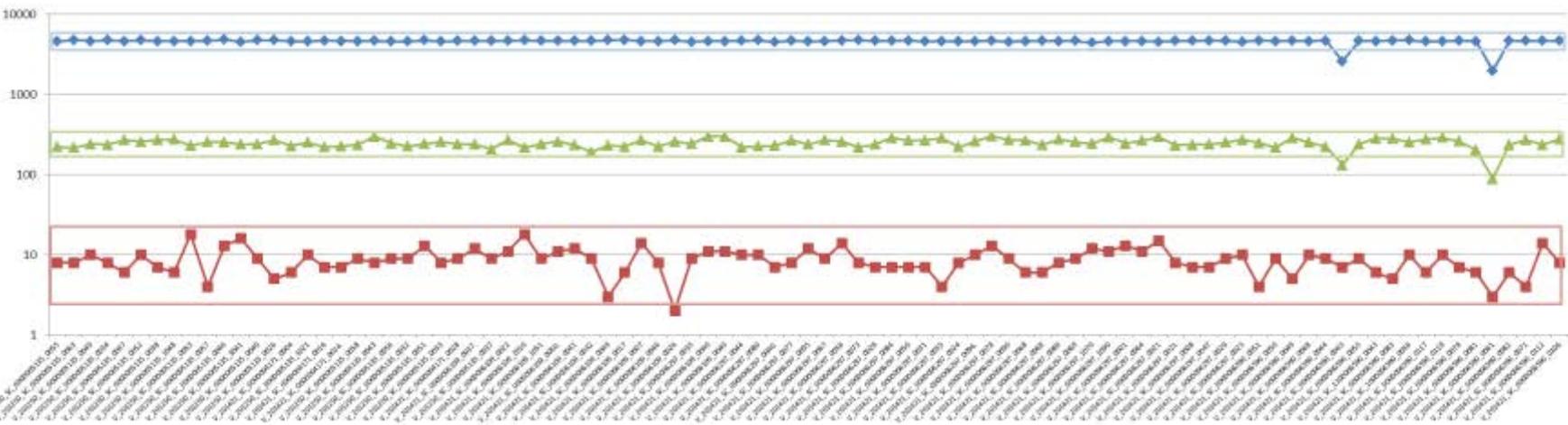
Sentosa SQ Oncology Panels

Sentosa SQ NSCLC Panel (CE-IVD), Sentosa SQ CRC Panel (CE-IVD)
Sentosa SQ Melanoma Panel (CE-IVD) Sentosa SQ Thyroid Panel (CE-IVD)
Sentosa SQ Leukemia Panel (Launch: 2016)*

FFPE validated - High Coverage – 5% detection threshold control on several Variants

QC follow-up across analysis batches

Coverage Depth X – first 100 batches of analysis



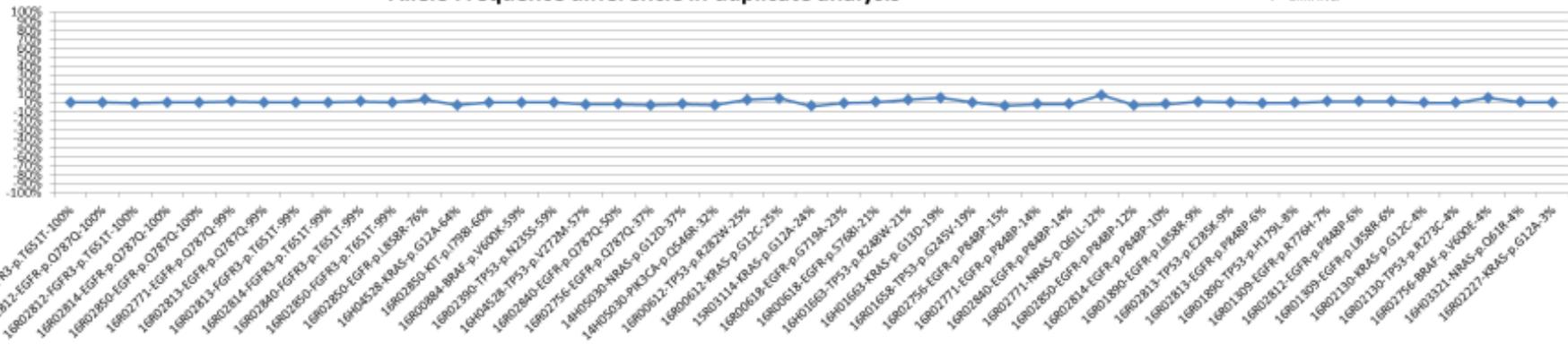
Moyenne	4611 X
M+2ET	5329X
M-2ET	3893X
CV%	8%

Moyenne	248 X
M+2ET	312X
M-2ET	184X
CV%	13%

Moyenne	8,7 X
M+2ET	15 X
M-2ET	2,6 X
CV%	35%

Replicates comparison

Allele Fréquence différencie in duplicate analysis

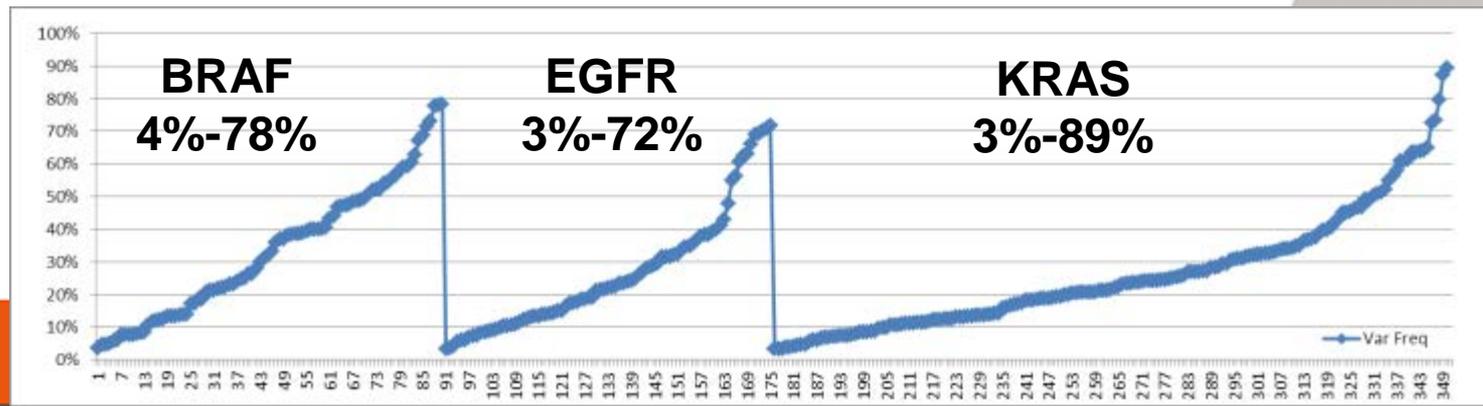


Gustave Roussy Standard screening data (n=949)

Cancers type	Mut. Status	Nb	Comm.
Lung Cancers (n=513)	WT	152	
	EGFR	69	Activating mutation
		12	Activating + T790M
		7	Rare
	BRAF	4	exon 11
		6	exon 15
	KRAS	114	
	NRAS	10	
	PIK3CA	19	
	FGFR3	3	
	CTNNB1	3	
	PTEN	1	
	TP53	53	co-occurente
		51	unique

Cancers type	Mut. Status	Nb	Comm.
Melanomas (n=183)	WT	30	
	BRAF	61	V660E
		10	V600K
		4	rare T599-K601
	NRAS	44	
	KRAS	3	
	KIT	3	
	CTNNB1	7	
	PIK3CA	5	
	PTEN	3	
	TP53	8	co-occurente
3		unique	
Colons (n=210)	WT	41	
	KRAS	61	
	NRAS	15	
	BRAF	11	
	PIK3CA	13	
	CTNNB1	6	
	PTEN	2	
	TP53	31	co-occurente
		27	unique
	Autres (N=33)	WT	16
Mut		17	

%age of mutated DNA



Tumeurs Solides

➤ 16 gènes

gène	Exons / hotspots
AKT1	3
ALK	23+24+25
BRAF	11+15
EGFR	18+19+20+21
ERBB2 (HER2)	20
ERBB4	E452K et R393W
FGFR2	S252, N549, K659
FGFR3	7+9+14 (R248 à S249 et G370 à Y373)
HRAS	2+3+4
KIT	8+9+11+13+17+18
KRAS	2+3+4
MAP2K1 (MEK1)	2
MET	2 + 14 à 20
NRAS	2+3+4
PDGFRA	12+14+18
PIK3CA	9 + 20

Lymphomes / SMP

➤ 14 gènes

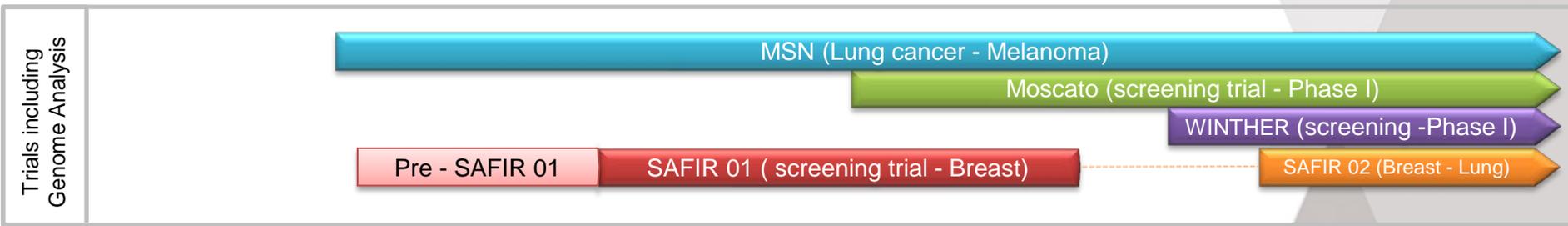
gène	Pathologie	Exons / hotspots
ATM	CLL	full
BIRC3	CLL, SMZL	6+7+8+9
BRAF	DLBCL, HCL	15
CARD11	DLBCL	4+5+6+7+8+9
CD79A	DLBCL	5
CD79B	DLBCL	5
EZH2	DLBCL	17
FBXW7	CLL, SMZL	9+10+11
MYD88	WM, DLBCL, SMZL, CLL	3+4+5
NOTCH1	CLL, MCL, DLBCL, SMZL	34
NOTCH2	SMZL, DLBCL	26+27+28+34
SF3B1	CLL	14+15+16+17+18
TNFAIP3	MCL, WM, DLBCL	full
TP53	CLL, DLBCL	full sauf exon1

Leucémies

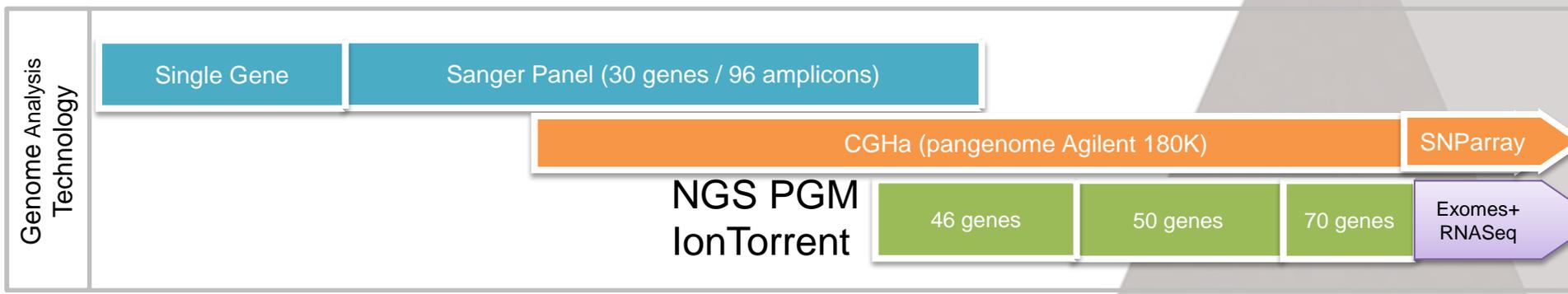
➤ 24 gènes

gène	Pathologie	Exons / hotspots
ASXL1	AML, MDS, MPN, CMML	12
CBL	AML, MDS, MPN, CMML	8+9
CEBPA	AML	Full
DNMT3A	AML, MDS, ALL?	Full
ETV6	AML	Full
EZH2	MPN, MDS	Full
FBXW7	ALL	9+10+11
IDH1	AML, MDS	4
IDH2	AML, MDS	4
JAK2	AML, MPN, MDS	12+14
KIT	Mast cell Neoplasm, MPN, AML	9+10+11+17
KRAS	AML, MDS, CMML, ALL	2 + 3
MPL	MPN, MDS	10
NOTCH1	ALL	26+27+28+34
NPM1	AML, MDS	12
NRAS	AML, MDS, JCMML, ALL	2+3
PTEN	ALL, AML	5+7
PTPN11	AML, JCMML	3+13
RUNX1	AML, MDS, CMML, ALL	full sauf 1 et 2
SF3B1	AML, MDS, MPN, CMML	13+14+15+16
SRSF2	AML, MDS, CMML	1
TET2	AML, MDS, MPN, CMML	Full
TP53	AML, MDS, MPN, CMML, ALL	Full sauf exon1
U2AF1	AML, MDS, CMML	2+ 6

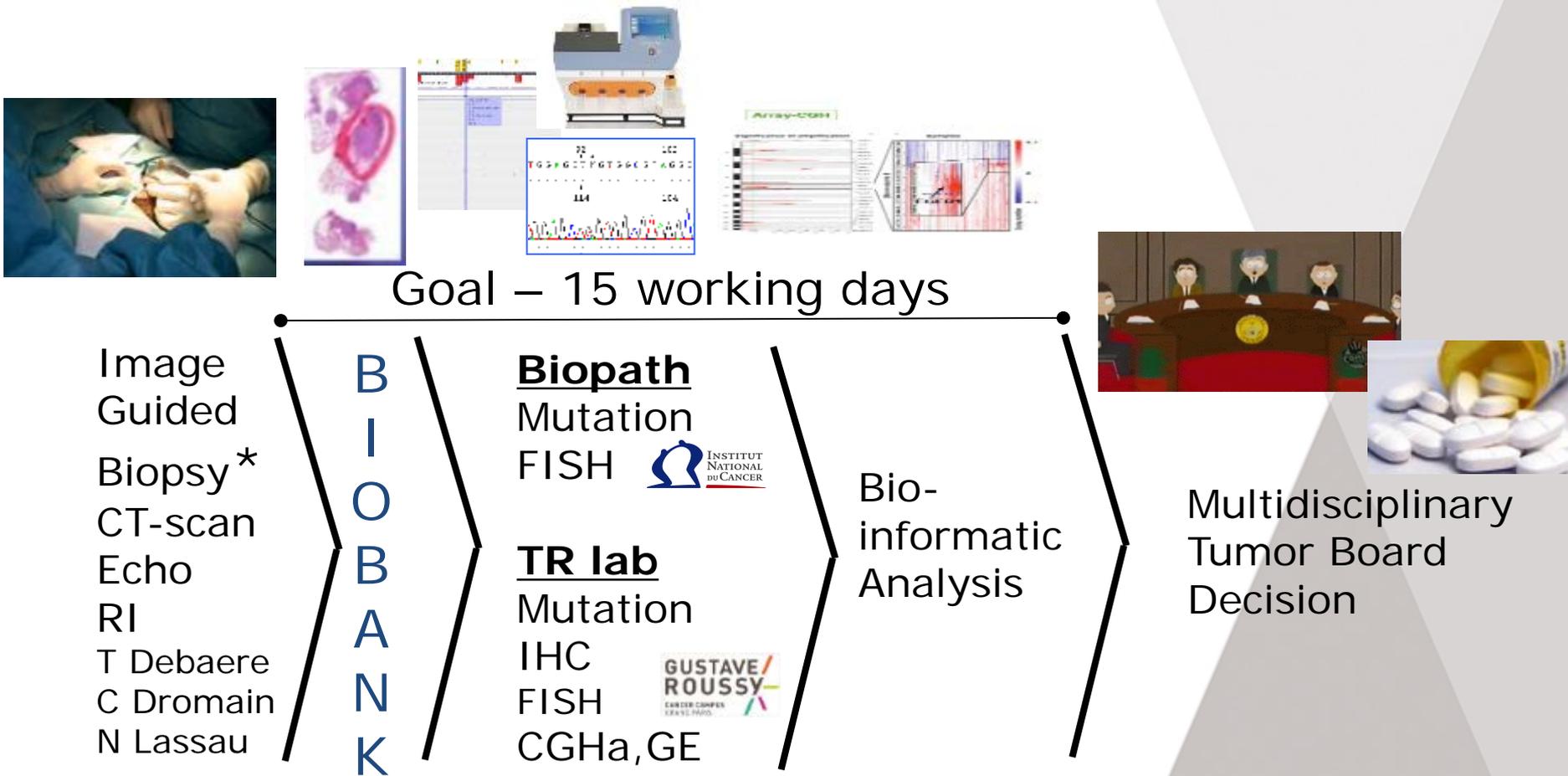
Gustave Roussy PCM Program :



1330 patients with molecular portrait in 4 years (2011-2014)



1st Challenge... Building the workflow to perform Tumor molecular portraits for therapeutic decision

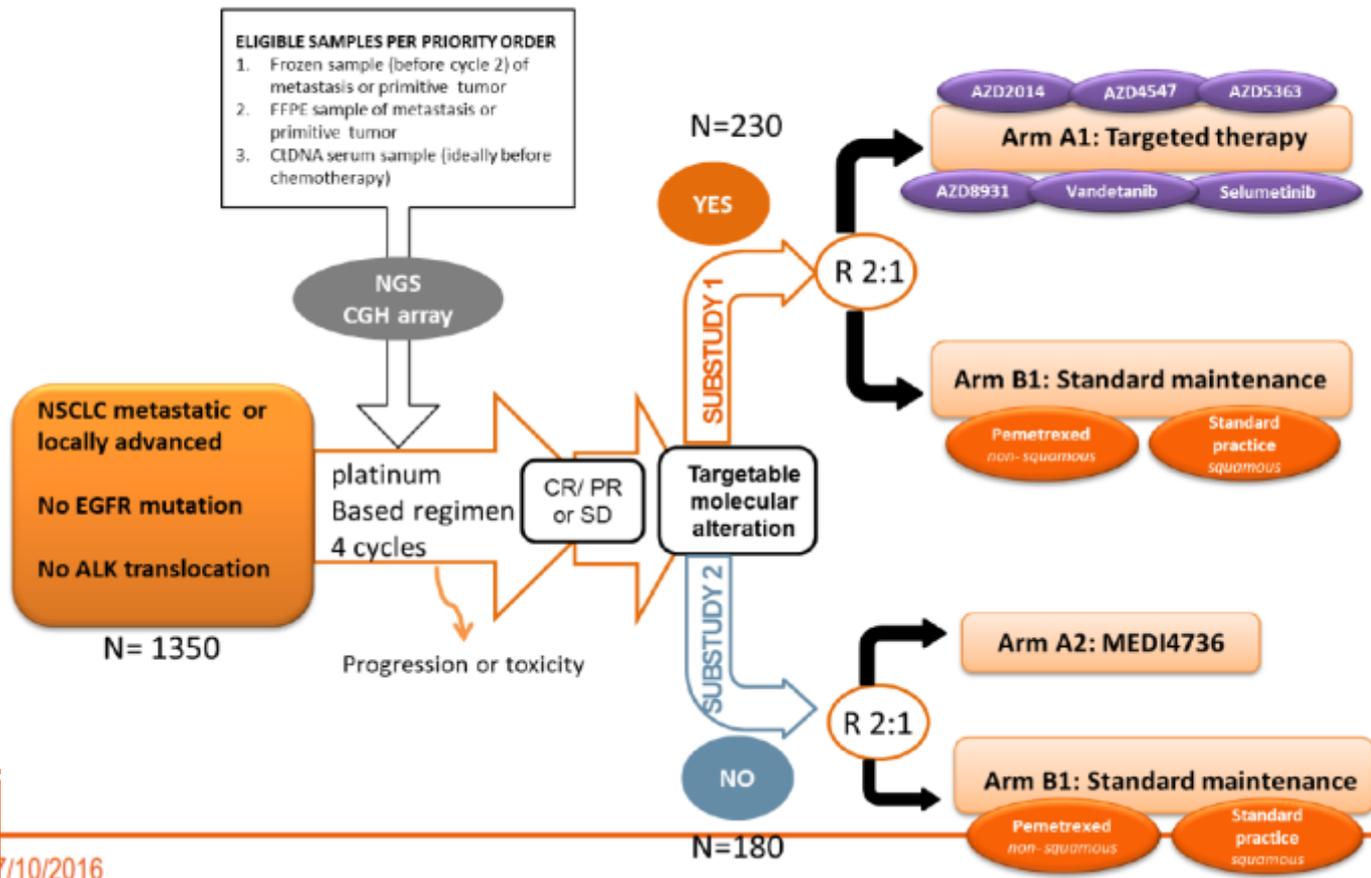


*including sequential biopsies and biopsies at PD

SAFIR02 studies : A Randomized trials in Lung and Breast metatstacic cancers (PI. JC Soria for Lung & F.André for Breast)

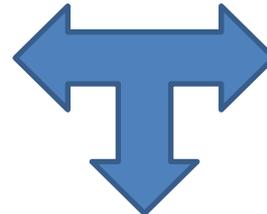
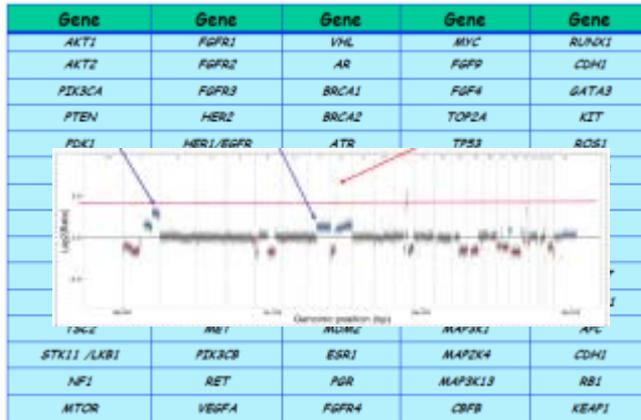
Study : Randomized, Multicentric phase II Biology-driven treatment VS standard maintenance CT.
Molecular analysis: CGHa (180K) and NGS (PGM – 50 genes – 300X)
Timelines: 3 years(Lung) / 2 years(Breast) **Follow-up:** 12 month
Treatment duration : until progression or toxicity

SAFIR02 Lung : STUDY DESIGN

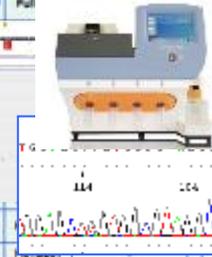


Mutation -50 gene panel NS – PGM - IonTorrent

Amplification / deletion
CGHa / SNPa pangenomic



Gene	Mutation	Comm.	Gene	Mutation	Comm.	Gene	Mutation	Comm.
AKT1	HotSpot	p.E17K	FGFR4	HotSpot	based on Cosmic	NOTCH1	HotSpot	based on Cosmic
AKT2	HotSpot	p.E17Klike	FLT1	FullCDS	vegfr1	NOTCH2	HotSpot	based on Cosmic
AKT3	HotSpot	p.E17Klike	HRAS	HotSpot	Ex2,3,4	NOTCH3	HotSpot	based on Cosmic
ALK	HotSpot	Ex 20 to 26	ZNF48	Full		RAS	HotSpot	Ex 2,3,4
BRAF	HotSpot	Ex 11 & 15				PRF4	HotSpot	12-14-18
BRCA1	FullCDS	-				SC4	HotSpot	Ex 10&21 + CYP2
BRCA2	FullCDS	-				SR1	HotSpot	based on Cosmic
CTNNA1	HotSpot	Ex3				TR1A	HotSpot	based on Cosmic
DDR2	FullCDS	-				TEN	FullCDS	-
EGFR	HotSpot	Ex 18-21 +				TPN21	HotSpot	based on Cosmic
HER2	HotSpot	Ex 8 + 19				RET	HotSpot	Ex 11-16
HER3	FullCDS	-	ABL1	HotSpot	-	ROB1	HotSpot	mut Resist
HER4	FullCDS	-	ALL3	HotSpot	-	STK11	FullCDS	-
FBXW7	FullCDS	-	MTOR	FullCDS	-	TP53	FullCDS	-
FGFR1	HotSpot	p.S46 p.664	NF1	FullCDS	-	TSO1	FullCDS	-
FGFR2	HotSpot	4 Exons	NF2L2	HotSpot	based on Cosmic	TSO2	FullCDS	-
FGFR3	HotSpot	based on Cosmic				VHL	FullCDS	-

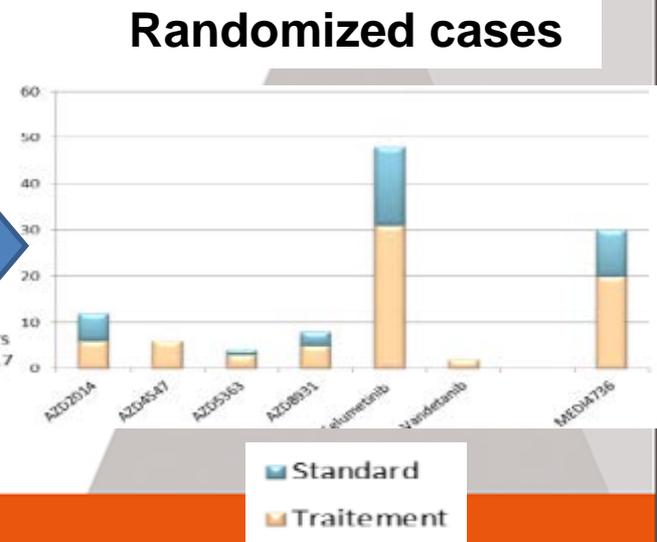
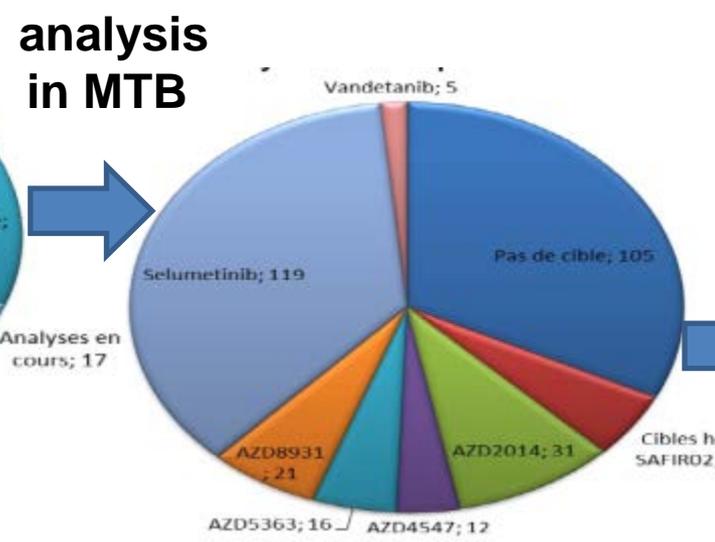
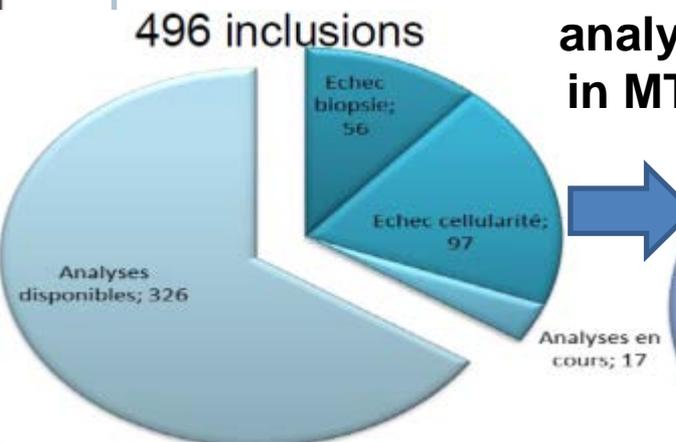
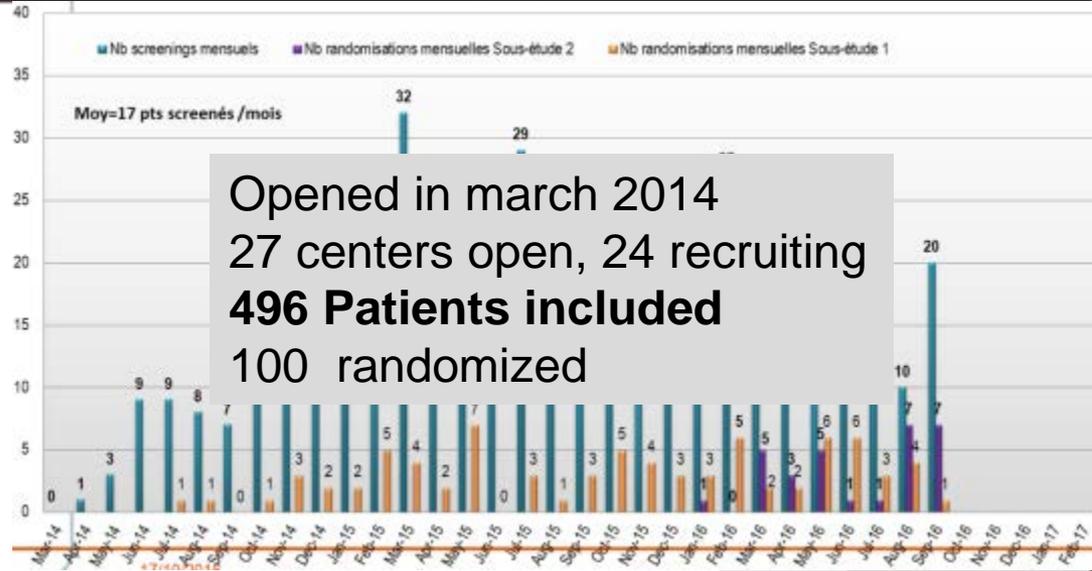
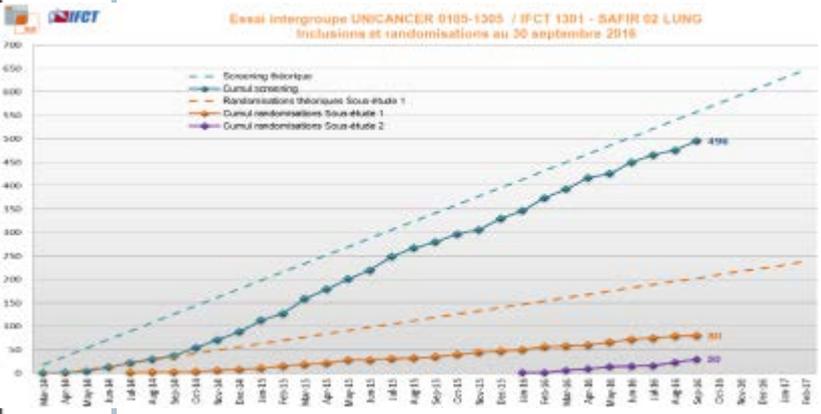


SAFIRO2 Lung - Order of preference for treatment

Or de	Genetic abnormality	Expected frequency in squamous cell carcinoma	Expected frequency in adeno-carcinoma	Therapeutic intervention
1	RET translocation	0%	1%	Vandetanib
2	HER2 amplification	0-1%	6%	AZD8931
	HER2 mutation	0%	2%	AZD8931
3	KRAS mutation	6%	21%	Selumetinib
	BRAF mutation	2%	1-3%	Selumetinib
4	PIK3CA amplification	33%	6%	AZD5363 (or AZD2014 if the number of pts with this abnormality is high)
	PIK3CA mutation	6%	3%	AZD5363 (or AZD2014 if the number of pts with this abnormality is high)
	PTEN loss	16%	1%	AZD5363 (or AZD2014 if the number of pts with this abnormality is high)
	PTEN mutation	10%	2%	AZD5363(or AZD2014 if the number of pts with this abnormality is high)
	AKT1 mutation	1%	Very rare	AZD5363
5	F6FR1 amplification	22%	1%	AZD4547
	F6FR2 mutation	4%	0%	AZD4547
	F6FR3 mutation	3%	0%	AZD4547
6	LKR1 mutation	5%	22%	AZD2014

6 targeted therapies available (AZD Pipeline)

Defined algorithm to oriented decision in weekly molecular tumor board



Update fall 2016

Whole Exome Sequencing (WES) vs Target Gene Sequencing (TGS) for Clinical Research ?

- **Could we generate results in the same delay than TGS Seq +CGHa ?**
- **Could we have same results on target SNV and CNV with WES ?**
for SNV, TGS list 75 genes
- **What is the added value of WES/RNASeq for**
 - Are the additional info information useful for clinical research ?
 - Other SNV ; 271 genes list (Van Allen et al. + Litt.)
 - Mutation profile
 - Mutational Load
 - Estimation of tumor percentage
 - HLA prediction / epitope calculation ..
 - Is RNAseq efficient to detect translocation ?
 - IS RNA quantification efficient for target markers ?

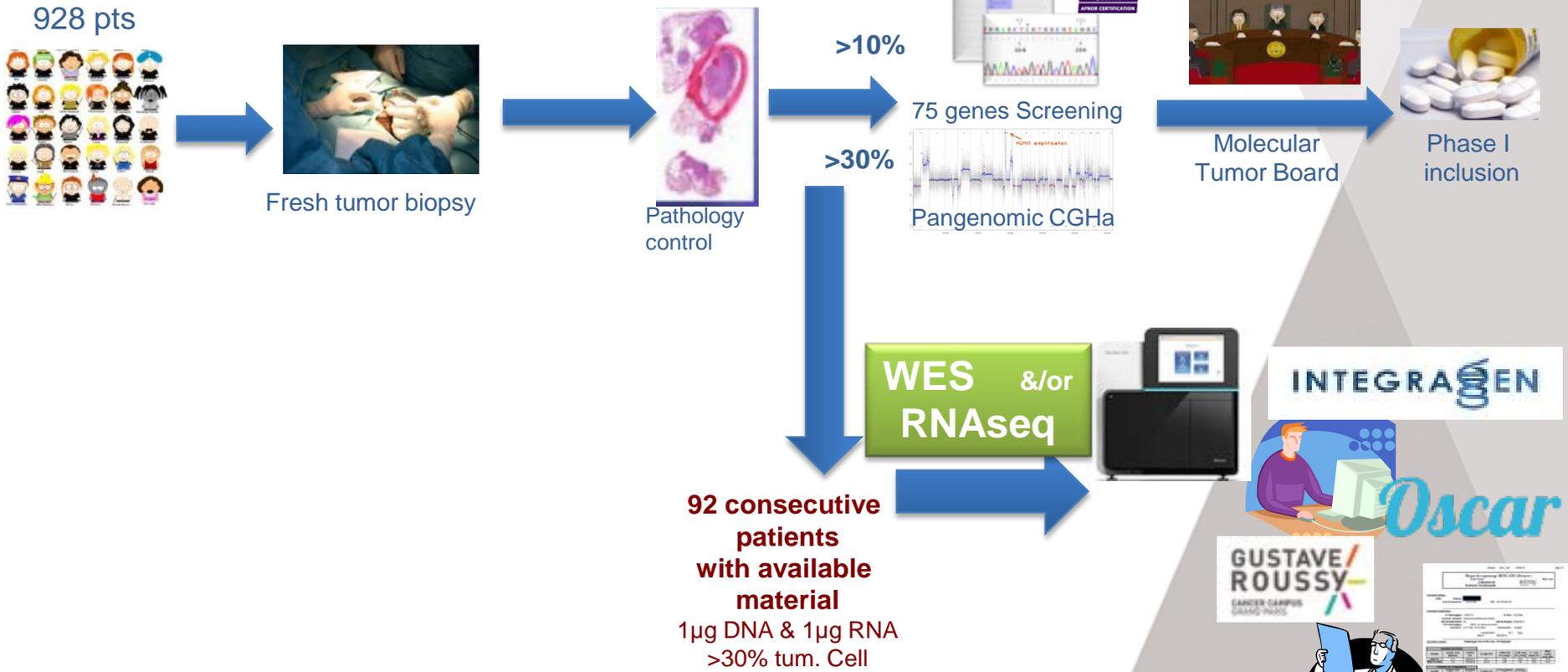
Molecular Screening for Cancer Treatment Optimization (MOSCATO 01): a prospective molecular triage trial.

Hollebecque A et al. ASCO 2013

Geogger B. et al. ASCO 2014

Ferté C et al. ASCO 2014

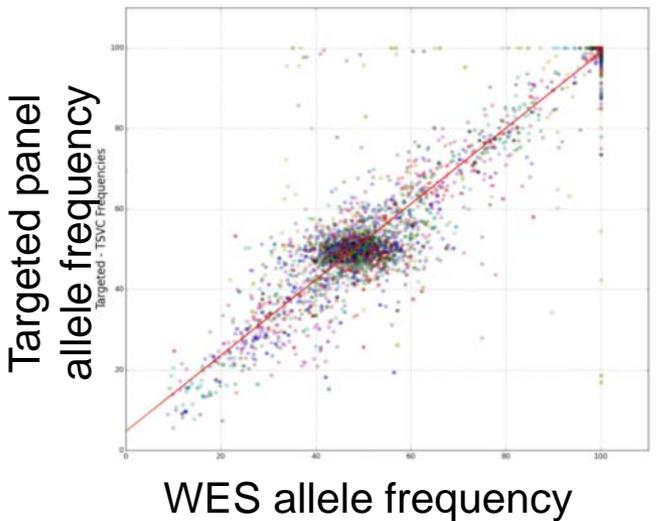
Charles Ferté, Christophe Massard, Ecaterina Ileana, Antoine Hollebecque, Ludovic Lacroix, Samy Ammari, Maud Ngo-Camus, Rastislav Bahleda, Anas Gazzah, Andrea Varga, Sophie Postel-Vinay, Yohann Loriot, Nathalie Auger, Valerie Koubi-Pick, Bastien Job, Thierry De Baere, Frederic Deschamps, Philippe Vielh, Vladimir Lazar, Marie-Cécile Le Deley, Catherine Richon, Vincent Ribrag, Eric Deutsch, Eric Angevin, Gilles Vassal, Alexander Eggermont, Fabrice André, Jean-Charles Soria



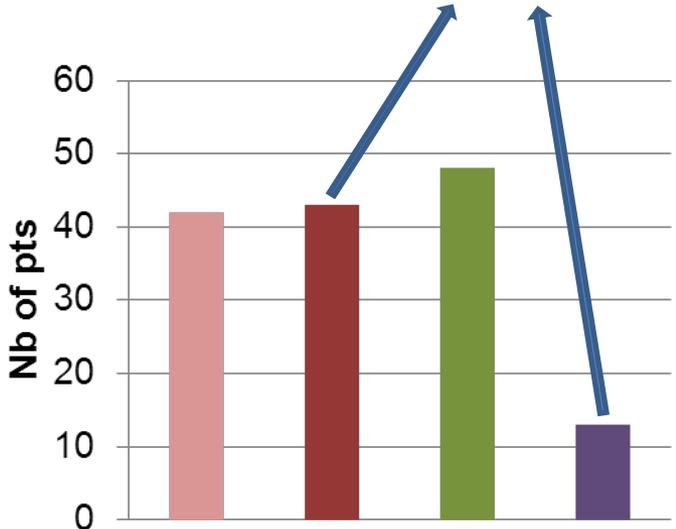
Sample ID	Gene	Expression	Copy Number
PT001	BRCA1	0.5	1.0
PT002	BRCA2	0.8	1.0
PT003	ERBB2	1.2	1.0
PT004	ERBB3	0.9	1.0
PT005	ERBB4	0.7	1.0

Comparison of SNV detection between WES and TGS :

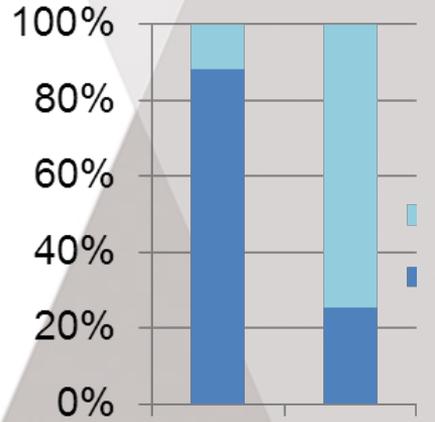
Correlation between Targeted - TSVC and Exome - GATK frequencies. slope=0.942. cor=0.910. n=4826.



Additional Information with WES for 38% cases



Mutation TGS	Yes	Yes	None	None
Additional Mutation WES	None	Yes	None	Yes



Mutation In TGS & WES (Green)
Mutation only in WES (Red)

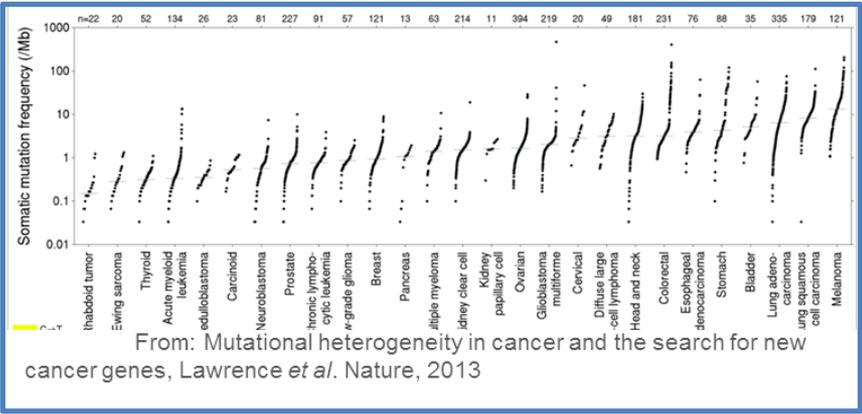
WES sensitivity: 94% (121/129)

Good correlation between WES and TGS AF

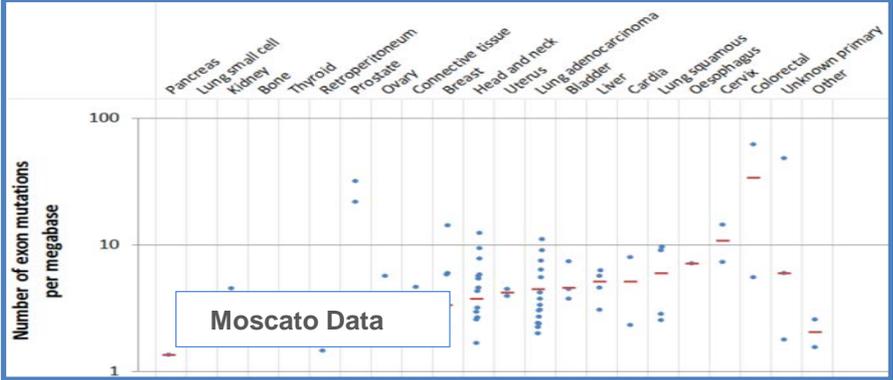
- Only low Allele Frequency are not detected 4.8 to 13.4%
- Information about somatic / constitutional Status

VP Pathogenic variant
VPI Variant of unknown pathogenicity

WES additional informations :



Mutational load

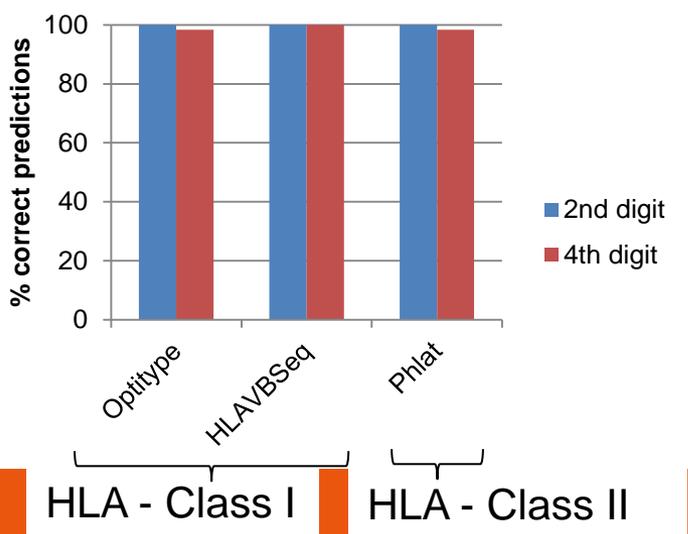


Sequenza analysis => in silico %age of tumor cells

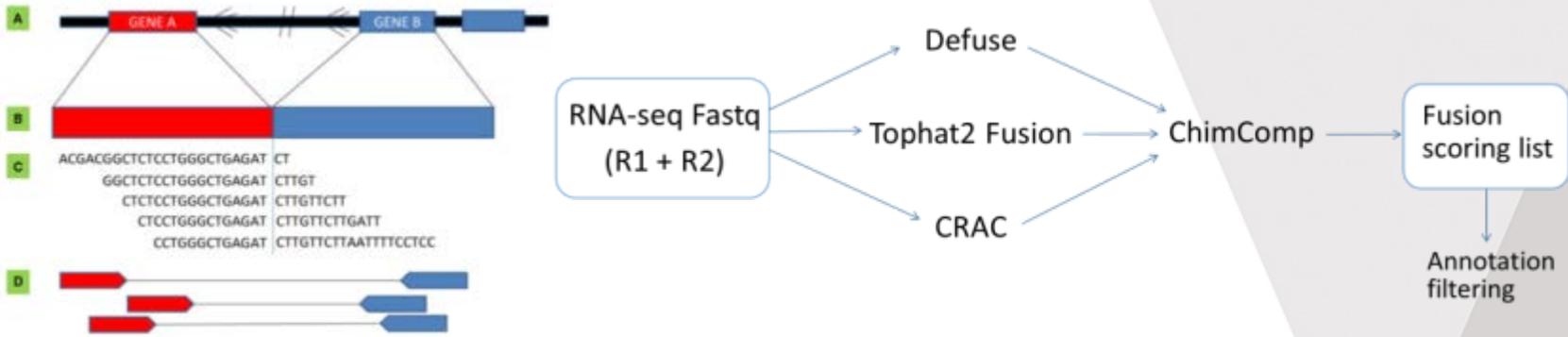
WES additional informations : and neo-epitope prediction

HLA typing reference:

- 10 MOSCATO patients
- HLA typing with gold standard method (Saint Louis)
 - Class I : locus A, B and C
 - Class II : locus DR, DQ and DP



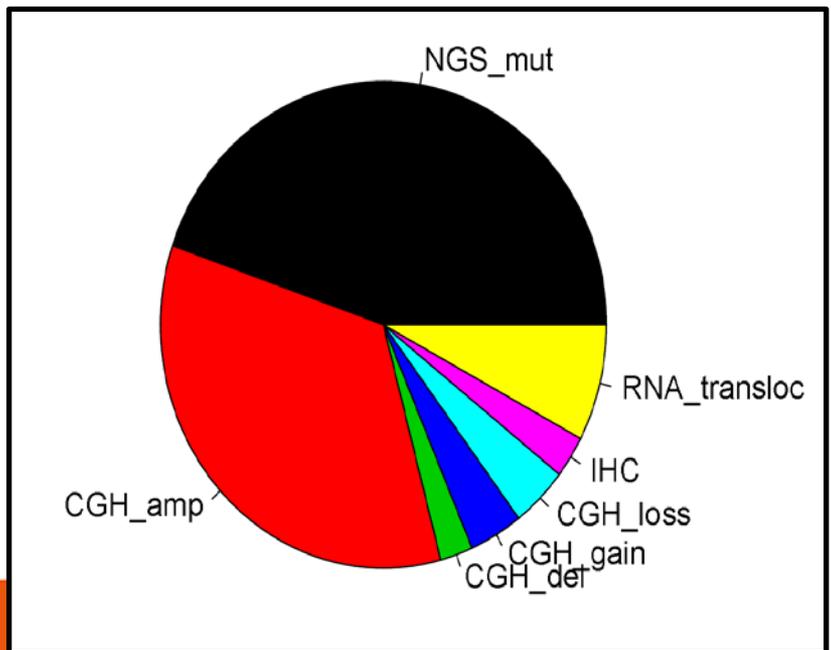
RNASeq: Fusion transcripts detection



- Identification of 15 fusions in 92 patients described in the literature as associated with the pathology.

Technics to drive treatments

- CD74/ROS1 (M656, Adenocarcinoma, Lung)
- KIF5B/RET (M545, Adenocarcinoma, Lung)
- FGFR3/TACC3 (M521, Transitional cell carcinoma, Bladder)
- TMPRSS2/ETV5 (M689, Adenocarcinoma, Prostate)
- EWSR1/FLI1 (M591, Ewing sarcoma)
- EWSR1/FLI1 (M639, Ewing sarcoma)
- PAX7/FOXO1 (M644, Alveolar Rhabdomyosarcoma)
- DNAJB1/PRKACA (M569, Hepatocellular carcinoma)
- MYB/NFIB (M574, Adenoid cystic carcinoma, Parotid gland)
- SLC45A3/ERG (M674, Adenocarcinoma, Prostate)
- COL1A2/COL1A1 (M686, Adenocarcinoma, Lung)
- SLC45A3/ELK4 (M689, Adenocarcinoma, Prostate)
- TRIO/DNAH5 (M725, Adenocarcinoma, Lung)
- AZGP1/GJC3 (M575, Adenocarcinoma, Prostate)
- CREB3L2/FUS (M640, Undifferentiated sarcoma, lower limb)



30yrs Cholangiocarcinoma Patient with FGFR2-CCAR1 fusion treated with FGFR inhibitor

Mosaic MD05 14H08290 page 1/2

Analyse Exome - RNASeq (Paf Integragen)
Laboratoire de Recherche Translationnelle GUSTAVE ROUSSY MD05

Information Patient :

NOM : Prénom : DROKLER Nadège (RE BICPSE)
Date de Naissance : 25/04/1961 NP : 2013-05005 EK

Information Délégement :

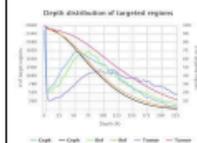
N° Histologique : 14H08290 N° Bloc : K01
Données Cliniques : cholangiocarcinome

Conclusion :
Présence d'un translocation impliquant FGFR2 et CCAR1 (cell division cycle and apoptosis regulator 1)

MD05-T1-ADN 13.4 26170904/26.22 0.56 0.55 20.05 14.8

Sample	Sample Yield (Gb/seq)	#Clusters (PF)	% Align (PF)	% Pair Aligned (PF)	# Fusion Reads (PF)
MD05-T1-ADN	9.92	118656106	90.81%	97.85%	1503

Exome Summary



Depth & coverage Whole Exome						
Sample	Agene	% of no coverage	% of 10X coverage	% of 25X coverage	Mean depth	
MD05-N	nb genes	3.21	23.53	60.77	94.51	
MD05-T1-ADN	nb genes	2.7	25.17	60.6	133.06	

Depth & coverage Favorite Genes						
Sample	Agene	% of no coverage	% of 10X coverage	% of 25X coverage	Mean depth	
MD05-N	74	2.24	24.87	60.5	94.73	
MD05-T1-ADN	74	1.83	26.42	64.06	133.9	

SNV detection statistics

Sample	Pharmac ²	Agemine ²	#all SNV	#B-SNP132	#nonans total	#nonans not in DB	#nonans total	#nonans not in DB
MD05-N-MD05-T1-ADN	76	307	53306/30201 (83 %)	550 (1 %)	470 (1 %)	20107 (36 %)	909 (17 %)	

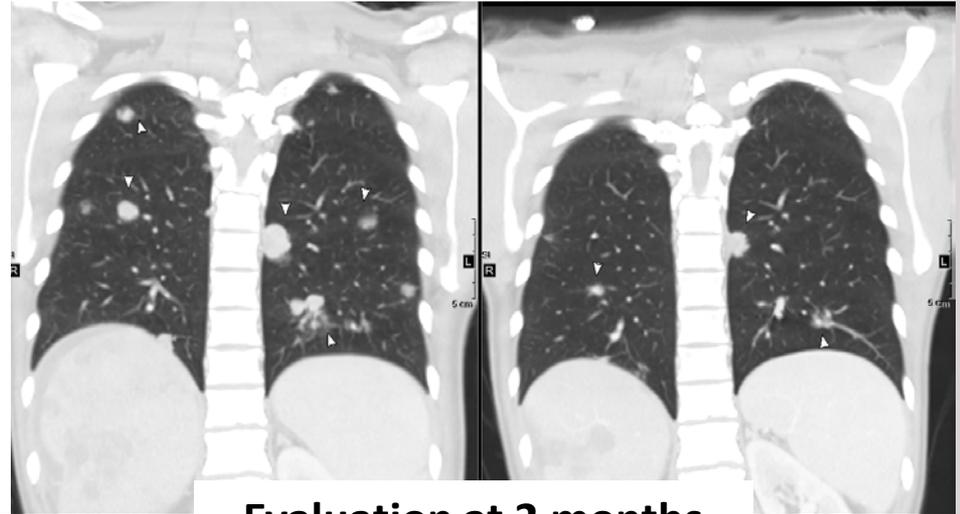
Indels detection statistics

Sample	Pharmac ²	Agemine ²	#all Indels	#B-SNP132	#COS	#COS not in DB
MD05-N-MD05-T1-ADN	7	36	1806/901 (54 %)	523 (29 %)	63 (3 %)	

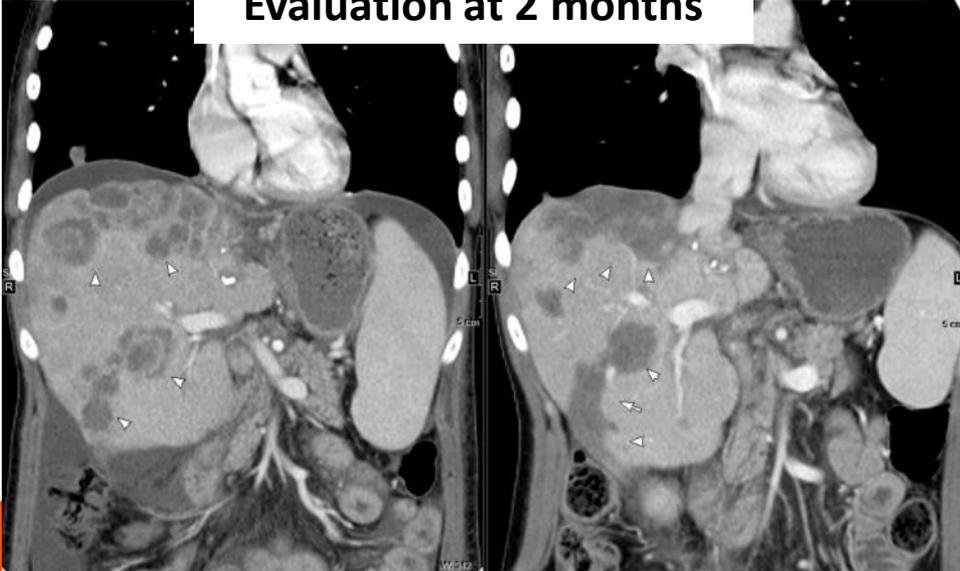
Fusion detection statistics

Sample	# potential	# fusion
MD05-T1-ADN	4	4

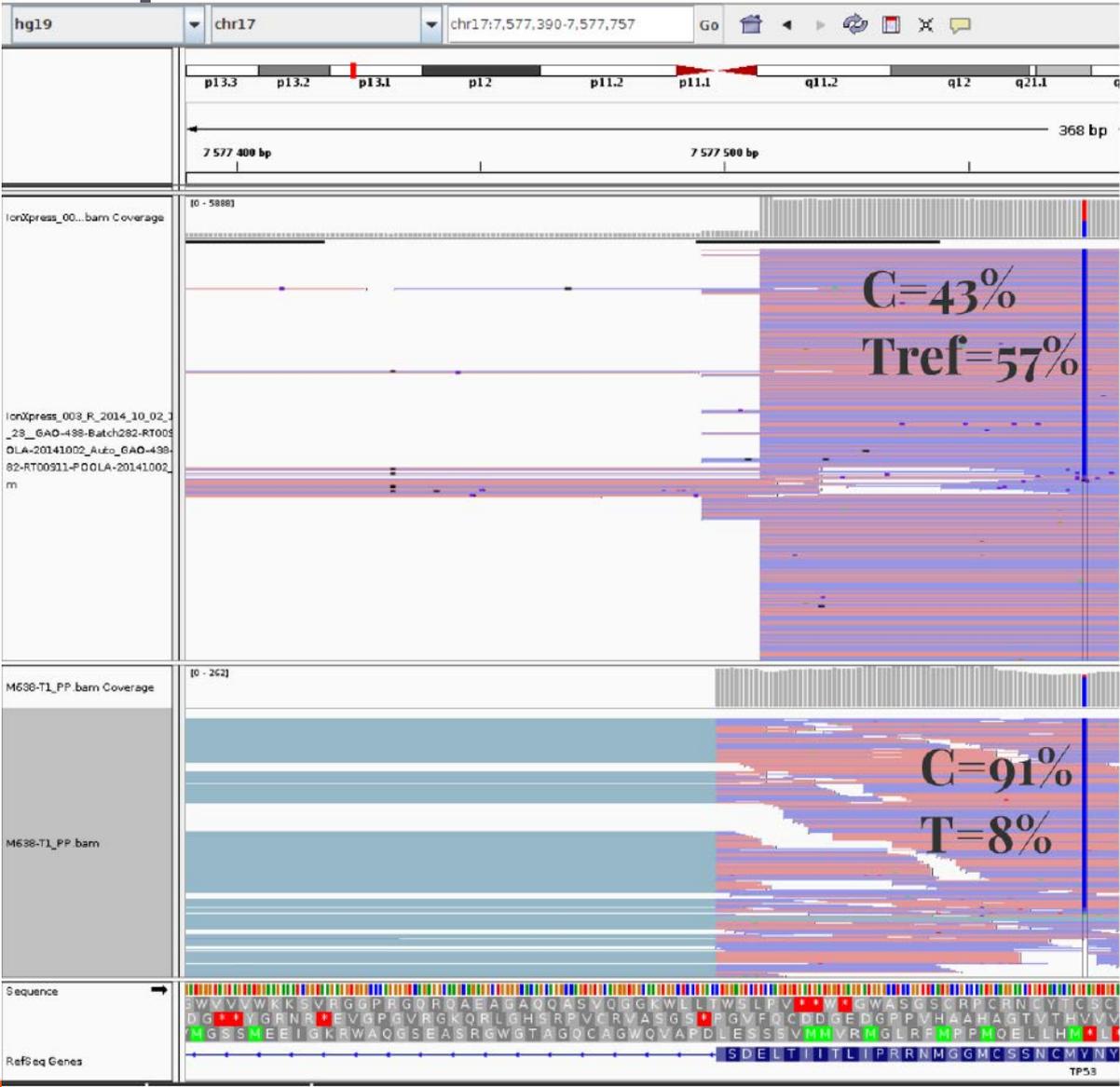
InFusions detection statistics				
Sample	# RefSeq Gene	% Expressed	# RefSeq	% Expressed
MD05-T1-ADN	24750	77.49%	36056	52.4%

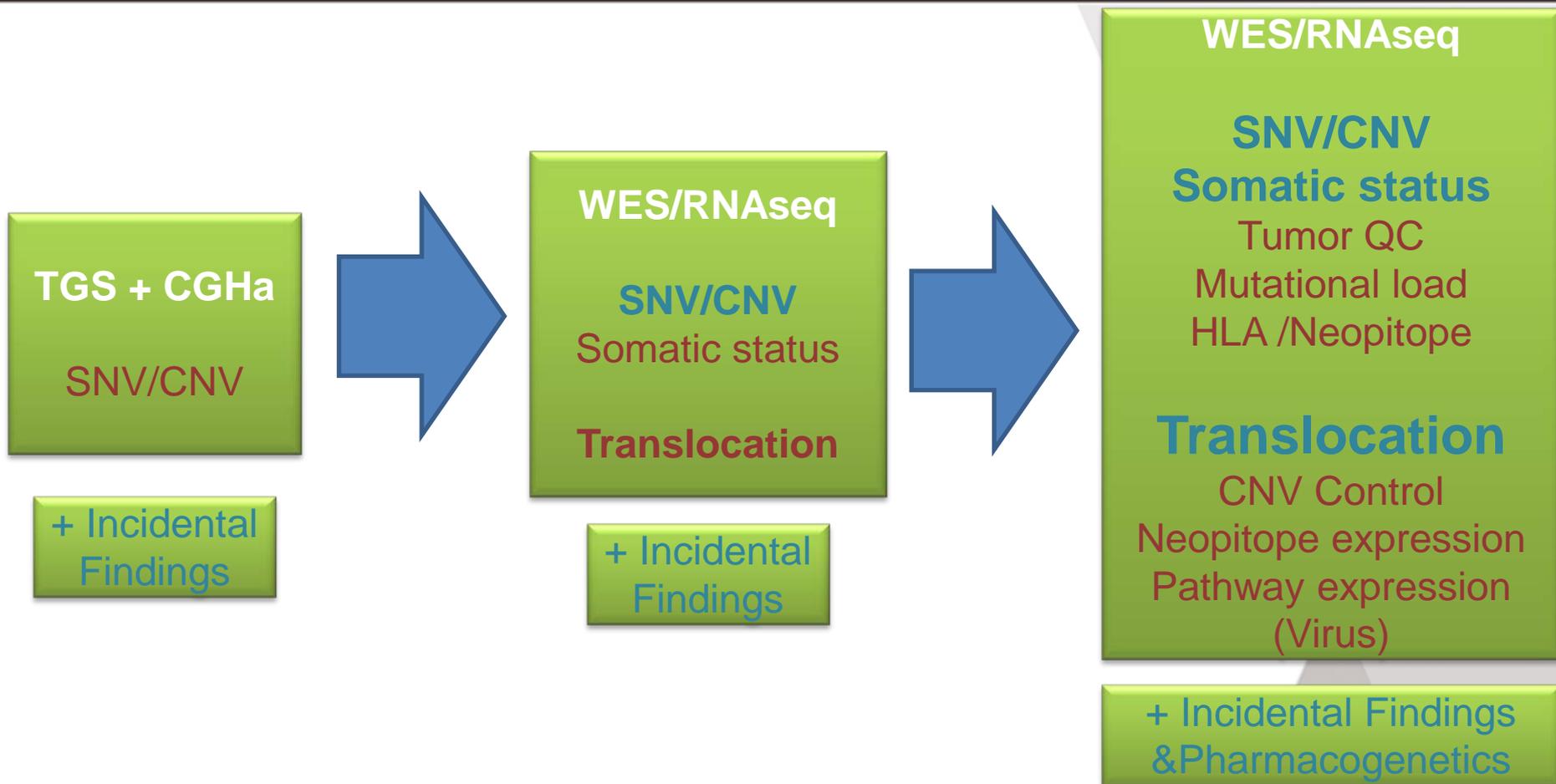


Evaluation at 2 months



Monoallelic expression





● **Limitation/Improvement WES/RNAseq for clinical use .**

- > Need of Improvement of the analysis TAT and sensitivity (sples <30% Tum.Cells)
- > Reinforce the quality of Annotation leading to clinical report for rare variant
- > Simplified the process to achieve efficient clinical report for molecular tumor board
- > Cost

Risk with whole exome/genome individual test

There is a big gap between large number of studies and the real usefulness/level of proof of “actionable” molecular biomarkers

NGS = global analysis giving information for all biomarkers, whatever is the level of proof.

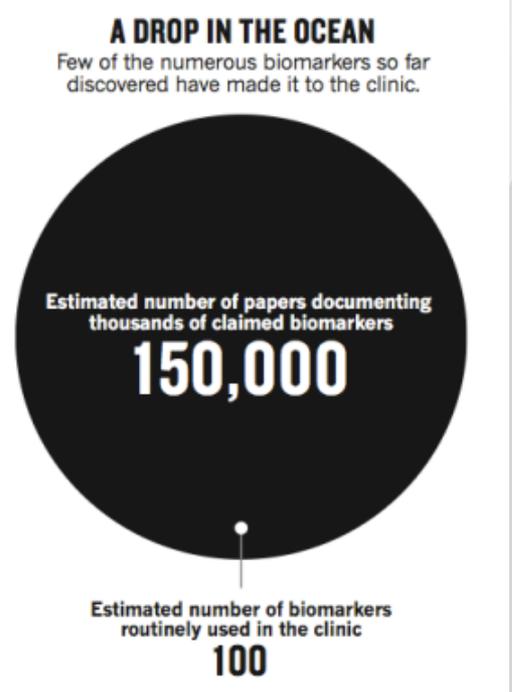


Table 1. Definition^a and classification of potentially actionable alterations

Category	Classification
Category 1	Gene variant predicts sensitivity to approved therapy in a particular malignancy.
Category 2	Gene variant predicts sensitivity for an approved therapy in any malignancy, but data for efficacy is lacking in that tumor type.
Category 3	Gene variant is an eligibility criterion for a clinical trial, or there is published evidence of clinical efficacy with an investigational agent.
Category 4	Gene variant with only preclinical support for use of an investigational therapy.

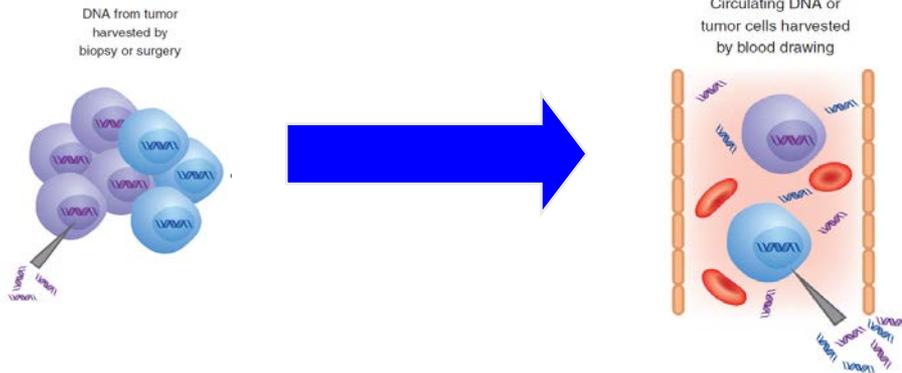
^aPotentially actionable alteration is defined as genetic alteration that is predicted to confer sensitivity to either approved targeted agent or experimental targeted agent in clinical trials.

NGS pour les applications cliniques

- **Technique très prometteuse**
- **Possibilité de multiplexer les marqueurs actuels (connus et robustes)**
- **Beaucoup d'application pour la recherche et l'innovation ...**
- **Nouveaux métiers (bioinformatique)**
- **Analyse haut débit encore difficile pour les applications cliniques**
 - > qualité de matériel
 - > Temps
 - > sensibilité
 - > sur-information
 - > Cout...
- **...application de panels ciblés pour la clinique uniquement**

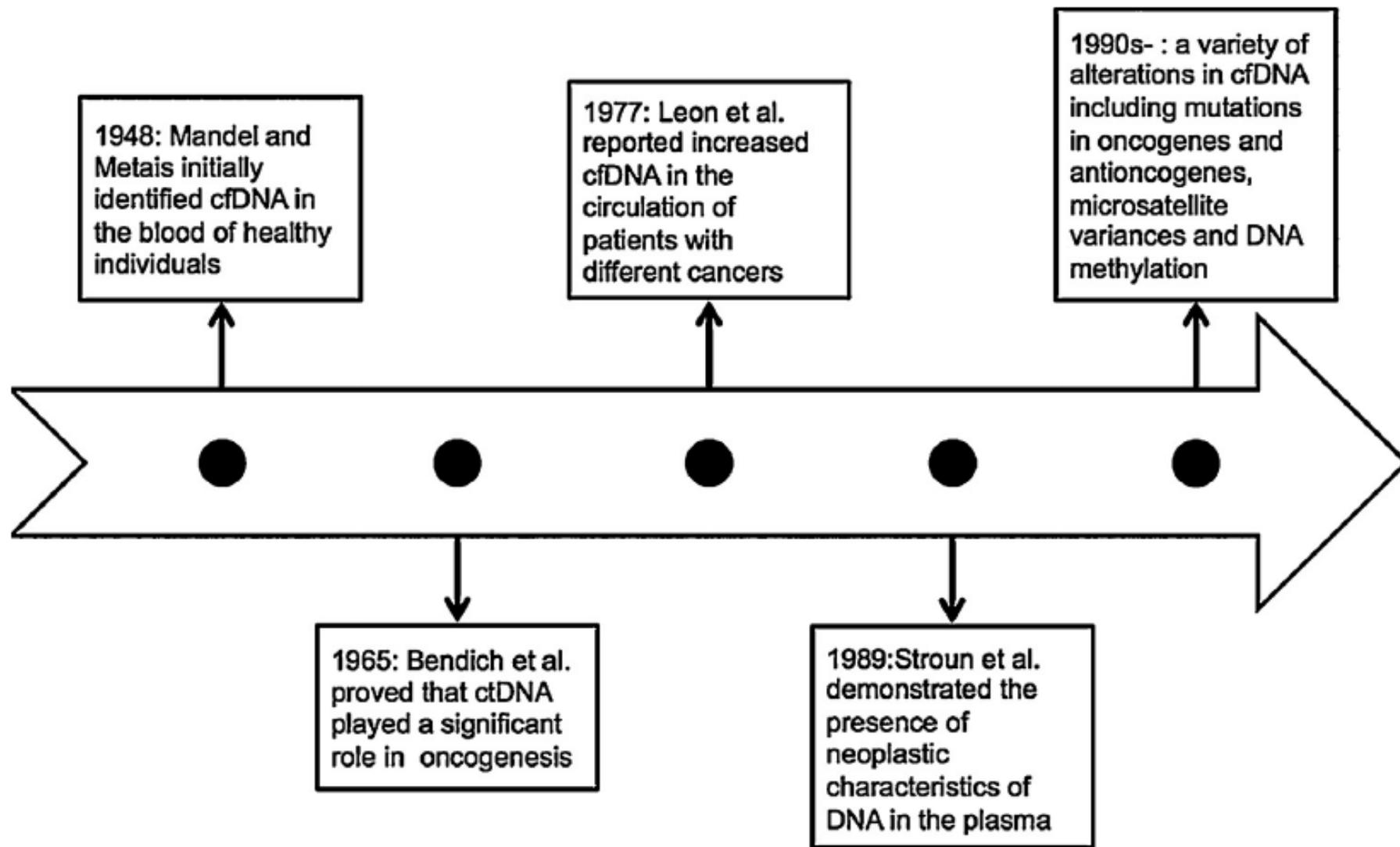
Les approches Émergentes,

Biopsie Liquide : ADN circulant Libre



**Avantage du prélèvement
non invasif**

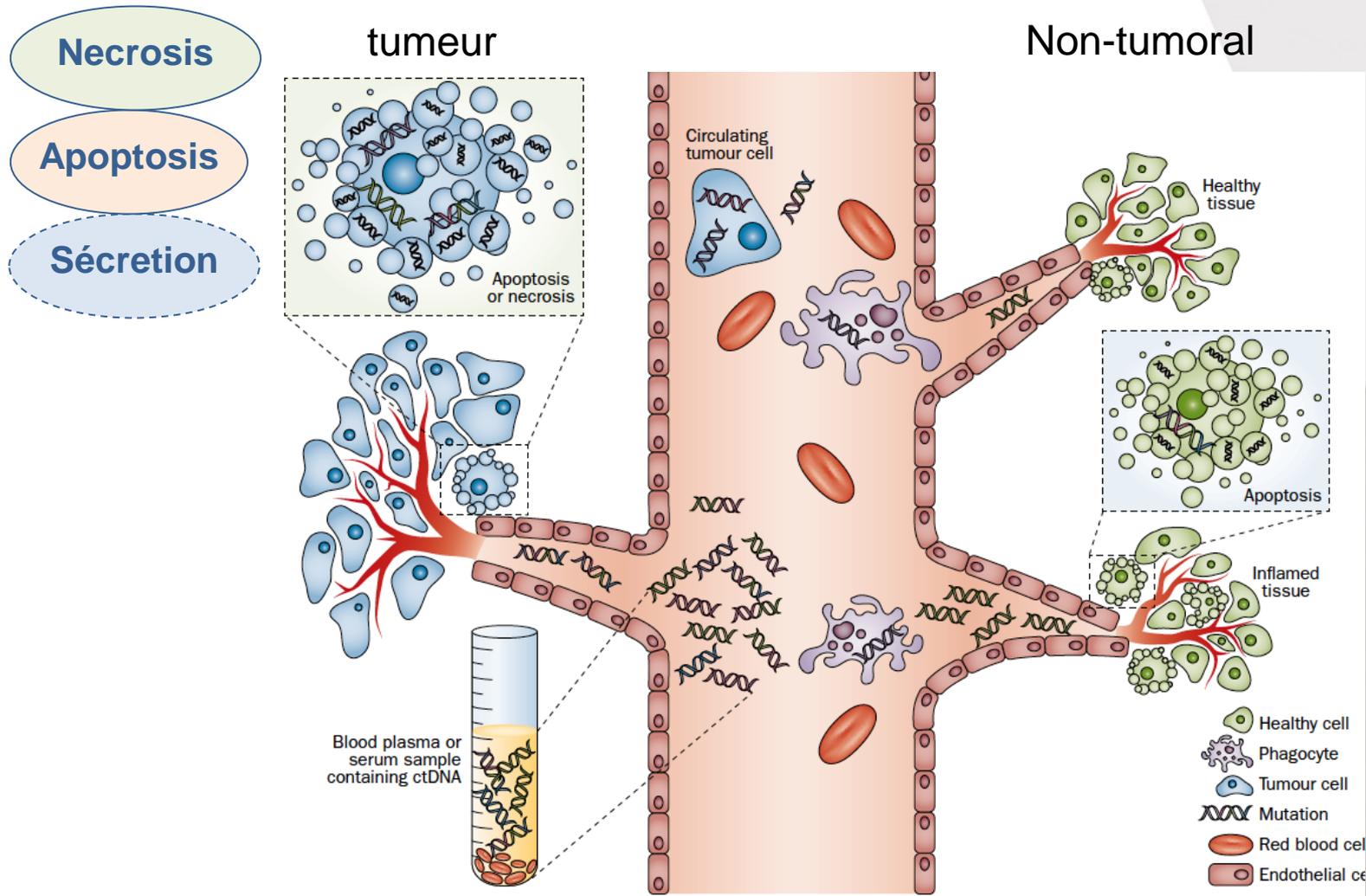




Introduction

- Origine de l'ADN libre circulant (cfDNA, circulating-free DNA)

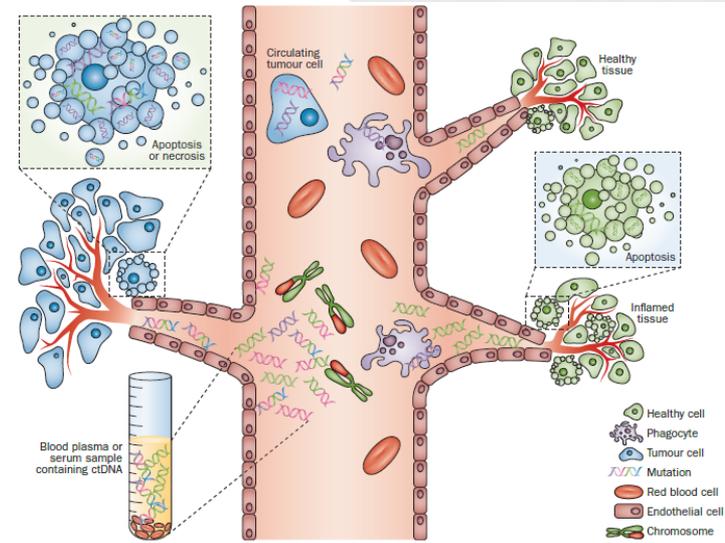
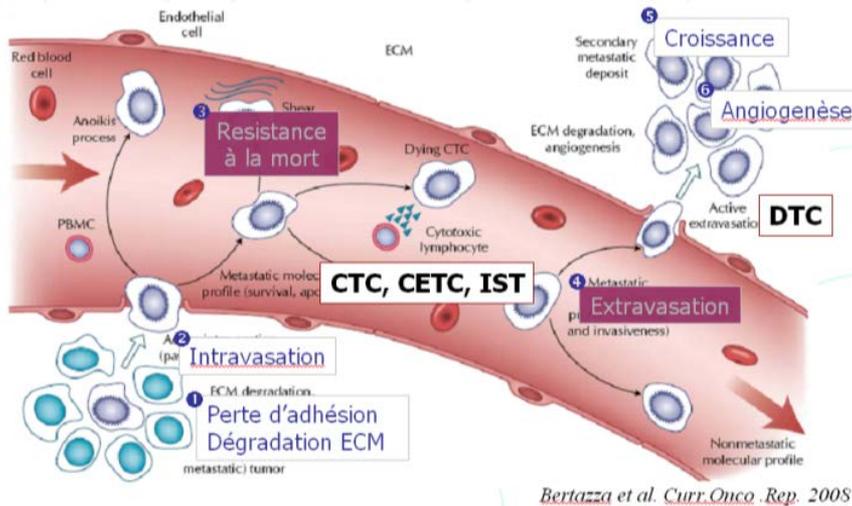
Présence dans le sang de petits fragments d'ADN libres



concept de Biopsie liquide

CTC

cfDNA



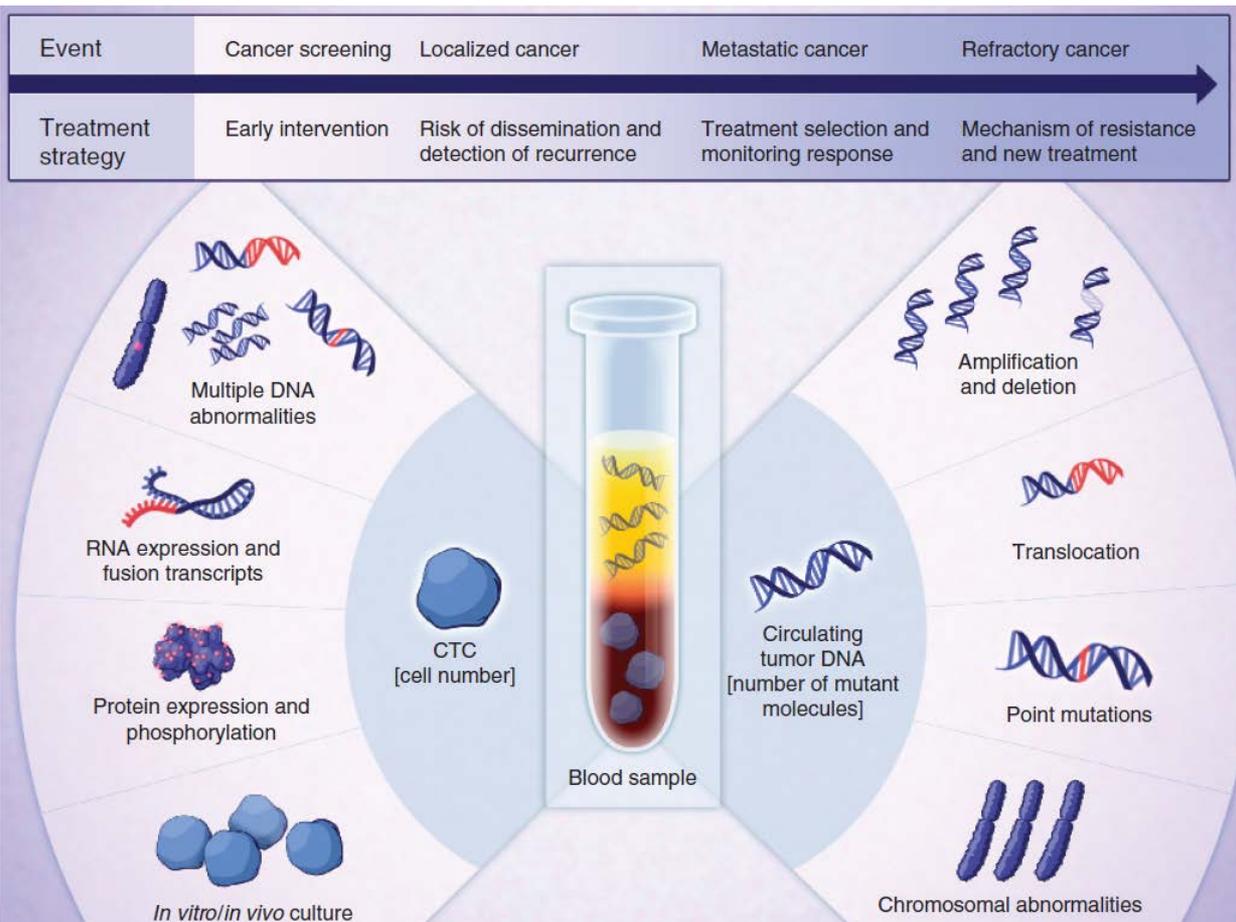
- Première description in 1869
- Libération active (+passive)
- ~1-5 cells/1mL
- Absent du sujet sains
- Nécessite enrichissement+détection.
- Cellules viables

- Première description in 1948
- Libération passive (+active)
- ~1-100 ng/mL
- Présent en cas d'inflammat°, traumatisme..
- Contam. par de l'ADN normal (*in vitro* lysis)
- Présence dans urine, ascite, ...

- Provenance de toutes les localisations/métastases
- Corrélation avec la masse tumorale

Test à partir d'une simple prise de sang, facile à répéter

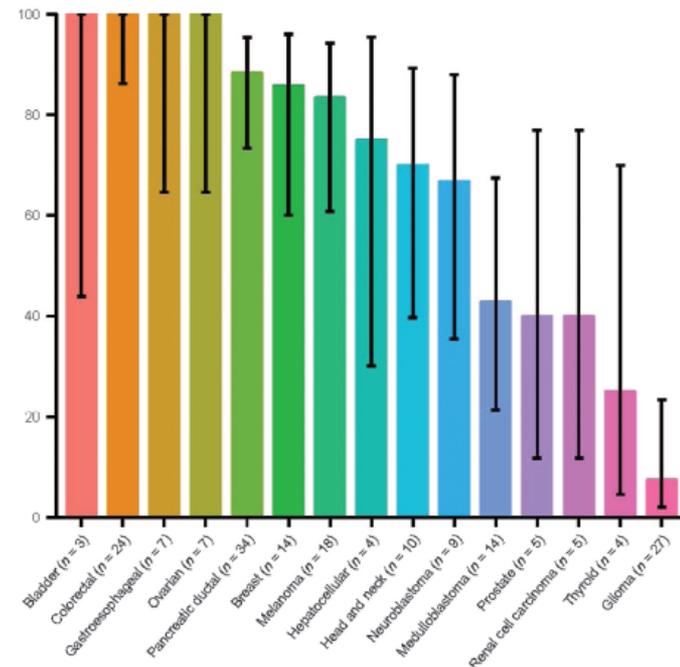
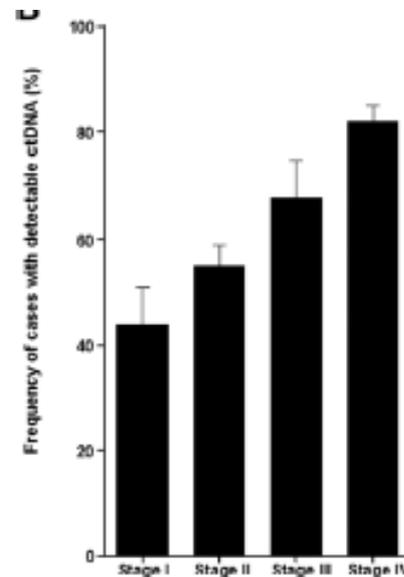
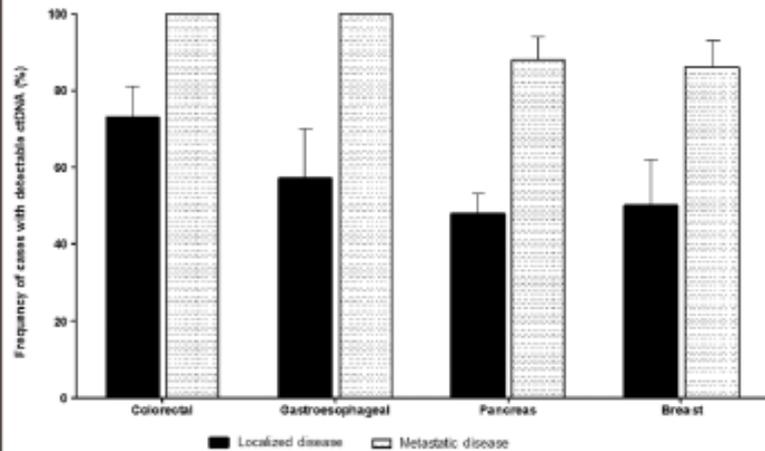
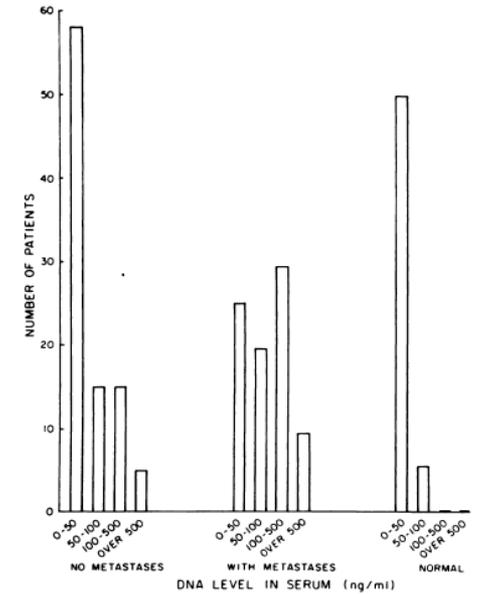
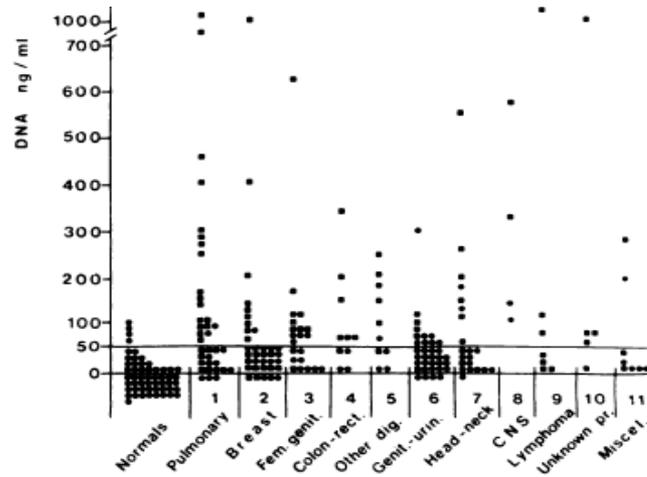
=> beaucoup d'enthousiasme



- Surrogate of biopsy for tumor genotyping
- Surrogate of drug response with serial samples
- Detection of Early relapse
- High-risk localized cancer
- Early detection of cancer
- Cancer biological study

Présence de cfDNA dans les cancers

Free DNA in the Serum of Cancer Patients and the Effect of Therapy
 S. A. Leon, B. Shapiro, D. M. Sklaroff, and M. J. Yaros
 [CANCER RESEARCH 37, 646-650, March 1977]



Quantification of CAN

● Concentration

- > Healthy Av. 15 ng/mL
(0 to 128 ng/ml)
- > Cancer Av. 137 ng/mL
(0 to 4738 ng/ml)

● Also in begin disease...

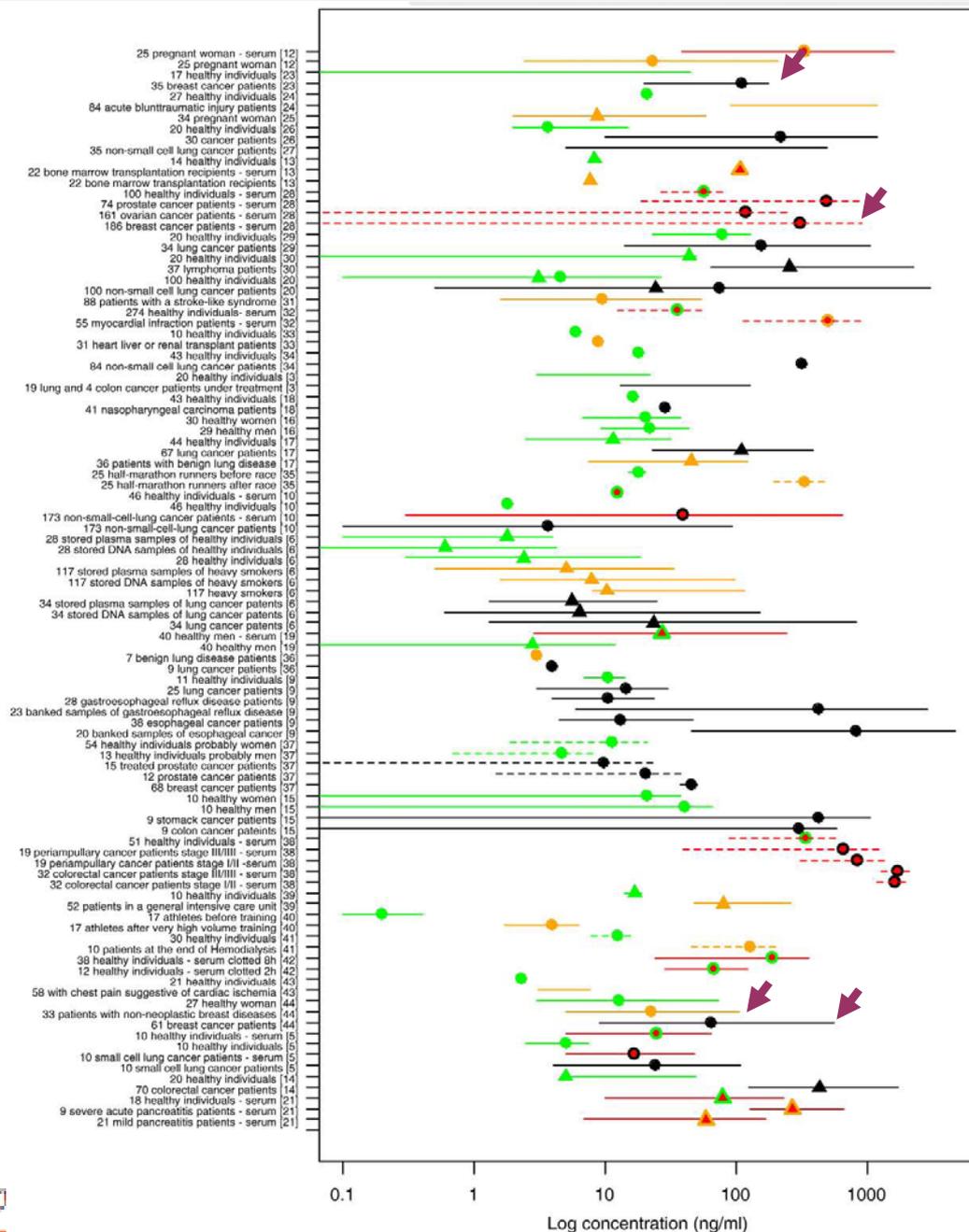
- > Hyperplasia, inflammation, pancreatitis...
- > Limitation as diagnostic tool

● CAN variation as Marker

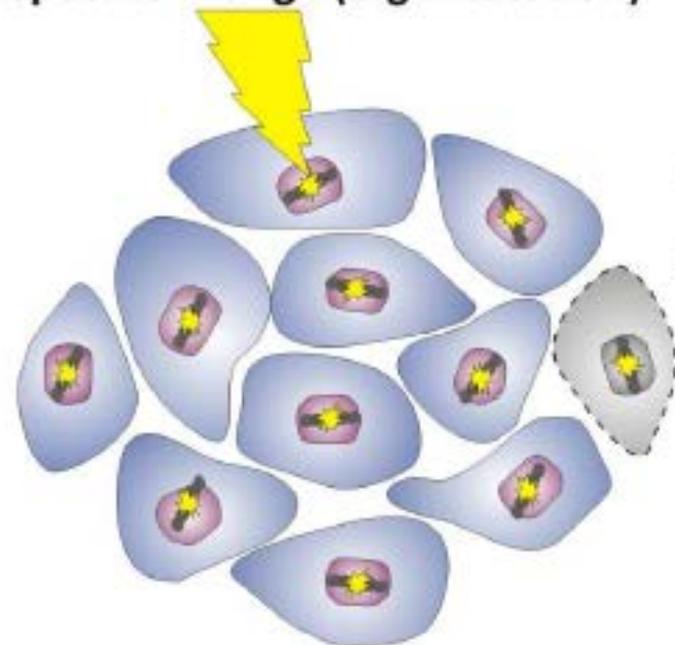
- > Prognosis (OS/DFS)
- > Follow-up, relapse

=>controversial

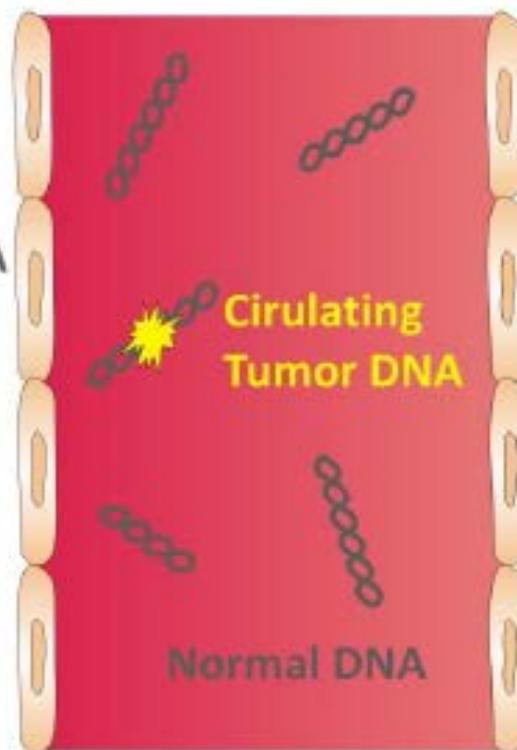
Healthy	—	Minimum
Benign	—	Maximum
Cancer	—	& Mean
serum	—	



Tumor specific change (e.g. Mutation)



**Tumor cell
release DNA**



Tumor

Blood

First evidence

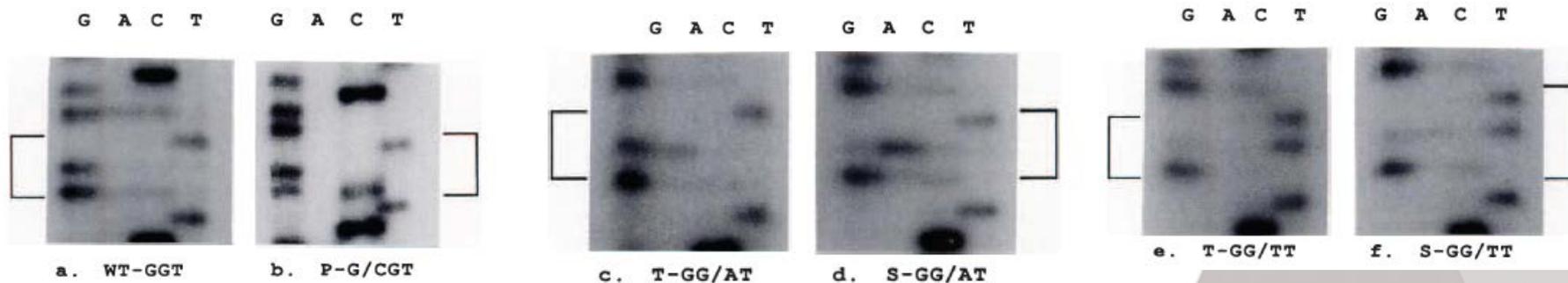
Soluble Normal and Mutated DNA Sequences from Single-Copy Genes in Human Blood¹

Vol. 3, 67-71, January/February 1994 *Cancer Epidemiology, Biomarkers & Prevention*

George D. Sorenson² Donna M. Pribish,
Frank H. Valone, Vincent A. Memoli, David J. Bzik, and
Siu-Long Yao

Table 1 Patients with pancreatic adenocarcinoma

Patient No.	Age	Sex	Plasma DNA mutation ^a (ASA)	Plasma DNA sequence	Tumor DNA sequence
1 (LC696)	74	F	CGT	G/C GT	N/A ^c
2 (HWO49)	69	M	GAT	GG/AT ^d	GG/AT
3 (HP385)	57	M	GTT ^d	GG/TT ^d	GG/TT



3 patients bearing pancreas cancers
ASA PCR and Sequencing

Quelques caractéristiques

- **AcNucl. Libre et stable**

- > Association avec : protéine & lipide, micro-vesicule ...
- > Stable,
- > Adsorption à la surface cellulaire (?)

- **Serum ou plasma ?**

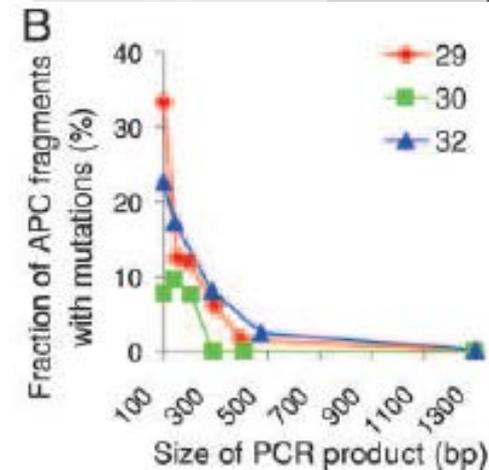
- > Les 2 dans la littérature mais plasma +++
- > « Contamination » in vitro par lyse et coagulation

- **Fragments courts d'ADN**

- > Petit fragment DNA associés à l'apoptose. (<300bp)
- > fragment long plus souvent "wildtype"
- > Extraction adaptée (pour les petits fragments <300bp)

- **élimination**

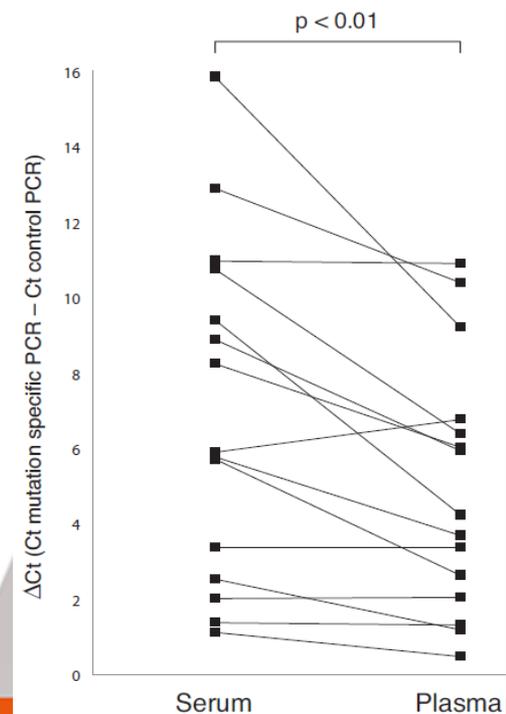
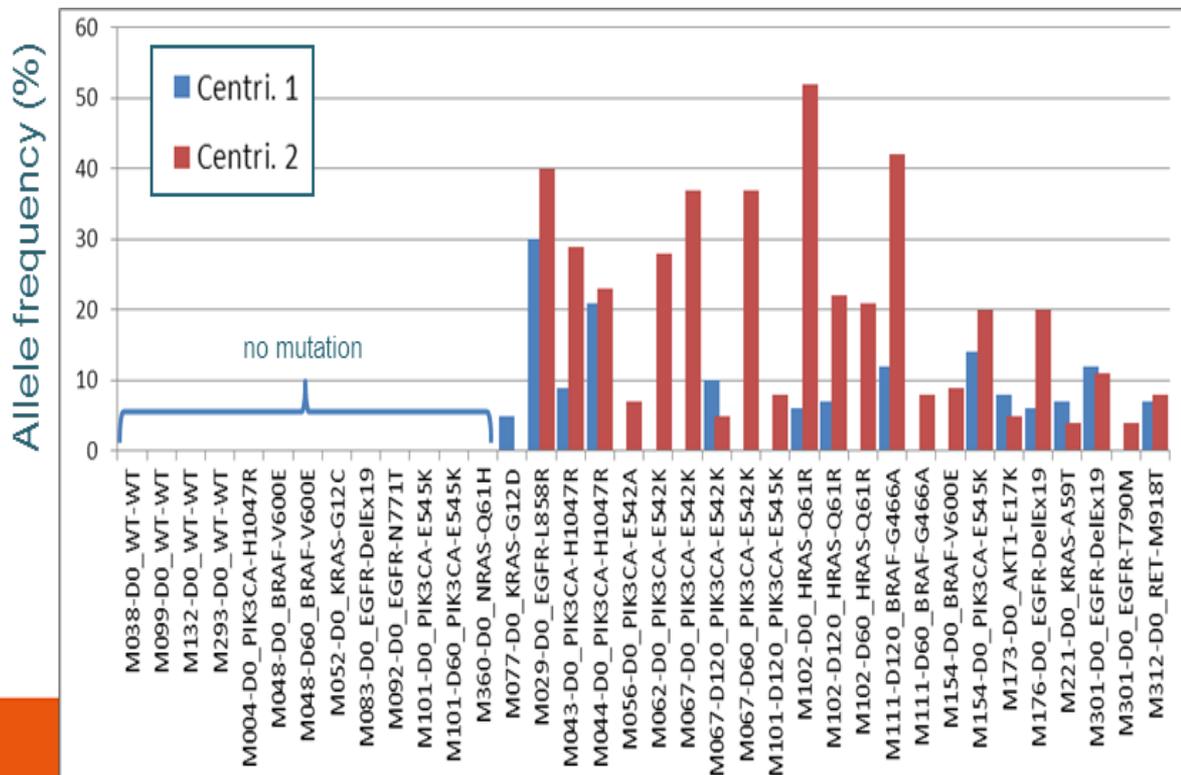
- > digestion, hépatique and urinaire



Diehl F *et al.* PNAS 2005

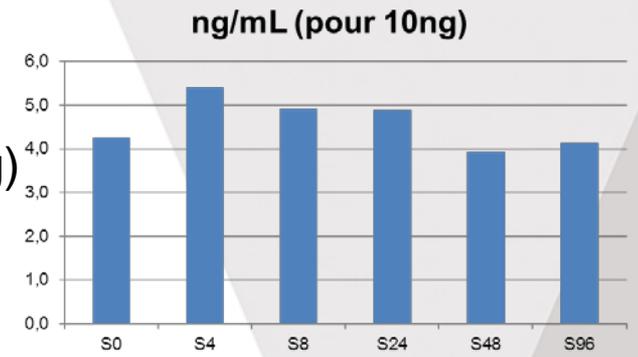
point clé : le pré-analytique.

- 5-10mL plasma-EDTA >>> serum, (proscrire l'héparinate de Li)
(ou tubes spécifiques Anticoag.+ conservateur)
- Collection & centrifugation en 2-4hrs (éviter la lyse *in vitro*)
& congélation à -80°C (ou tubes spécifiques)
=> notion d'échantillon dédié.
- Seconde centrifugation nécessaire (immédiate or avant analyse)

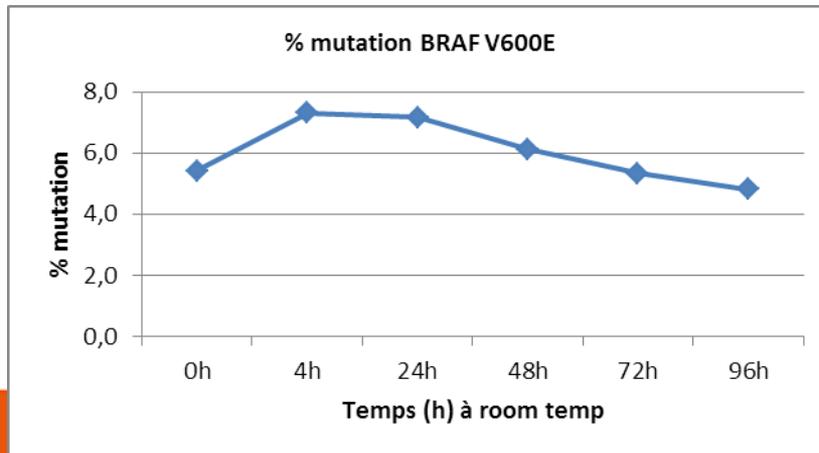
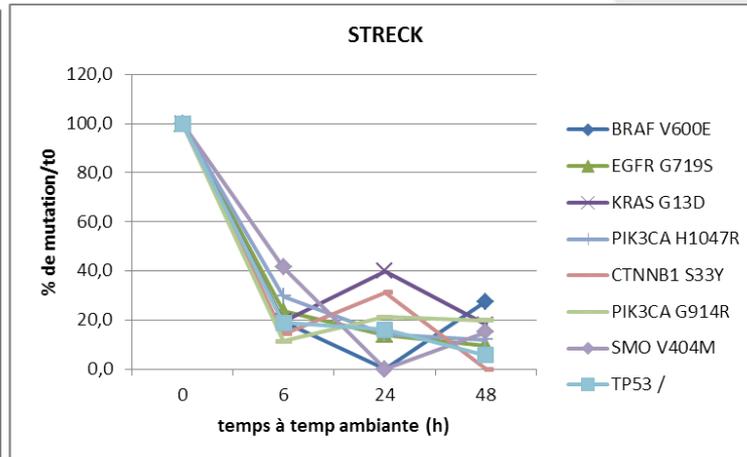
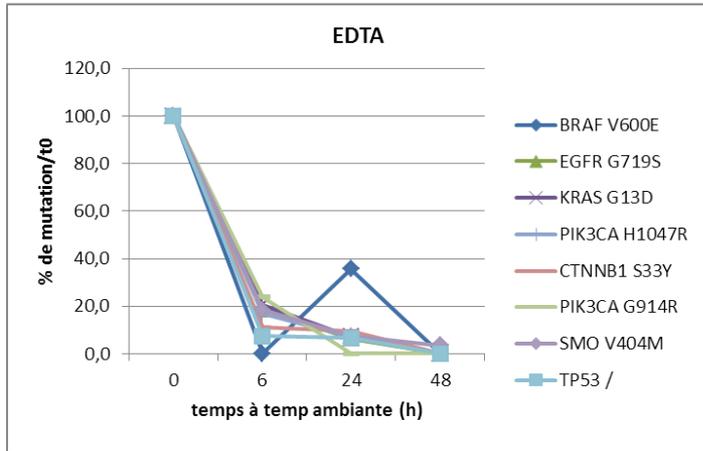


Test de conservation

Quantité totale (ng)



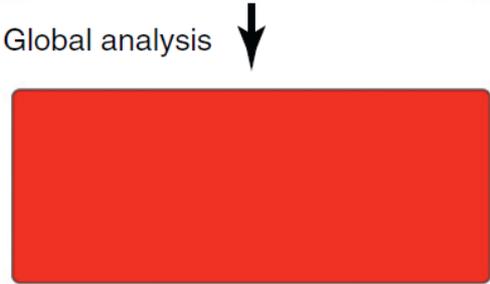
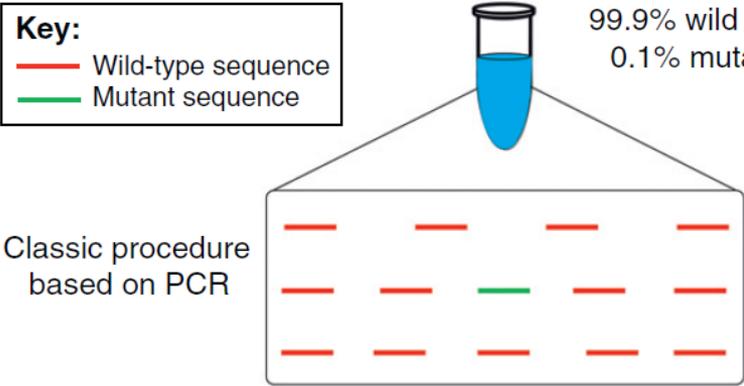
Fréquence allélique des mutations dans le sang total



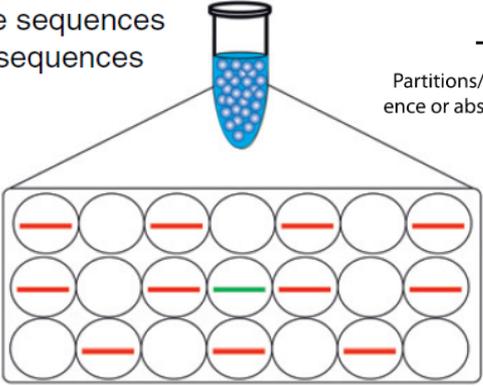
Fréquence allélique des mutations dans le plasma

Principe de la PCR digitale

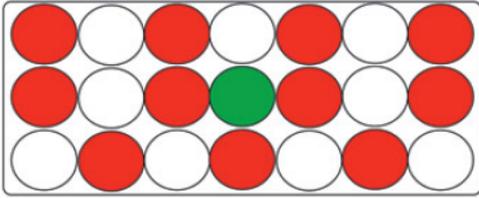
Key:
— Wild-type sequence
— Mutant sequence



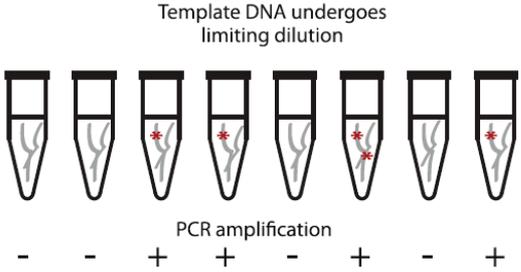
Low or no signal corresponding to mutant sequence



Analysis of each compartment individually



99.9% wild type sequences
0.1% mutant sequences



Partitions/aliquots are scored as positive or negative depending on the presence or absence of one or more copies of the locus of interest in each partition

Digital procedure compartmentalization of individual DNA

- Rare variant detection
- Estimating copy-number variation
- Minimal template requirements

Easy analysis

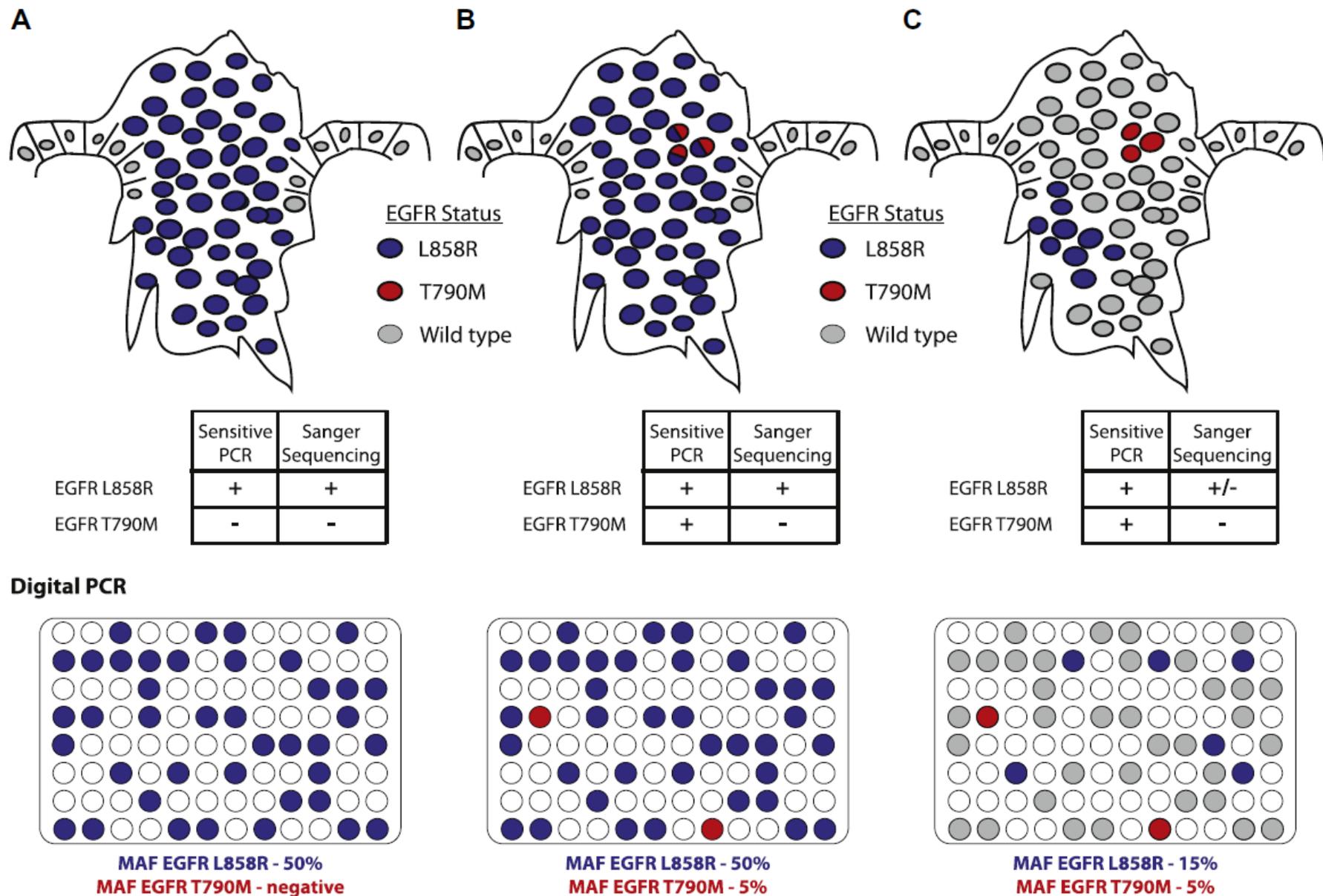
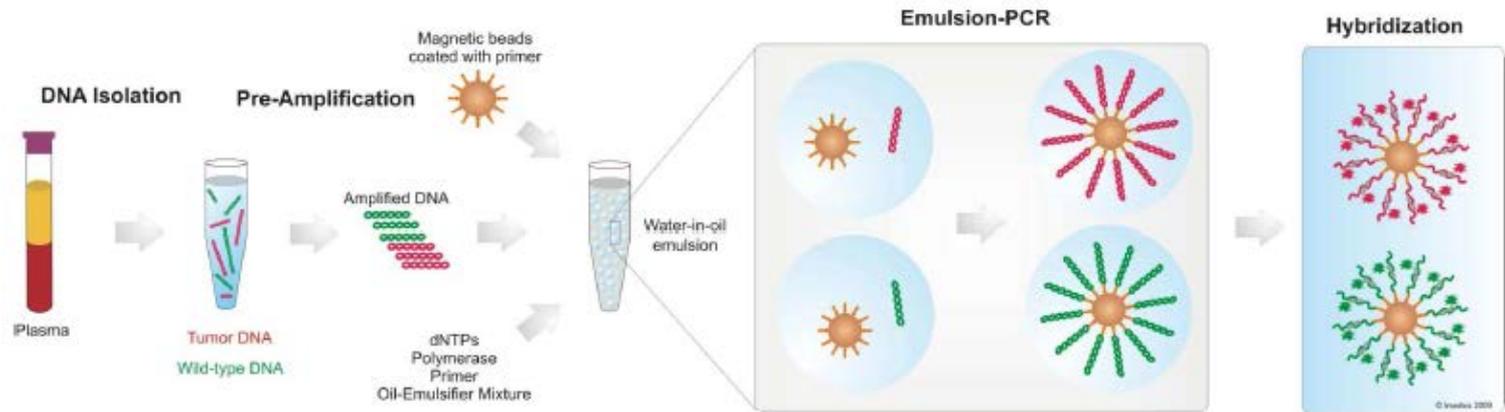


Fig. 2. Digital PCR, mutant allele frequency (MAF) and test sensitivity. The issue of sensitivity and mutant allele frequency in biopsy material may have a significant impact on the interpretation of molecular biomarkers and the delivery of personalized cancer medicine. Current (probe-based, COLD-PCR) report a sensitivity of approximately 1% for mutations. However the mutant allele frequency is not estimated

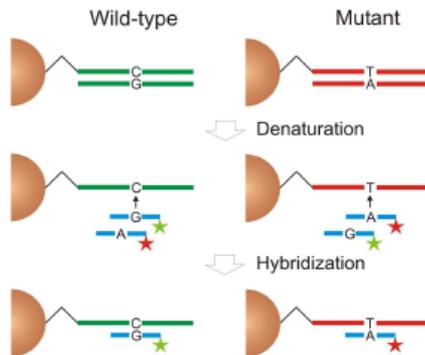
New technologies



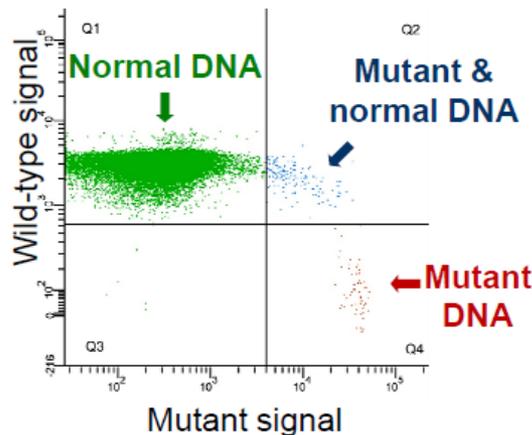
BEAMing (Beads, Emulsions, Amplification, and Magnetics)

Dressman *et al.* PNAS 2003

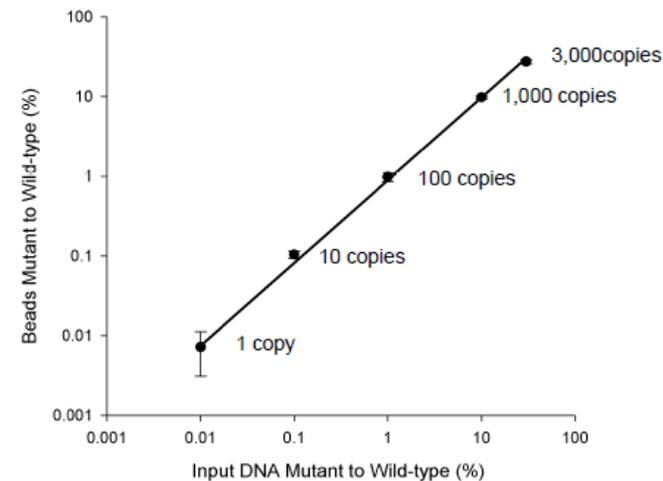
Allele-specific hybridization



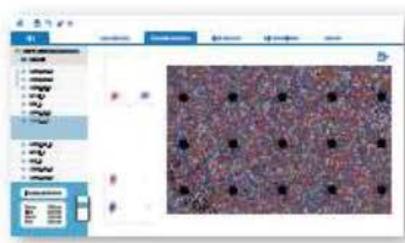
Flow Cytometry Analysis



Mutant genomic DNA spiked into wild-type genomic DNA



Passée

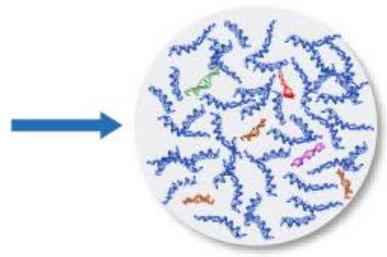


Extract nucleic acids

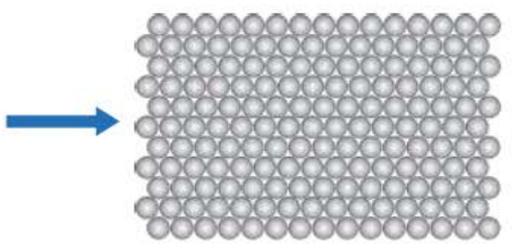
Partition sample into a droplet crystal

Amplify targets by PCR

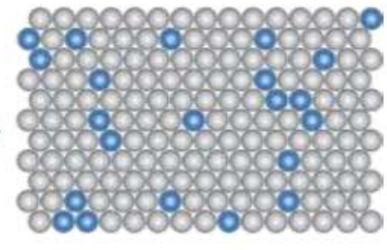
Detect & Quantify by counting positives



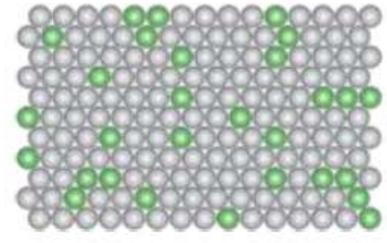
- Wild-type DNA
- GMO DNA 1
- GMO DNA 2
- miRNAs
- etc...



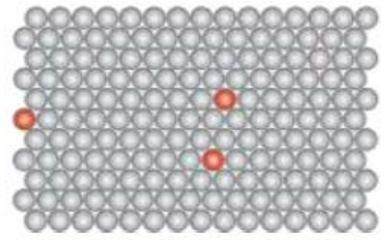
Droplet crystal:
Self-assembled 2D array of droplets



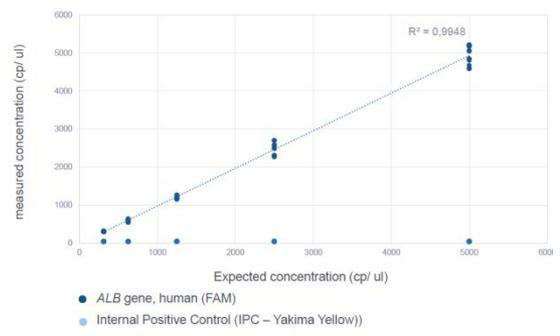
C = 102 cp/μL



C = 152 cp/μL



C = 8 cp/μL

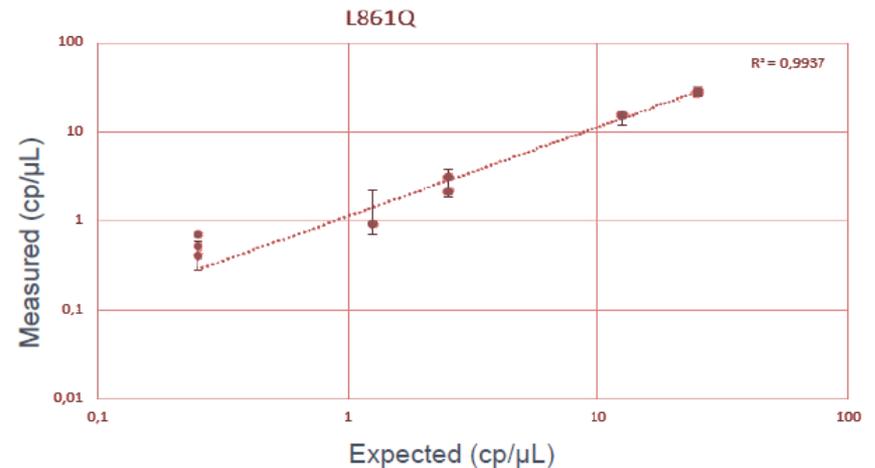
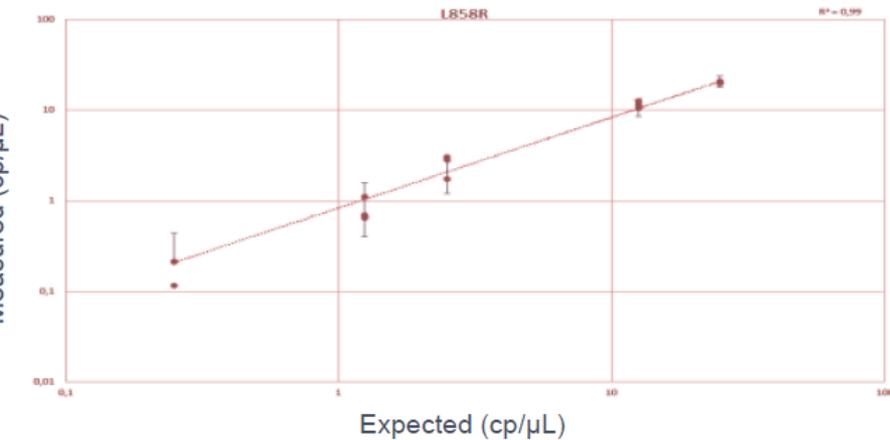
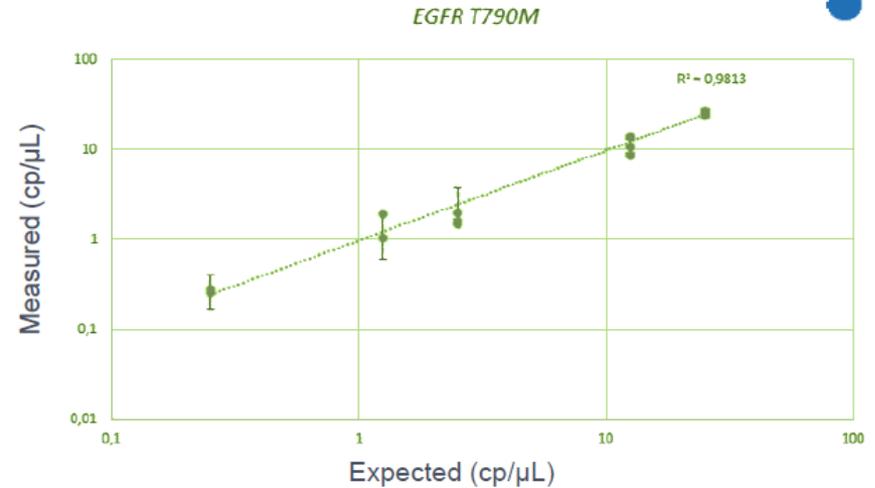


3-color multiplex detection of *EGFR* mutations

Assay: Hydrolysis probes

- EGFR WT** (FAM)
- EGFR T790M** (Cy3)
- EGFR L858R** (Cy5)
- EGFR L861Q** (Cy5)

0-fold dilution series in WT background (500 cp/μL)



Platform	Description	Number of reactions	Aliquot volume	Analysis	Published sensitivity for rare variants	Integrated thermocycling and analysis	Commercial availability	Published applications
<i>Microdroplets</i> ddPCR, BioRad	Microdroplets are generated in an emulsion and transferred to 96 well plates for cycling then to the custom analysis unit (QX100 droplet reader). The reader unpacks the emulsion to single droplets for analysis.	20,000 per 20 µl sample	1 nL	Automated droplet flow cytometer (two colors) with Taqman probes	0.001%	No	Yes QX100 Droplet digital PCR System	Genotyping absolute quantification [9,11]
RainDrop, raindance technologies	Microdroplets, are generated in an emulsion, collected and transferred for thermocycled. The emulsion is then injected onto a microfluidic device and each droplet is analysed	Continuous flow	9 pL	End point analysis with TaqMan probes	0.0005%	No	Yes RainDrop digital PCR system	Genotyping[65] Absolute quantification[63]
BEAMing (beads, emulsion, amplification, magnetics)	Microdroplets containing magnetic beads are generated in an emulsion and transferred to 96 well plates for thermocycling. The emulsion is dispersed and the beads separated. A circularizable probe is hybridized to the sequences on the beads and the changes of interest are labeled with fluorescently labeled dideoxynucleotide terminators	5×10^7 beads	9 µm diameter	Labeled beads are analyzed by flow cytometry	0.01%	No	No	Genotyping Absolute quantification [13]
<i>Microfluidic chambers</i> MegaPixel digital PCR	Surface tension based sample partitioning creates aliquots that are thermocycled and analyzed on the device. Fluorescent probes are annealing during thermocycling to enable analysis	1×10^6	10 pL	Microarray scanner	0.001%	Yes	No	Genotyping [20]
Spinning disk platform	Aliquots are generated by passive compartmentalization through centrifugation. These are thermocycled and analysed on the device	1,000	33 nL	CCD camera – end point melting curve analysis	–	Yes	No	Copy number variation and absolute quantification [61]
OpenArray Life technologies/ ABI	Microfluidic reaction chambers are loaded, thermocycled and analysed using the OpenArray system. Chambers may be preloaded with the assay of choice	3,072	33 nL	CCD camera – real time PCR end point melting curve analysis	–	Yes	Yes OpenArray Real-Time PCR platform	
Digital array chip, fluidigm	Microfluidic reaction chambers are loaded, thermocycled and analysed using the BioMark system	9,180 (12 × 765) Prototype 2 × 100,893	6 nL	CCD camera – real time PCR end point melting curve analysis		Yes	Yes BioMark HD system	microRNA expression [53] Single cell gene expression [66] Genotyping [35] Targeted resequencing [67] Copy number variation[19,21,60] Absolute quantification [25]



exploration cfDNA–Techniques

Method of analysis	Description	Limit of detection (%) [†]	Ref.
HRMA	PCR-based method that provides a specific sequence-related pattern, allowing a sensitive identification of mutations. Further sequencing is needed to identify the specific mutation	0.1–10	[64]
ARMS	Mutations are identified through the use of probes matching specific target sequences. ARMS technology is a sensitive and rapid method, although do not allow the detection of unknown mutations	0.5–1	[55]
PNA-clamp	PNA-clamp technology consists in the selective amplification of mutant alleles through the use of specific PNAs that suppress wild-type sequence. Mutations must be known in advance	0.01	[65]
Digital PCR	Digital PCR allows the detection and quantification of small amounts of mutant DNA, by partitioning a DNA sample into many single and parallel PCRs. Mutations must be known in advance	0.005–0.01	[62]
NGS	NGS provides the detection and quantification of known and unknown mutations in different genes. It is a sensitive method and allows samples multiplexing, but it requires good expertise	1–5	[62]
DHPLC	Sensitive method to detect mutations based on the differential retention of homo and heteroduplex DNA partially denaturated. DHPLC only gives information of presence/absence of mutations	1–5	[25]
Mutant-enriched PCR	Not expensive PCR-based assay for mutation detection through digestion of PCR products, that are then amplified again and sequenced. Only specific alterations can be analyzed	0.2	[66]

[†]Lower fraction of mutant DNA detected in a background of wild-type DNA.
ARMS: Amplified refractory mutation system; DHPLC: Denaturing HPLC; HRMA: High-resolution melting analysis; NGS: Next-generation sequencing; PNA-clamp: Peptide nucleic acids clamp.

Fenzia et al. Fut.Onco. 2015

Importance de la sensibilité analytique Mais pas uniquement

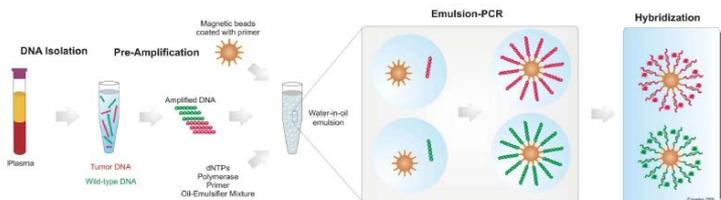
Concentration Cf DNA moyenne 1-100ng/mL de plasma dans les maladies progressives

=> soit soit 330 à 33 000 copies d'ADN

Question de la sensibilité mais aussi du volume de plasma

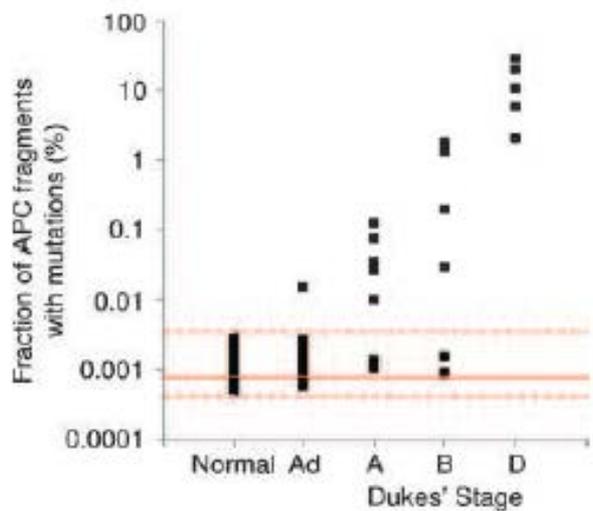
Recommandation GFCO => technique sensible à minimum 2%

et sensibilité précisée sur le CR d'analyse/considéré dans la conclusion.

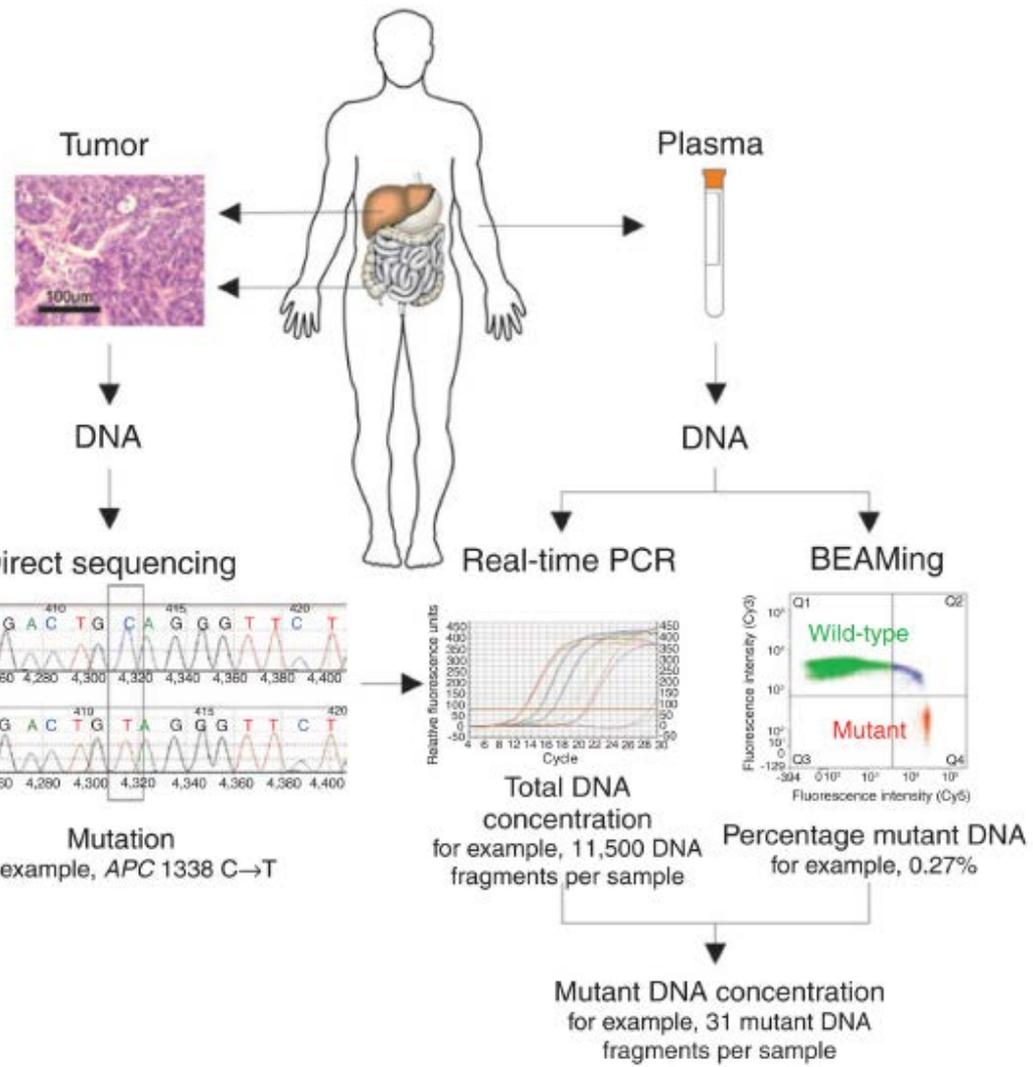


BEAMing (Beads, Emulsions, Amplification, and Magnetics)

Dressman *et al.* PNAS 2003



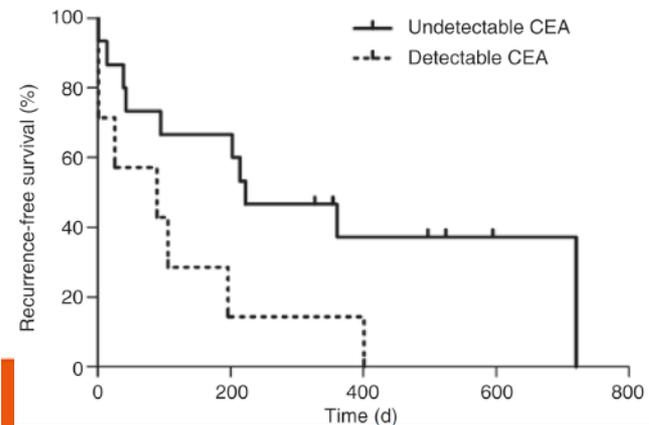
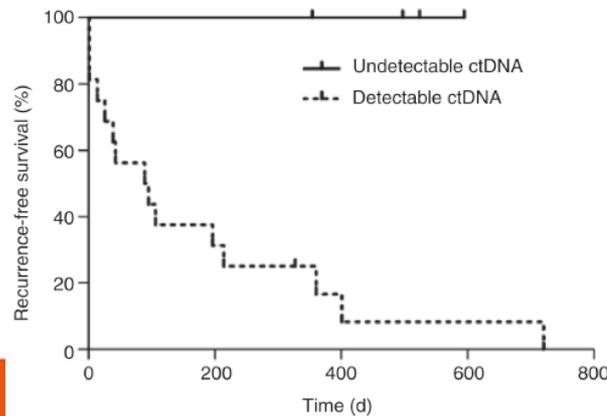
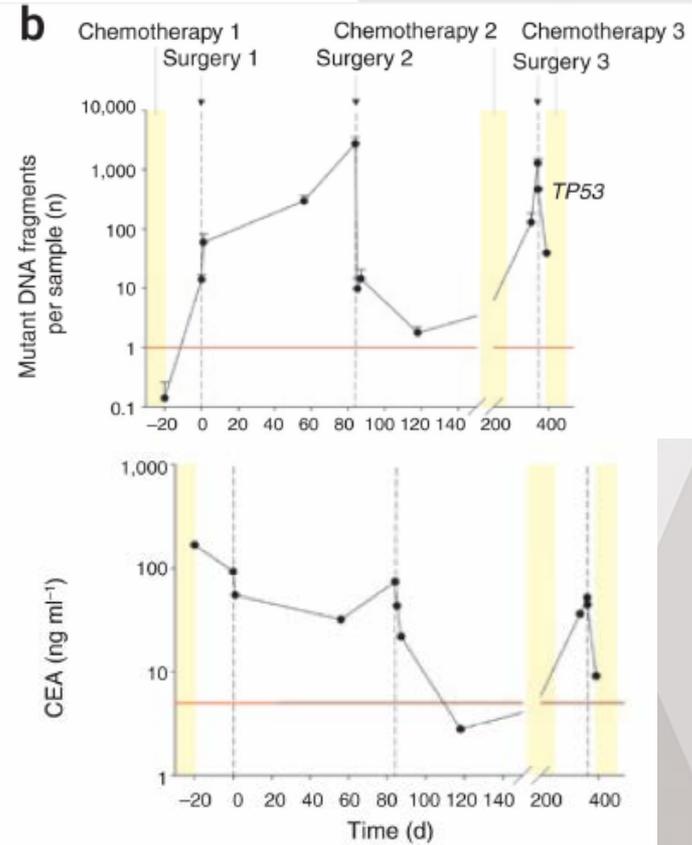
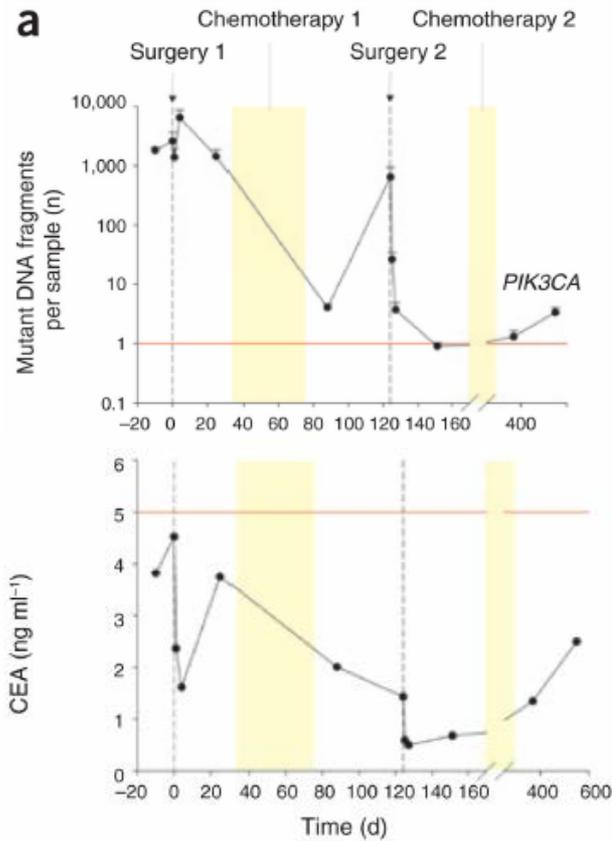
Diehl F *et al.* PNAS 2005



Diehl F *et al.* Nat Res 2008

cfDNA pour le suivi dans le cancer du colon

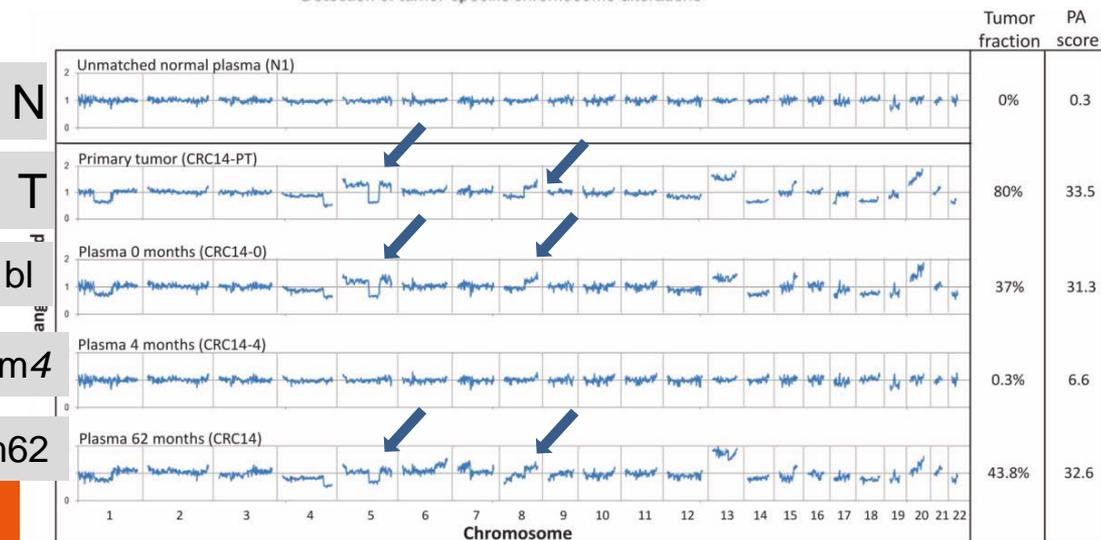
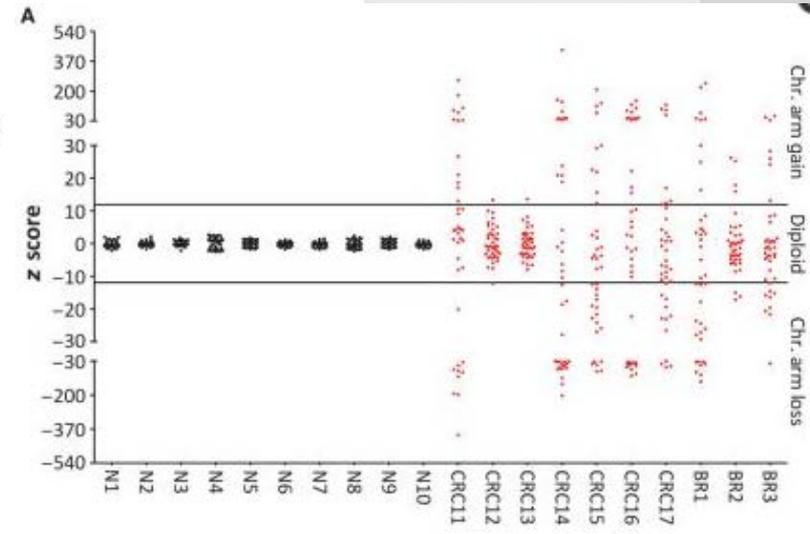
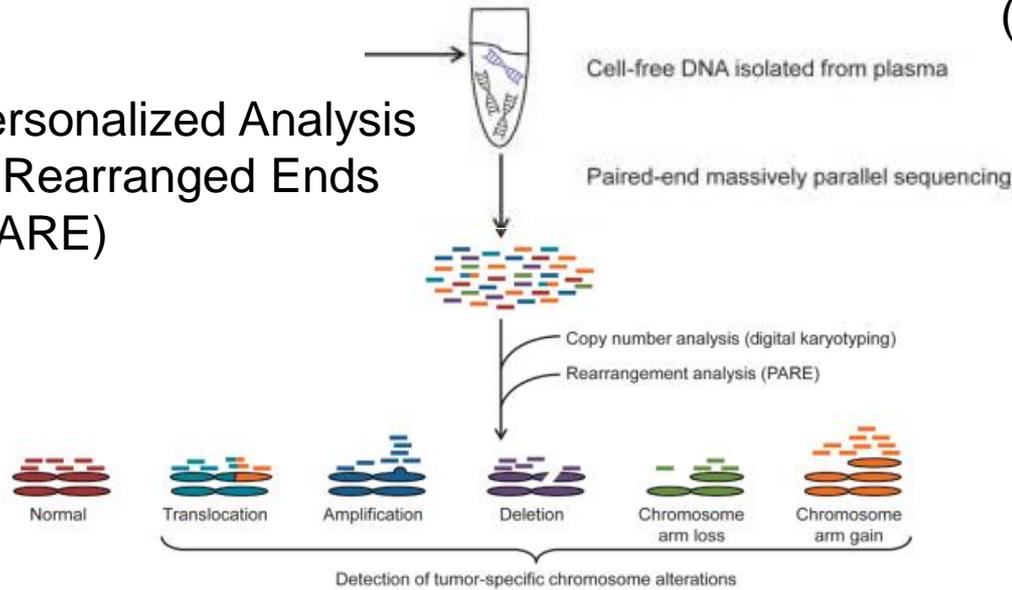
18 cas
162 plasma



cfDNA application– WES/Chr alterations

Compare WGS for advanced cancer & healthy patient
(4 to 18mL plasma)

Personalized Analysis
of Rearranged Ends
(PARE)



Demonstration of the feasibility
of direct detection of
chromosomal alterations
in the plasma of cancer patients

cfDNA for tumor follow-up

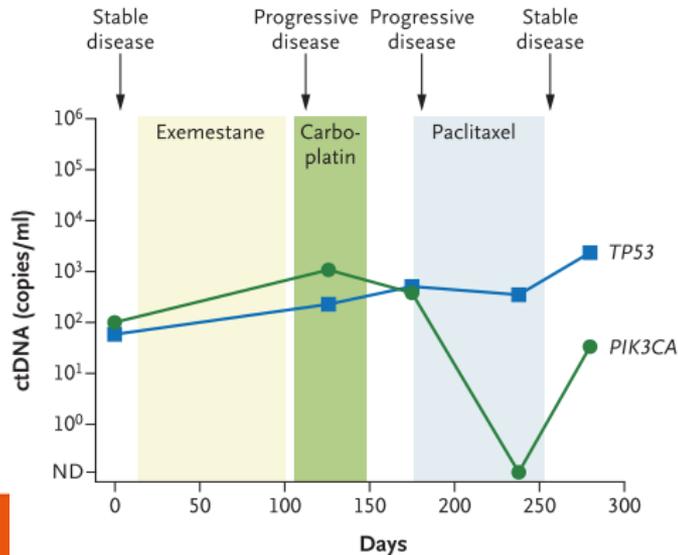
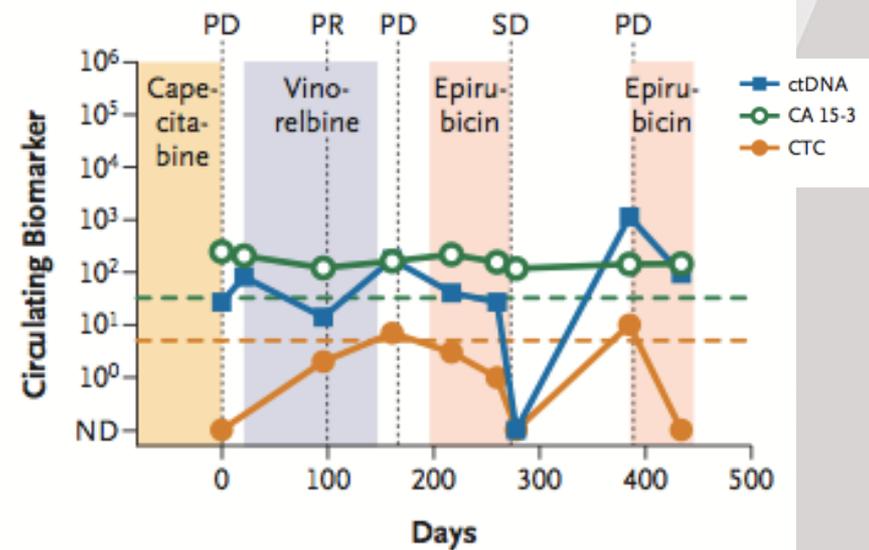
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

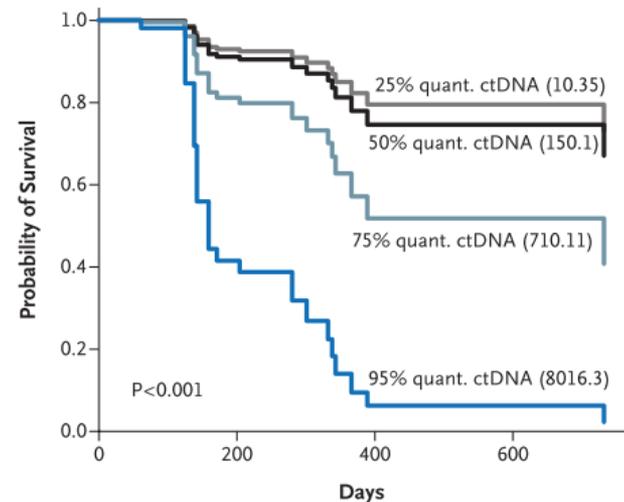
Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer

Sarah-Jane Dawson, F.R.A.C.P., Ph.D., Dana W.Y. Tsui, Ph.D.,
 Muhammed Murtaza, M.B., B.S., Heather Biggs, M.A.,
 Oscar M. Rueda, Ph.D., Suet-Feung Chin, Ph.D., Mark J. Dunning, Ph.D.,
 Davina Gale, B.Sc., Tim Forshew, Ph.D., Betania Mahler-Araujo, M.D.,
 Sabrina Rajan, M.D., Sean Humphray, B.Sc., Jennifer Becq, Ph.D.,
 David Halsall, M.R.C.Path., Ph.D., Matthew Wallis, M.B., Ch.B.,
 David Bentley, D.Phil., Carlos Caldas, M.D., F.Med.Sci.,
 and Nitzan Rosenfeld, Ph.D.

30 mutated MBC, 126 blood Samples

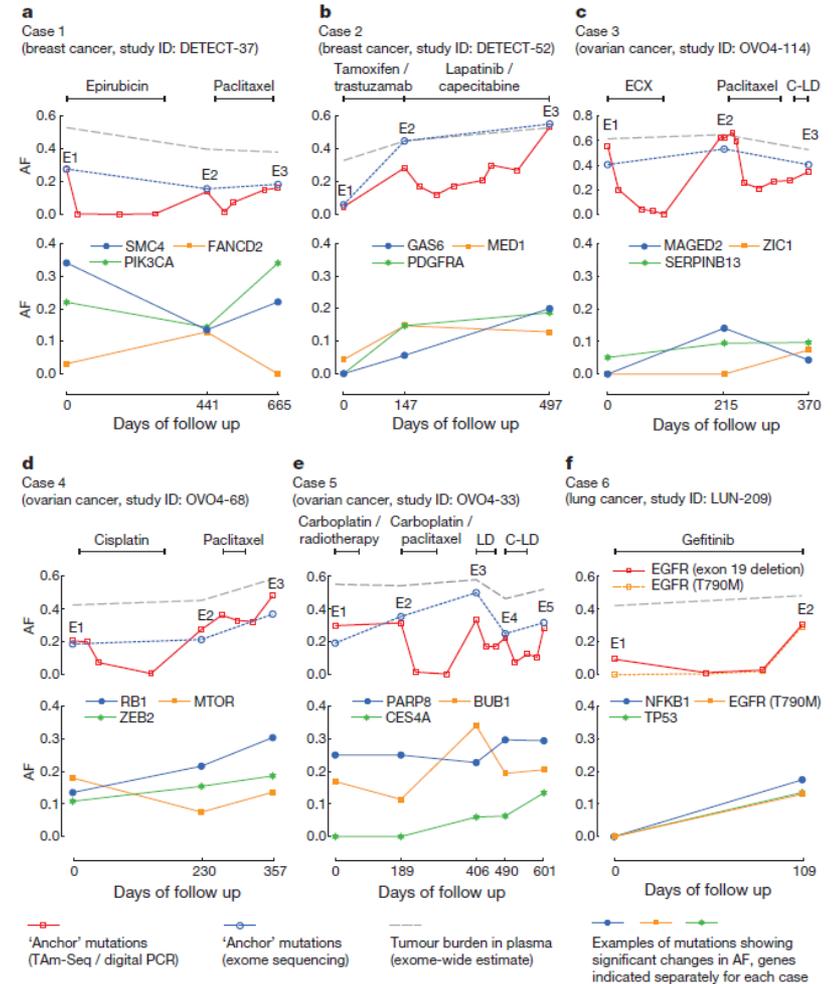
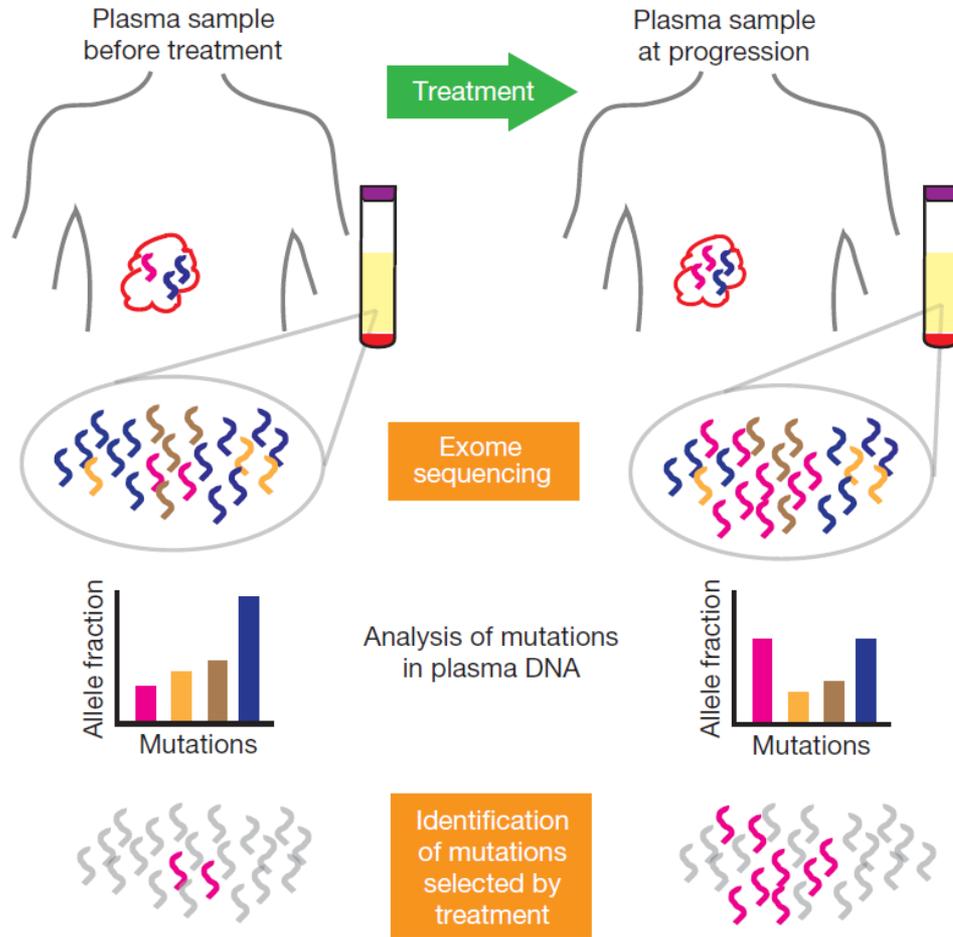


E Quantiles of ctDNA and Overall Survival



Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA

Muhammed Murtaza^{1*}, Sarah-Jane Dawson^{1,2*}, Dana W. Y. Tsui^{1*}, Davina Gale¹, Tim Forshew¹, Anna M. Piskorz¹, Christine Parkinson^{1,2}, Suet-Feung Chin¹, Zoya Kingsbury³, Alvin S. C. Wong⁴, Francesco Marassi¹, Sean Humphray³, James Hadfield¹, David Bentley³, Tan Min Chin^{4,5}, James D. Brenton^{1,2,6}, Carlos Caldas^{1,2,6} & Nitzan Rosenfeld¹



Application Clinique – Cancer Bronchique

Epidermal Growth Factor Receptor Mutation Status in Circulating Free DNA in Serum

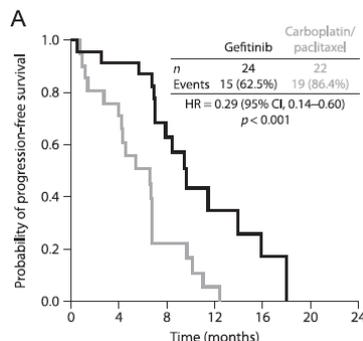
From IPASS, a Phase III Study of Gefitinib or Carboplatin/Paclitaxel in Non-small Cell Lung Cancer

Journal of Thoracic Oncology • Volume 7, Number 1, January 2012

Koichi Goto, MD, PhD,* Yukito Ichinose, MD,† Yuichiro Ohe, MD, PhD,‡

Sensitivity = **43.1%** (22 cfDNA M+ out of 51 tumor M+).^b

Specificity = **100%** (all 35 tumor M- were cfDNA M-).^b



cfDNA EGFR mutation-positive
DxS EGFR Mutation Test Kit

2mL of serum

Original Investigation

Association of EGFR L858R Mutation in Circulating Free DNA With Survival in the EURTAC Trial

1,2mL of serum & plasma

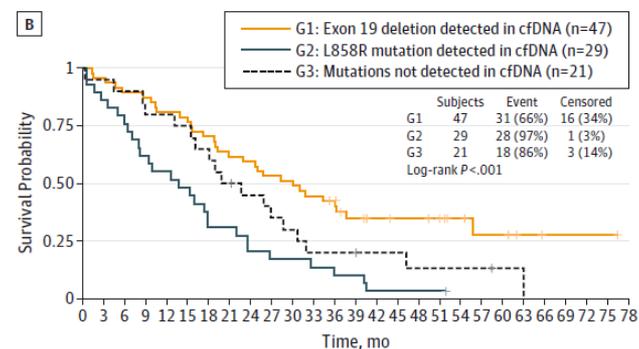
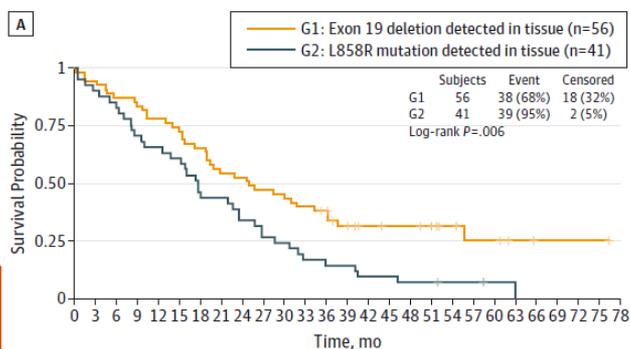
Niki Karachaliou, MD; Clara Mayo-de las Casas, PhD; Cristina Queral, PhD; Itziar de Aguirre, PhD;

JAMA Oncol. 2015;1(2):149-157.

PNA clamp

Sensitivity = **78%** (76/97)

Figure 2. Overall Survival According to Epidermal Growth Factor (EGFR) Mutation Status in Tissue and in Circulating Free DNA (cfDNA)



Application Clinique – Cancer Bronchique

21(14) July 15, 2015

Clinical
Cancer
Research

Detection and Dynamic Changes of *EGFR* Mutations from Circulating Tumor DNA as a Predictor of Survival Outcomes in NSCLC Patients Treated with First-line Intercalated Erlotinib and Chemotherapy

FASTACT-2

Tony Mok¹, Yi-Long Wu², Jin Soo Lee³, Chong-Jen Yu⁴, Virote Sriuranpong⁵,

2 mL of blood
cobas 4800 blood test

Sensitivity = 75% (72/96)
Specificity = 96% (137/142)

Highly Sensitive Droplet Digital PCR Method for Detection of *EGFR*-Activating Mutations in Plasma Cell-Free DNA from Patients with Advanced Non-Small Cell Lung Cancer



Guanshan Zhu,* Xin Ye,* Zhengwei Dong,* Ya Chao Lu,* Yun Sun,* Yi Liu,† Rose McCormack,† Yi Gu,* and Xiaoqing Liu†

The Journal of Molecular Diagnostics, Vol. 17, No. 3, May 2015

EGFR E19-Del

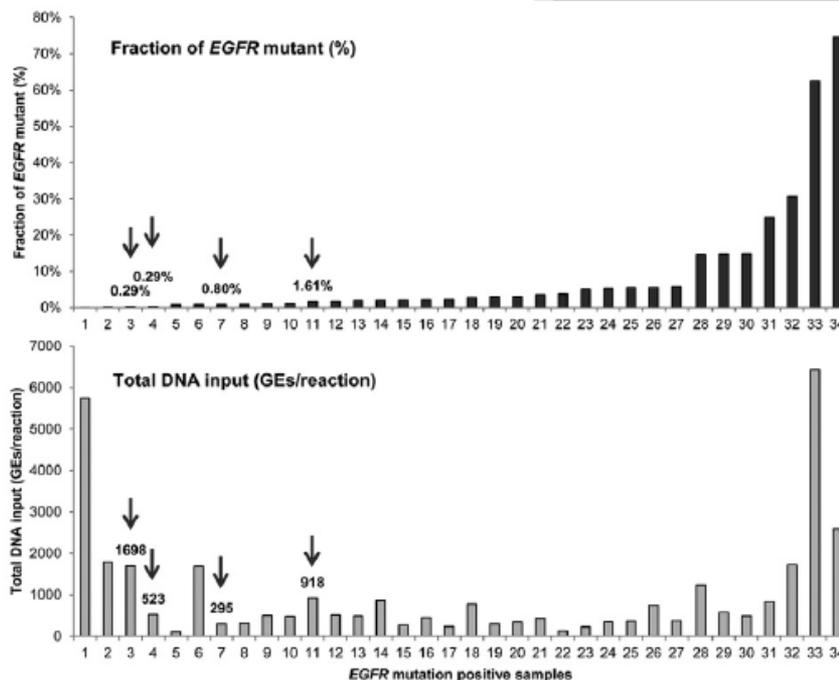
sensitivity of 81.82% (95% CI, 59.72%–94.81%)

specificity of 98.44% (95% CI, 91.60%–99.96%)

EGFR L858R

sensitivity of 80.00% (95% CI, 51.91%–95.67%)

specificity of 95.77% (95% CI, 88.14%–99.12%)



Résultats des études cfDNA sur cohortes cliniques CBNPC

Etude	Echant.	Effectif	Méthode	Sens	Spe	ref
IPASS	2mL serum	96	DxS EGFR	43%	100 %	Goto JTO 2012
EURTAC	1,2 mL Serum ou plasma	97	PNA Clamp	78%	N.A	Karachalio u JAMA 2015
FASTAC-2	2mL Plasma	238	Cobas	75%	96%	Mok CCR 2015
BioCAST	plasma	107 (68)	NGS Panel	58%	87%	Couraud CCR 2014
N.A	plasma	34	dPCR	82% _{del} 80% _{L858R}	98% 96%	Zhu JMD 2015

First author	Country	Year	Detection methods	Female (%)	Adenocarcinoma (%)	Ever smokers (%)	No. of samples	Sensitivity (%)	Specificity (%)
Kimura H (37)	Japan	2006	ARMS	37.3	85.2	NA	11	75	40
Kimura H (38)	Japan	2007	ARMS	33.3	73.8	66.7	42	75	97
He C (46)	China	2009	ME-PCR	36.6	75.4	53	18	89	100
Yung TK (26)	China	2009	Digital PCR	NA	NA	NA	29	100	94
Kuang Y (47)	USA	2009	ARMS	81.5	NA	NA	43	70	85
Bai H (48)	China	2009	DHPLC	46.5	74.3	44.8	230	97	92
Sriram KB (49)	Australia	2011	ME-PCR	33.9	56.3	93.7	64	50	100
Jiang B (50)	China	2011	ME sequencing	31	72.4	62.1	58	78	100
Taniguchi K (51)	Japan	2011	BEAMing	65.9	95.5	NA	44	73	0
Brevet M (52)	USA	2011	Sequenom	51.6	96.8	54.8	31	44	85
Goto K (33)	Japan	2012	AS-APEX	87.6	NA	9	86	43	100
Nakamura T (53)	Japan	2012	I-PCR-QPM	51.3	100	46.2	70	45	100
Hu C (54)	China	2012	HRM	50	58.3	45.8	24	100	0
Huang Z (55)	China	2012	DHPLC	46.7	78	41.4	822	64	85
Xu F (56)	China	2012	ARMS	39.2	84.3	NA	34	50	100
Yam I (57)	China	2012	AS-APEX	60	94.3	14.3	35	100	80
Jing CW (58)	China	2014	HRM	42.5	58.3	NA	120	64	97
Liu X (59)	China	2013	ARMS	34.9	98.8	54.7	86	68	100
Lv C (60)	China	2013	DHPLC	54.5	NA	45.5	6	0	100
Zhang H (61)	China	2013	MEL	43	75.6	51.2	86	68	100
Kim ST (62)	Korea	2013	PNA-LNA PCR clamp	38.6	70.2	56.1	57	66	93
Zhao X (39)	China	2013	ME-PCR	31.5	65.8	51.4	111	35	98
Kim HR (63)	Korea	2013	PNAClamp	NA	NA	NA	40	17	100
Li X (plasma) (64)	China	2014	ARMS	42.5	78	46.8	141	48	95
Li X (serum) (64)	China	2014	ARMS	44	79.6	43.5	108	40	96
Weber B (41)	Denmark	2014	Cobas EGFR blood test	49	95	91	196	61	96
Douillard JY (42)	Europe	2014	ARMS	NA	NA	NA	652	66	99
Wang S (65)	China	2014	ARMS	48.5	80.6	46.3	74	22	97

● Meta Analyse

- > 1-Luo et al. 2014 _ 20 études Asie (+1US)
 - **Sens 0,674** (0,517-0,800) ; **Spé 0,935** (0,888-0,963)
- > 2-Qiu et al. 2015 _ 20 études id + 7 dt caucasians pop.
 - **Sens 0,620** (0,513-0,716) ; **Spé 0,959** (0,929-0,977)

● 3- recent data (real life setting) European Lung Cancer Conference 2015

Parameter	ASSESS trial				IGNITE trial			
	Overall (n=1,162)		Same method (n=254)		Asian pacific patients (n=1,687)		Russian patients (n=894)	
	n/N (%)	95% CI	n/N (%)	95% CI	n/N (%)	95% CI	n/N (%)	95% CI
Concordance	1,035/1,162 (89.1)	<u>87.1-90.8</u>	221/254 (87.0)	<u>82.2-90.9</u>	1,310/1,687 (77.7)	<u>75.6-79.6</u>	767/894 (85.8)	<u>83.3-88.0</u>
Sensitivity	87/189 (46.0)	<u>38.8-53.4</u>	25/56 (44.6)	<u>31.3-58.5</u>	343/692 (49.6)	<u>45.8-53.4</u>	33/109 (30.3)	<u>21.8-39.8</u>
Specificity	948/973 (97.4)	<u>96.2-98.3</u>	196/198 (99.0)	<u>96.4-99.9</u>	967/995 (97.2)	<u>96.0-98.1</u>	734/785 (93.5)	<u>91.5-95.1</u>
PPV	87/112 (77.7)	68.8-85.0	25/27 (92.6)	75.7-99.1	343/371 (92.5)	89.3-94.9	33/84 (39.3)	28.8-50.5
NPV	948/1,050 (90.3)	88.3-92.0	196/227 (86.3)	81.2-90.5	967/1,316 (73.5)	71.0-75.8	734/810 (90.6)	88.4-92.5

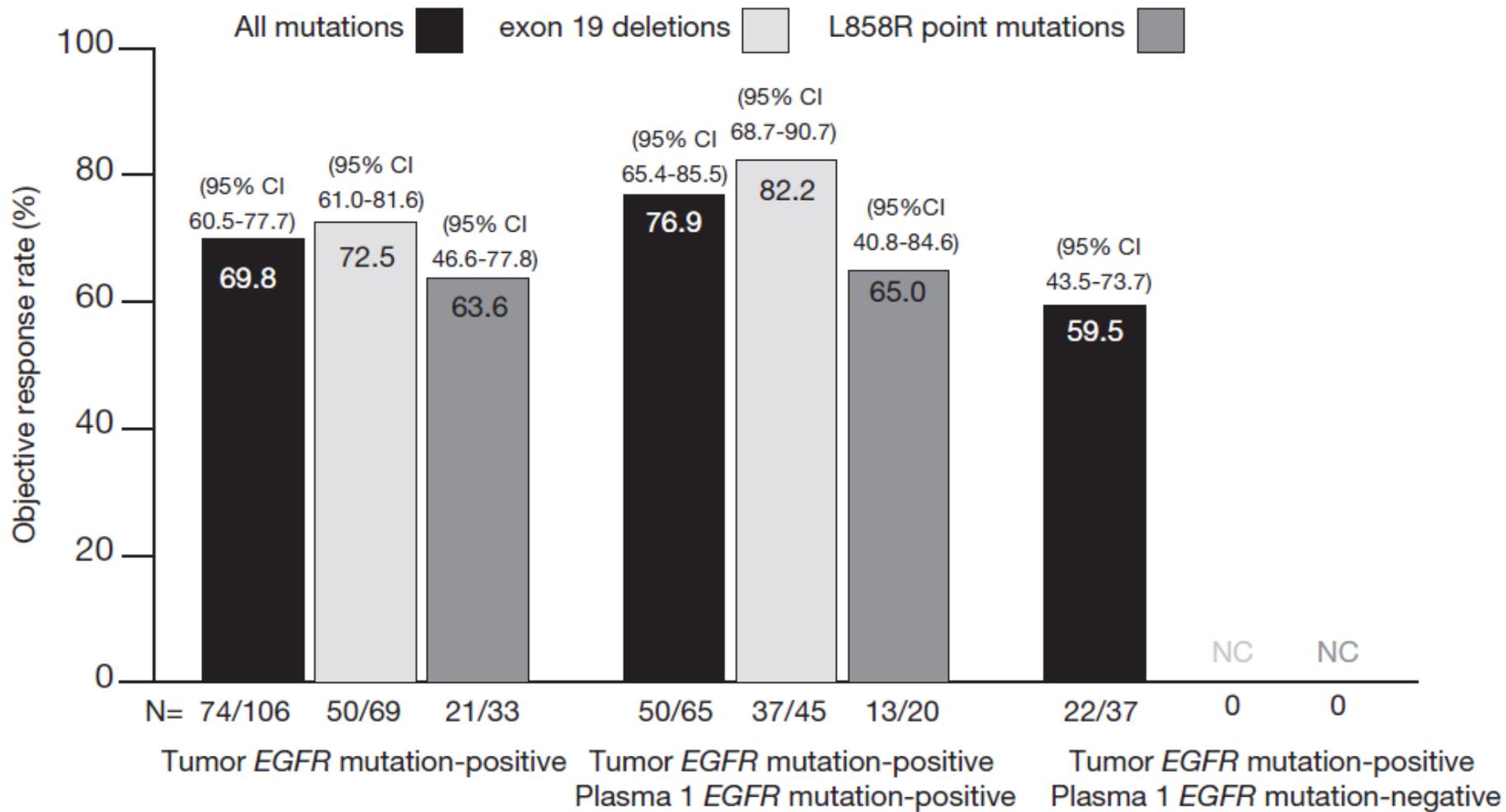
n, numerator value for each parameter; N, denominator value for each parameter; PPV, positive predictive value; NPV, negative predictive value.

Gefitinib Treatment in EGFR Mutated Caucasian NSCLC

Circulating-Free Tumor DNA as a Surrogate for Determination of EGFR Status

(*J Thorac Oncol.* 2014;9: 1345–1353)

Jean-Yves Douillard, MD, PhD,* Gyula Ostoros, MD,† Manuel Cobo, MD,‡ Tudor Ciuleanu, MD,§
 Rebecca Cole, PhD, || Gael McWalter, MSc, || Jill Walker, PhD, || Simon Dearden, MSc, ||
 Alan Webster, MSc, || Tsveta Milenkova, MD, || and Rose McCormack, PhD ||



26/09/2014 le médicament (CHMP) a validé une variation de l'autorisation de mise sur le marché (AMM) d'Iressa (géfitinib).

« Lorsque l'utilisation d'IRESSA est envisagée en tant **que traitement du CBNPC** localement avancé ou métastatique, il est important que la mutation de l'EGFR à partir du tissu tumoral soit recherchée pour tous les patients. **Si un échantillon de la tumeur n'est pas analysable, de l'ADN tumoral circulant (ADNct) obtenu à partir d'un échantillon de sang (plasma) peut alors être utilisé.**

Seul(s) **un/des test(s) robuste(s), fiable(s) et sensible(s)**, ayant démontré son/leur habilité à déterminer le statut de la mutation de l'EGFR au sein la tumeur ou de l'ADNct, doivent être utilisés pour éviter les déterminations de faux négatifs ou de faux positifs ».

17/02/2016 autorisation de mise sur le marché (AMM) de Tagrisso (osimertinib).

« TAGRISSO est indiqué dans le traitement des patients adultes atteints d'un cancer bronchique non à petites cellules (CBNPC) localement avancé ou métastatique, avec mutation EGFR T790M (récepteur du facteur de croissance épidermique). »

idem Xegafri (rociletinib) 3/5/2016

● **Problématique**

- > Standardisation des méthodes pré-analytique
- > Standardisation des méthodes de détection
- > Complémentaire – analyse de recourt attractive
- > Attention à ne pas minimiser l'apport de l'approche tissulaire

Recommandations du GFCO pour l'utilisation diagnostique des analyses génétiques somatiques sur l'ADN tumoral circulant.

Antoinette Lemoine, Sébastien Couraud, Frédéric Fina, Sylvie Lantuejoul, Pierre-Jean Lamy, Marc Denis, Étienne Rouleau

- **Importance du préanalytique :**

Ech. Dédié/tube spécifique, 2-4mL plasma, délais d'acheminement, double centrifugation, conservation -80°C, sensibilité de la technique...

- **Recommandation sur les méthodes :** La technique choisie doit être suffisamment sensible : minimul 2% mieux si <1%. La quantité d'ADN initial doit être correctement évaluée, soit sur qPCR soit sur dPCR.

- **-Pour la recherche de mutation activatrice de sensibilité: La biopsie reste la règle.** L'ADN tumoral circulant doit rester une **solution par défaut**, en l'absence d'ADN tumoral disponible, du mois en routine. Indication : recherche la mutation activatrice de l'EGFR dans l'ADN tumoral circulant.

- **Pour la recherche de T790M, l'approche plasma en 1ere intention** mais doit être compléter par un approche tissulaire si négatif et possible

- Le suivi et le dépistage restent du domaine de la recherche.

- **Toute technique confondue (en moyenne) sensibilité = 70% ; spécificité = 92%.** L'absence de résultat positif ne signifie pas que le patient ne présente « aucune anomalie ». Les faux-négatifs devraient également être ré-interprétés selon l'histoire du patient

Recommandations du GFCO
A.Lemoine et al. 2016

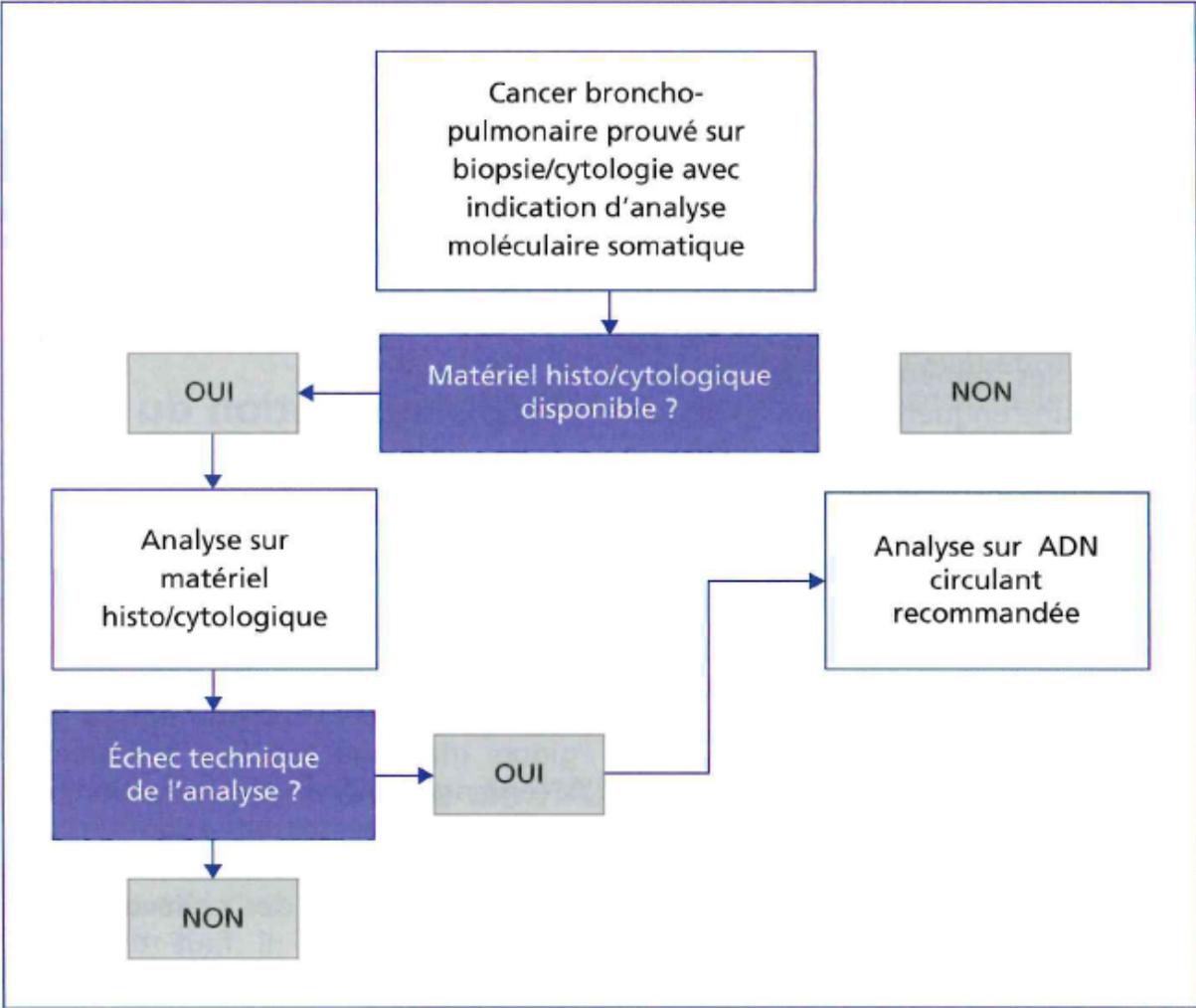


Figure 1. Indication de l'analyse moléculaire sur ADN circulant en situation de diagnostic moléculaire initial.
Figure 1. Indication for molecular analysis of circulating DNA during initial molecular diagnosis.

Recommandations du GFCO
A.Lemoine et al. 2016

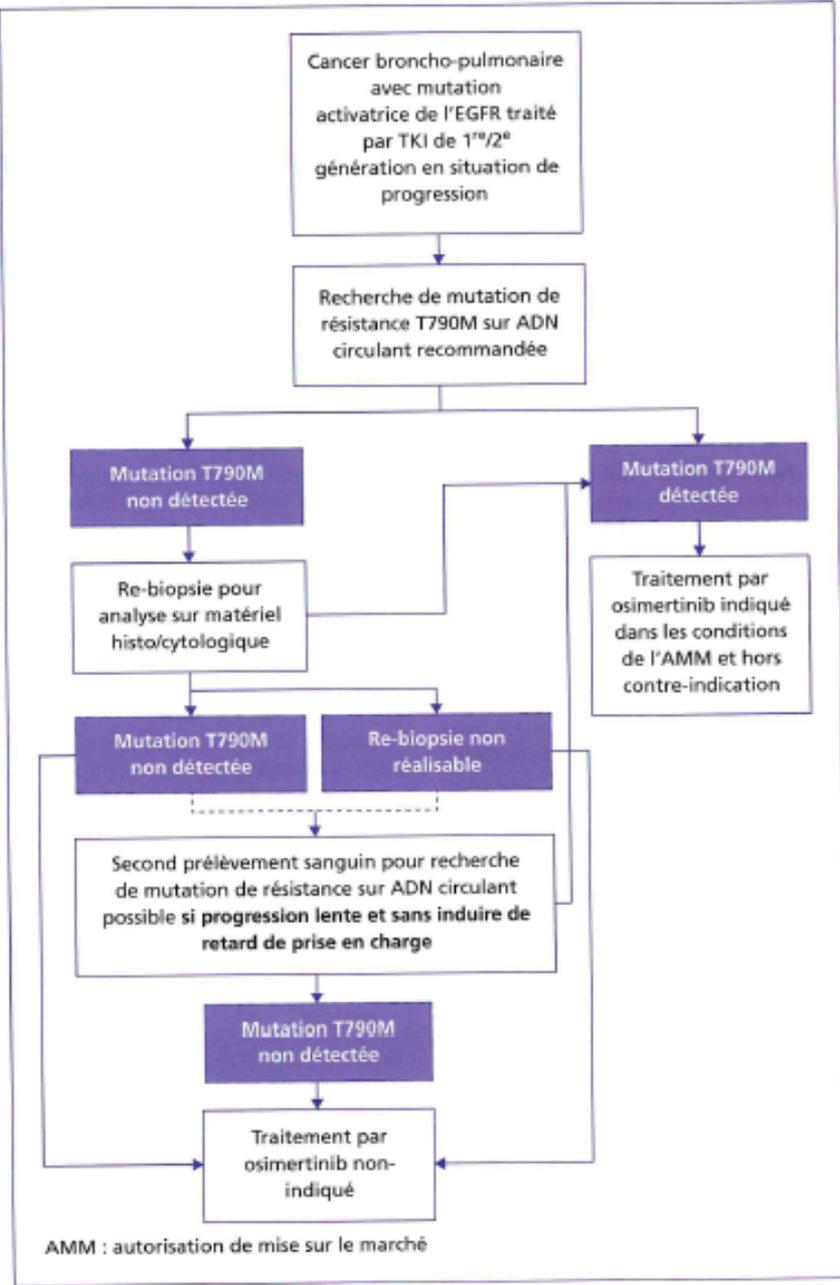


Figure 2. Stratégie d'indication de l'analyse moléculaire sur ADN circulant en situation de diagnostic moléculaire de résistance aux TKI d'EGFR de première ou seconde génération.

Figure 2. Strategy for indication for molecular analysis of circulating DNA during molecular investigation of resistance to first or second generation EGFR TKIs.

Autre application

First author	Year	Methodic	No. of patients	EGFR determination	EGFR variation levels	T790M determination (timing)	T790M variation levels	Others
Murtaza M (36)	2013	Digital PCR	1	√	√	√ (R)	√	p53, NFKB1
Oxnard GR (81)	2014	dd-PCR	9	√	√	√ (R)	√	–
Sorensen BS (82)	2014	Cobas EGFR blood test	23	√	√	√ (R)	√	–
Marchetti A (83)	2015	Cobas EGFR blood test	57	√	√	–	–	–
Ahn MJ (84)	2015	dd-PCR	60	√	√	√ (R)	–	–
Wang Z (85)	2014	Digital PCR, ARMS	135	–	–	√ (D)	√	–
Nakamura T (86)	2011	MBP-PQ	49	–	–	√ (R)	√	–
Marcq M (87)	2014	ARMS	2	√	√	√ (R)	√	–
Piotrowska Z (88)	2015	BEAMing	12	√	√	√ (R)	√	–
Sequist LV (89)	2015	BEAMing	113	–	–	√ (R)	√	–
Thress KS (90)	2015	NGS, dd-PCR	19	√	√	√ (R)	√	EGFR C797S

dd-PCR, digital droplet-PCR; ARMS, amplification refractory mutation system; MBP-PQ, mutation-biased PCR quenching probe; BEAMing, beads, emulsion, amplification and magnetics; (R), at resistance; (D), at first diagnosis.

Bordi et al. Role of circulating DNA in EGFR mutated NSCLC

Diagnostic Accuracy of Noninvasive Genotyping of EGFR in Lung Cancer Patients by Deep Sequencing of Plasma Cell-Free DNA

Junji Uchida,¹ Kikuya Kato,^{2*} Yoji Kukita,² Toru Kumagai,¹ Kazumi Nishino,¹ Haruko Daga,³ Izumi Nagatomo,⁴ Takako Inoue,¹ Madoka Kimura,¹ Shigeyuki Oba,⁵ Yuri Ito,⁶ Koji Takeda,³ and Fumio Imamura¹

Stage	
I	64
IA	45
IB	18
IA or IB	1
II	19
IIA	13
IIB	6
III	53
IIIA	27
IIIB	26
IV	146
Undetermined	6
Histology	
Adenocarcinoma	274
Squamous cell carcinoma	7
Adenosquamous cell carcinoma	2
Other	5

exon 19 del.

Sens. 50.9% (95% CI 37.9%–63.9%)

Spe. 98.0% (88.5%–100%)

L858R,

Sens. 51.9% (38.7%–64.9%)

Spe. 94.1% (83.5%–98.6%)

Table 4. Biopsy and plasma mutation types under the final threshold setting.

Biopsy	Plasma			
	Wild-type	Exon 19 deletion	L858R	Double
All cases				
Wild-type	163	5	15	2
Exon 19 deletion	23	25	2	1
L858R	23	0	26	1
Double	1	1	0	0
Stage I-III A				
Wild-type	65	0	8	1
Exon 19 deletion	13	2	2	0
L858R	15	0	3	1
Double	0	0	0	0
Stage IIIB-IV				
Wild-type	93	5	7	1
Exon 19 deletion	10	23	0	1
L858R	7	0	23	0
Double	1	1	0	0

stages IA–IIIA
sensitivity 22.2%
(11.5%–38.3%)

stages IIIB–IV
sensitivity 72.7%
(60.9%–82.1%)

« Because sensitivity was low in early-stage NSCLC, the detection system is preferred for stage IIIB–IV NSCLC »

Detection and Dynamic Changes of *EGFR* Mutations from Circulating Tumor DNA as a Predictor of Survival Outcomes in NSCLC Patients Treated with First-line Intercalated Erlotinib and Chemotherapy

Clin Cancer Res; 21(14) July 15, 2015

Tony Mok¹, Yi-Long Wu², Jin Soo Lee³, Chong-Jen Yu⁴, Virote Sriuranpong⁵, Jennifer Sandoval-Tan⁶, Guia Ladrera⁷, Sumitra Thongprasert⁸, Vichien Srimuninnimit⁹, Meilin Liao¹⁰, Yunzhong Zhu¹¹, Caicun Zhou¹², Fatima Fuerte¹³, Benjamin Margono¹⁴, Wei Wen¹⁵, Julie Tsai¹⁵, Matt Truman¹⁶, Barbara Klughammer¹⁷, David S. Shames¹⁸, and Lin Wu¹⁵

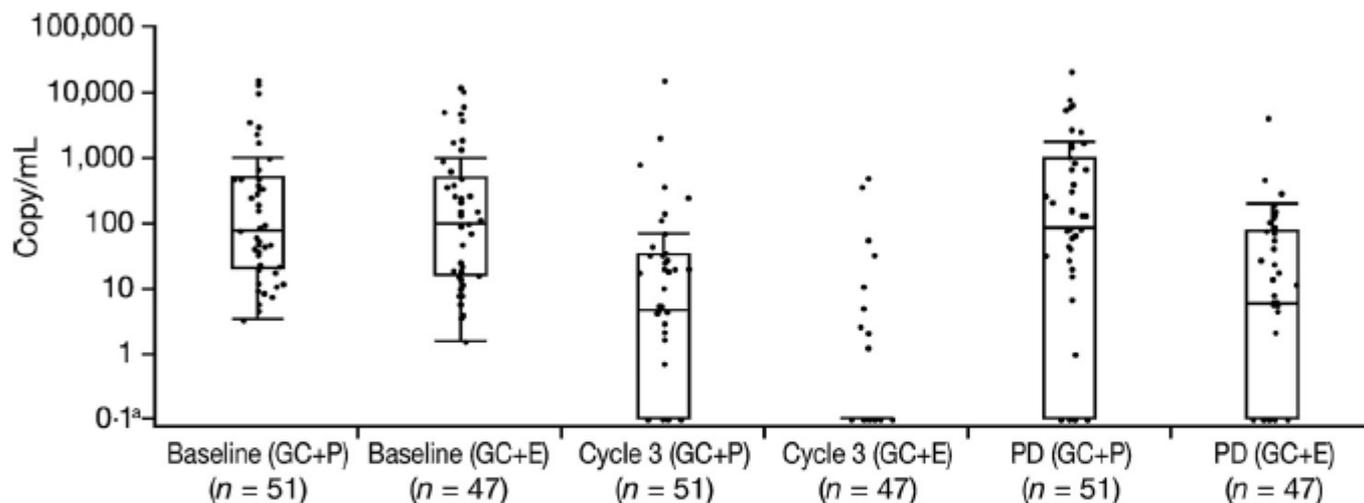
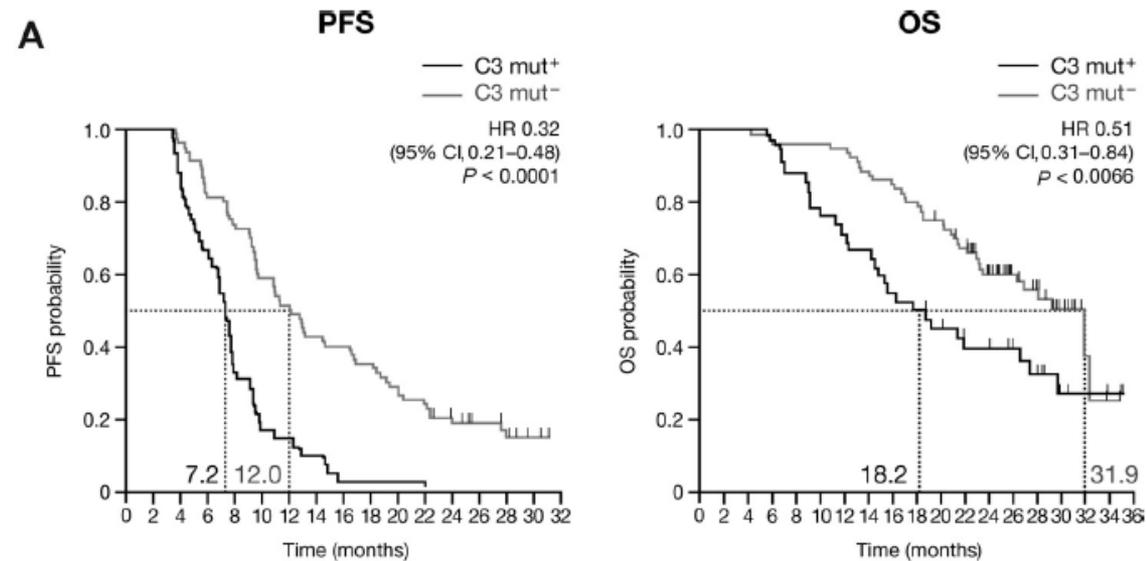


Figure 2. Dynamic quantitative change in *EGFR* mut⁺ cfDNA at baseline, C3, and PD. GC+E, erlotinib plus chemotherapy; GC+P, placebo plus chemotherapy. ^acopy/mL ≤ 0.1 were undetectable.

Median *EGFR* mut⁺ cfDNA (copy/mL of blood)

	GC+P	GC+E
Baseline	78	94
C3	5	0
PD	83	6



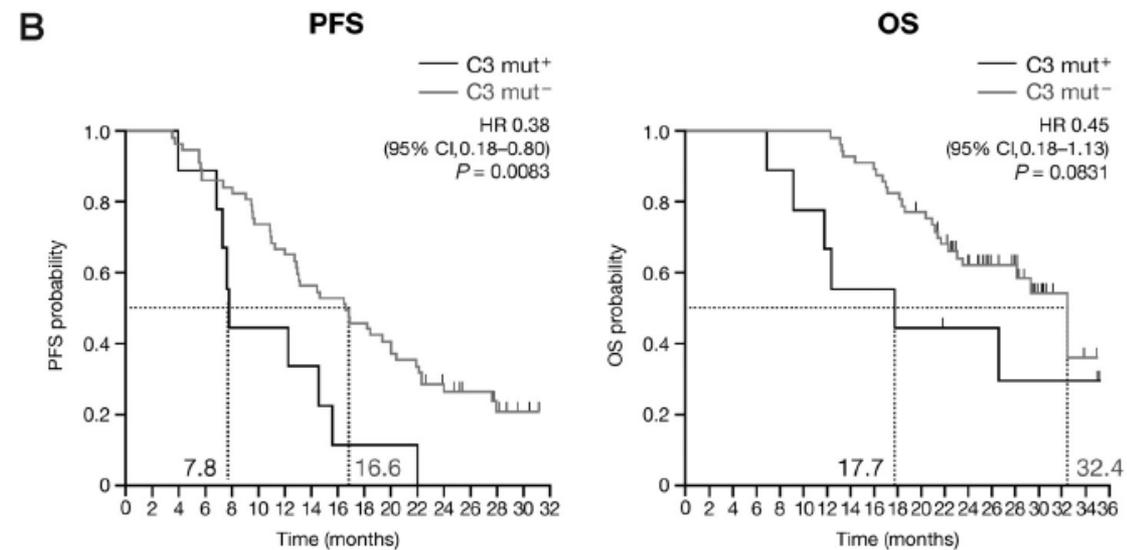
Patients, n

C3 mut ⁺	42	42	35	28	14	7	6	4	1	1	1	0	0	0	0	0	0
C3 mut ⁻	80	80	77	65	59	47	40	34	32	28	23	19	13	10	7	3	0

Patients, n

C3 mut ⁺	42	42	41	37	32	30	26	23	21	18	14	14	12	9	4	3	2	0	
C3 mut ⁻	80	80	80	77	77	77	76	71	68	64	59	52	38	29	22	12	3	1	0

Figure 3. PFS and OS for baseline cfDNA mut⁺ patients stratified by C3 cfDNA EGFR mutation status in both treatment arms combined (A) and in the GE+E arm only (B).



Patients, n

C3 mut ⁺	9	9	8	8	4	4	4	3	1	1	1	1	0	0	0	0	0
C3 mut ⁻	57	57	55	49	48	42	37	32	30	26	23	19	13	10	7	3	0

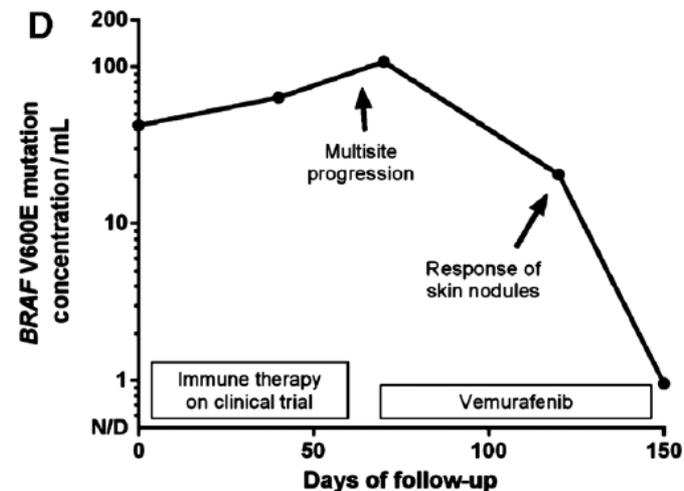
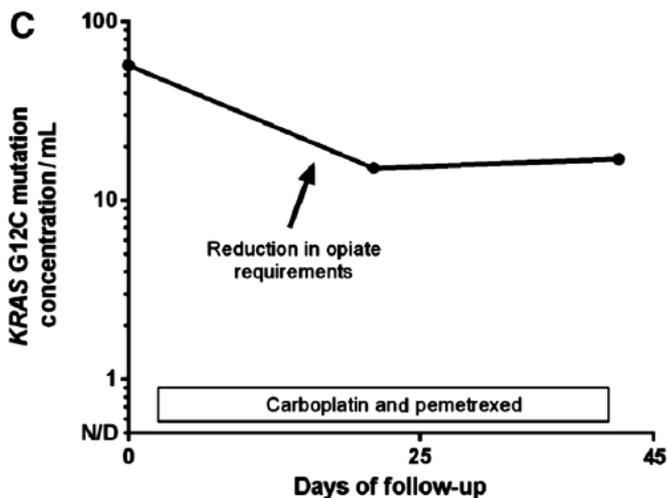
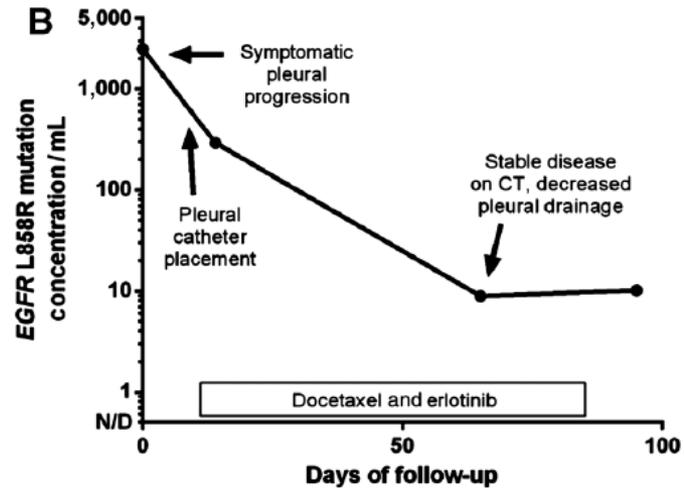
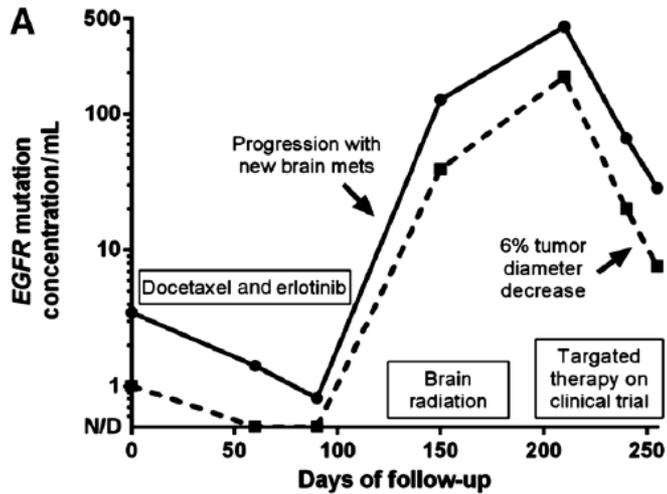
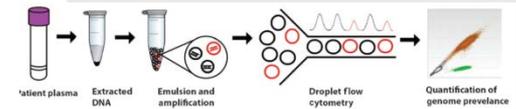
Patients, n

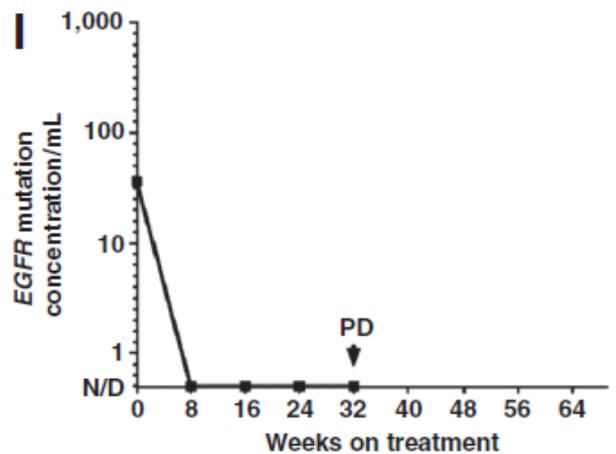
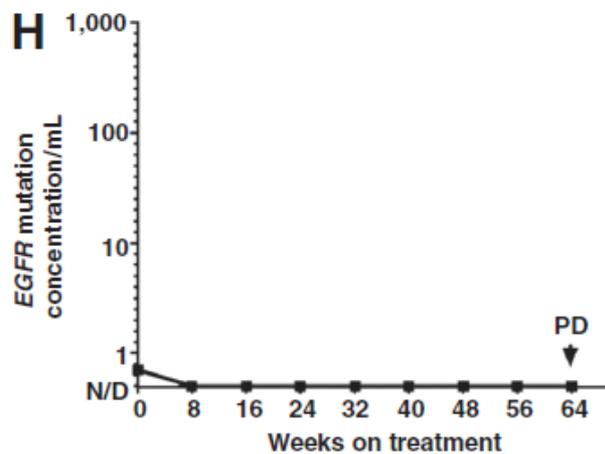
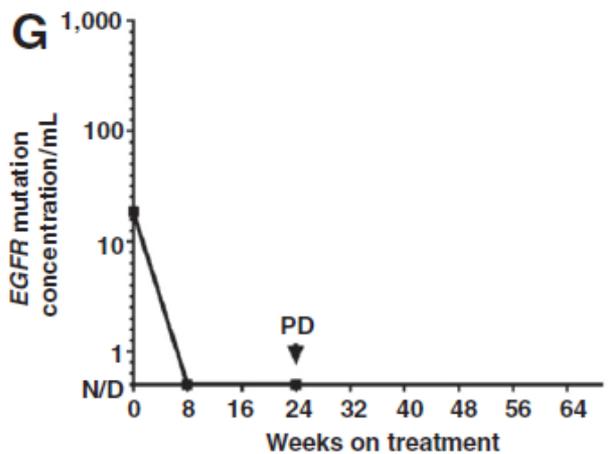
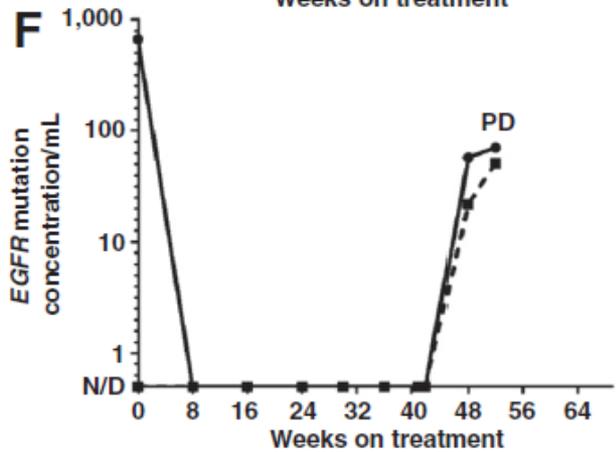
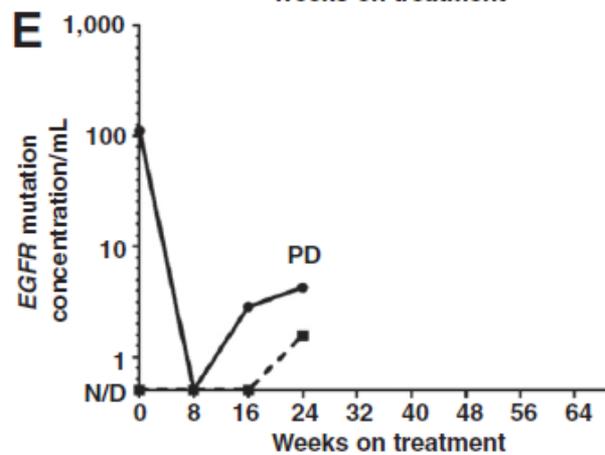
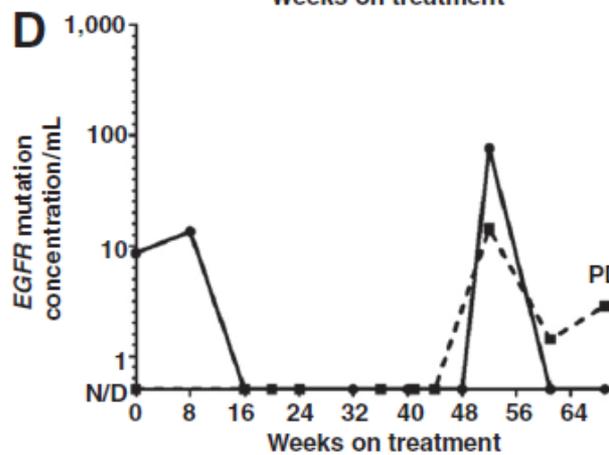
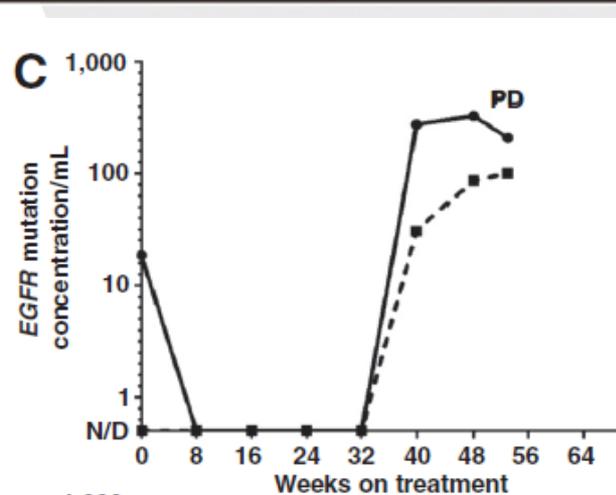
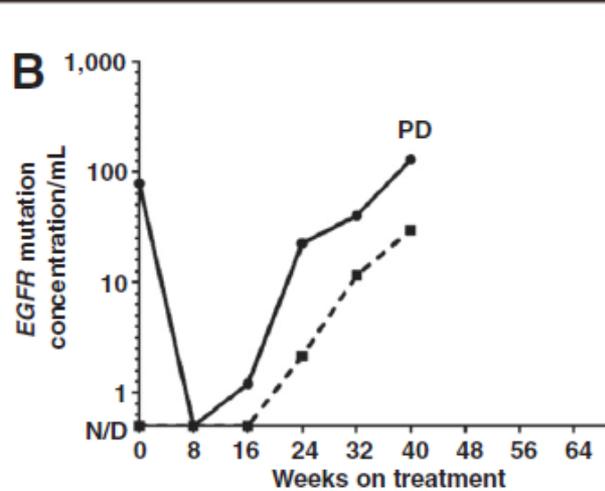
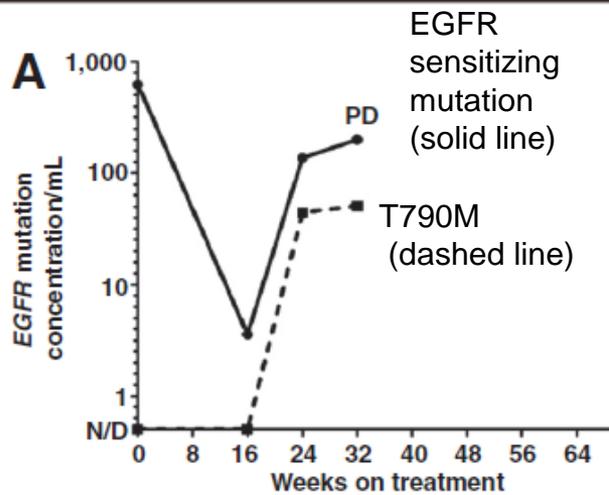
C3 mut ⁺	9	9	9	8	7	6	5	5	4	4	3	3	3	2	2	2	2	0
C3 mut ⁻	57	57	57	57	57	57	53	51	47	43	37	28	22	18	8	3	1	0

Noninvasive Detection of Response and Resistance in *EGFR*-Mutant Lung Cancer Using Quantitative Next-Generation Genotyping of Cell-Free Plasma DNA

Clin Cancer Res; 20(6); 1698–705. ©2014 AACR.

Geoffrey R. Oxnard^{1,3}, Cloud P. Paweletz^{1,2}, Yanan Kuang^{1,2}, Stacy L. Mach¹, Allison O'Connell^{1,2}, Melissa M. Messineo^{1,2}, Jason J. Luke^{1,3}, Mohit Butaney¹, Paul Kirschmeier^{1,2}, David M. Jackman^{1,3}, and Pasi A. Jänne^{1,2,3}



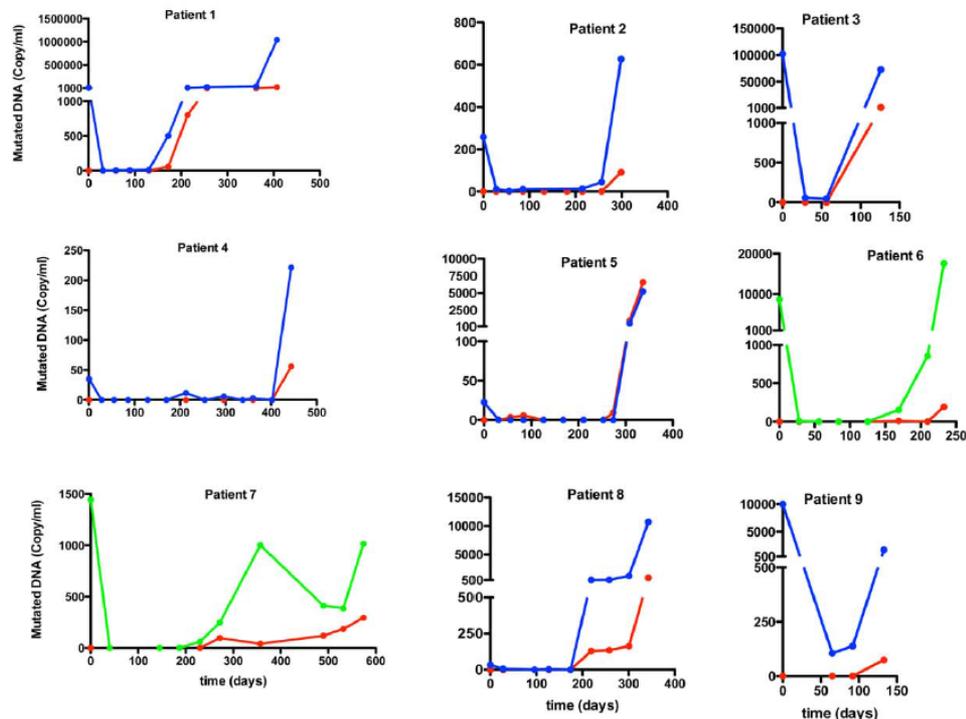


Monitoring of Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor-Sensitizing and Resistance Mutations in the Plasma DNA of Patients With Advanced Non-Small Cell Lung Cancer During Treatment With Erlotinib

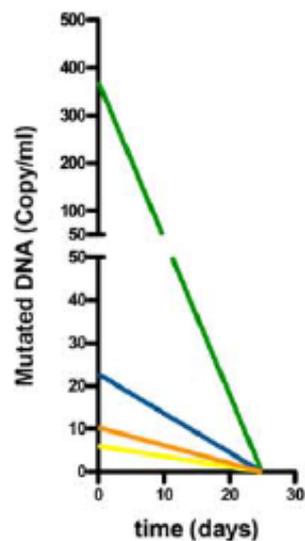
Boe S. Sorensen, MS, PhD¹; Lin Wu, MS, PhD²; Wen Wei, MS, PhD²; Julie Tsai, BS²; Britta Weber, MD, PhD^{1,3}; Ebba Nexø, MD, PhD¹; and Peter Meldgaard, MS, PhD³

— T790M mutation
— EGFR Ex 19 deletion
— EGFR L858R.

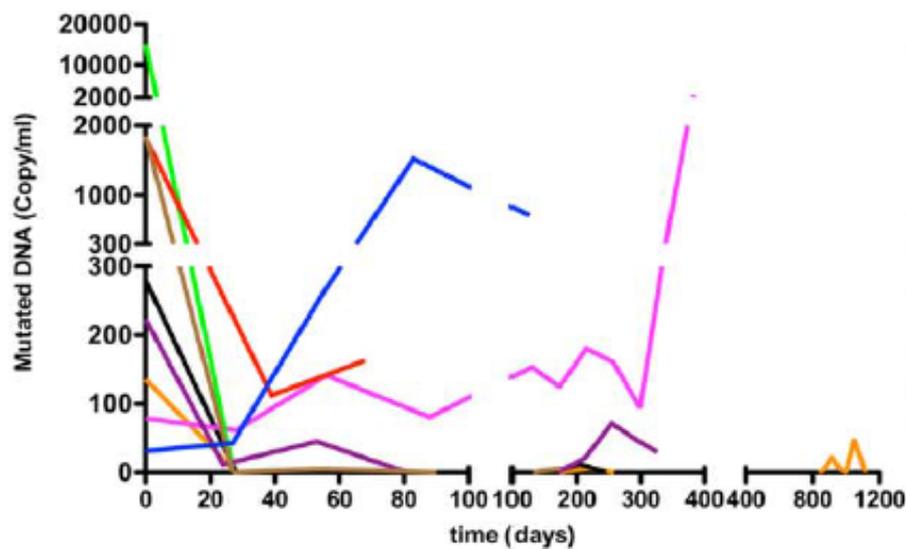
Cancer December 15, 2014



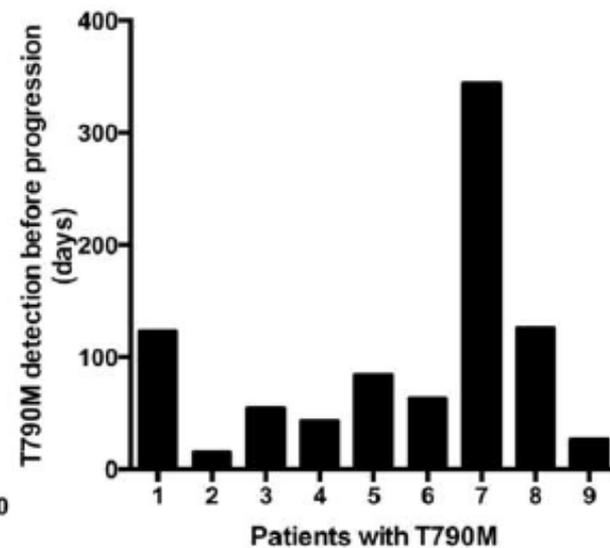
B.



C.



D.



Quantification and Dynamic Monitoring of *EGFR* T790M in Plasma Cell-Free DNA by Digital PCR for Prognosis of *EGFR*-TKI Treatment in Advanced NSCLC

Zhijie Wang, Rui Chen, Shuhang Wang, Jia Zhong, Meina Wu, Jun Zhao, Jianchun Duan, Minglei Zhuo, Tongtong An, Yuyan Wang, Hua Bai*, Jie Wang*

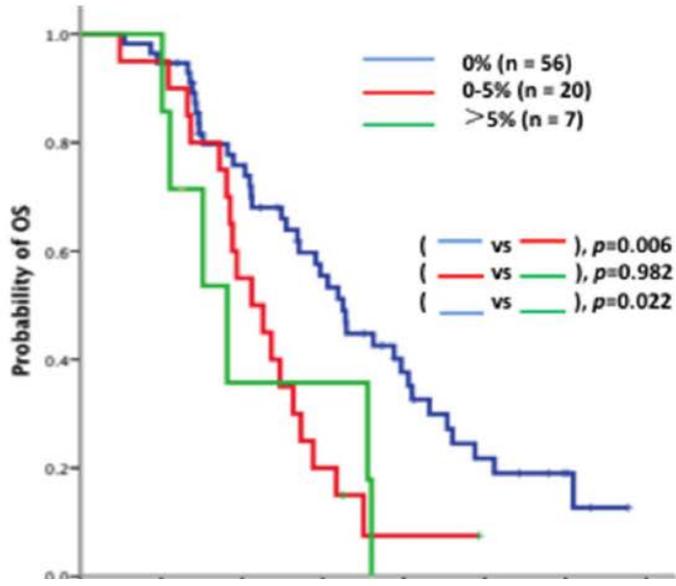
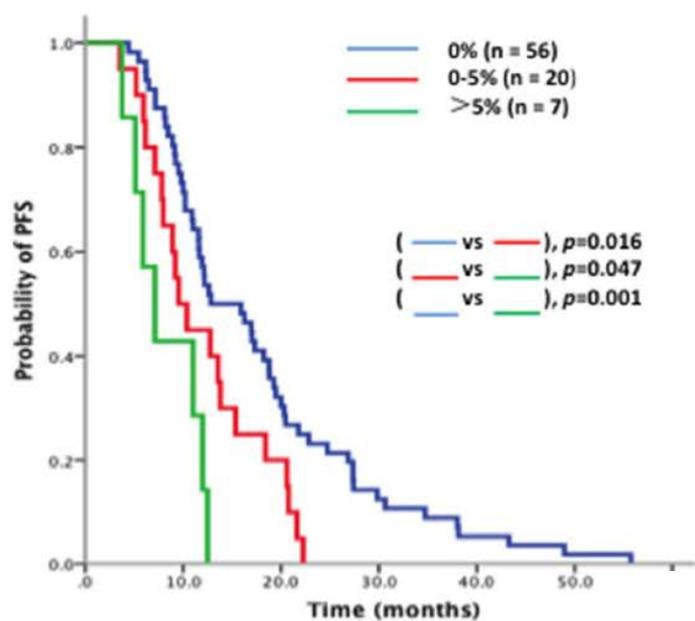
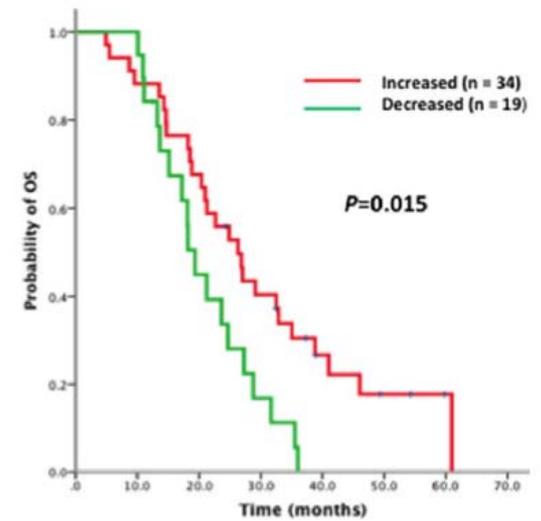
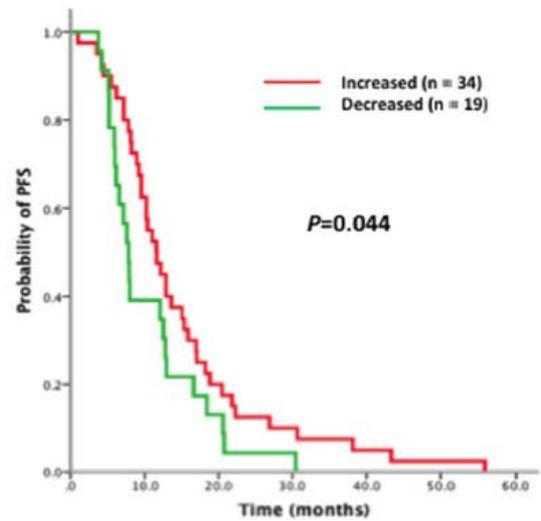


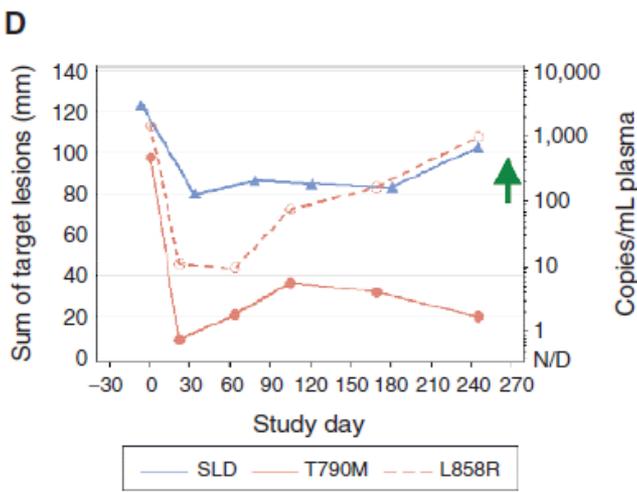
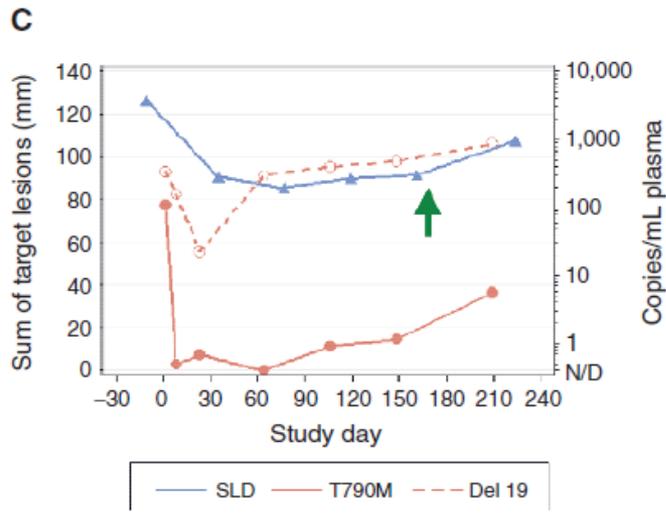
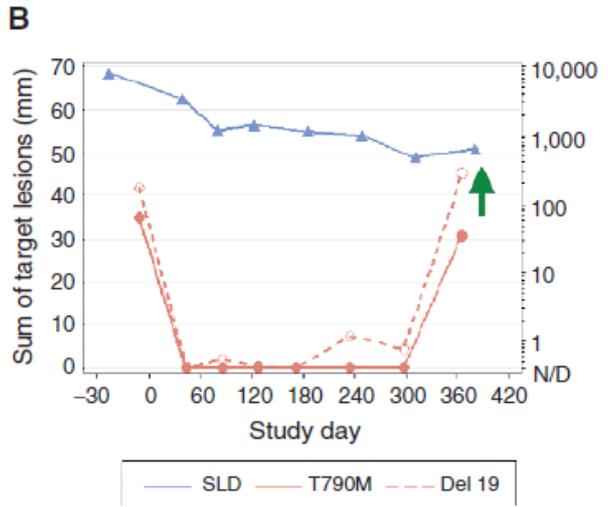
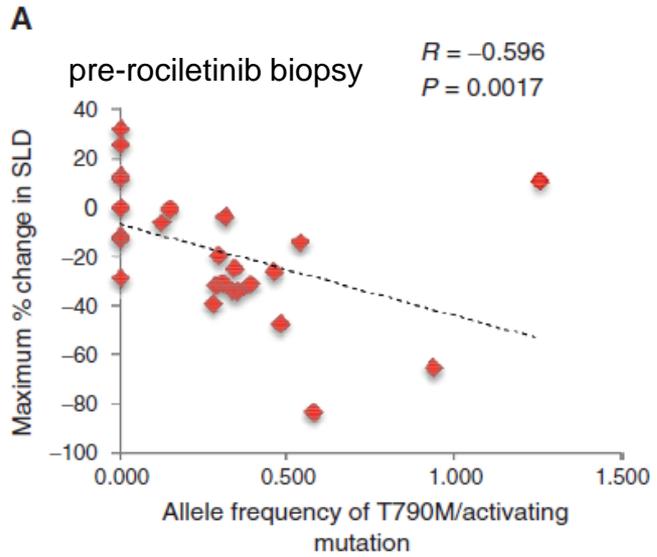
Figure 2. Survival analysis on *EGFR*-TKI according to the qu



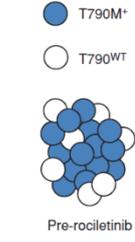
Heterogeneity Underlies the Emergence of EGFR^{T790} Wild-Type Clones Following Treatment of T790M-Positive Cancers with a Third-Generation EGFR Inhibitor

Zofia Piotrowska^{1,4}, Matthew J. Niederst^{1,4}, Chris A. Karlovich², Heather A. Wakelee³, Joel W. Neal³, Mari Mino-Kenudson^{1,4}, Linnea Fulton¹, Aaron N. Hata^{1,4}, Elizabeth L. Lockerman¹, Anuj Kalsy¹, Subba Digumarthy^{1,4}, Alona Muzikansky^{1,4}, Mitch Raponi², Angel R. Garcia¹, Hillary E. Mulvey¹, Melissa K. Parks¹, Richard H. DiCecca¹, Dora Dias-Santagata^{1,4}, A. John Iafrate^{1,4}, Alice T. Shaw^{1,4}, Andrew R. Allen², Jeffrey A. Engelman^{1,4}, and Lecia V. Sequist^{1,4}

rociletinib



Copies/mL plasma



Mechanisms of acquired resistance to AZD9291 in EGFR T790M positive lung cancer

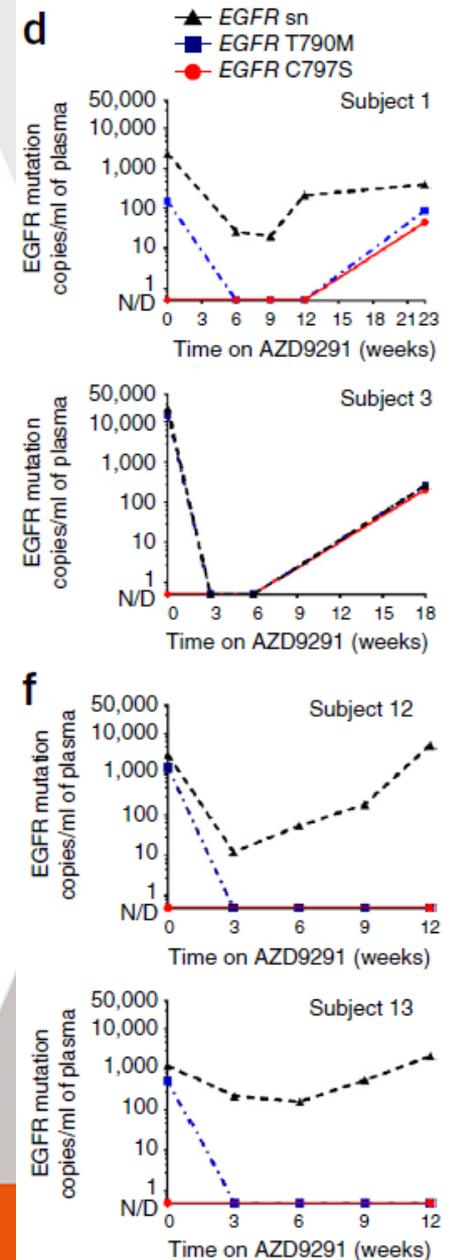
Geoffrey R. Oxnard¹, et al.

AZD9291- osimertinib (TAGRISSO) irreversible, mutant-selective EGFR-TKI developed to have potency against both *EGFR* sensitizing mutations and T790M. Phase I study of AZD9291 (AURA, NCT01802632), the response rate in patients with T790M was >60%

We recently reported that acquired resistance to AZD9291 mediated by acquired EGFR C797S or loss of the EGFR T790M.

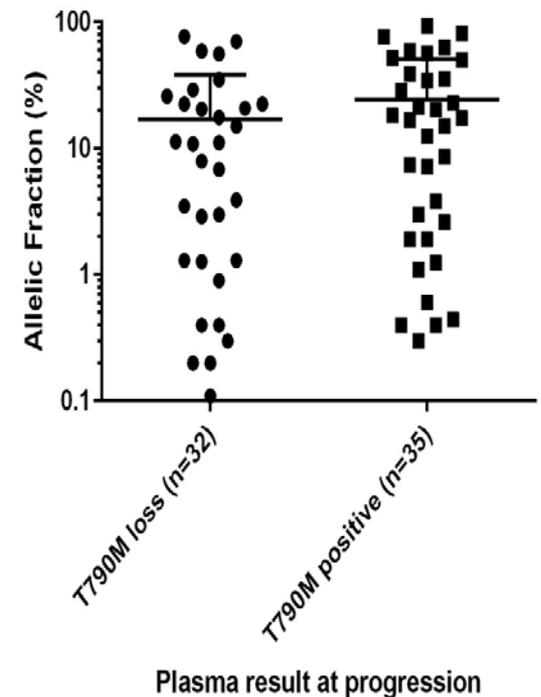
(Thress KS, et al. Acquired, Nat Med 2015;21:560-2.)

⇒ 67 patients enrolled in the study.



- 67 pts were eligible for acquired resistance and T790M positive on plasma or tumor genotyping at enrollment. Of those, 15 had detectable C797S on ddPCR (22%), all with detectable T790M.
- 32 of 67 (47%) had no detectable T790M in plasma despite presence of the EGFR-TKI sensitizing mutation, suggesting overgrowth of an alternate resistance mechanism.
- Loss of T790M at progression may be mediated by overgrowth of a competing resistance mechanism eg. HER2, BRAF, MET

Allelic fraction of EGFR sensitizing in T790M loss vs T790M positive plasma at progression



Results: HER2 amplification

LUNG BIOPSY

EGFR del19
T790M negative



SCAPULAR BIOPSY

EGFR del19
T790M negative



LUNG BIOPSY

EGFR del19 and
T790M positive
No HER2 amplification
(CGH)

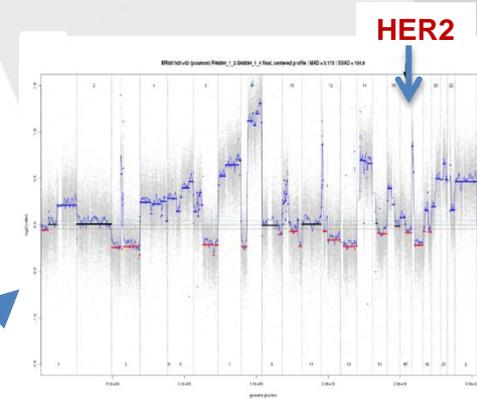


SCAPULAR BIOPSY

EGFR del19
T790M negative

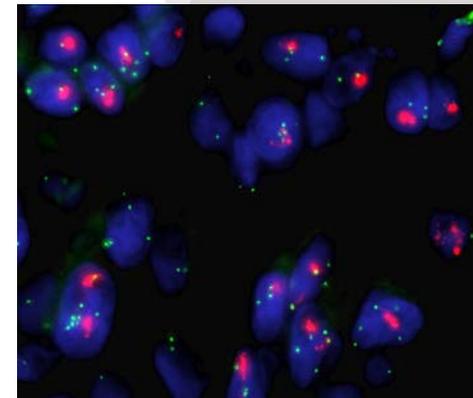
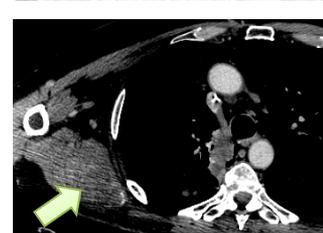
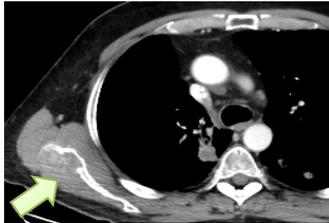
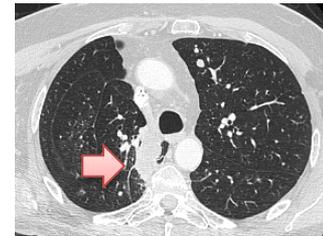
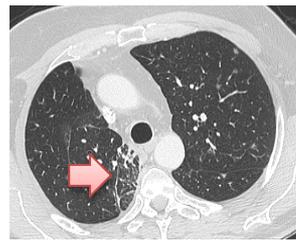
LUNG BIOPSY

EGFR del19
T790M negative
C797S negative
HER2 amplification



HER2 amplification by CGH:
log ratio x3.32

- 54 year-old man,
- Former smoker (20 PY)
- Adenocarcinoma
- Metastatic to brain, bone
- EGFR exon 19 del



HER2/CEP17: 6.65
HER2 in red; centromere 17 in green

Gefitinib

AZD9291 (80 mg)

NGS: Ion Torrent PGM
CGH: Agilent technology
HER2-FISH: Dako DNA probe kit

Développement de l'analyse cfDNA pour la recherche clinique à Gustave Roussy.

- **Programme de Médecine personnalisée**

Proposition de traitement en fonction du profil moléculaire du patient

Certaines altérations génomiques = cibles thérapeutiques

- **Standard = biopsie à l'inclusion**

- > Challenge organisationnel
- > Geste invasif, coût, accessibilité variable de la tumeur
- > ¼ Cas <10-30% cellules tumorales dans la cassette de biopsie: NC

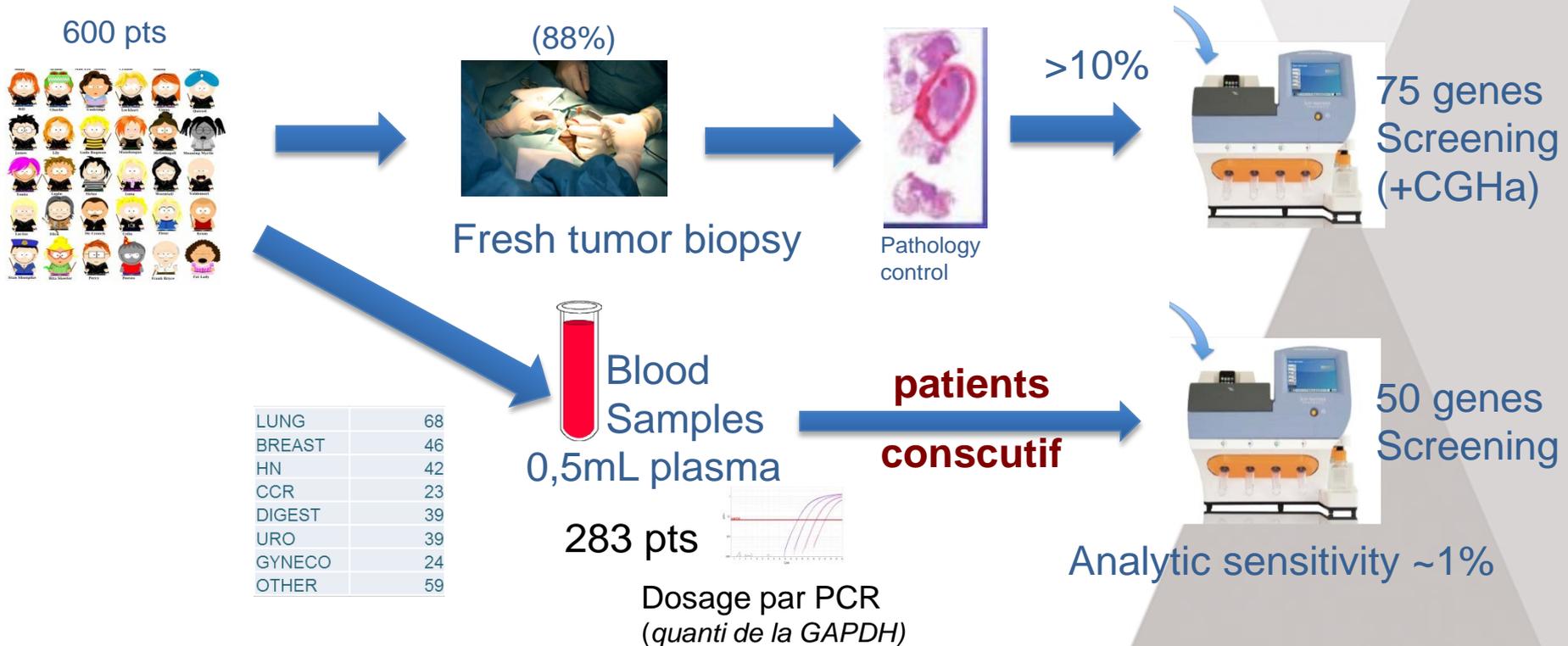
- **La biopsie liquide peut elle être une alternative à la biopsie de tissu pour une caractérisation moléculaire ?**

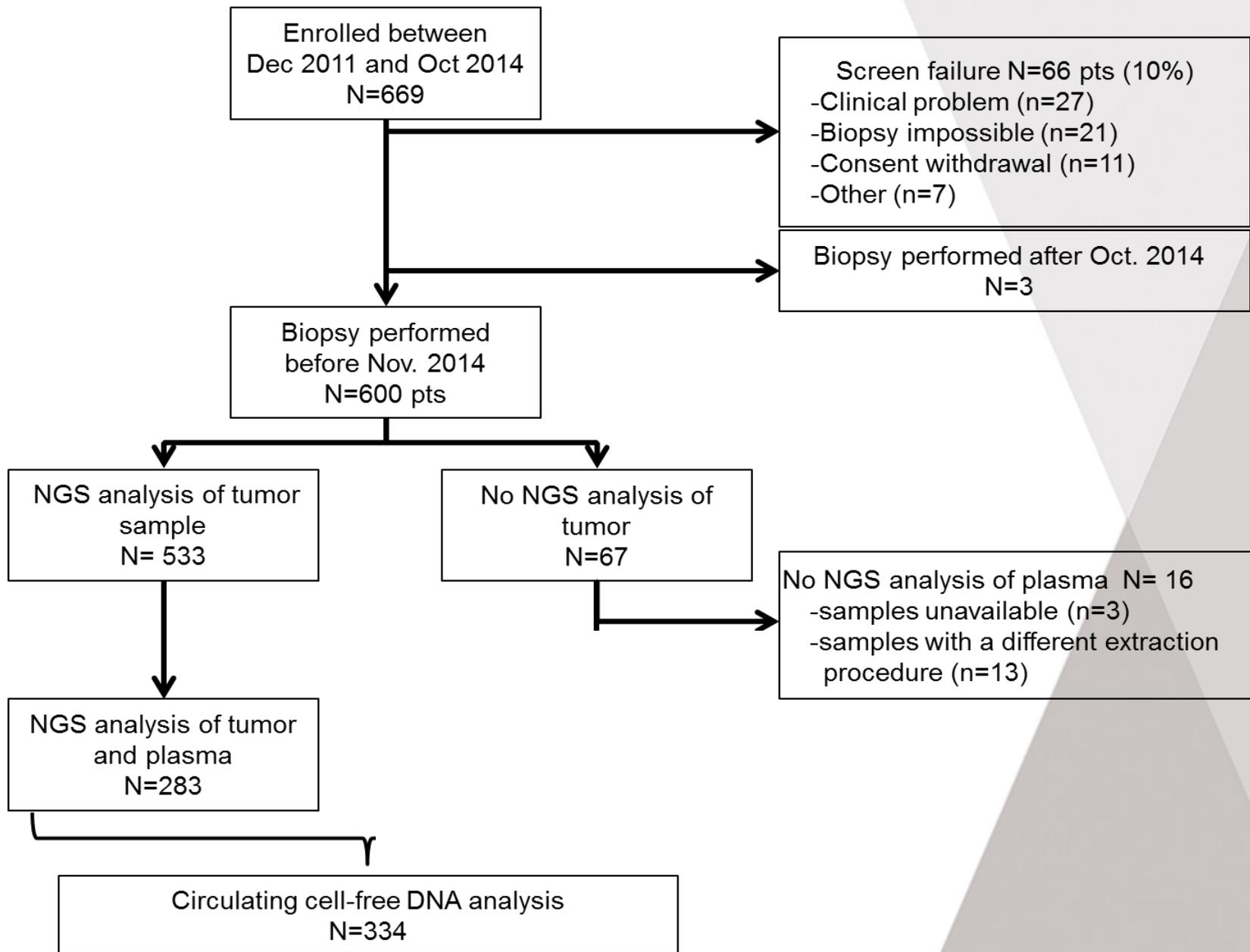
Evaluation de la concordance des mutations retrouvées dans le plasma à celles retrouvées dans la biopsie

GR project (AACR 2015 – CCR 2016)

Circulating cell-free tumor DNA (cfDNA) analysis of 50-genes by next-generation sequencing (NGS) in the prospective MOSCATO trial

Cécile Jovelet, Ecaterina Ileana¹, Marie-Cécile Le Deley, Nelly Motté, Silvia Rosellini, Alfredo Romero, Céline Lefebvre, Marion Pedréro, Noémie Pata-Merci, Nathalie Droin, Marc Deloger, Christophe Massard, Antoine Hollebecque, Charles Ferté, Amélie Boichard, Sophie Postel-Vinay, Maud Ngo-Camus, Thierry De Baere¹⁰, Philippe Vielh, Jean-Yves Scoazec, Gilles Vassal, Alexander Eggermont, Fabrice André, Jean-Charles Soria, Ludovic Lacroix





Séquençage:

Analyse de plasma de 283 patients MOSCATO collectés le jour de la biopsie

- 500µL de plasma
- Dosage QPCR (GAPDH) des ADN circulant
- Technique: NGS – ion torrent PGM (id. Tumeur)
- Panel utilisé: panel CHP2
 - > 50 oncogènes couverts, 207 amplicons
 - > Principaux hotspots
 - > Validation avec des Spick d'ADN ctr
- Analyse en aveugle
 - > Référence: human genome hg19
 - > Variant Caller 4.2.1.0 – Galaxy
 - > Variants retenus:
 - Fréquence allélique > 1%
 - Nombre de reads pour le variant > 5
 - Couverture minimale > 50

Panel : Ion AmpliSeq™ Cancer Hotspot Panel v2			40 Gènes			207 amplicons		
Gene	RefSeq	Exons testés (partiel)	Gene	RefSeq	Exons testés (partiel)	Gene	RefSeq	Exons testés (partiel)
ABL1	NM_007313.2	4 à 7	ATM	NM_00005.3	8-9-12-17-26-34a36-39-50-54-59-61-63			
ALK	NM_004304.3	23&25	AKT1	NM_005163.2	3&6			
APC	NM_000038.5	16 (partiel)	BRAF&15	NM_004333.4	11			
CDKN2A	NM_00077.4	2	CDH1	NM_004360.2	3-8-9			
CSF1R	NM_005211.3	7&22	CTNNB1	NM_001098209.1	3			
ERBB2	NM_004448.2	19a21	EGFR	NM_005228.3	3-7-15-18a21			
EZH2	NM_004456.4	16	ERBB4	NM_005235.2	3-4-6-a9-15-23			
FBXW7	NM_033632.2	5-8a11	FGFR3	NM_000142.4	7-9-14-16-18			
FGFR1	NM_023110.2	4&7	FLT3	NM_004119.2	11-14-16-20			
FGFR2	NM_022970.3	7-9-12	HNF1A	NM_000545.5	3&4			
GNA11	NM_002067.2	5	GNAS	NM_000516.4	8&9			
GNAQ	NM_002072.3	5&8	HRAS	NM_005343.2	2&3			
IDH1	NM_005896.2	4	JAK2	NM_004972.3	14			
IDH2	NM_002168.2	4	JAK3	NM_000215.3	4-13-16			
MLH1	NM_000249.3	12	KRAS	NM_003360.2	2a4			
MPL	NM_005373.2	1	KDR	NM_002253.1	6-7-11-19-21-26-27-30			
NOTCH1	NM_017617.3	26-27-34	KIT	NM_000222.2	2-9a11-13-15-17-18			
NPM1	NM_002520.6	12	MET	NM_001127500.1	2 (partiel)-11-14-16-19			
PTEN	NM_000314.4	1-3-5-6-a8	NRAS	NM_002524.3	2a4			
PTPN11	NM_002834.3	3&13	PDGFRA	NM_006206.4	12-14-15-18			
SMAD4	NM_005359.5	3a6-8a12	PIK3CA	NM_006218.2	2-5-7-8-10-14-19-21			
SMARCB1	NM_003073.2	2-4-5-9	RB1	NM_000321.2	4-6-10-11-14-17-18-20a22-			
SRC	NM_005417.3	14	RET	NM_020975.4	10-11-13-15-16			
STK11	NM_000455.4	1-4-6-8	SMO	NM_005631.4	3-5-6-9-11			
TP53	NM_000546.4	2-4a8-10	VHL	NM_000551.2	1a3			

Résultats

Dosage

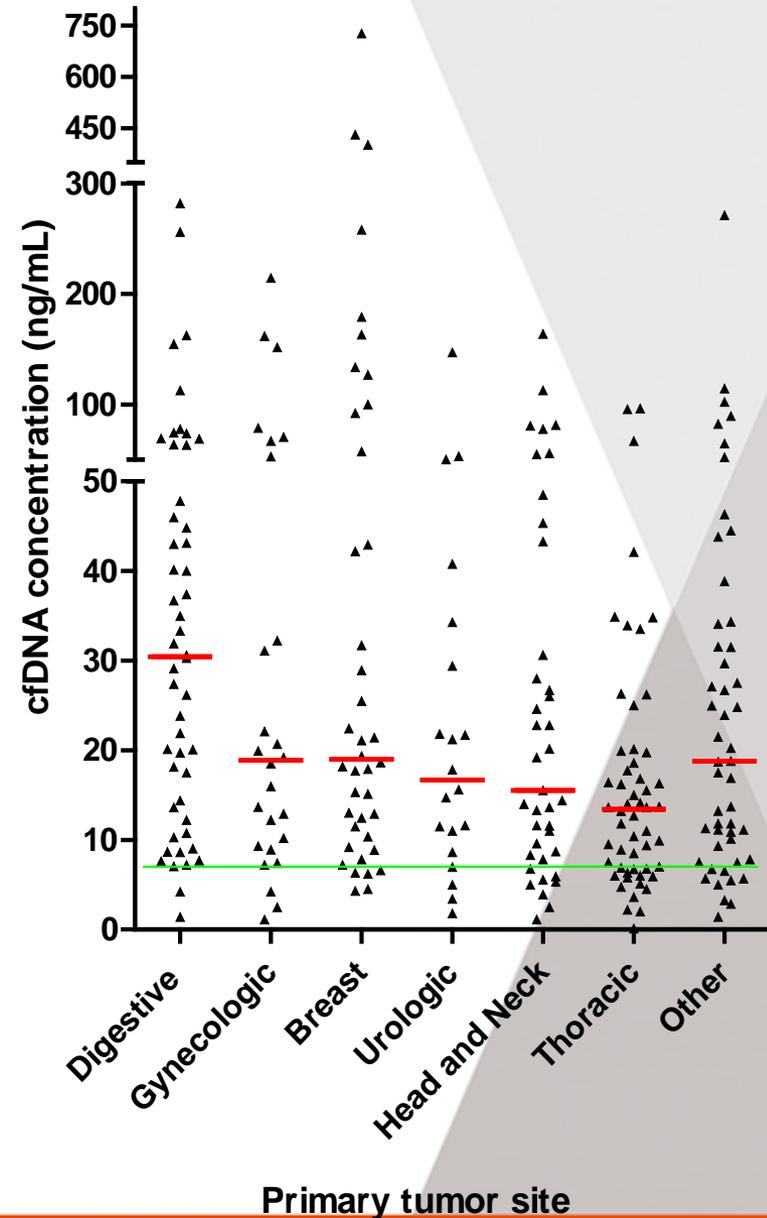
Dosage du cfDNA dans le plasma:

- Conc (toutes pathologies):

de 0.1 ng/mL à 727 ng/mL.

moyenne : 18 ng/mL

- Différence significative en fonction des pathologies



Séquençage et comparaison à la tumeur (calcul par gène)

- **TUMEUR tDNA :**

367 mutations chez les 283 patients

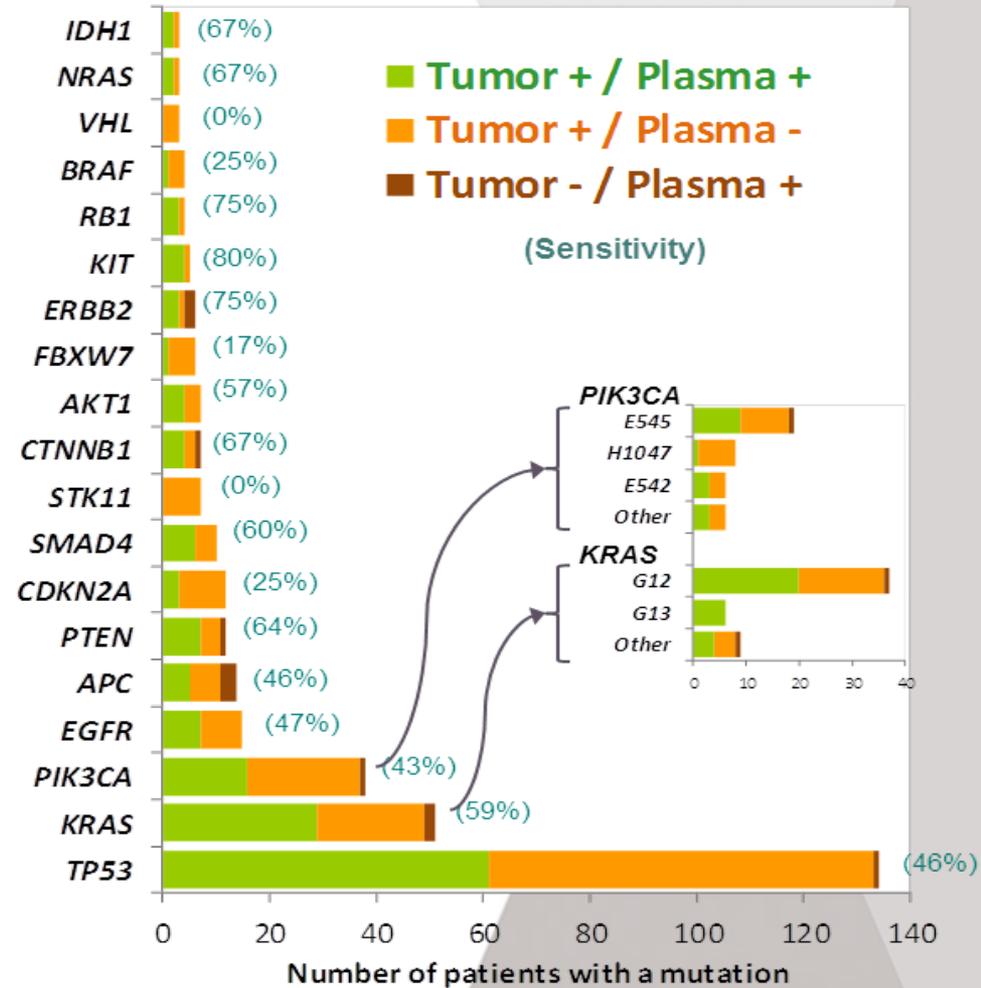
- **Plasma (cfDNA)**

204 mutations dont 179 mutations identiques à celles de la tumeur

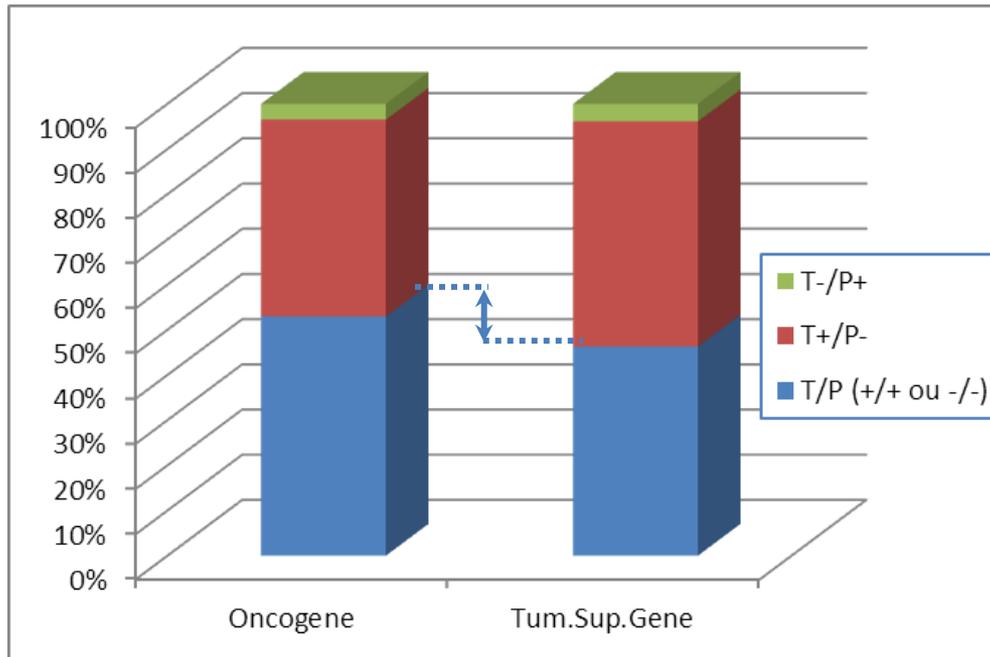
- Sensibilité : 49,9%
(95%CI, 44.6 to 55.1%)
- Spécificité : 99,8%
(95%CI, 99.8 to 99.9%)
- VPP 98% / VNP 91%
- Global Kappa coefficient : 0,6
(95%CI, 0.50 to 0.69)

- **Par gène**

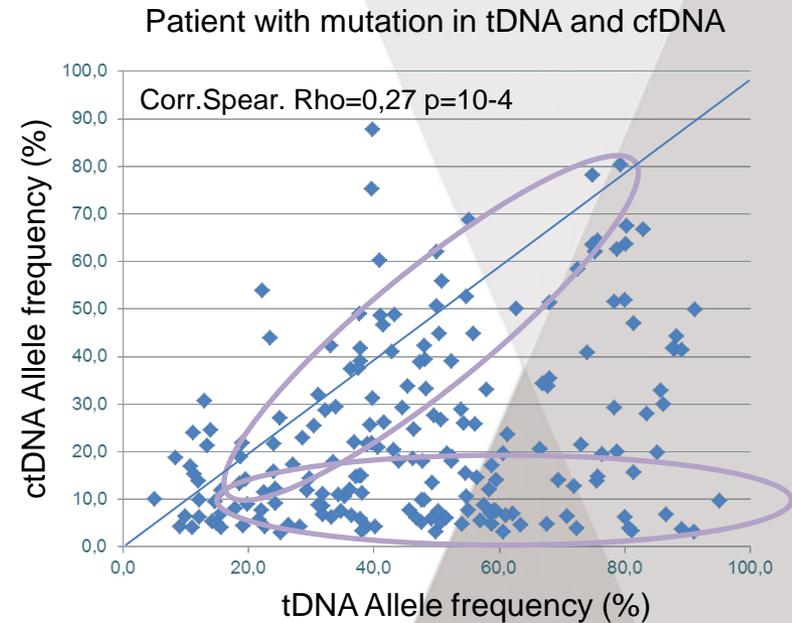
- TP53, 47%,
- KRAS, 59%,
- PIK3CA, 43%,
- EGFR, 53%
- APC, 46%
- PTEN, 60%



Comparison tDNA and cfDNA at D0



- tDNA & cfDNA concordant
- Mut in tDNA only
- Mut in cfDNA only



No correlation was observed between allele frequency observed in tumor and in plasma

Détail pour les 283 patients:

- 215 patients avec des mutations détectées dans la tumeur (1 ou plusieurs)
 - Toutes ces mutations ont été retrouvées dans le plasma pour 87 patients (32%)
 - Une partie des mutations a été retrouvée dans le plasma pour 34 patients (12%)
 - Aucune des mutations n'a été retrouvée dans le plasma pour 94 patients (34%)

Sensibilité estimée est de 55.0% (95%CI, 48.4%-61.6%)

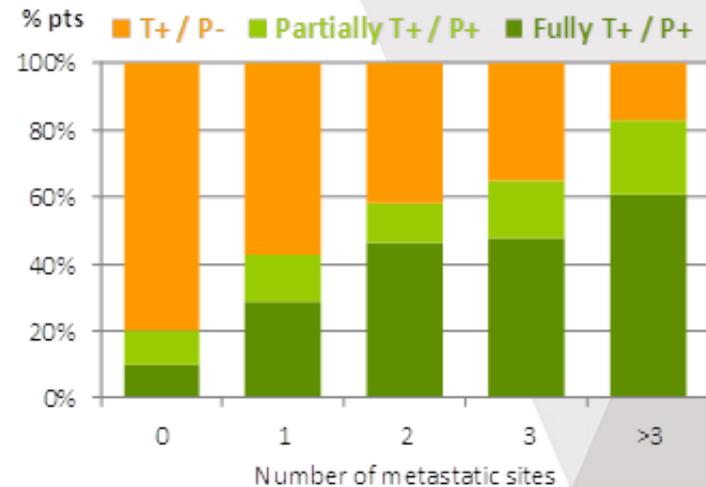
- 58 patients (20%) sans mutation détectée (tumeur & plasma WT)
- 5 patients (2%) avec mutations détectées dans le plasma mais pas dans la tumeur –fournis des informations complémentaires / biopsie
 - Qq nouvelles mutations => peut fournir des informations complémentaires / biopsie

Taux de concordance en fonction:

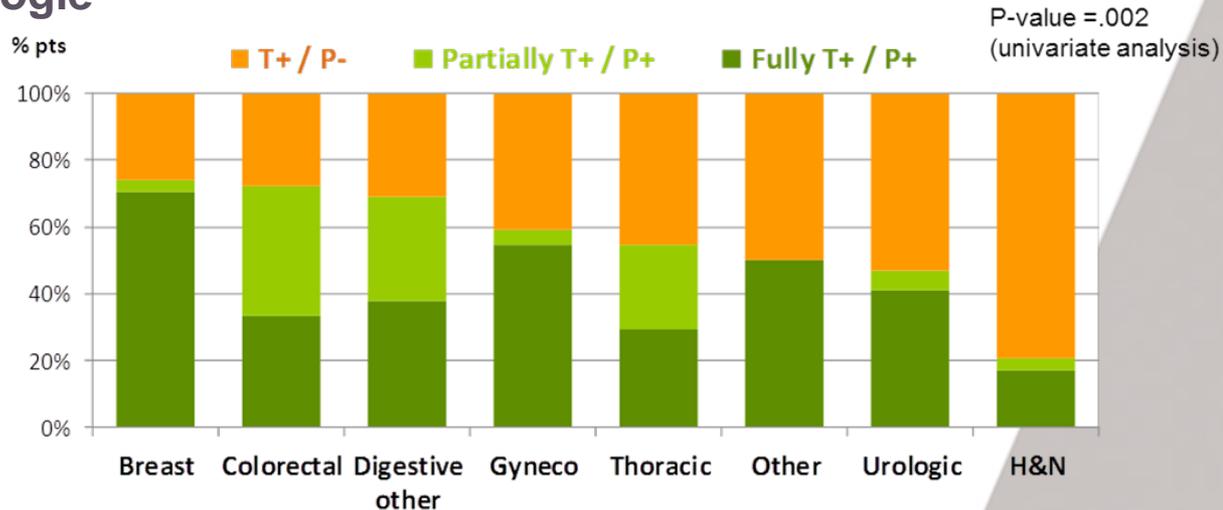
- Association avec :
 - Nb de sites métastatiques
 - Conc. Albumine / LDH
 - Conc. cfDNA
 - Type de tumeur
 - Nombre de ligne de ttt
 - RMH score

(NS avec Age/ECOG/Genre)

● Du nombre de métastases

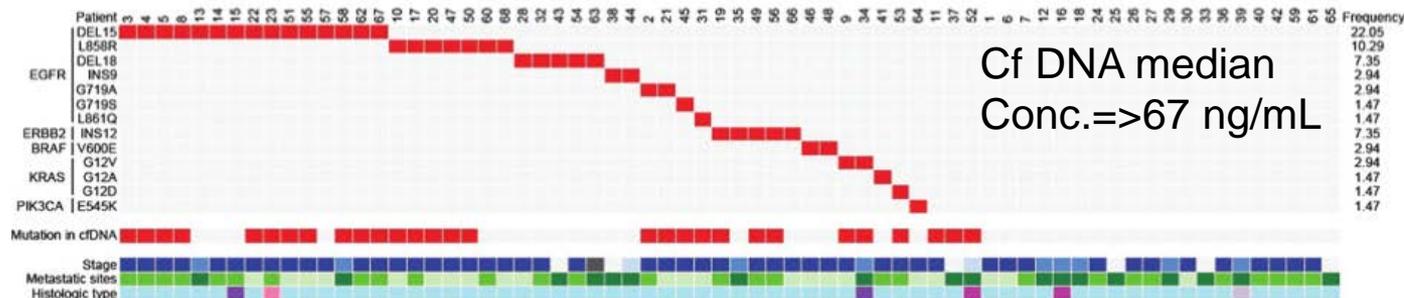


● De la pathologie



cfDNA and NGS in clinical research context.

Couraud et al. BioCAST/ IFCT-1002 CCRes. 2014
107 patients, 68 comparisons Biopsie vs cfDNA J0



Sensitivity : 58%
 (IC95 43%–71%)
Specificity : 87%
 (IC95 62%–96%)

Mosc.Lung
 55% (n=57)

Rothé F et al. In breast cancer. Ann Oncol. 2014
17 patients (31 plasma) Biopsie vs cfDNA J0

Sensitivity : 82%

Mosc.Breast
 74% (n=41)

Frenel J-S, et al. Phase I . CCRes. 2015
39 patients,

Sensitivity : 59%

Mosc.01
 55%

Lebofsky R et al. SHIVA-18 types tumoraux . Mol Oncol. 2014
34 patients, covering Biopsie vs cfDNA J1_J494 (consécutifs?)

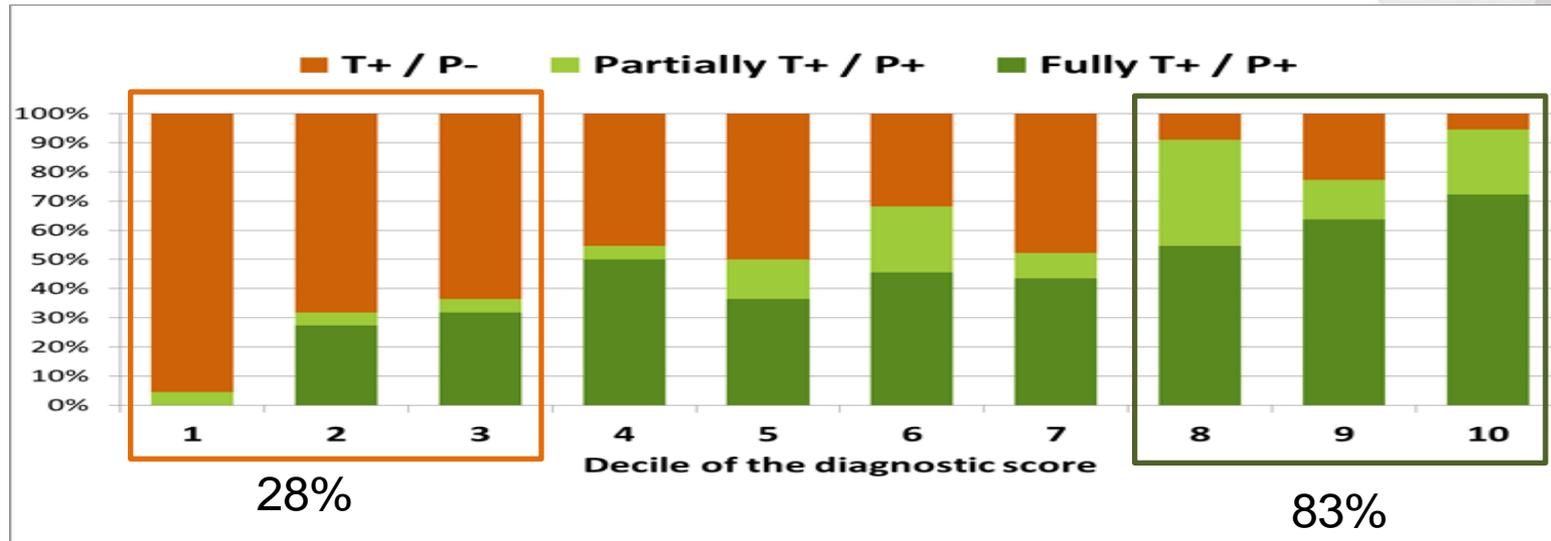
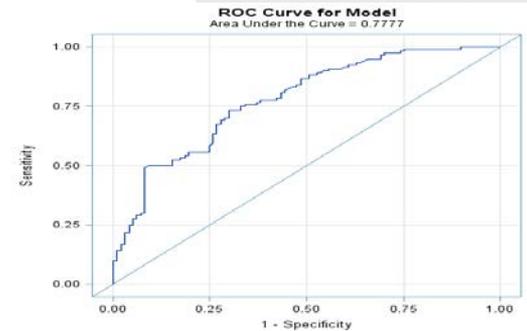
Sensitivity : 98%

=> Peu de données cliniques pour comparer les cohortes

Création d'un score:

- Pronostiquer la concordance entre mutations trouvées dans la tumeur et dans le plasma à partir de critères cliniques :

- Taux d'albumine
- Type de tumeur
- Nombre de métastases
- Nombre de lignes de traitements



- Sélection des patients pour lesquels la biopsie liquide pourrait être intéressante

> Patients avec un sensitivity prediction score ≥ 8 ? => à valider

Mise en place du test systématique pour les patients inclus dans MOSCATO avec une biopsie NC (< 10% cellules tumorales):

- **Nombre de patients concernés: 113 patients**
 - > 66 patients sans mutation (58%)
 - > 47 patients avec 68 mutations (42%)
 - Dont 39 pathogènes
 - Dont 16 avec des conséquences thérapeutiques (cibles thérapeutiques, sensibilité à des traitements, résistance...)

Donc apport de l'approche pour la 37% des cas analysés avec une implication thérapeutique pour 14%.

- **Déploiement dans les autres essais de MP (Poumon, Mélanome...)**
- **Pré-screening pour les inclusions dans des essais ciblés.**

Conclusion:

POUR CETTE ETUDE :

- Etude la plus large à ce jour en NGS/panel dans le cadre de screening multi-tumeur,
- Première description d'un score permettant d'identifier l'apport potentiel de l'analyse basée sur le cfDNA.
- Résultats globalement concordant entre les différentes études basées sur des « vraies » cohortes cliniques avec des sensibilités quand même inférieures aux études « preuves de concept ».

POUR LA RECHERCHE CLINIQUE :

- La biopsie liquide peut-elle servir alternative à la biopsie tissulaire, pour l'analyse de panel?
 - > Peut être un bon compromis quand la biopsie tissulaire n'est pas analysable,
 - > Mais pas chez tous les patients: mise en place du Score (à valider prospectivement) pourrait être un argument pour re-biopsier (au suivre à différents temps)

EN CLINIQUE:

- Une seule application recommandée: «recherche de mutation activatrice pour un traitement TKI en première ligne de CBNPC métastatique si le tissu n'est pas disponible,
 - Sensibilité encore perfectible (70%)
 - Manque de standardisation (préanalytique – méthode)
 - Valeur pour les mutations de résistance ? (T790M)
 - Pas de recommandation pour le suivi

Conclusion

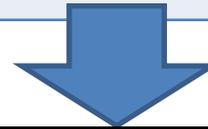


Cadre d'application des tests biologiques

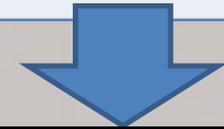
	Recherche	Transfert/R.Translationnelle	Clinique
Objectif	Examen à visée recherche	Examen à visée recherche, perspective d'application clinique	Examen de biologie médicale, acte médical (CSP L. 6211-1)
Phase développement	Concept, faisabilité	Evaluation, Validation	Routine
Structure	EPST (INSERM, CNRS, EA, ...)	<ul style="list-style-type: none"> •EPST, laboratoires hospitaliers, •Laboratoires dédiés RT 	laboratoires hospitaliers et secteur privé
Réglementaire	NA	NA	Loi 2013-442 du 30 mai 2013
Assurance Qualité	NA	Non réglementaire : ISO 9001 (CLIA??) – BPL (GLP)	Réglementaire : Accréditation COFRAC ISO 15189 (EEQ, métrologie) (CLIA - US)
Méthodes	« In-house »	« In-house » / RUO	<ul style="list-style-type: none"> • « In-house » accrédité COFRAC • Equipements, réactifs : marquage CE-IVD
Financement	<ul style="list-style-type: none"> •Dotation EPST • Ressources externes (caritatif, industriel, ...) 	<ul style="list-style-type: none"> •Ressources externes (caritatif, industriel, ...) • MIG non reproductible, PHRC, PSTIC 	<ul style="list-style-type: none"> •T2A (CNAMTS) •MIG reproductible (DGOS)
Personne habilitée Responsable	•Chercheur, enseignant-chercheur	<ul style="list-style-type: none"> •Chercheur, enseignant-chercheur •Praticien hospitalier 	•Praticien hospitalier qualifié (DES Bio Med)



Recherche
Exploration
NGS/RNaseq...



Utilisation Recherche Clinique
Tests ciblés et NGS



Utilisation Clinique validée
Tests ciblés
NGS Constit. (et somatique)

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) initiative: methods of the EGAPP Working Group

Steven M. Teutsch, MD, MPH¹, Linda A. Bradley, PhD², Glenn E. Palomaki, BS¹, James E. Haddow, MD¹, Margaret Piper, PhD³, Ned Calonge, MD, MPH⁴, W. David Dotson, PhD^{2,6}, Michael P. Douglas, MS^{2,6}, and Alfred O. Berg, MD, MPH¹, Chair, on behalf of the EGAPP Working Group

Validité analytique

Validité analytique

Mesure fiable et précise d'un biomarqueurs

(performance du test, reproductibilité, répétabilité, robustesse, control qualité...)

Validité Clinique

Validité Clinique

Association fiable et précise du biomarqueurs avec une situation clinique

(valeur Pronostique ou Prédictive)

Validé généralement au travers d'études rétrospectives ...

Utilité Clinique

Utilité Clinique

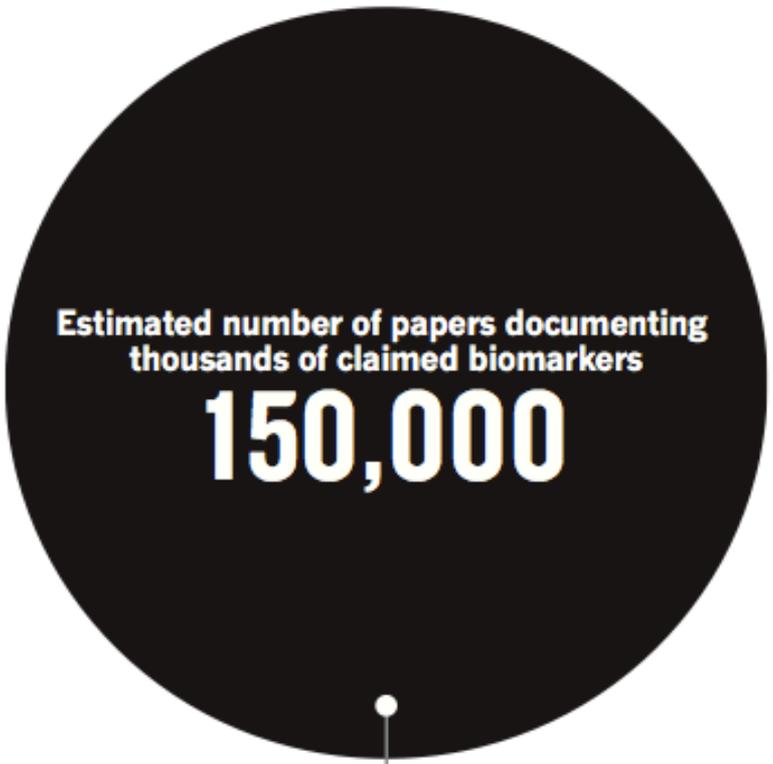
Association avec une décision pour la prise en charge thérapeutique basée sur les résultats d'un biomarqueurs

Validé généralement au travers d'études prospectives ... d'essais randomisés prospectifs ...ou de méta-analyse d'essais.

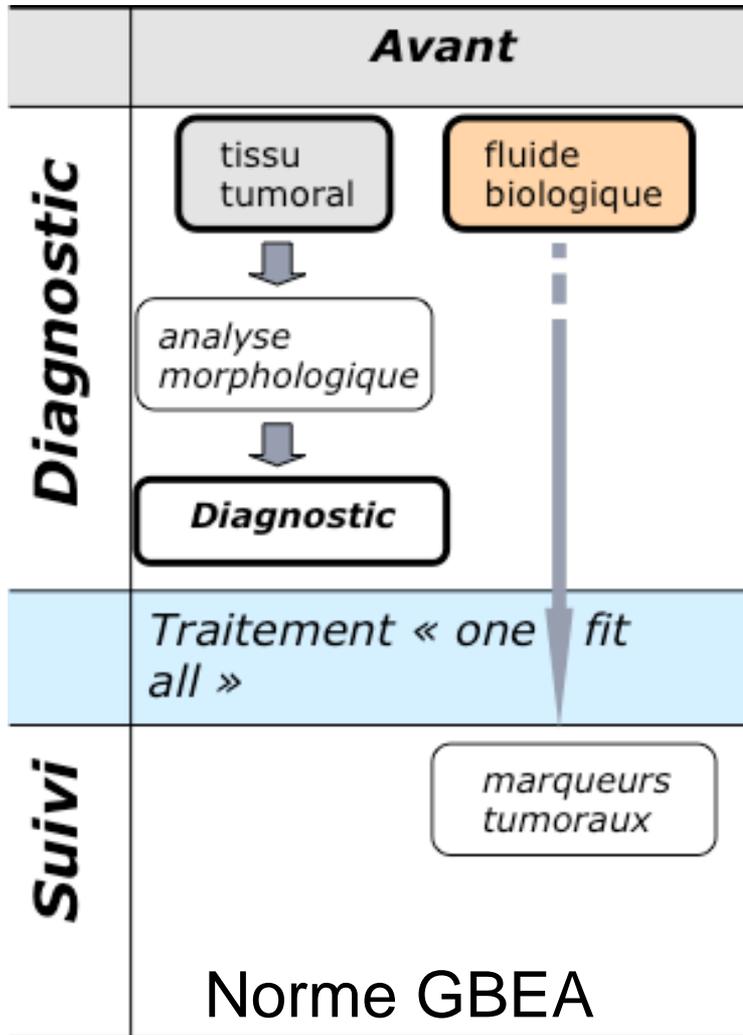
Une grosse différence entre le nombre de marqueurs publiés et le nombre de marqueurs utilisable en clinique

A DROP IN THE OCEAN

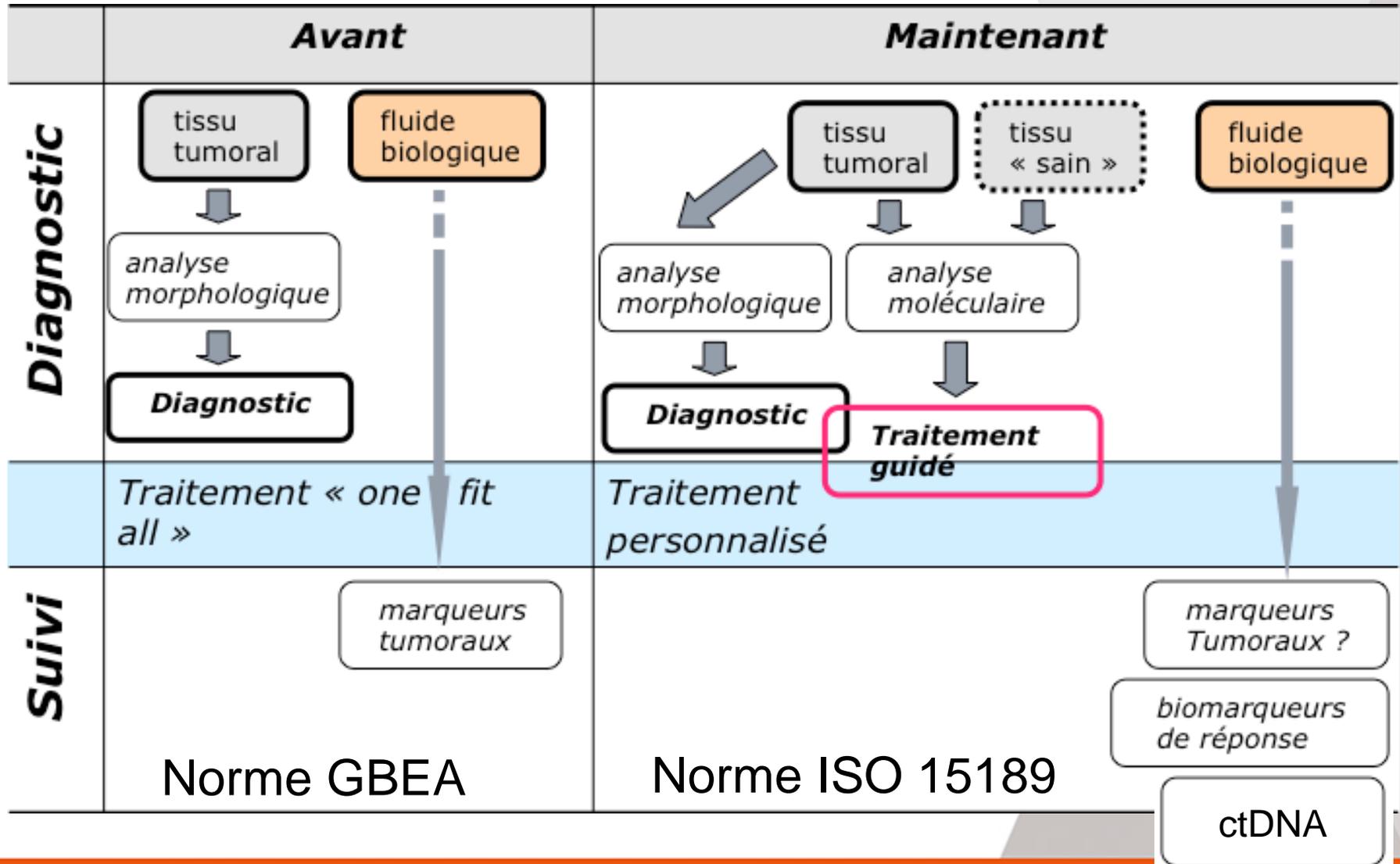
Few of the numerous biomarkers so far discovered have made it to the clinic.



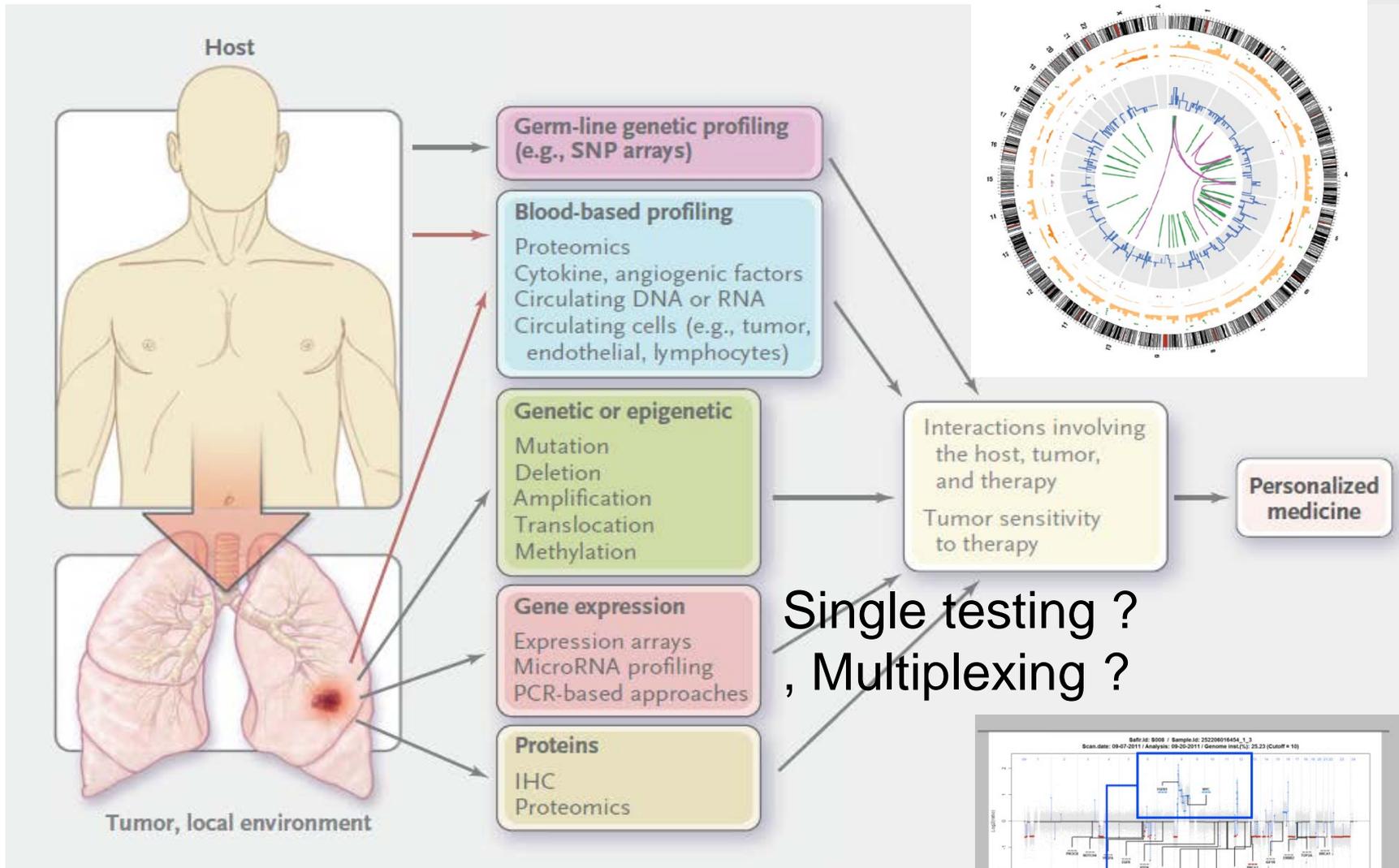
➤ *Change of Paradigm.*



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Witch Algorithm is the good one for daily practice?





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