



11H15

INTÉRÊT DES ANALYSES GÉNOMIQUES HAUT DÉBIT DANS LE CADRE DE L'INNOVATION CLINIQUE : EXPÉRIENCE DE GUSTAVE ROUSSY

16^e Journée
annuelle
du réseau
PROGRAMME



Réseau de cancérologie Centre-Val de Loire

6 DÉCEMBRE
2018

Espace Béraire
La Chapelle-Saint-Mesmin

OncoGériatrie
Centre-Val de Loire



GUSTAVE
ROUSSY
CANCER CAMPUS
GRAND PARIS

UNIVERSITÉ
PARIS
SUD

Instituts
thématisques

Inserm
Institut national
de la santé et de la recherche médicale

GFCO
www.gfco.fr

Dr Etienne Rouleau

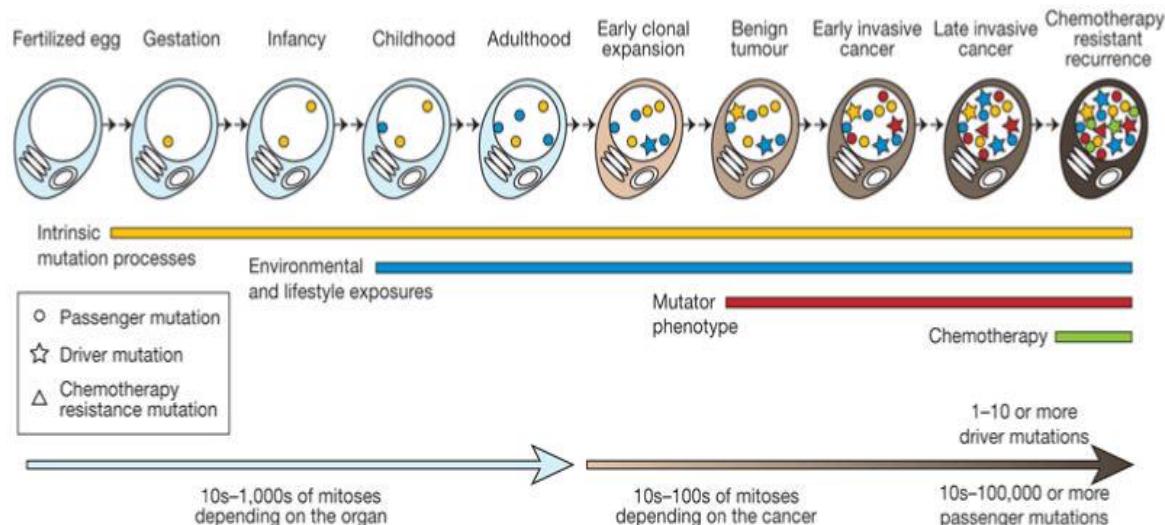
Chef du Service de Génétique des Tumeurs
Département de Biologie et Pathologie Médicales
Plateforme de Biopathologie Moléculaire, Unité AMMIca UMS3655-US23



Analyses génomiques haut débit

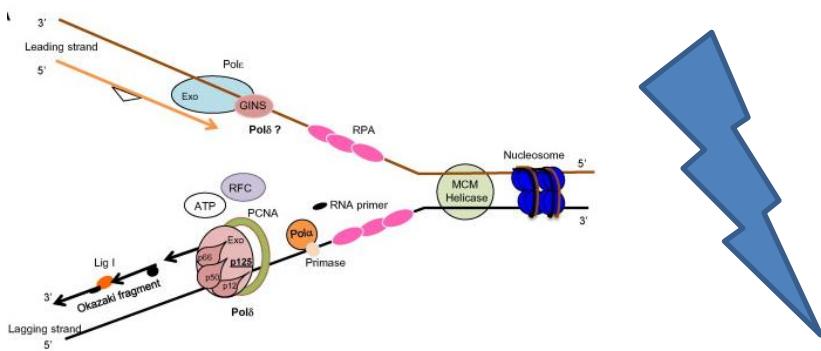
Pourquoi étudier la génomique dans le cancer ?

- La tumorigenèse concerne toutes les étapes menant à la formation des tumeurs.
- Cette progression est essentiellement dépendante d'une accumulation séquentielle de mutations dans les cellules tissulaires, une partie d'entre elles ayant une capacité de transformation maligne.

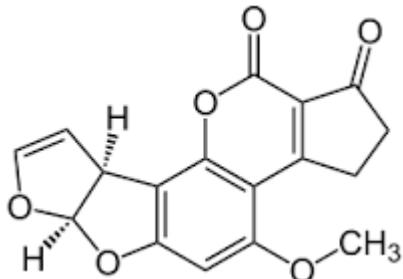


Tumorigénèse

Causes de mutations

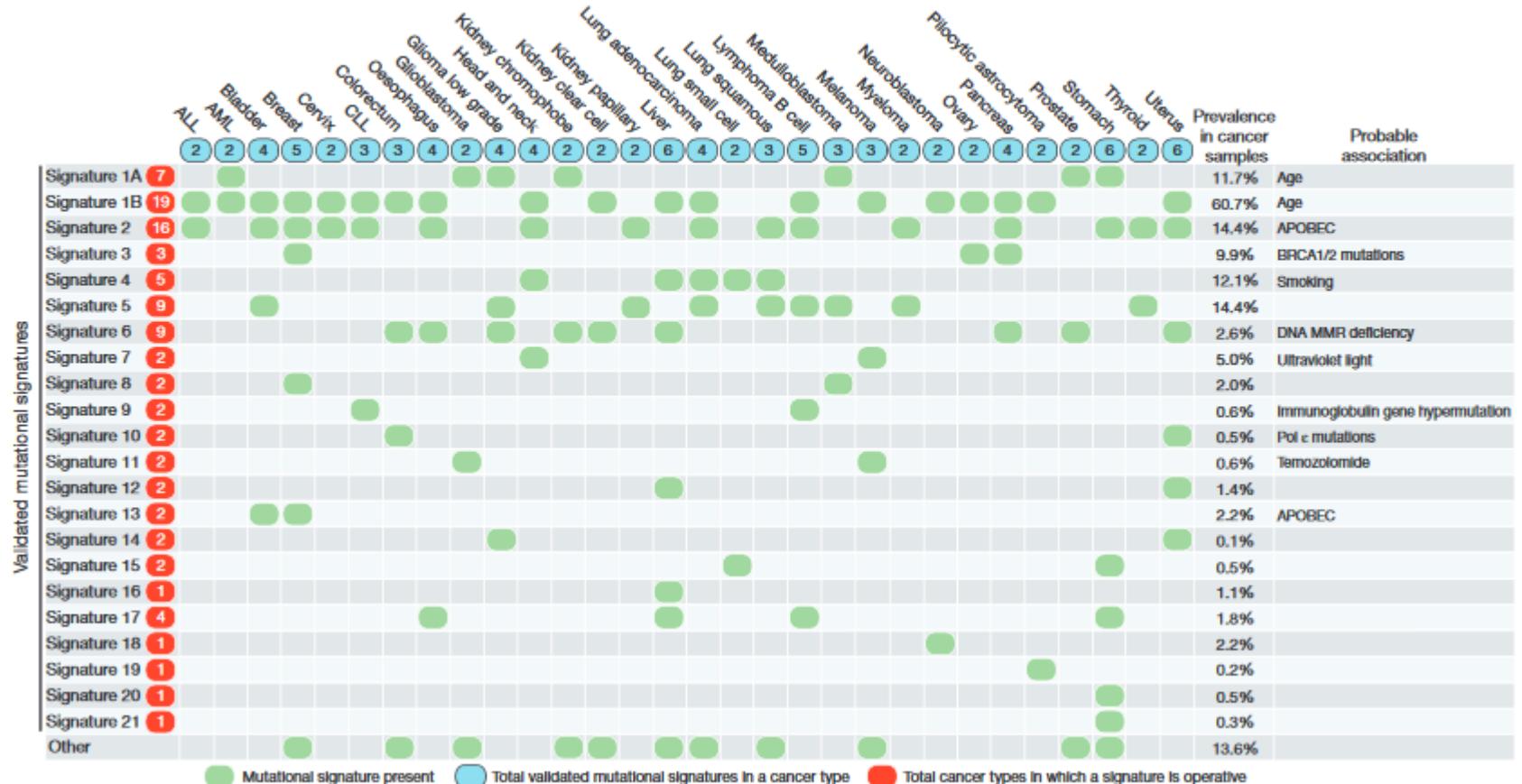


TAAGGACTACCCGTCTAATGTCAACCCTGTTCTTAAACCTAGA...



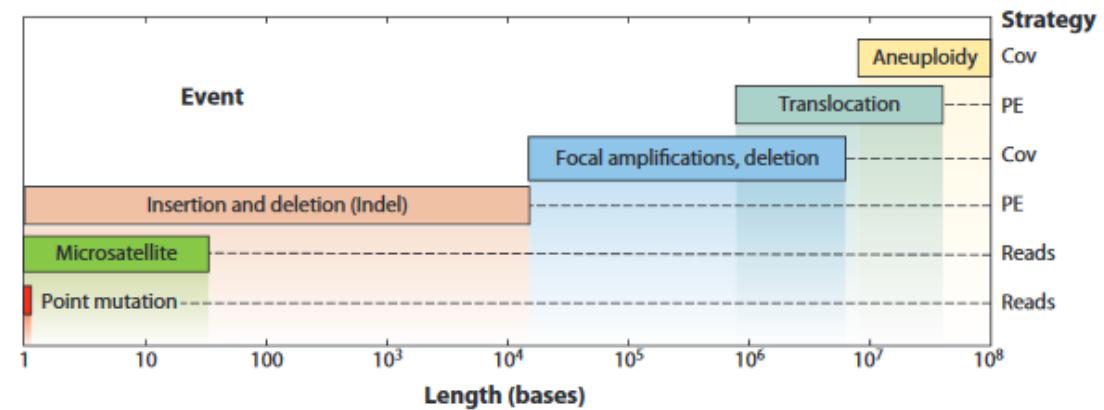
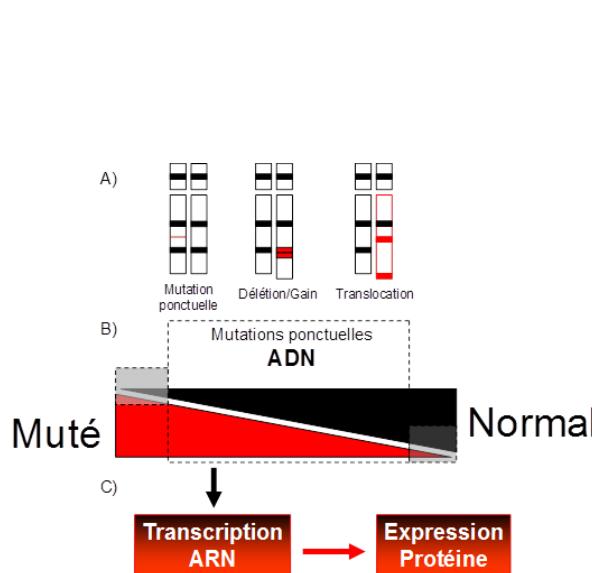
Signatures mutationnelles

ARTICLE RESEARCH



Multiplicité des altérations génomiques

- L'altération de quelques gènes suffit à déclencher la tumorigénèse
- Il peut exister un terrain génétique favorable au déclenchement du processus tumoral
- Certains virus peuvent aussi favoriser ces développements (ex. HPV)



Wang, Linghua, et David A. Wheeler. « Genomic Sequencing for Cancer Diagnosis and Therapy ». *Annual Review of Medicine* 65, n° 1 (2014): 33-48.

Découverte des mutations

Séquençage de première génération

Séquençage de 1ère génération

Approche Sanger – gènes candidats

Identification des altérations majeures
(drivers)

Mutations majoritaires / hotspot

Séquençage des exons (« exomes »)

BRAF mélanome

EGFR poumon

JAK2, ALK, PIK3CA,

IDH1 glioblastome

KRAS cancer du pancréas



1973 Dr Janet Rowley LMC

Rowley, J. D. « Letter: A New Consistent Chromosomal Abnormality in Chronic Myelogenous Leukaemia Identified by Quinacrine Fluorescence and Giemsa Staining ». *Nature* 243, n° 5405 (1 juin 1973): 290-93.

1982 HRAS et cancer de la vessie

Reddy, E. Premkumar, Roberta K. Reynolds, Eugenio Santos, et Mariano Barbacid.
Nature 300, n° 5888 (11 novembre 1982): 149-52.

A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene

E. Premkumar Reddy, Roberta K. Reynolds, Eugenio Santos & Mariano Barbacid

Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

The genetic change that leads to the activation of the oncogene in T24 human bladder carcinoma cells is shown to be a single point mutation of guanosine into thymidine. This substitution results in the incorporation of valine instead of glycine as the twelfth amino acid residue of the T24 oncogene-encoded p21 protein. Thus, a single amino acid substitution appears to be sufficient to confer transforming properties on the gene product of the T24 human bladder carcinoma oncogene.

Découverte des mutations

Séquençage de première génération

- 26 juin 2000 Consortium international public de séquençage *Human Genome Project (HGP)* et son concurrent privé lancé par M. Craig Venter, *Celera Genomics Corp*
- Avril 2003 - premier génome humain entièrement séquencé.
- *HGP* sur 15 ans : 2,7 milliards de dollars
- Passage à la nouvelle génération
 - Microfluidique
 - Informatique

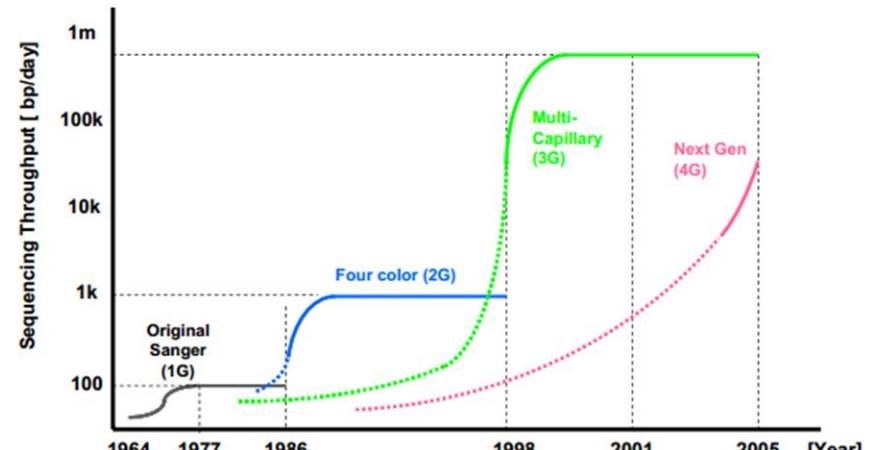


Figure 2. S-curves for the genome sequencing tool industry.

Découverte des mutations

“Séquençage de masse”

Séquençage génome entier

Identification de réarrangements

Capacité : 90Gb

Ex. Prostate translocation TMPRSS2-ERG

Tomlins SA et al Science 2005 644-648

Ex. Poumon translocation EML4-ALK

Soda M et al Nature 2007 561-566

Séquençage de l'exome

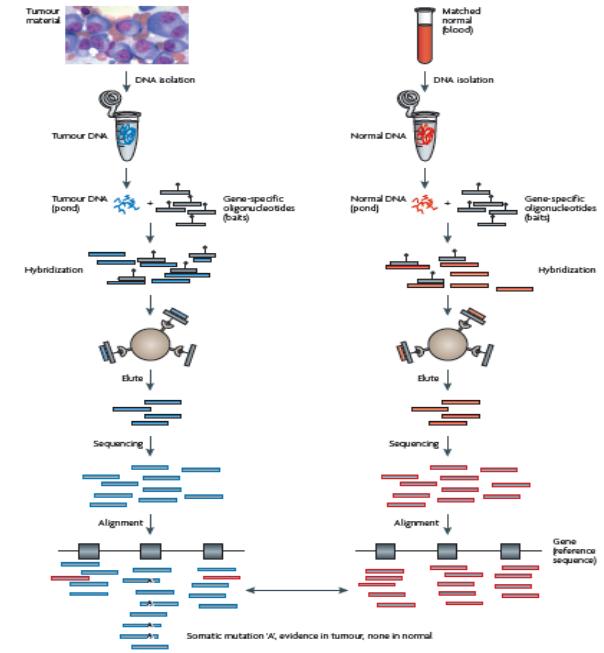
Sans sélection a priori des gènes

Capacité : 3Gb

Coût exome : **800€/exome**

RNASeq séquençage des ARN

Identification des fusions



Meyerson M, Gabriel S, Getz G.
Advances in understanding cancer genomes through second-generation sequencing.
Nat Rev Genet. 2010 Oct;11(10):685-96.

« Next Generation Sequencing – NGS »

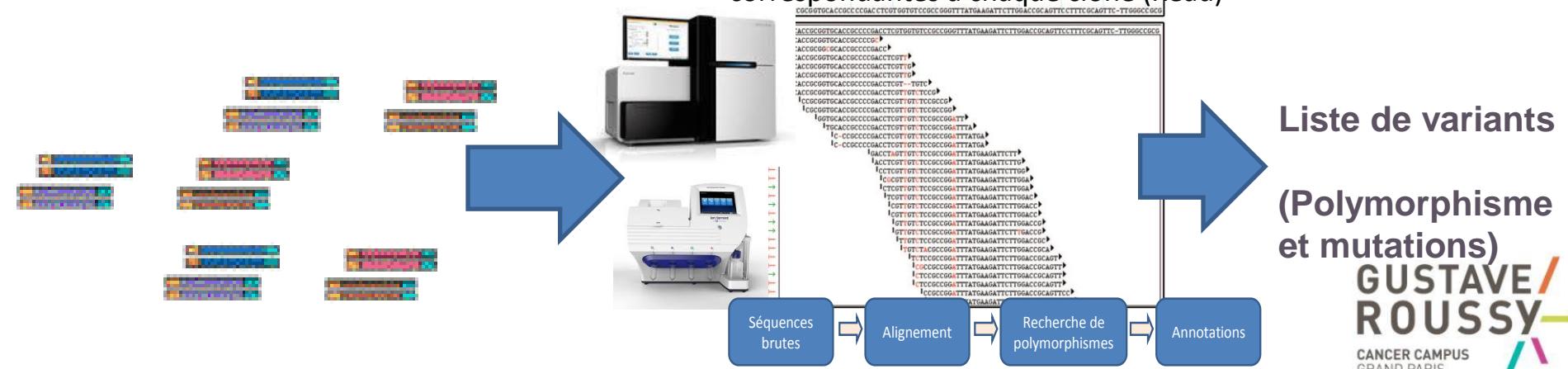
2005 première étude NGS, en 2016 = outil de base

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

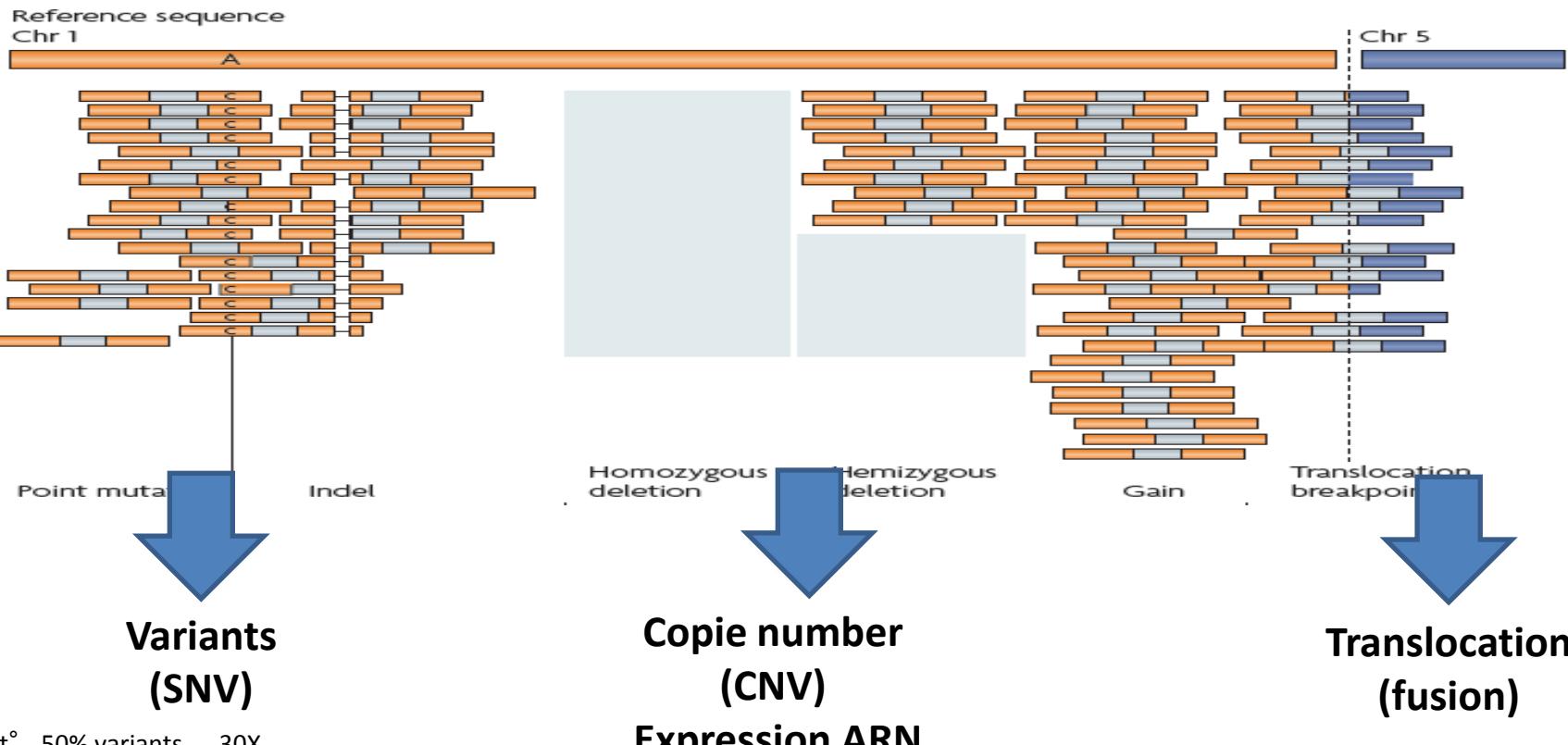
- 4 étapes: 1-Préparation de “library”
 2-Sequencage
 3-Bioinformatique
 4-Compte rendu clinique

ShotGun => Whole Genome / RNAseq
Capture => Exome / Panel / RNAseq ciblé
PCRmultiple=> Panel / fusion Ciblé

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

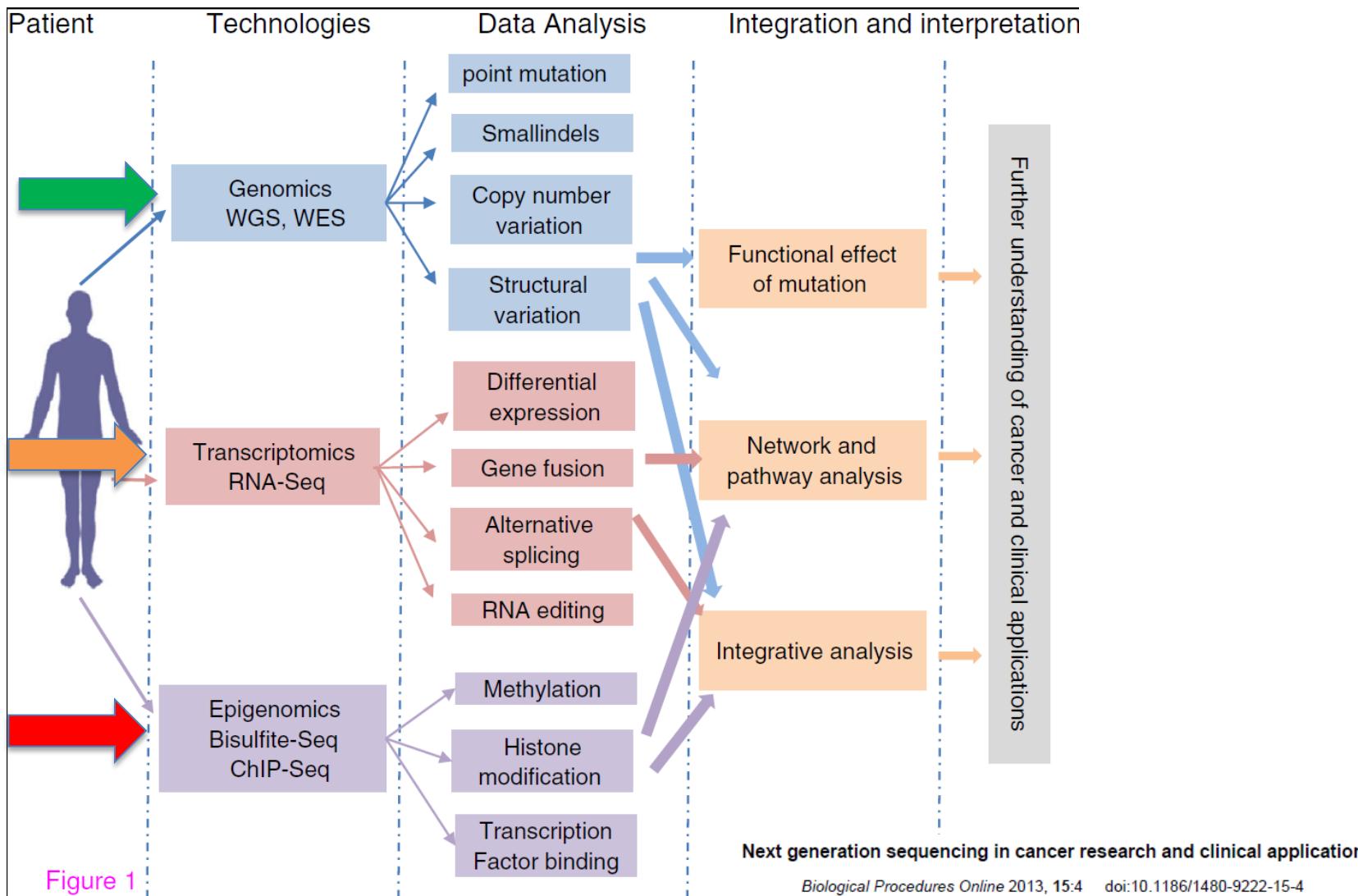


Next Generation Sequencing /Massive Parallel Sequencing



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



Biological Procedures Online 2013, 15:4 doi:10.1186/1480-9222-15-4

Outils et plateformes à disposition

Analyses moléculaires à GR

CLINICAL

Pathology and Medicine Laboratory department – **ISO15189**

Molecular Pathology and Genetic Services (Dr E. Rouleau)

- NGS (PGM / Illumina)
- qPCR / FISH / Caryotype



CLINICAL RESEARCH

Biomolecular Unit – **ISO 9001**(Dr. L.Lacroix)

- Sanger, Q-PCR, ddPCR, NGS (S5), Affy...
- Activity by projet (gene panel)
- GR and Collaboration Funding
- National EQ organisation



Integragen:
Next Seq500
Exome – RNAseq
-ICE/Mercury



RESEARCH

Genomic Platform (Dr.N.Droin)

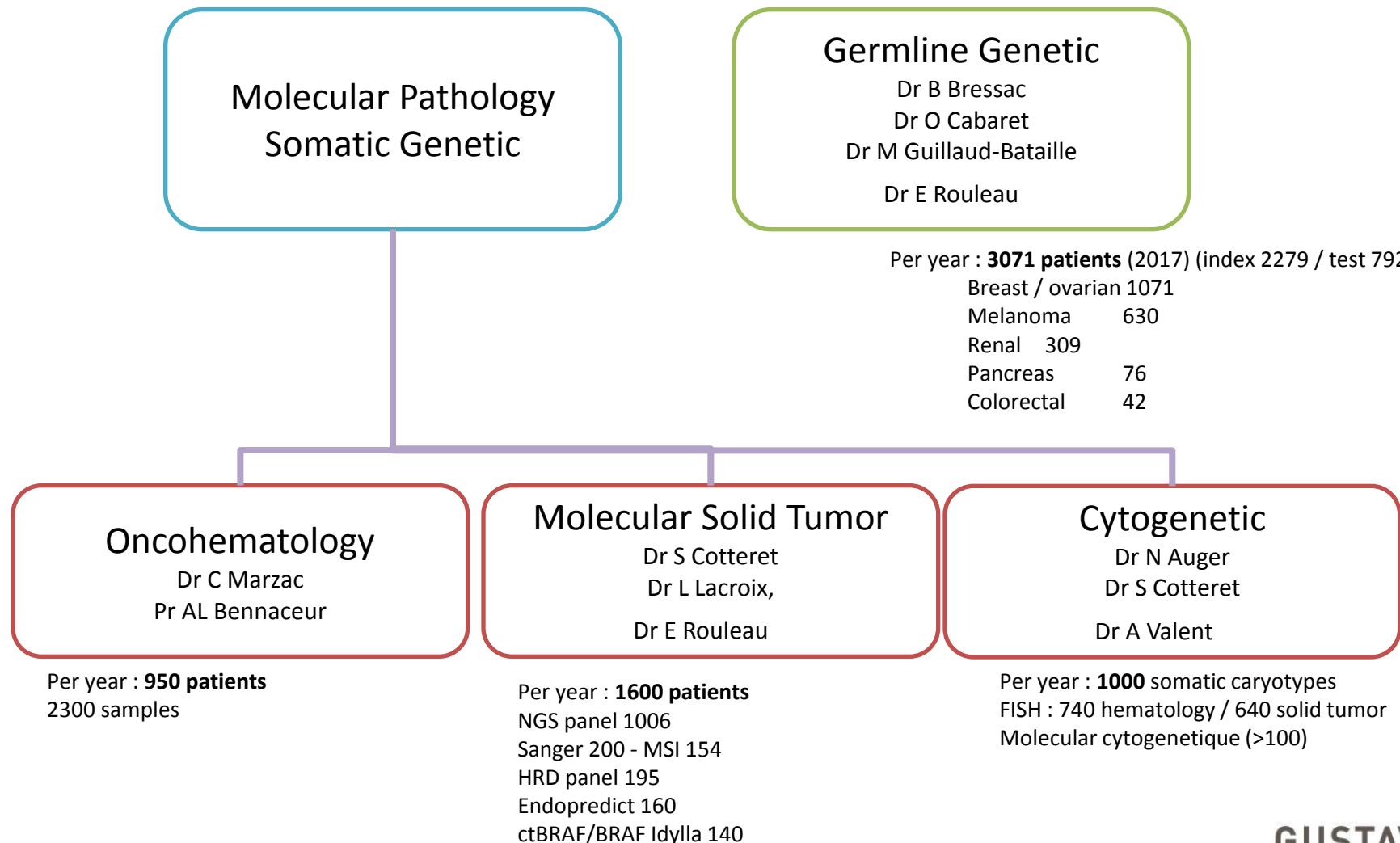
- PCR, GE, CGHa, NGS (NovaSeq/Myseq)
- Activity by projet (gene panel)
- GR and Collaboration Funding

Ptf AMMICa
US23/UMLS3655



Service de Génétique des Tumeurs

Clinical activities



40 persons with 9 biologists, 1 bioinformatic person, 3 engineers and 27 technicians

CLINICAL RESEARCH : RESEARCH platform

● Technologies



❖ PLATEFORME de MICROARRAY (Affymetrix™)

- **SNParrays** Cytoscan/Oncoscan (fixé / congelé / circulant)
- **Gene Expression** - HTA / MTA / RTA (Homme, Souris)
 - Puce Epissage
 - Puce Pico FFPE



❖ PLATEFORMES DE SEQUENCAGE NOUVELLE GENERATION (NGS)

- **Séquençage Haut débit** (HiSeq , Illumina)
 - Exome (Homme, Souris)
 - RNA-Seq (Homme, Souris)
 - ChIP-Seq (Homme, Souris)
- **Séquençage Ciblé** Ion-Torrent (Life) ou MiSeq (Illumina)
 - AmpliSeq (CHP2, Oncomine, ADN circulant, design à façon...)
 - Amplicons (produits de PCR) / Capture



❖ PLATEFORME Q-PCR / DIGITALE-PCR

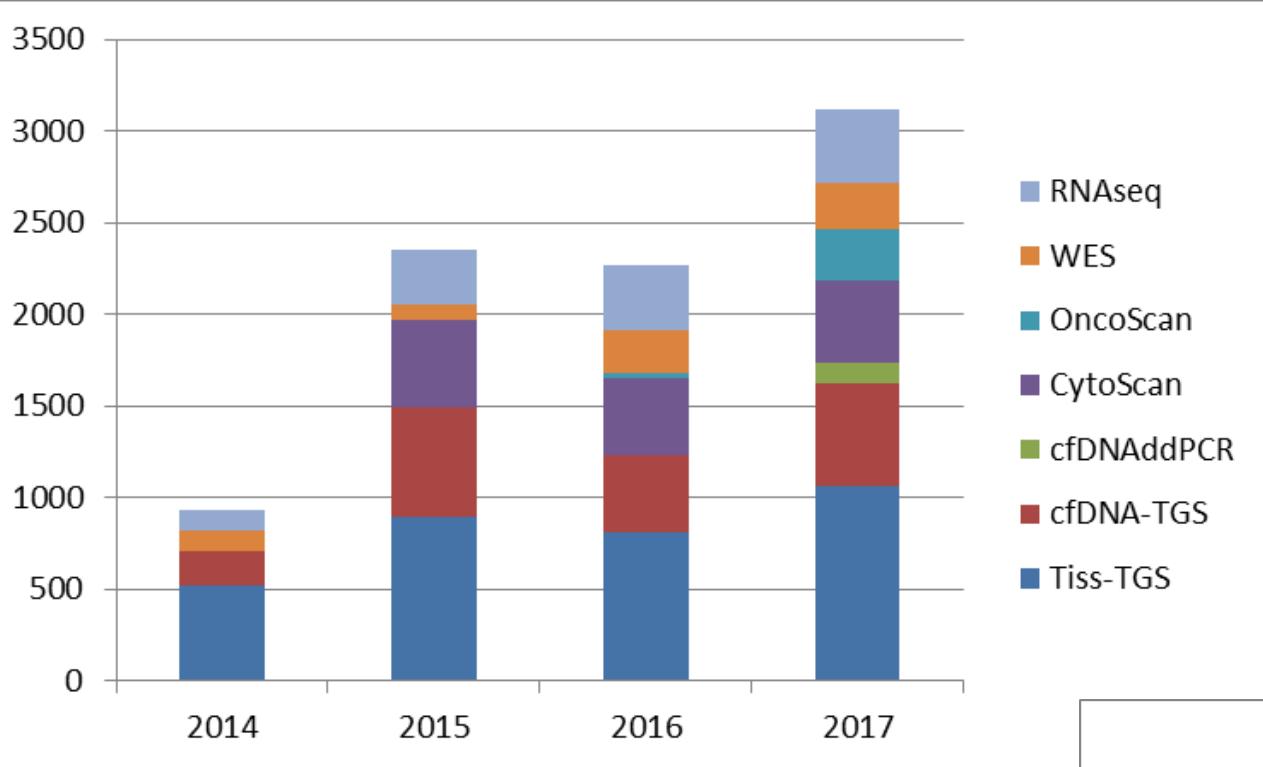
- 7900 / VIA VII (Life Technologies)
- Quant Studio 3D (Qs3D Life technologies)
- Biomark (Fluidigm)
- Stillal Technologies (Cristal DigitalPCR)



❖ SINGLE CELL (Fluidigm™)

- C1
 - STA
 - DNA-seq
 - RNA-seq
 - Eva Green
 - Taqman
 - 96:96 / 192:24 / 48:48
- Biomark

MP tumeurs solides



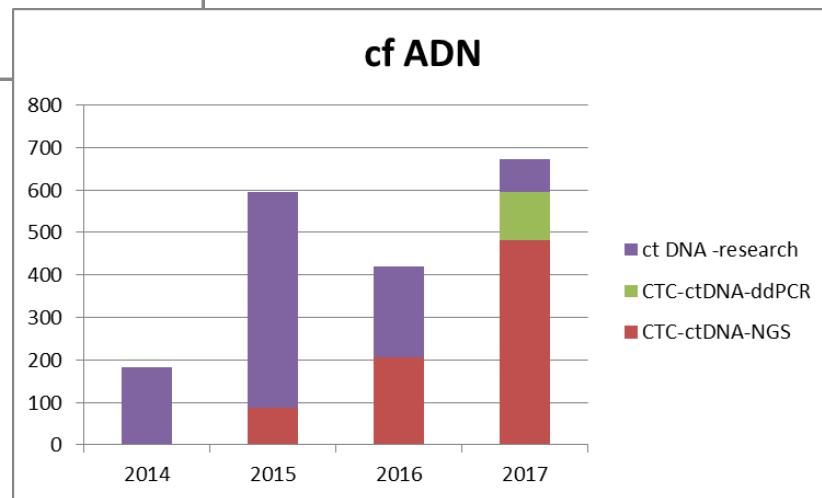
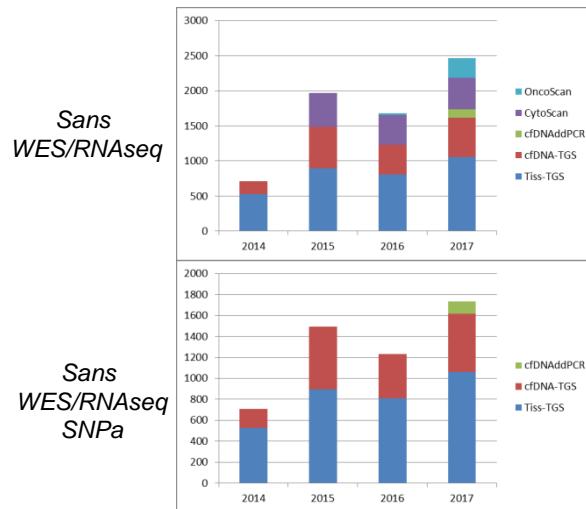
Délai < 10jrs (8,2 pour moscato)

Responsable : Ludovic Lacroix

Biologistes : S Cotteret, N Auger, E Rouleau

15 personnes : 4 biologistes, 9 techniciens, 2 ingénieurs

	2014	2015	2016	2017
Tiss-TGS	526	895	811	1059
cfDNA-TGS	182	596	420	560
cfDNAAddPCR	0	0	0	114
CytoScan	0	479	424	450
OncoScan	0	0	24	281
WES	114	83	238	255
RNAseq	111	296	349	395



MP Hématologie

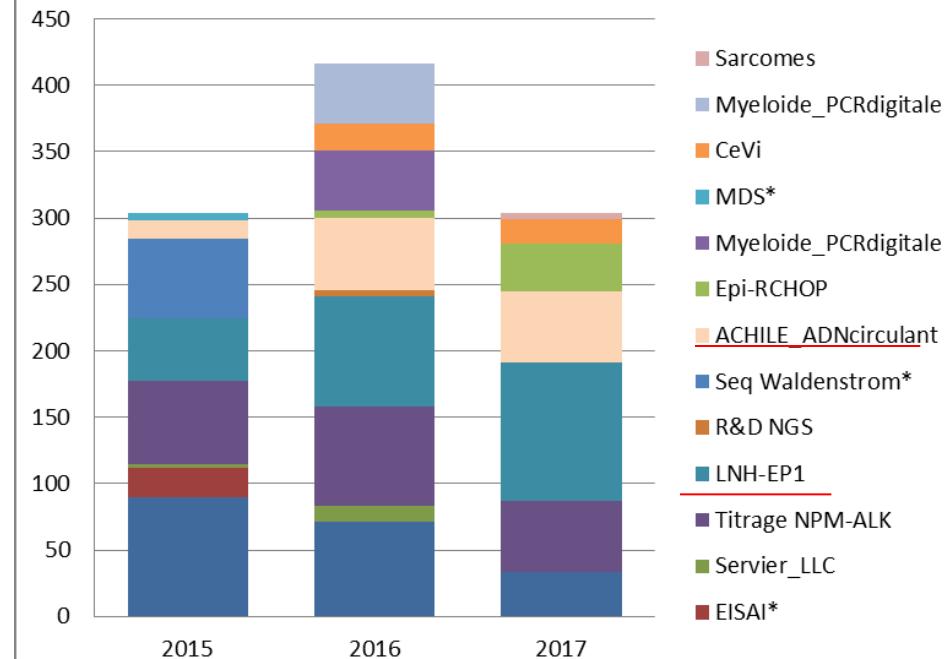
n° RT	Projet	2015	2016	2017
RT09413	S-Hemo	90	71	33
RT09613	EISAI*	22	/	/
RT15313	Servier_LLC	3	12	0
RT07614	Titrage NPM-ALK	62	75	54
RT11514	LNH-EP1	47	83	104
RT11814	R&D NGS	0	5	0
	Seq Waldenstrom*	60	/	/
RT12014	MDS*	6	/	/
RT05716	ACHILE_ADNcirculant	14	54	54
RT09216	Epi-RCHOP	/	6	36
RT09216	CeVi	/	20	18
RT09516	Sarcomes	/	/	5
RT01017	Myeloide_PCRdigitale	/	45	3
TOTAL:		304	371	307

* projet académique et industriel de séquençage

Contrat Industriel :

n° RT	Projet	2015	2016	2017
RT11015	MCL CD70/CD27	42 (1 exp)	42 (1 exp)	84 (2 exp)

Suivie de l'évolution du nombre d'échantillons réceptionnés dans les différentes projets du module d'Hématologie.



Activité de médecine personnalisée en Héma

Secteur 7 / 12

- 158 réceptions
- 104 LNH-EP1
- 54 Achile

Secteur 8

- 166 extractions d'ADN
- 56 échantillons à + 30% CT
- 52 cfDNA
- 58 échantillons (non tumoral)

Secteur 9

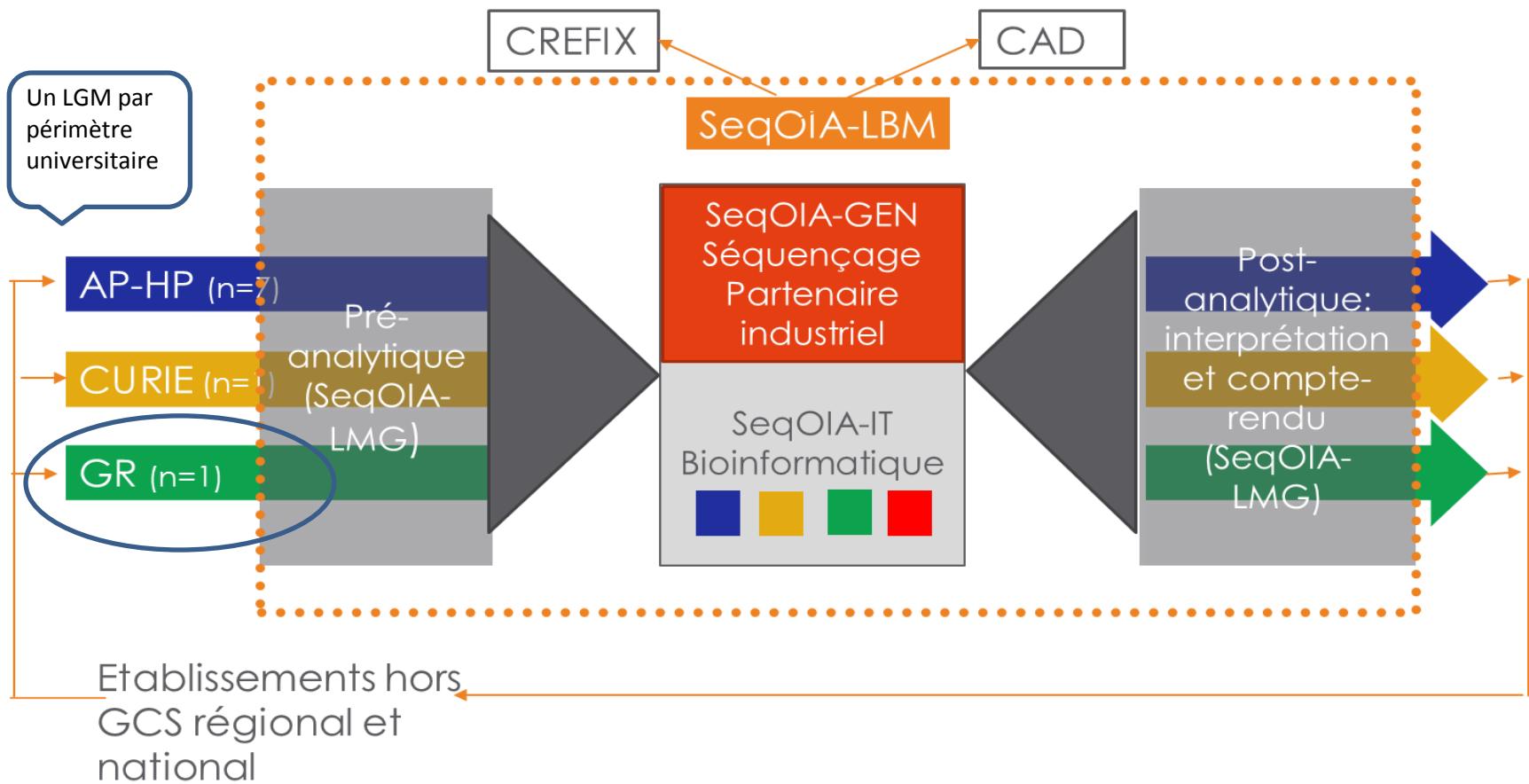
- 141 librairies validées
- 68 librairies tumorales
- 25 librairie « normales »
- 48 librairies sur cfDNA

Secteur 14

- 55 CR générés pour LNH-EP1

Dr. V.Camara

Implication dans le projet France Génomique



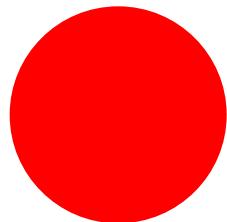
CrefIX= Centre de référence technologique, d'innovation, et de transfert / CAD= Collecteur analyseur de données

Genome
3,000,000,000 base pairs

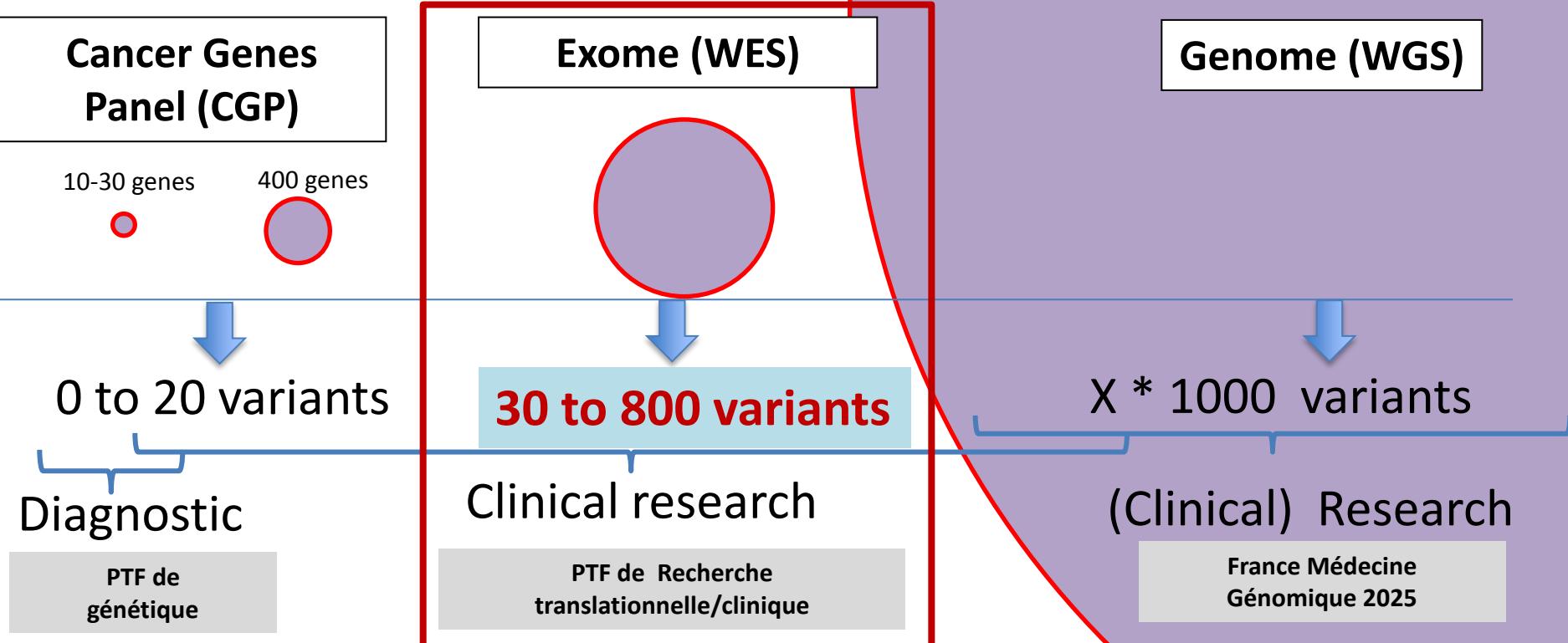
Exome
30,000,000 base pairs
(20,000 protein-coding genes)

Etude ciblée
Qq gènes

GENOME : 3,4 billion bases (Gb)
EXOME : 1% du génome 30Mb
>26 000 GENES



Outils



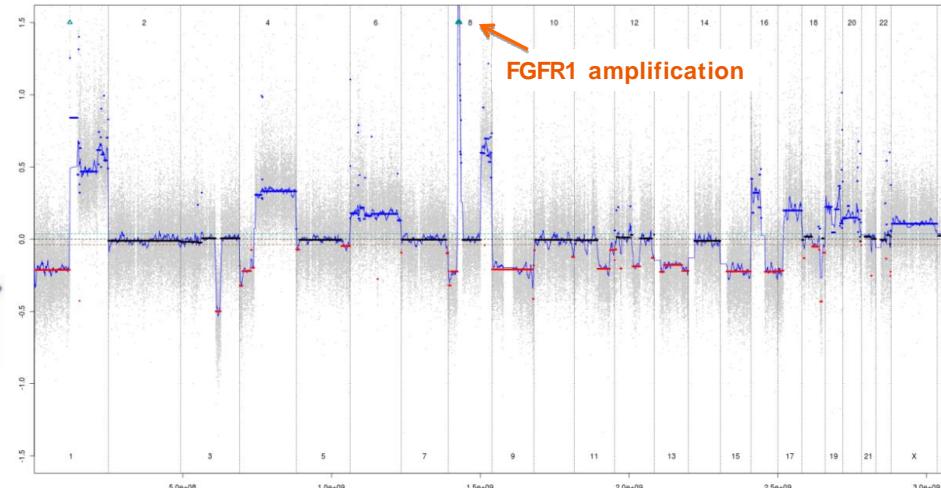
➤ ***Production de nombreuses informations génomiques***

Outils

Hotspot genes				Full-length genes			Copy number genes		Gene fusions (inter- and intragenic)		
AKT1	FOXL2	MET	AKT2	ATM	TP53	MSH6	AKT1	PPARG	ALK	RET	NF1
ALK	GATA2	MTOR	AKT3	BAP1	TSC1	NBN	AR	TERT	AXL	ROS1	NOTCH1
AR	GNA11	MYD88	AXL	BRCA1	TSC2	NOTCH2	CCND1	AKT2	BRAF	AKT2	NOTCH4
ARAF	GNAQ	NFE2L2	CCND1	BRCA2	ARID1A	NOTCH3	CCNE1	AKT3	EGFR	AR	NRG1
BRAF	GNAS	NRAS	CDK6	CDKN2A	ATR	PALB2	CDK4	ALK	ERBB2	BRCA1	NTRK2
BTK	HNF1A	PDGFRA	ERCC2	FBXW7	ATRX	PMS2	CDK6	AXL	ERG	BRCA2	NUTM1
CBL	HRAS	PIK3CA	FGFR4	MSH2	CDK12	POLE	EGFR	BRAF	ETV1	CDKN2A	PDGFRB
CDK4	IDH1	PPP2R1A	H3F3A	NF1	CDKN1B	RAD50	ERBB2	CCND2	ETV4	ERB84	PIK3CA
CHEK2	IDH2	PTPN11	HIST1H3B	NF2	CDKN2B	RAD51	FGFR1	CCND3	ETV5	ESR1	PRKACA
CSF1R	JAK1	RAC1	MAP2K4	NOTCH1	CHEK1	RAD51B	FGFR2	CDK2	FGFR1	FGR	PRKACB
CTNNB1	JAK2	RAF1	MDM4	PIK3R1	CREBBP	RAD51C	FGFR3	CDKN2A	FGFR2	FLT3	PTEN
DDR2	JAK3	RET	MYC	PTCH1	FANCA	RAD51D	FGFR4	CDKN2B	FGFR3	JAK2	RAD51B
EGFR	KDR	RHEB	MYCN	PTEN	FANCD2	RNF43	FLT3	ESR1	NTRK1	KRAS	RB1
ERBB2	KIT	RHOA	NTRK1	RB1	FANCI	SETD2	IGF1R	FGF19	NTRK3	MDM4	RELA
ERBB3	KNSTRN	SF3B1	NTRK2	SMARCB1	MLH1	SLX4	KIT	FGF3	PDGFRA	MET	RSP02
ERBB4	KRAS	SMO	PDGFRB	STK11	MRE11A	SMARCA4	MDM2	NTRK1	PPARG	MYB	RSP03
ESR1	MAGOH	SP0P	PIK3CB				MDM4	NTRK2	RAF1	MYBL1	TERT
EZH2	MAP2K1	SRC	ROS1				MET	NTRK3			
FGFR1	MAP2K2	STAT3	SMAD4				MYC	PDGFRB			
FGFR2	MAPK1	U2AF1	TERT				MYCL	PIK3CB			
FGFR3	MAX	XPO1	TOP1				MYCN	RICTOR			
FLT3		MED12					PDGFR	TSC1			
							PIK3CA	TSC2			

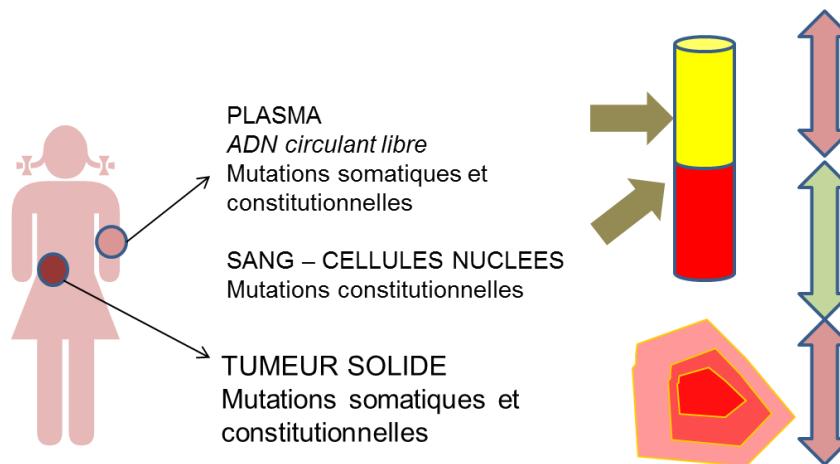


MERCURY

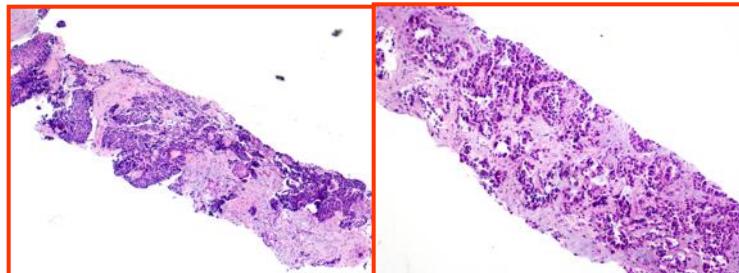
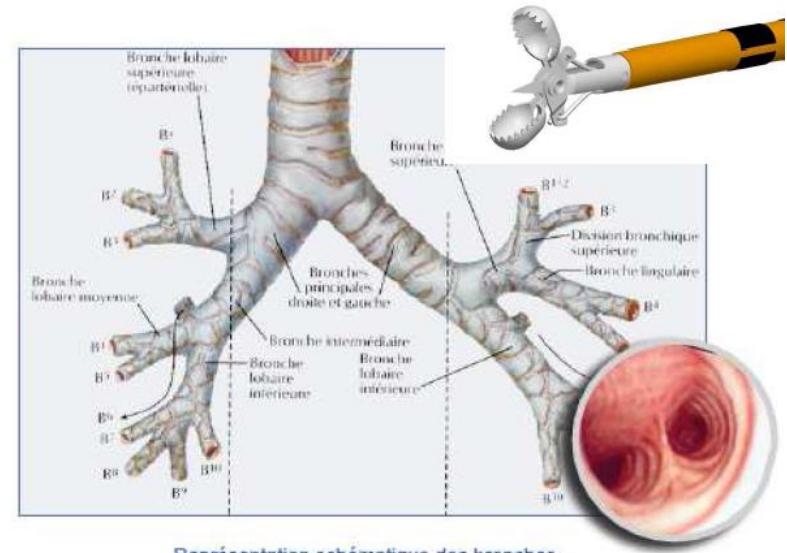
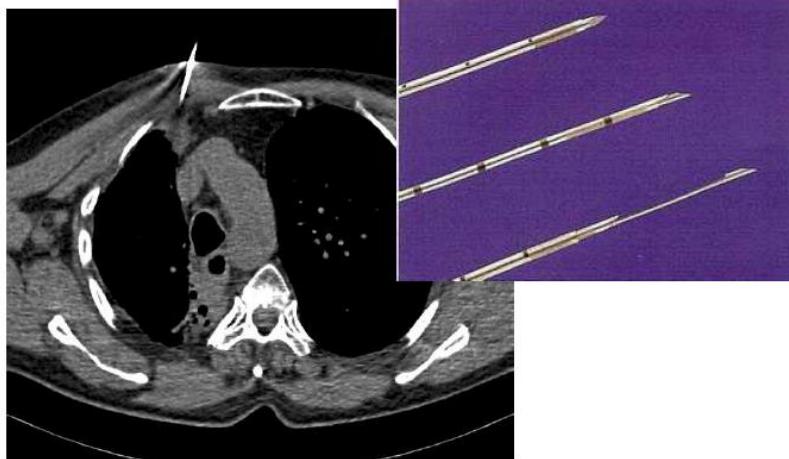


Nature du matériel étudié

- Tissu congelé – biopsie dédiée
- Tissu fixé et inclus en paraffine (FFPE)
- Prélèvement sanguin – constitutionnel
- Plasma – ADN circulant



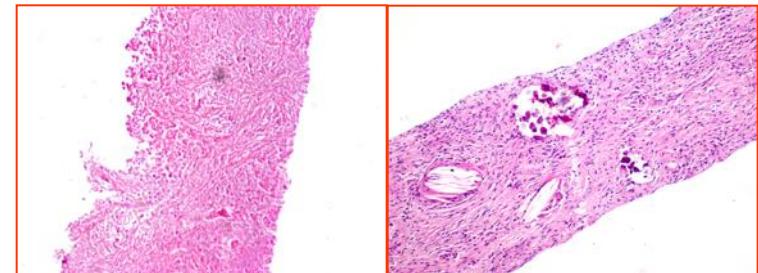
Importance du prélèvement



Squamous Cell Ca

Adenocarcinoma

Adequate Biopsies

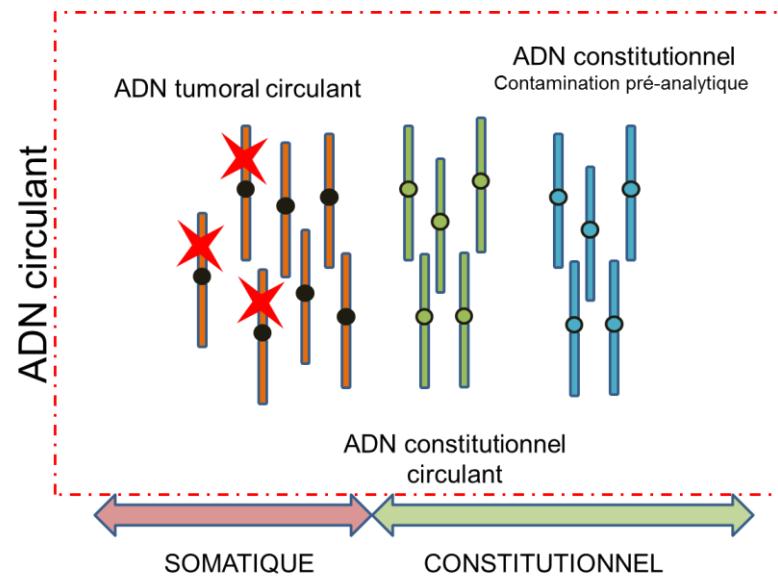
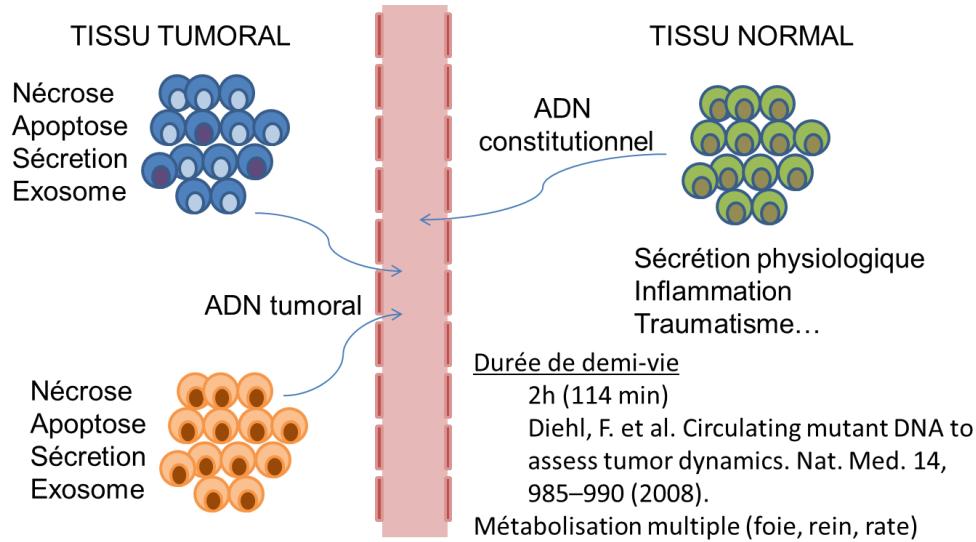


Necrosis

Fibrosis

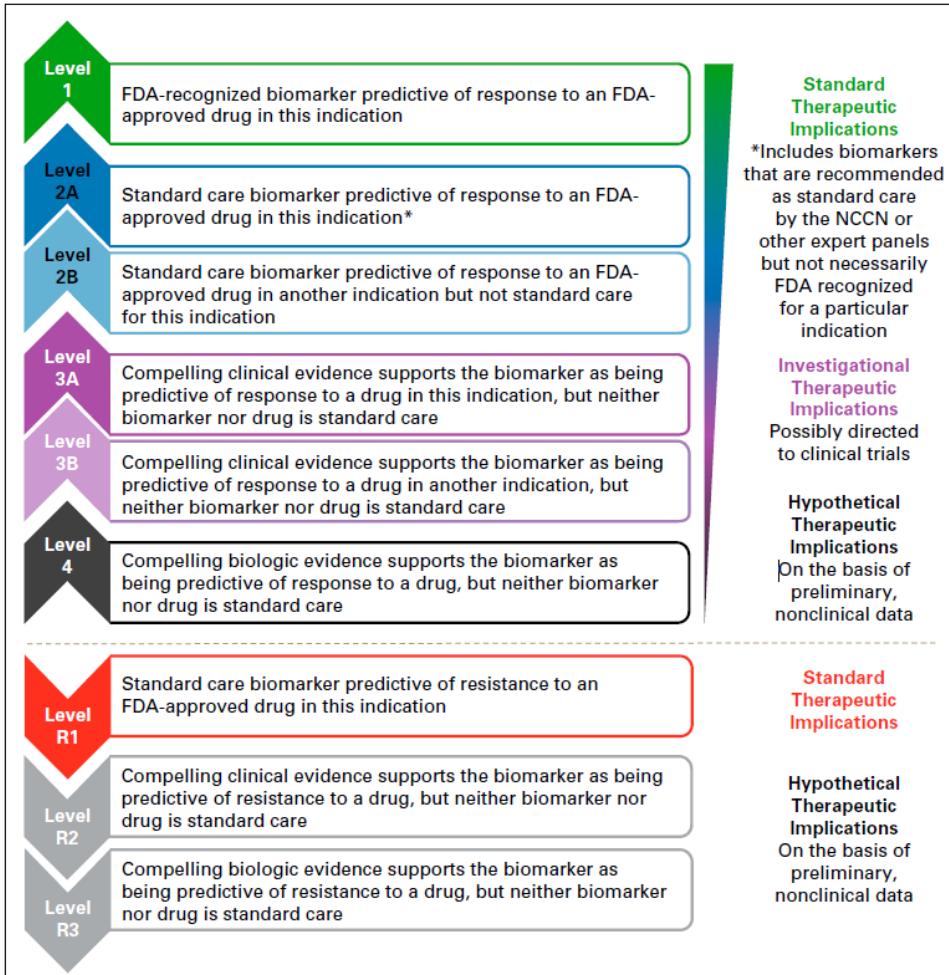
Inadequate Biopsies

ADN circulant



Principe de la médecine personnalisée

Interprétation des variants « actionabilité »

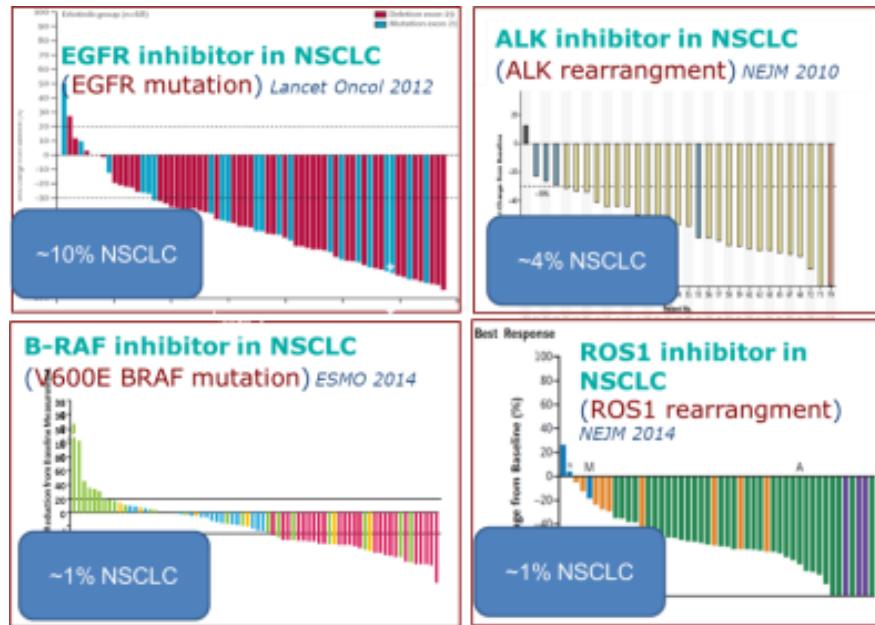
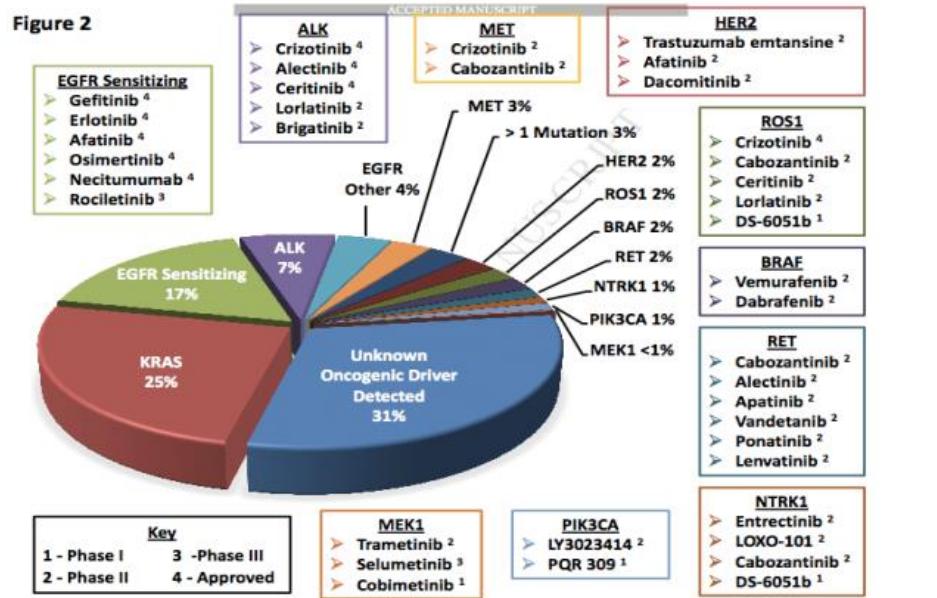


Gene alterations were considered ‘actionable’ based on predicted function, taking into account of gain or loss of function and when targetable directly or their pathway upstream or downstream



Modèle du cancer du poumon

Figure 2



Tsao – J Thorac Oncol 2016

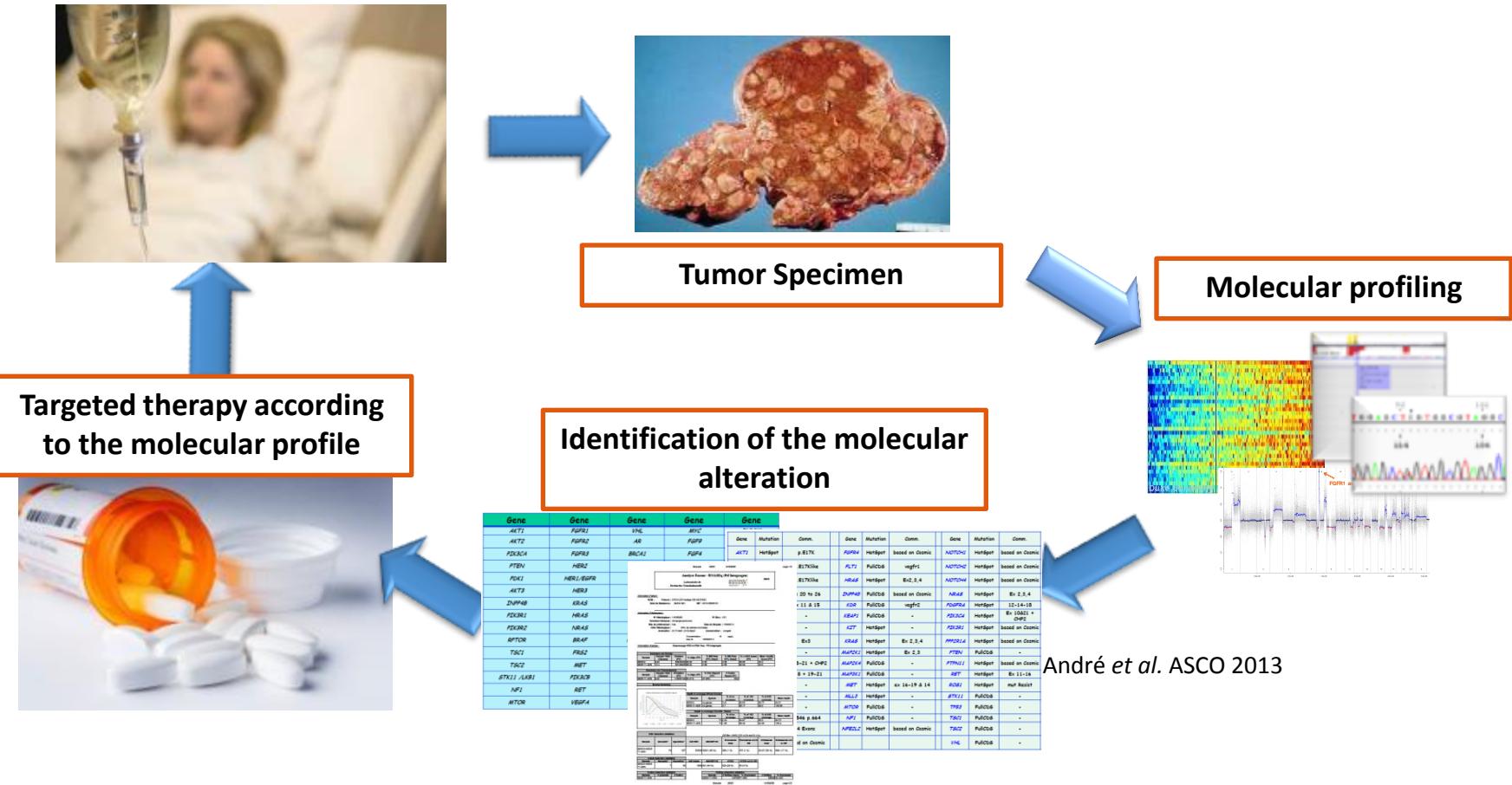
Biomarker Need for standard drug :

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

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

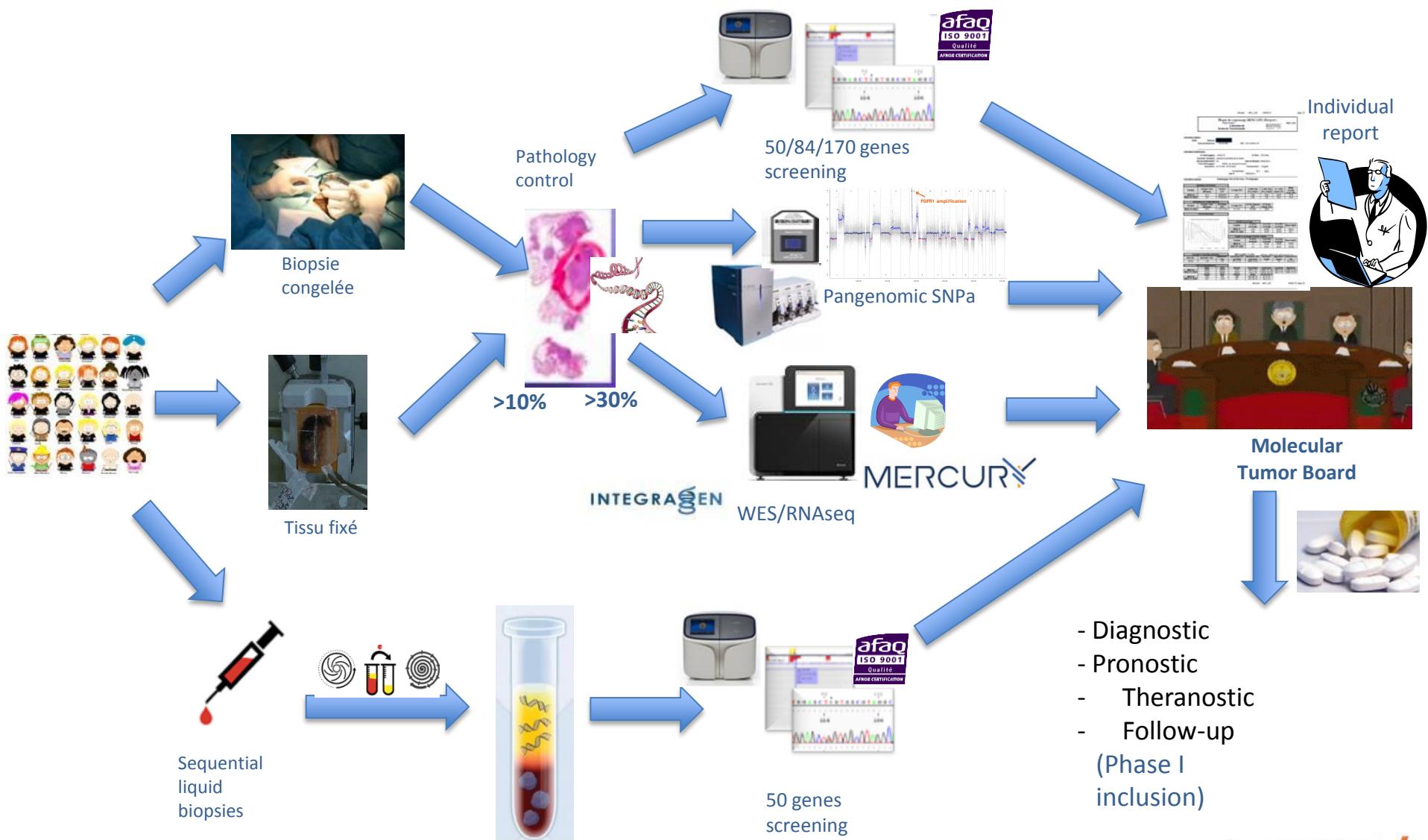
Concept de Médecine Personnalisée

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



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

Criblage moléculaire en recherche clinique



Trial or program name and origin	Genomics analysis techniques	Cancer types	Tumor sample analyzed	Nb patients	Clinical trial reference
MOSCATO 01/02 Gustave Roussy, France	TGS, CGHa, WES, RNAsq	Solid tumors eligible for Phase I trials	Fresh tissue biopsy	2000	NCT01566019
MULTIPLI Bergonié (pilot for Genomic Medicine Plan 2025, France)	WES	Sarcoma and advanced colon	Recent FFPE block (<3 mon) or fresh tissue biopsy	100 (goal: 235,000 genomes/yr)	-
PERMED Paoletti, France	WES	Advanced solid tumors	Fresh tissue biopsy	460	NCT02342158
ProfiLER 01/02 Leon Berard, France	TGS, WES	Advanced Solid Tumor	Recent FFPE block (<3 mon) or fresh tissue biopsy	2000	NCT 01774409 /NCT03163732
MATCHR Gustave Roussy, France	TGS, CGHa, WES, RNAsq	Solid tumor relapse	Fresh tissue biopsy	600	NCT02517892
Profiling Breast Cancer Avera McKennan Hospital	RPMA, TGS, IHC, WGS, RNA-seq, WES	Metastatic Breast Cancer or Advanced Gynecological Malignancies	Fresh tissue biopsy	500	NCT02470819
MAPPYACTS	WES, RNAseq	Pediatric	Fresh tissue biopsy	600	NCT02613962
CoPPO	WES, SNP Array and RNA-seq		Fresh tissue biopsy	500	NCT02290522
BIOMEDE Gustave Roussy, France	WES, RNAseq	Diffuse Intrinsic Pontine Glioma	Fresh tissue biopsy	250	NCT02233049
MASTER NCT Heidelberg	WES, RNAseq, WGS	Advanced states of all histologies and rare tumors	Biopsy	>550	-
EXaCT-1 Weill Cornell Medical College	WES	metastatic cancers	fresh tissue biopsy	97	-

Impact du profilage génomique et orientation thérapeutique

Group	Sample Size	Platform	Fresh Biopsy vs FFPE	Germ-line Control	Number and % of "Matched" Patients in Genotype-Matched Clinical Trials
Gustave Roussy MOSCATO	1,035	40-75 gene panels (Life) + CGH (Agilent) + RNA Seq	Fresh biopsy	Yes	$199/1035 = 19\%$
Institut Curie	741	46 gene panel (Life) + CNA (Affymetrix) +IHC	Fresh biopsy	No	195 randomized/741 = 26%
BCCA	100	Whole genome	Fresh biopsy	Yes	$1/100 = 1\%$
MD Anderson	2,000	11-50 gene panels (Life)	FFPE	No	$83/2000 = 4\%$
Princess Margaret	1,640	23-48 gene panels (Illumina, Life)	FFPE	Yes	$92/1640 = 5.6\%$

CNA = Copy number alterations; IHC = Immunohistochemistry

Massard et al. Cancer Dis 2017; LeTourneau et al. Lancet Oncol 2015; Laskin et al. Cold Spring Harb Mol Stud 2015; Meric-Bernstam et al. J Clin Oncol 2015; Stockley, Bedard et al. Genome Med 2016.

Implémentation du WES / RNASeq en recherche clinique

Techniques

Exome sequencing

- Capture SureSelect XT Clinical Research Exome (54 Mb) (Agilent Technologies®)
- Sequencing paired-end 75 bases.

Bionformatic :

Image analysis and base calling : Illumina Real Time Analysis (2.7.3) with default parameters.

Elandv2e (Illumina, CASAVA1.8.2) (then GATK/Mutect)
+ In-house algorithm

Variants annotation + Variant Effect Predictor

Normal cov depth 80X

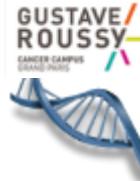
Tumor cov depth 100-150X

RNAseq

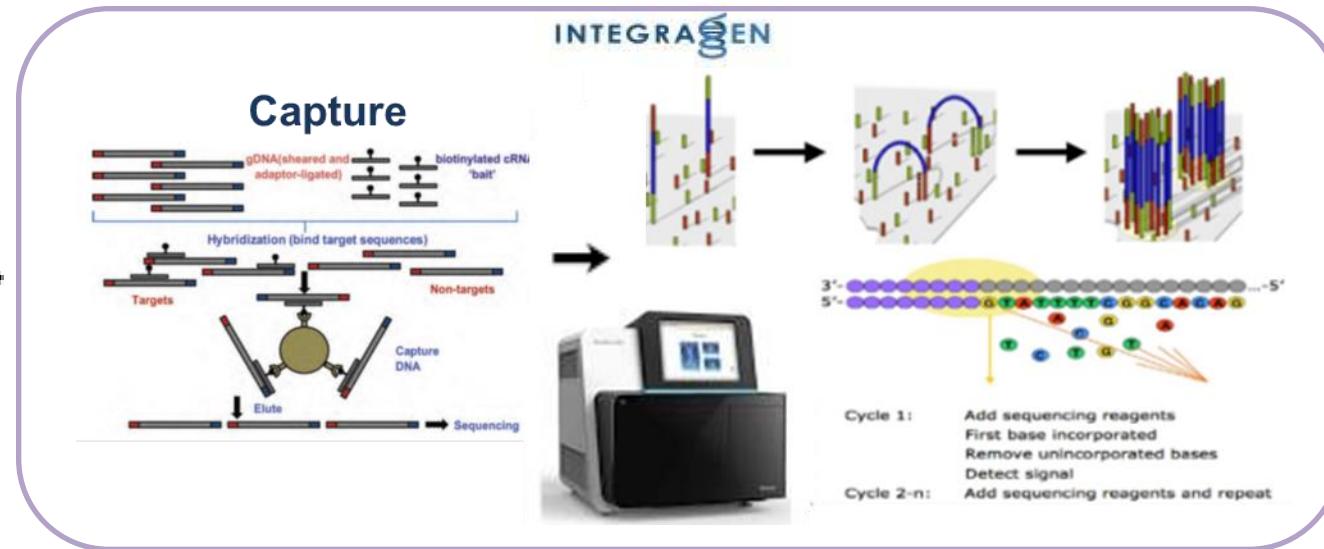
- TruSeq® Stranded mRNA kit (illumina)
- Sequencing paired-end 75 bases.
- Bionformatic :
 - TopHat2.1.0, including Bowtie2.
 - TopHatFusion—post algorithm + in-House script to annotate and translate the fusions+blast.
 - SNV & Indels : Samtools/BcfTools (Broad Institute).
 - Annotation idemDNA

RNASeq > 8 Gb (55 M PE)

Process intégré



Frozen
30% cellularity
DNA/RNA 500ng



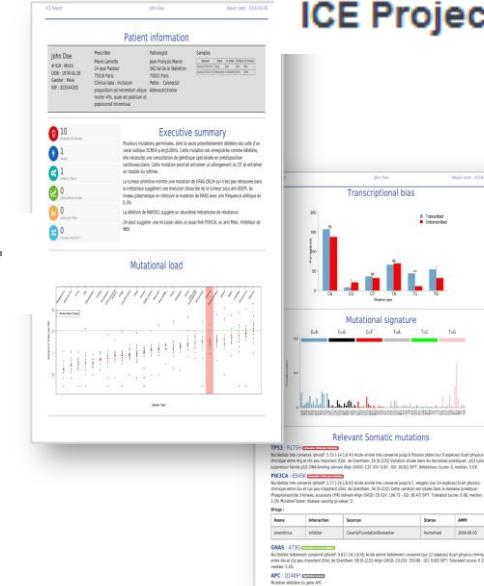
Oscar

INTEGRAGEN



06/12/2018

ICE Project

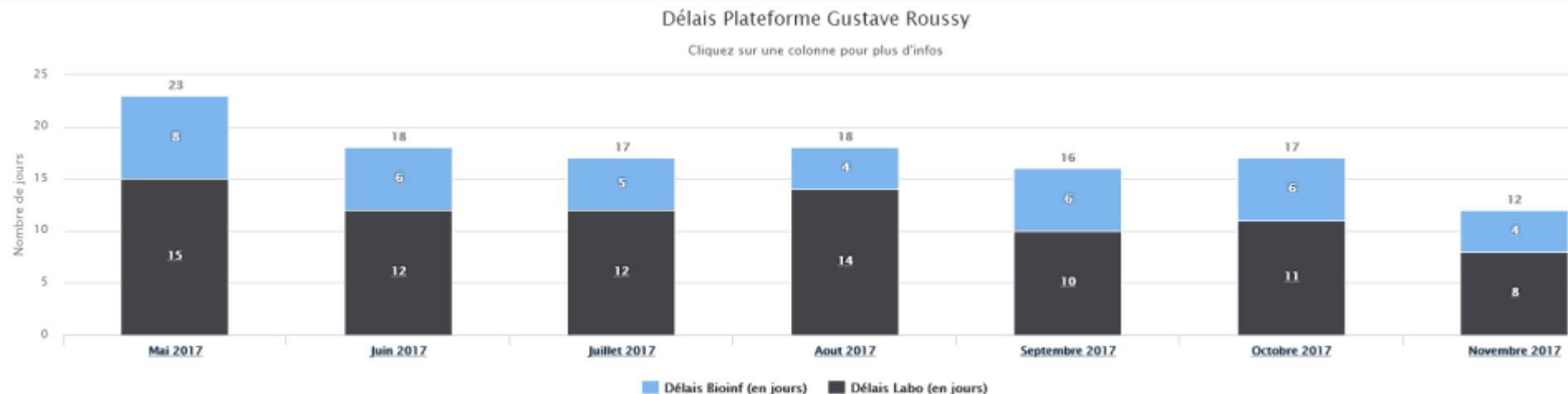


Normalized Report For Tumor board discussion

In silico Target.

Délai de rendu des résultats

12 to 23 days for libraries preparation+bioinformatique analysis

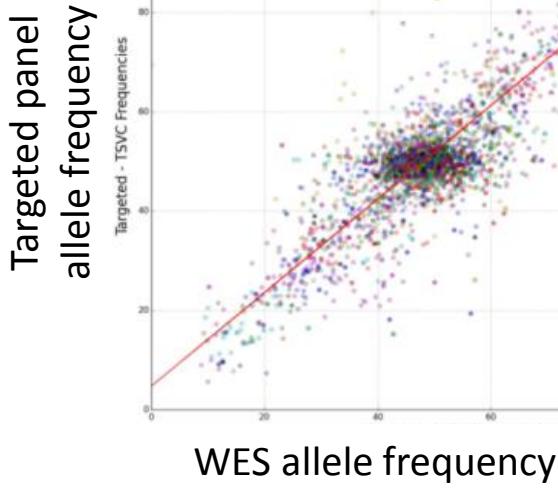


- + time between :
 - consent to biopsie (median 15 days)
 - biopsie / control / N.A extraction / transfert (5 days)
 - analyses (12 to 23 days)
 - reporting to molecular tumor board (5 days)

in total 1 to 1,5 month from « prescription » to discussion

Comparaison au Cancer Gene Panel - CGP

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



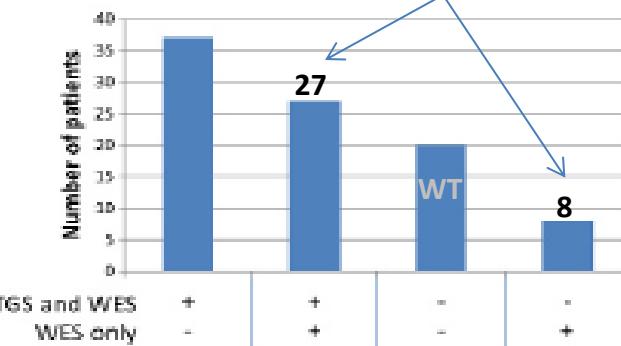
WES sensitivity: 94% (121/129)

Good correlation between WES and TGS AF

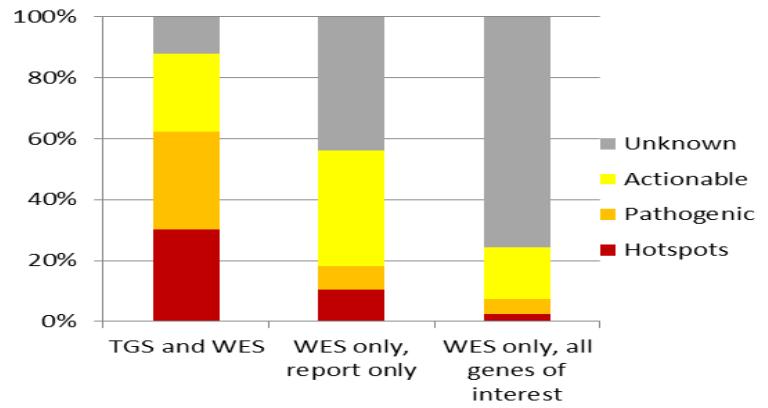
- Only low Allele Frequency are not detected 4.8 to 13.4%
- Information about somatic / constitutional Status
- Few additional targetable variants.

12/6/2018

Additionnal Information with WES for 35 of cases
Few actionable target (29%)

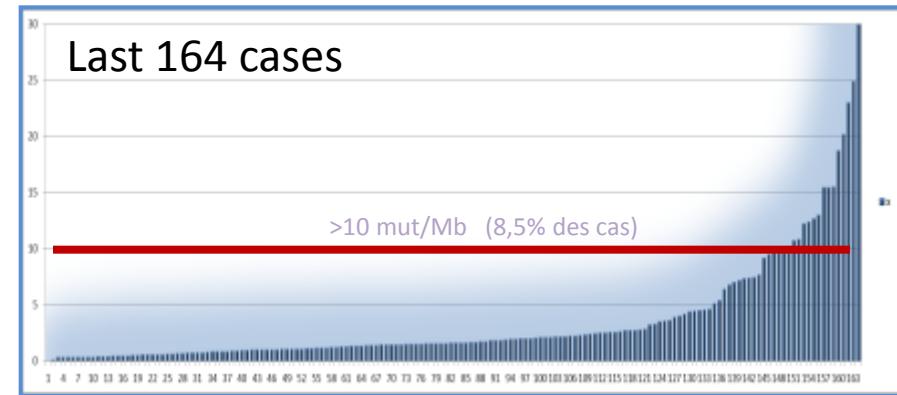
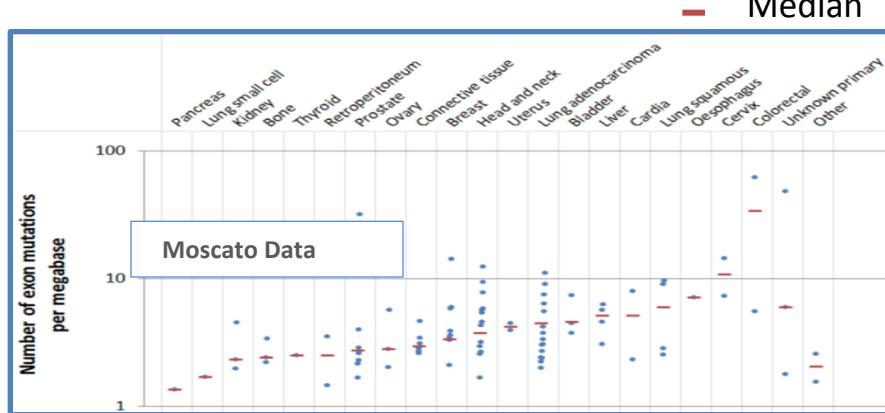


Additionnal Information with WES for 1/3 cases



Informations supplémentaires par rapport au CGP

- Mutational load



Nb of somatic mutations / Nb of bases >4X.

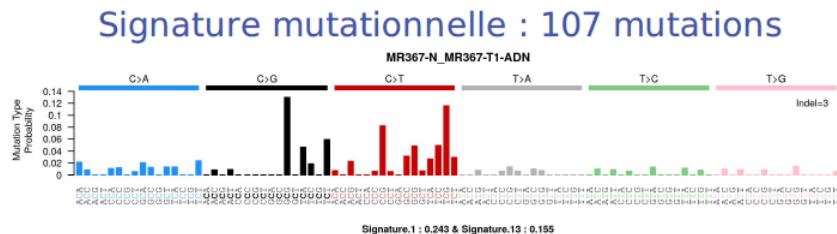
(Somatic mutations filters : Stop-Start-Missense-Splice SNPs and Inframe/Frameshift indels ; Somatic score > 4, Tum.VAF \geq 5% (<4% in N), Mut Count, Pop frequency \leq 1% IG DB & \leq 0.5% in EVS & 100G & Exac DB)

- Mutational signatures

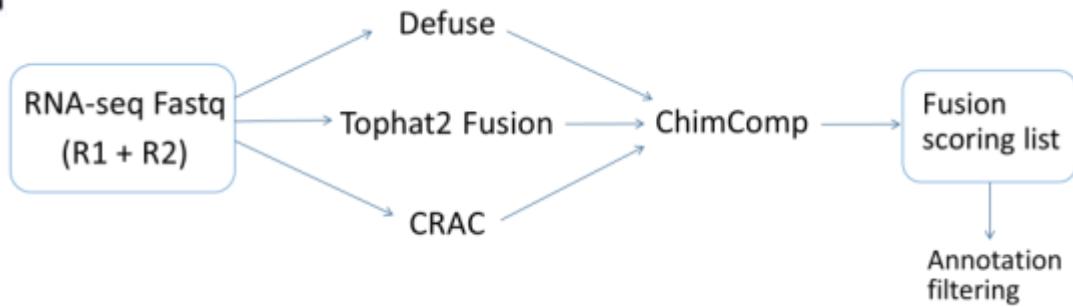
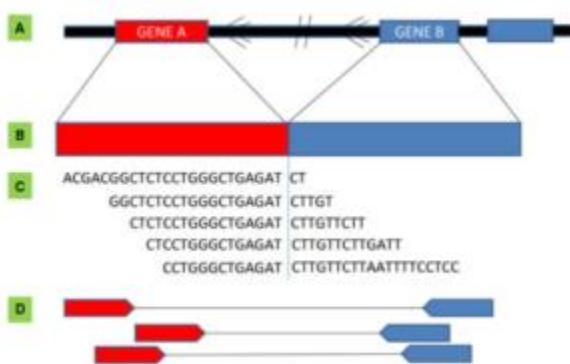
- MSI , HRD, etc...

- WES additional informations and neo-epitope prediction

- in silico percentage of tumor cells

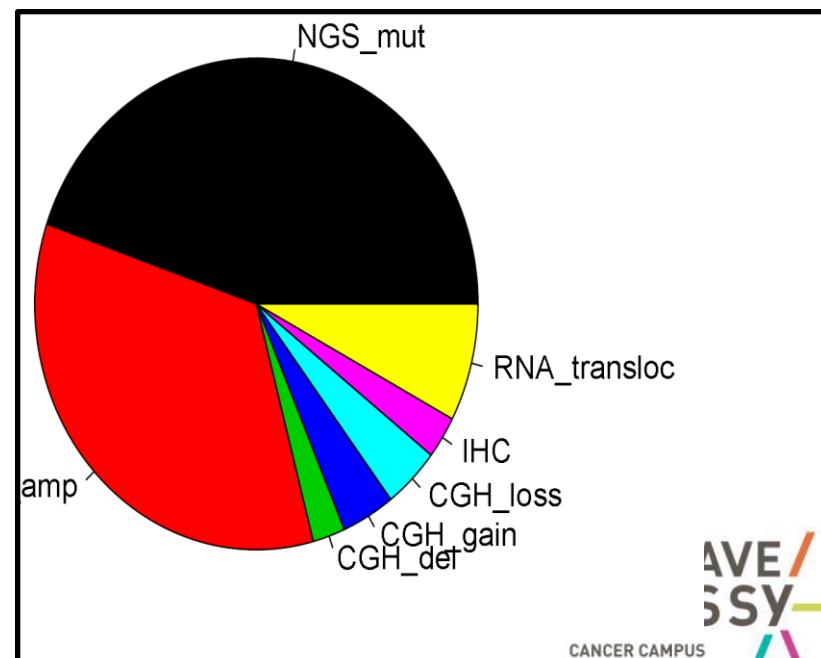


RNASeq: détection des fusions

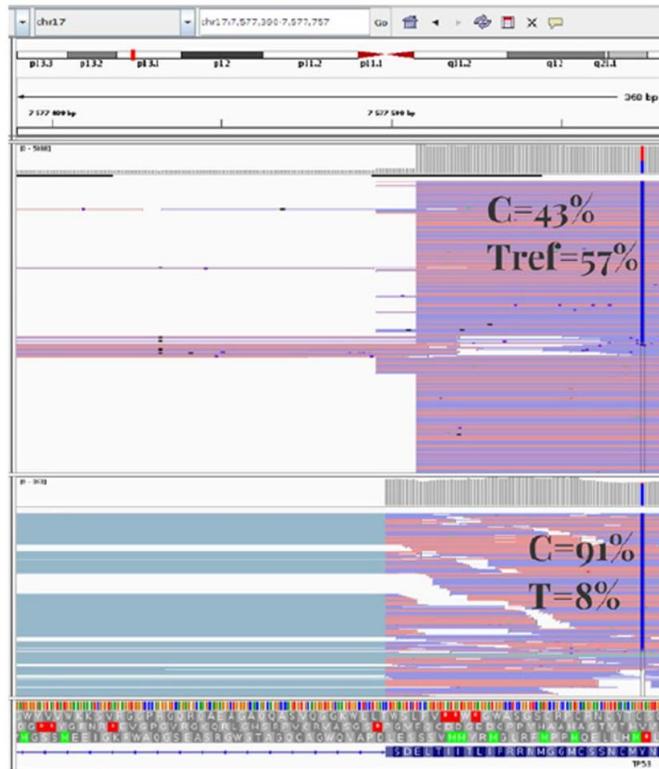


10-15% Cases with
actionnable Fusion

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



Détection des variants à partir de l'ARN



Monoallelic expression

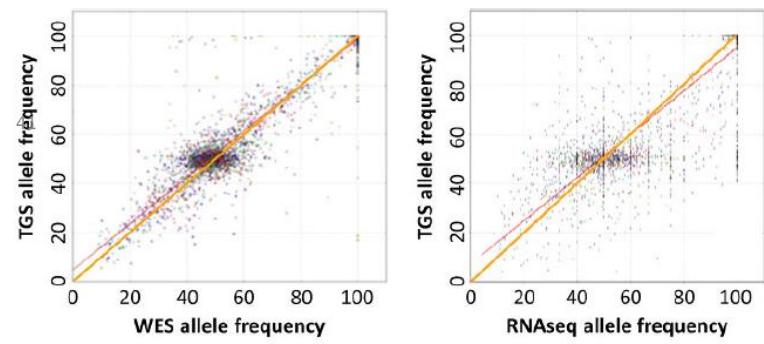
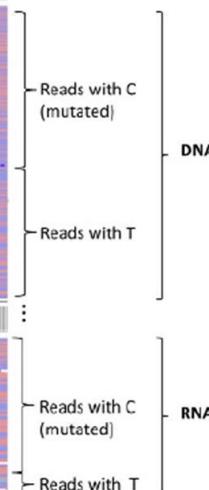


FIGURE 5. Comparison of WES versus TGS validated variants. Only variants validated by an expert geneticist were included. A, Number of patients with at least 1 variant found in both WES and TGS ("TGS and WES" line) or at least 1 variant found in WES and not TGS ("WES only" line). WES delivered additional information in 38% of patients (second and fourth bars). B, Classification of all variants validated by an expert geneticist, excluding nonpathogenic variants. Variants were classified as actionable when in an actionable gene or in a gene belonging to an actionable pathway. The first bar corresponds to variants found both in WES and TGS. The last 2 bars correspond to variants found in WES but not covered by the TGS panel. Among all the WES variants identified in a list of cancer-relevant genes (third bar), only a selection of variants was deemed relevant to be included in the clinical report (second bar).

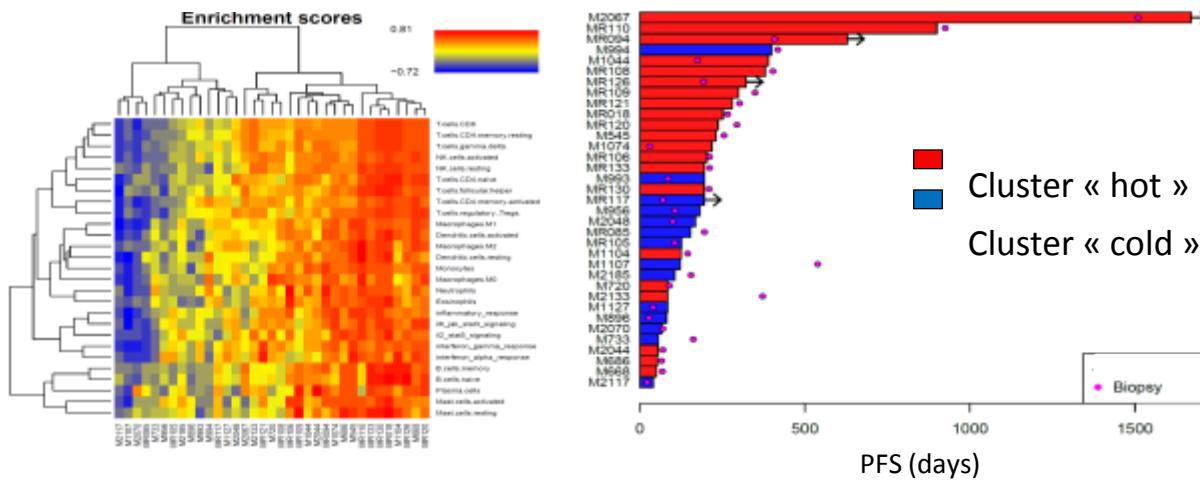
RNAseq – développement de signatures

Target expression

ID	Cancer type	ESR1 (IHC)	ESR1 (RNAseq)	ERBB2 (IHC)	ERBB2 (RNAseq)
654	Breast	+	+	-	-
658	Breast	+	+	-	-
662	Breast	+	+	-	-
678	Breast	-	-	+	+
681	Breast	+	+	-	-
694	Breast	?	+	?	-
702	Breast	+	+	-	-
723	Breast	+++	+	-	-

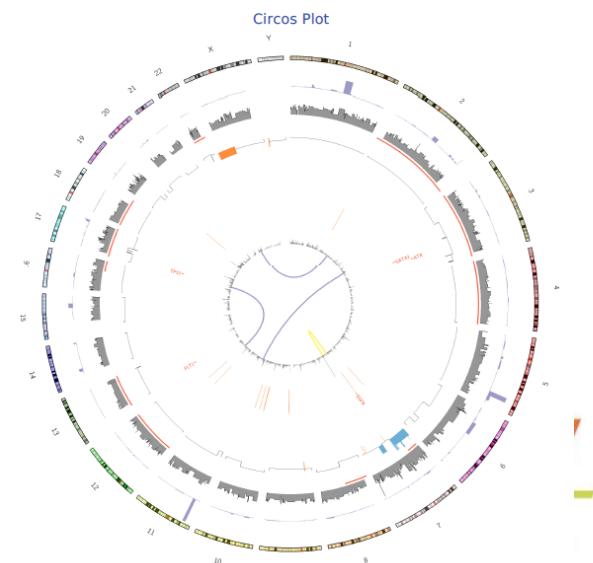
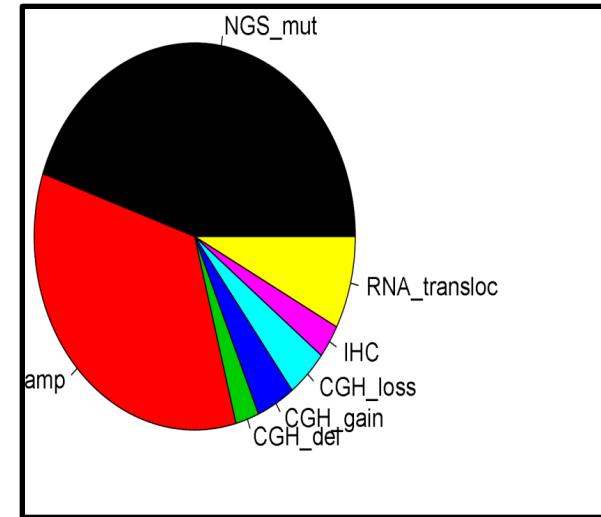
ID	Cancer type	PCR			RNAseq		
		HPV16	HPV18	HPV33	HPV16	HPV18	HPV33
370	H&N	-	-	-	-	-	-
536	H&N	-	-	-	-	-	-
646	H&N	-	-	-	-	-	-
653	H&N	379	-	-	14482	-	-
666	H&N	842	-	-	30421	-	-
670	H&N	-	-	-	-	-	-
683	H&N	-	-	-	-	-	-
708	H&N	-	-	-	-	-	-
721	H&N	-	-	-	-	-	-

Development of expression profile



Programme associé au WES / RNAseq

- MOSCATO-02
 - TGS + SNPa – short TAT
 - RNAseq for all cases (RIN>6,0 ; >500ng)
 - WES on selected cases.
- MATCH-R
 - TGS + SNPa – short TAT
 - WES/RNAseq for all cases (if QC ok)
- MAPPYACT
 - WES/RNAseq for all cases
- Other _ biomede-RARE-EXORARE
 - (WES/RNAseq)

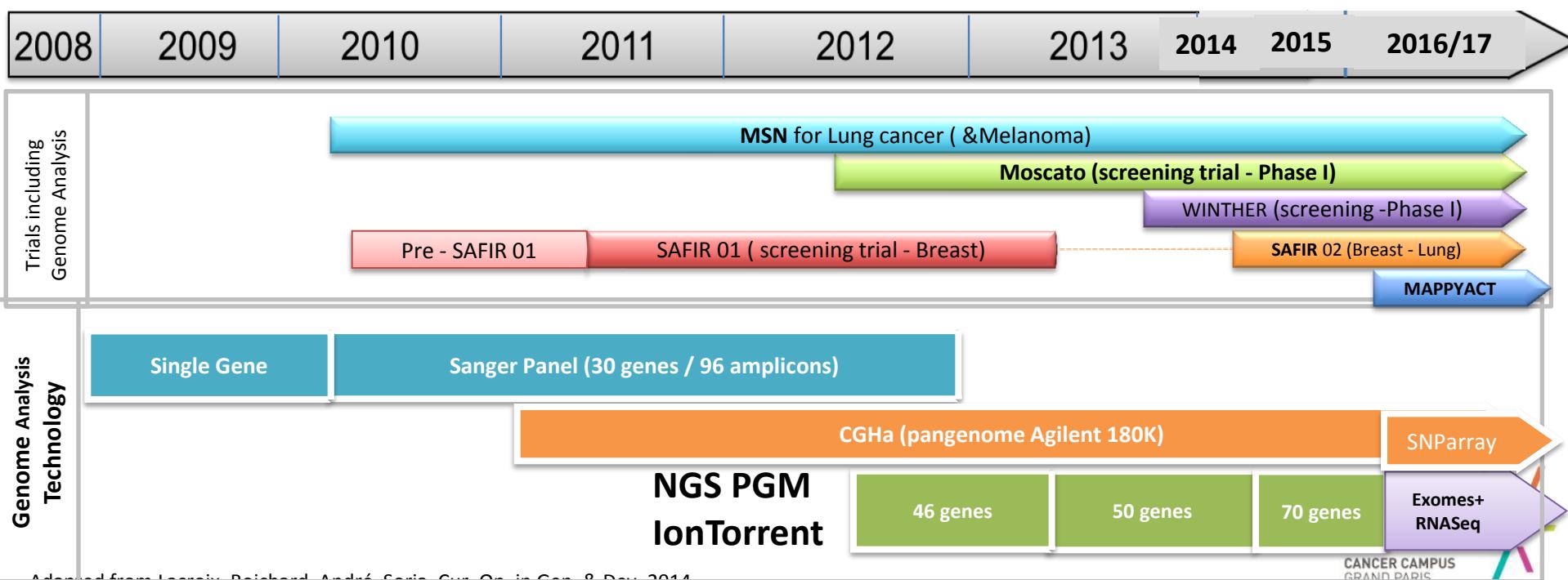
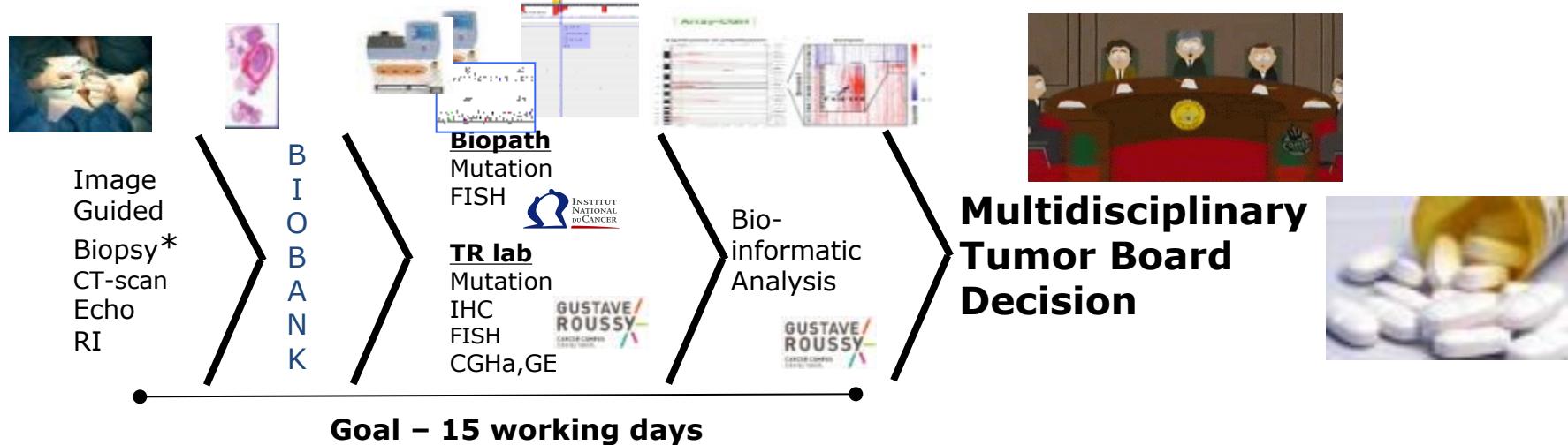


1219 RNAseq

805 WES in clinical research work flow

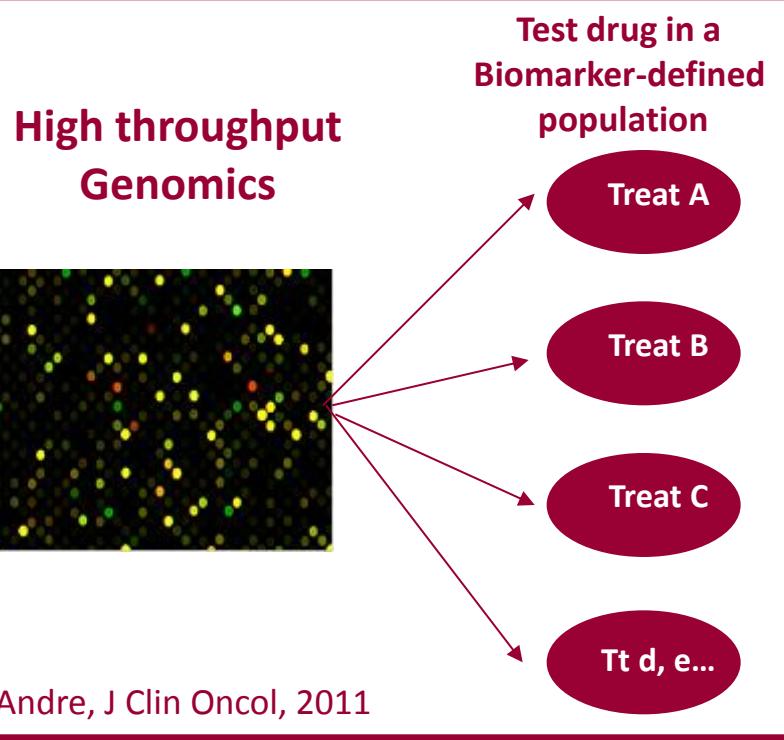
Exemples des programmes de médecine personnalisé

... Building the workflow to perform Tumor molecular portraits for therapeutic decision in Precision Med. Programs



From Molecular screening to Precision medicine

Molecular triage



Discovery phase:
Pick-up the winner targets
Give-up the looser targets

- SAFIR 01

André et al. 2014 Lancet Oncol.

MBC - 423pts

- MSN lung /MSN Melanoma

>1000pts / 300 Profiles

Planchard et al.
ESMO, 2012 [8]

Specta EORTC Prg

Completed by

- MOSCATO 01

Phase I - >1110pts

- MOSKIDO (part of MOSCATO)

Pediatric program

Massard et al.
Canc Discov 2017

- MOSCATO 02

- MAPPYACTS (WES/RNAseq)

Hartrampf et al.
CCR 2017

- MATCH-R (Resistance to Targeted TTT)

- EXPRESS (exceptional responder)

-

Programme MOSCATO-02

Molecular Screening Analysis Used as Decision Tool for Targeted Molecular Treatment

- Estimated Enrollment : 1050 participants
- Official Title: Study Start Date : October 2011
- Estimated Study Completion Date : October 2019

MOSCATO

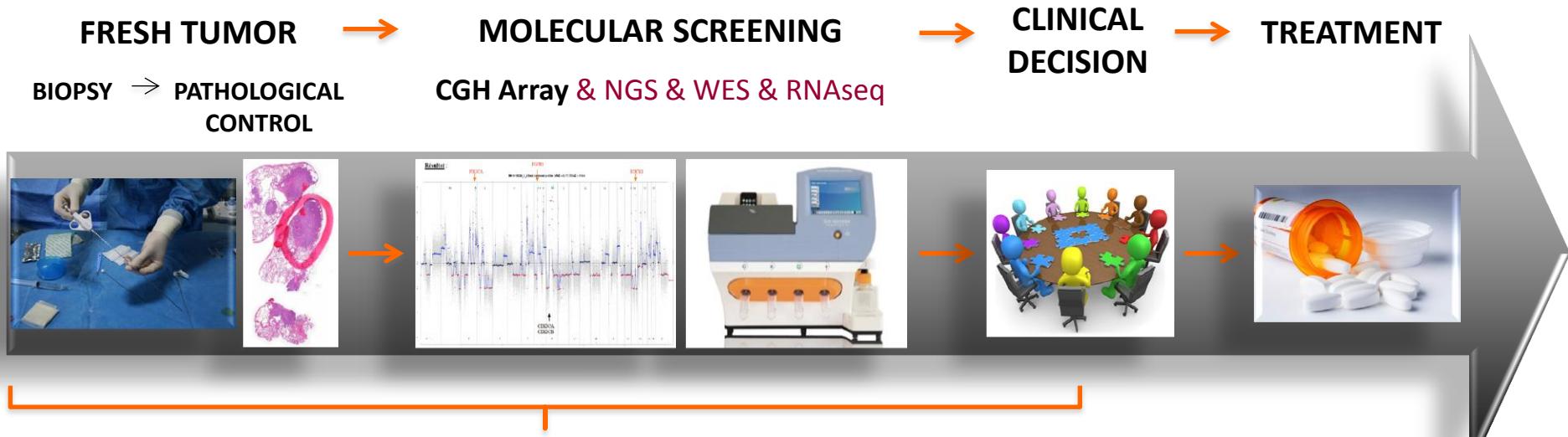
- Primary Outcome Measures :
 - **Progression free survival (PFS)** using a targeted treatment selected by molecular profiling compared to the PFS for the most recent regimen
 - Progression according to RECIST criteria or clinical progression or death of any cause
- Secondary Outcome Measures :
 - Number of patient who received a targeted treatment oriented by molecular profiling
 - Number of patient who received a targeted treatment oriented by molecular profiling
 - Progression free Survival, Overall Survival and Response Rate [Time Frame: Until progression, up to 1 year]
 - Comparison of Progression Free Survival, Overall Survival and Response Rate between patients with targeted treatment and others enrolled patients.

MOSCATO

- Inclusion Criteria:
 - **Solid tumors ; Stade IV ; Local relapse or metastatic ; Uncurable**
 - Age > 6 months
 - PS 0/1 or Lansky play scale $\geq 70\%$
 - **Minimum one treatment line, no limit in the prior number of treatment line**
 - Evaluable or measurable disease

MOSCATO01

- High through-put analysis in a high volume phase I center
- Monocentric
- Target accrual => 1000 patients



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

High-Throughput Genomics and Clinical Outcome in Hard-to-Treat Advanced Cancers: Results of the MOSCATO 01 Trial; Cancer Discovery 2017.



Christophe Massard, Stefan Michiels, Charles Ferté, Marie-Cécile Le Deley, Ludovic Lacroix, Antoine Hollebecque, Loic Verlingue, Ecaterina Ileana, Silvia Rosellini, Samy Ammari, Maud Ngo-Camus, Rastislav Bahleda, Anas Gazzah, Andrea Varga, Sophie Postel-Vinay, Yohann Loriot, Caroline Even, Ingrid Breuskin, Nathalie Auger, Bastien Job, Thierry De Baere, Frederic Deschamps, Philippe Vielh, Jean-Yves Scoazec, Vladimir Lazar, Catherine Richon, Vincent Ribrag, Eric Deutsch, Eric Angevin, Gilles Vassal, Alexander Eggemont, Fabrice André and Jean-Charles Soria

Hollebeque A
et al.ASCO 2013

Ferté C et al.
ASCO 2014

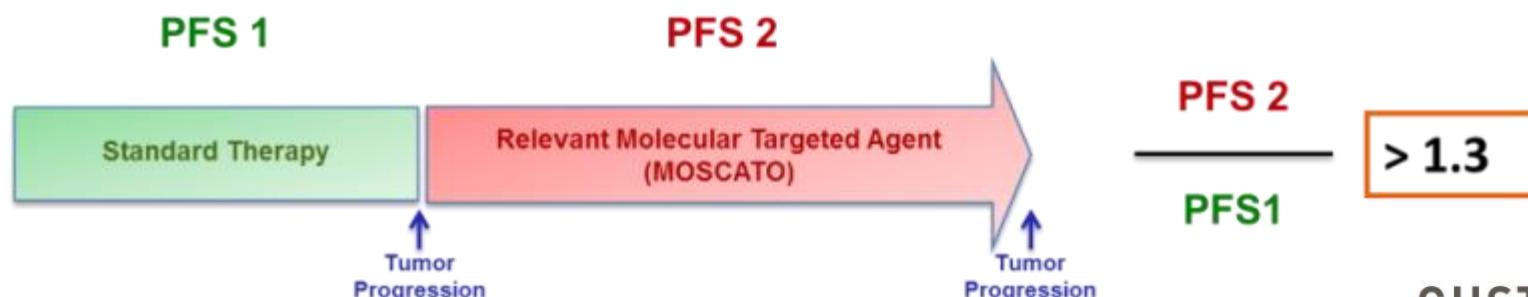
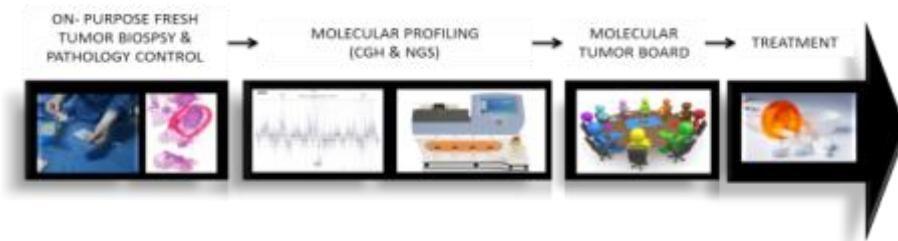
Geoeger B. et al.
ASCO 2014

Soria et al.
MAP 2015 /2016

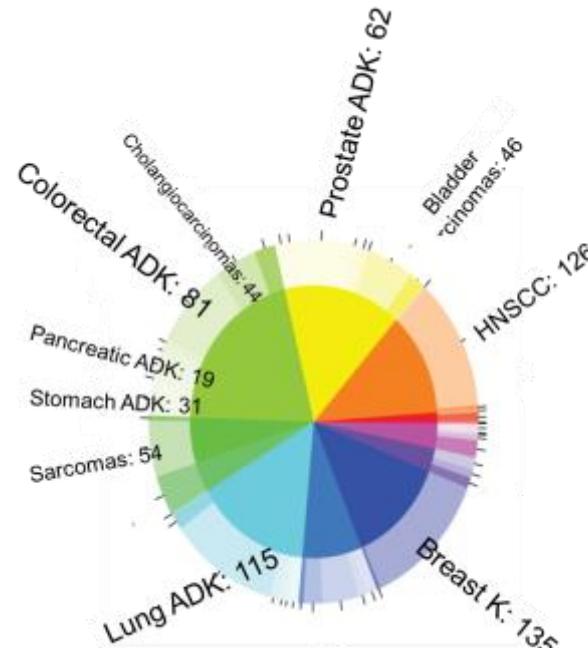
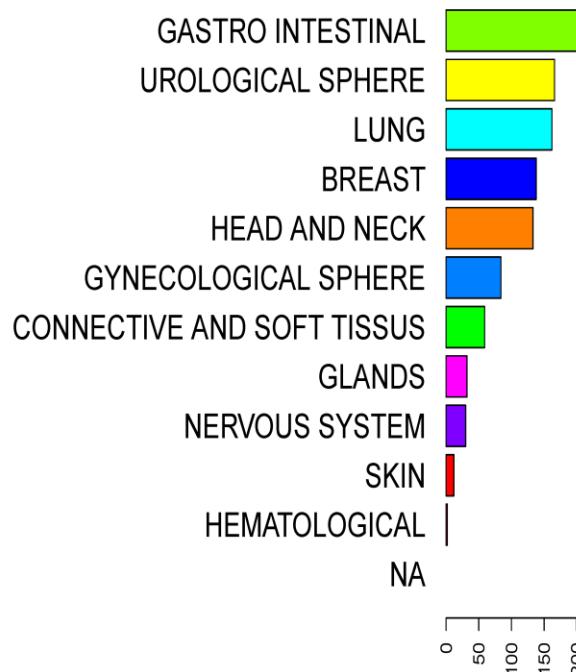
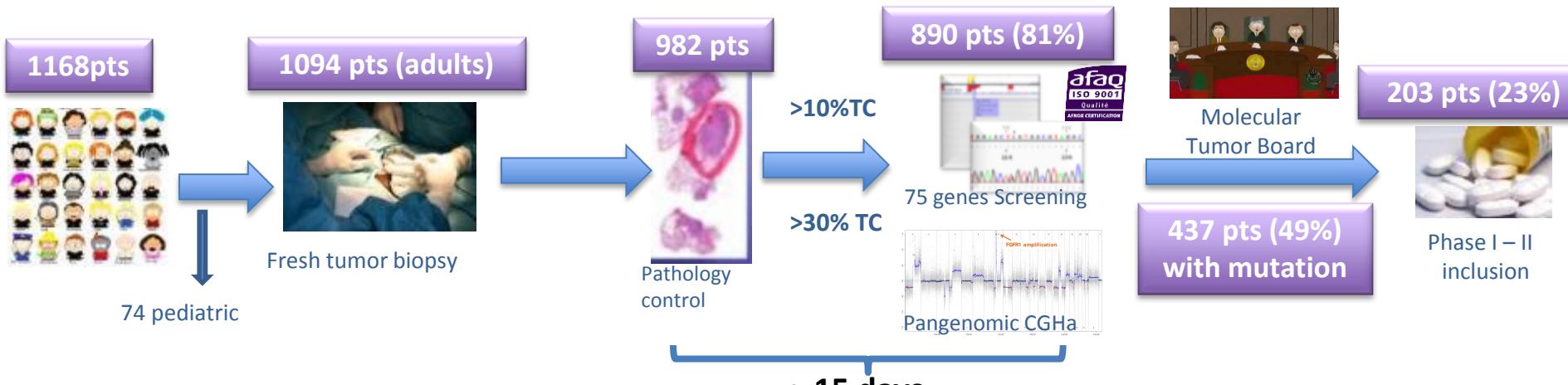
Hartrampf et al.
CCR 2017

Massard et al.
Canc Discov 2017

- Prospective molecular screening program to optimize the decision-making for patients susceptible to be enrolled in early clinical trials
- Monocentric (Gustave Roussy)
- 1110 patients



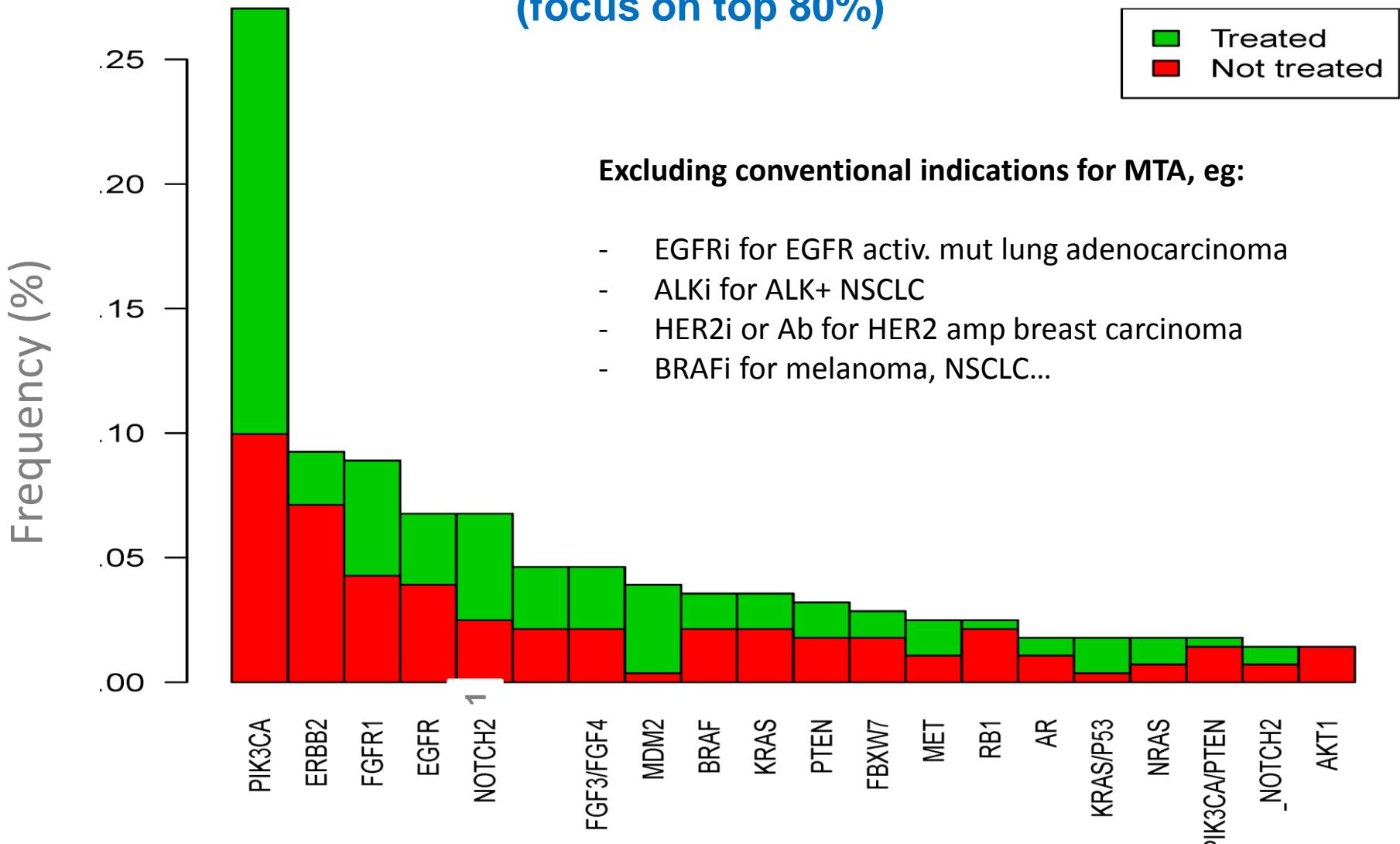
MOSCATO 01: a prospective molecular triage trial.



Biology driven orientations and treatments

Excluding standard therapies

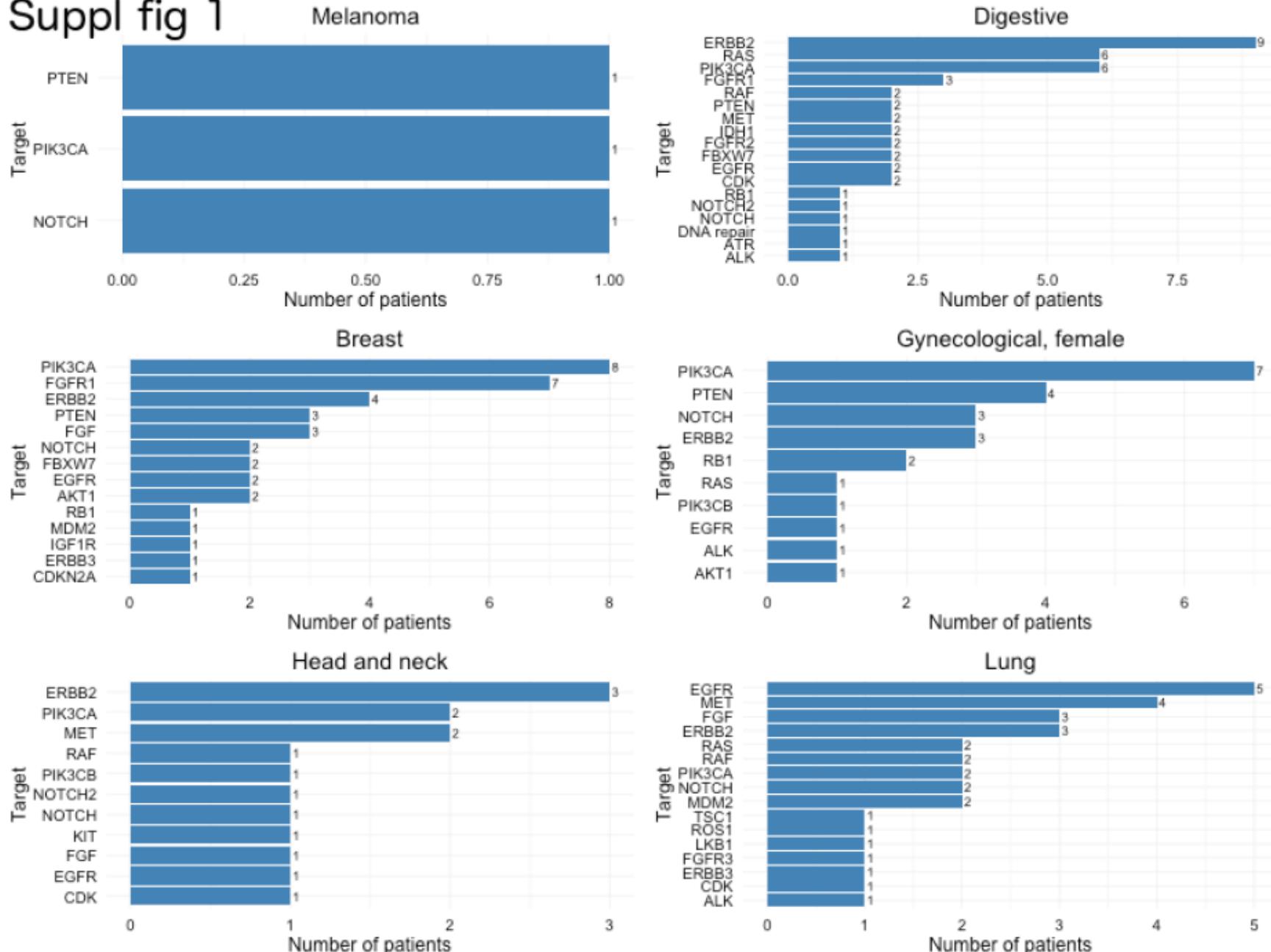
(focus on top 80%)



→ Top actionable aberrations belong to the PI3K/AKT, FGFRs/FGFs, HERs, NOTCH pathways

Top alterations per histological subtypes: examples

Suppl fig 1



Meilleure réponse (RECIST) et Ratio PFS1/PFS2 pour les patients orientés et traités (n=193)

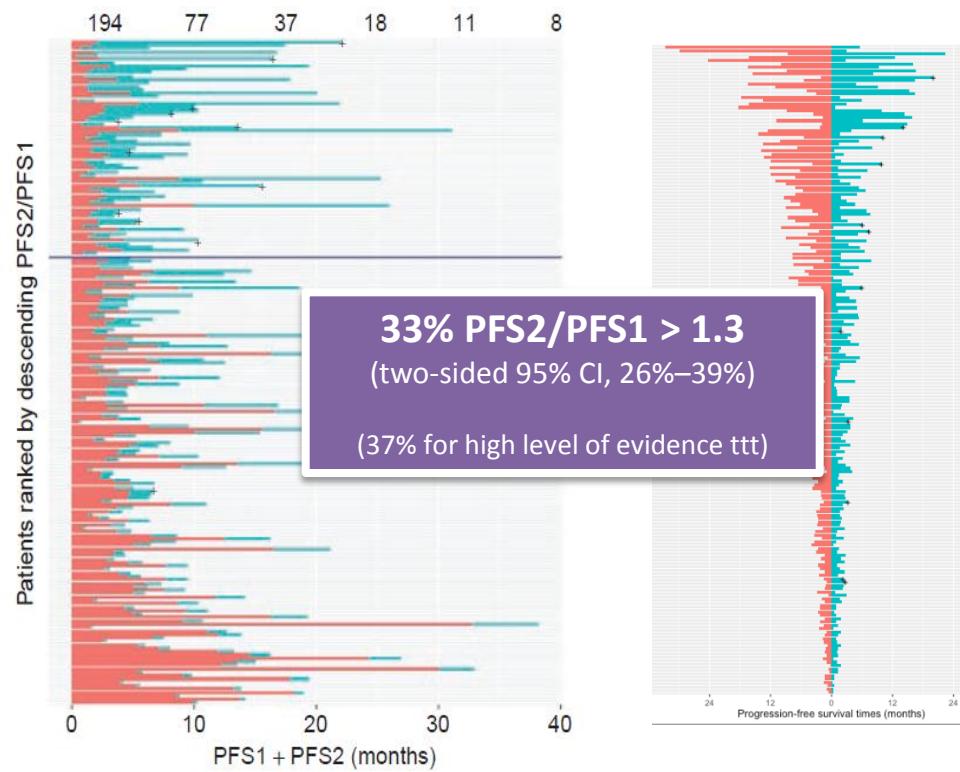
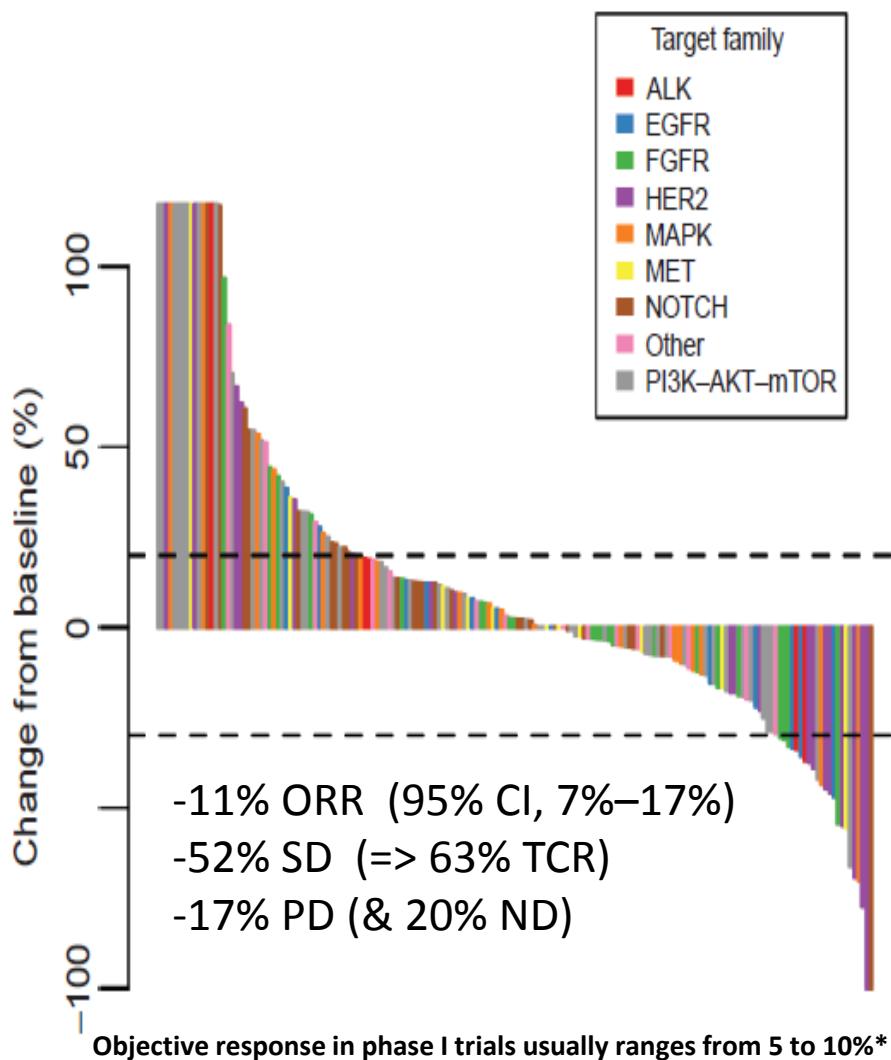
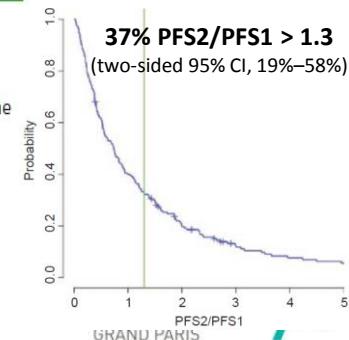


Figure 3. Efficacy on primary endpoint. A, Kaplan-Meier curve of PFS2/PFS1. Crosses denote censored data. Green line denotes PFS2/PFS1 > 1.3. B, Individual PFS1 and PFS2 times, ordered by descending PFS2/PFS1 ($n = 194$). Crosses denote censored data. Patients above the blue horizontal line have PFS2/PFS1 > 1.3.



* Horstmann et al, *N Engl J Med* 2005

Italiano et al, *Annals Oncol* 2007

Olmos et al, *J Clin Oncol* 2012

Programme SAFIR02

- Evaluation of the Efficacy of High Throughput Genome Analysis as a Therapeutic Decision Tool
 - SAFIR02_Breast Estimated Enrollment : 1460 participants
 - SAFIR02_Lung : Estimated Enrollment : 650 participants
- Actual Study Start Date : April, 2014
- Estimated Study Completion Date : December/February 2022

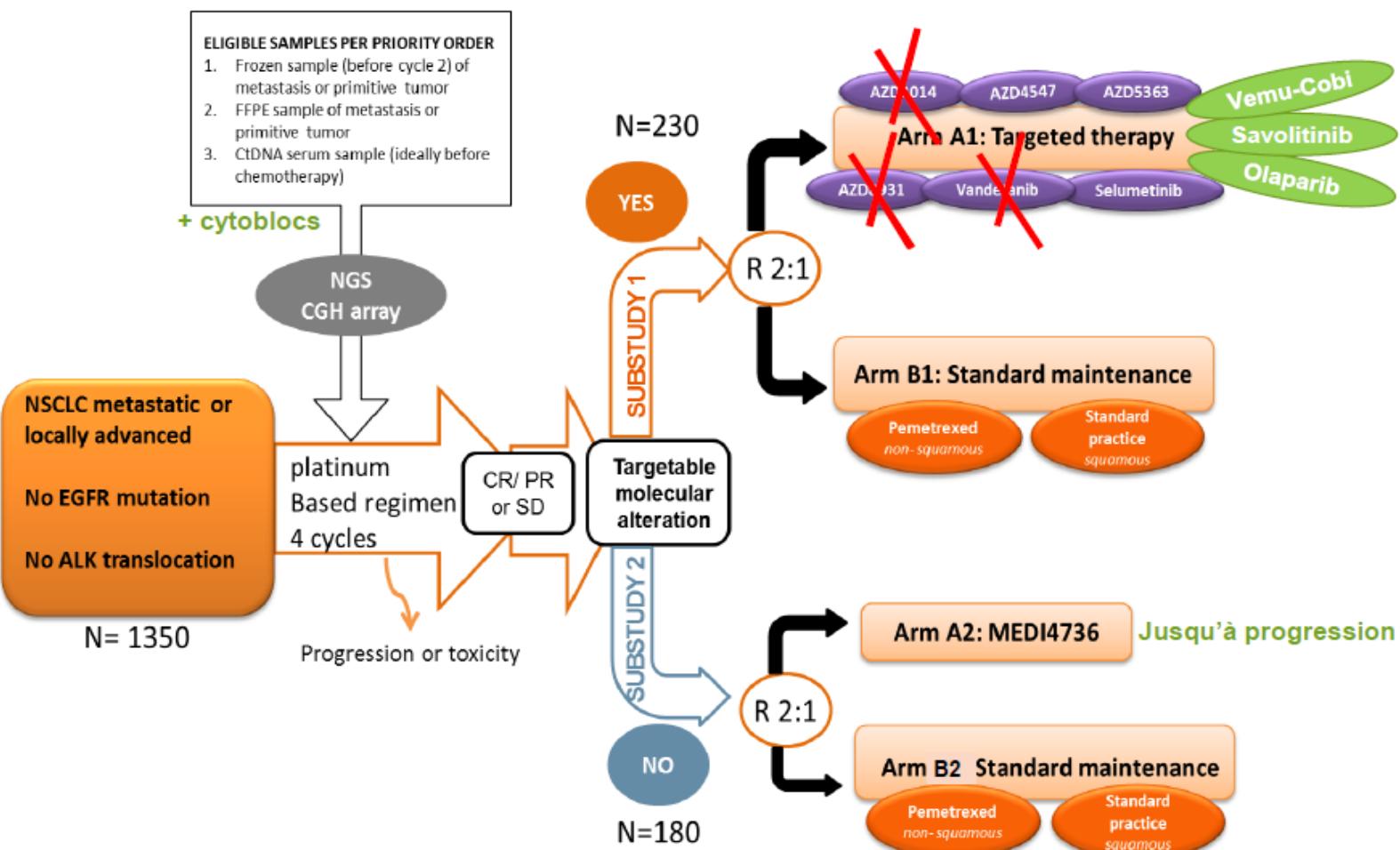
SAFIR02 studies : A Randomized trials in Lung and Breast metatstatic cancers

(PI. JC Soria for Lung & F.André for Breast)

Study : Randomized, Multicentric phase II Biology-driven treatment VS standard maintenance CT.

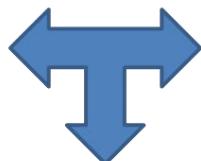
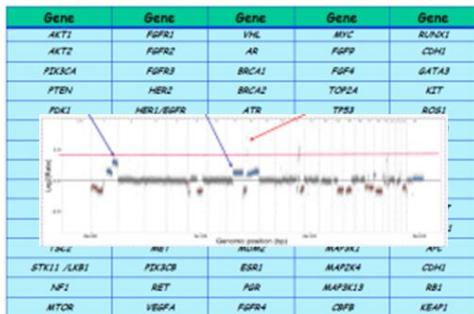
Molecular analysis: CGHa (180K) and NGS (PGM – 50 genes – 300X)

Timelines: 3 years(Lung) / 2 years(Breast) **Follow-up:** 12 month **TTT duration :** until progression or toxicity



SAFIRO2

Amplification / deletion
CGHa / SNP_a pangenomic



Mutation -50 gene panel
NS – PGM - IonTorrent

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	FLT3	FuLCoL	vegfr1	NOTCH4	HotSpot	based on Cosmic
AKT3	HotSpot	p.E17Klike	HRAS	HotSpot	Ex2,3,4	NOTCH4	HotSpot	based on Cosmic
ALK	HotSpot	Ex 20 to 26	ZMPTR1	FuL		MAB	HotSpot	Ex 2,3,4
BRAF	HotSpot	Ex 11 & 15				PTEN	HotSpot	12-14-16
BRCA1	FuLCoL	-				IGCA	HotSpot	Ex 10&11 + OHPC
BRCA2	FuLCoL	-				IDH2	HotSpot	based on Cosmic
CTNNB1	HotSpot	Ex3				ERIA	HotSpot	based on Cosmic
DORZ	FuLCoL	-				TPV1	FuLCoL	-
EGFR	HotSpot	Ex 18-21 +				TPN11	HotSpot	based on Cosmic
HER2	HotSpot	Ex 8 + 19...				RET	HotSpot	Ex 11-16
HER3	FuLCoL	-				EGFR	HotSpot	mut Recept
HER4	FuLCoL	-				STK11	FuLCoL	-
PIK3CB	FuLCoL	-				TP53	FuLCoL	-
PIK3R1	HotSpot	p.S46 p.s64	AKT1	FuLCoL	-	TP53	FuLCoL	-
PIK3R2	HotSpot	4 Exons	AKT2	FuLCoL	-	TP53	FuLCoL	-
PIK3R3	HotSpot	based on Cosmic	AKT3	FuLCoL	-	TP53	FuLCoL	-
			VHL	FuLCoL	-			

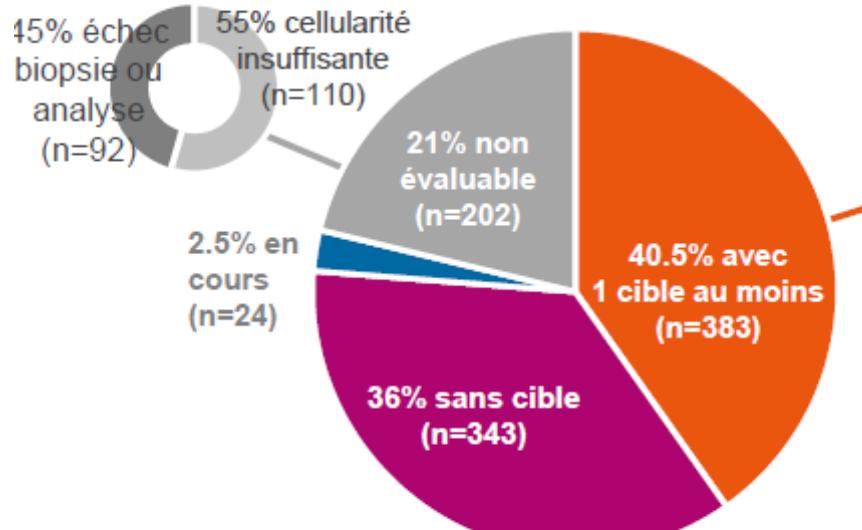
SAFIRO2 Lung - Order of preference for treatment

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%	AZD0891
3	HER2 mutation	0%	2%	AZD0891
	KRAS mutation	6%	21%	Schumertinib
	BRAF mutation	2%	1-3%	Selumetinib
4	PIK3CA amplification	33%	6%	AZD0363 (or AZD2014 if the number of pts with this abnormality is high)
	PIK3CA mutation	6%	3%	AZD0363 (or AZD2014 if the number of pts with this abnormality is high)
	PTEN loss	16%	1%	AZD0363 (or AZD2014 if the number of pts with this abnormality is high)
	PTEN mutation	10%	2%	AZD0363 (or AZD2014 if the number of pts with this abnormality is high)
	AKT1 mutation	1%	Very rare	AZD0363
5	FGFR1 amplification	22%	1%	AZD4547
	FGFR2 mutation	4%	0%	AZD4547
	FGFR3 mutation	3%	0%	AZD4547
6	LKB1 mutation	5%	23%	AZD2014

6 targeted therapies
available (AZD Pipeline)

Defined algorythm for
treatment
decision in weekly
molecular tumor
board

958 inclusions

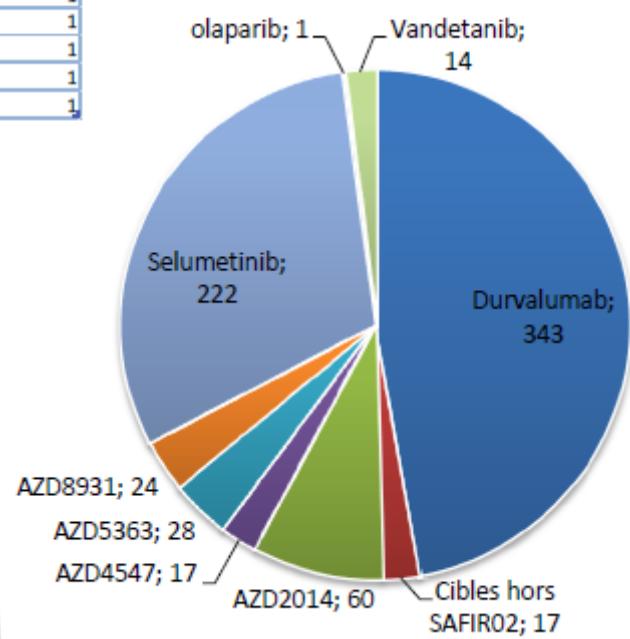


701 profils de patients revus en MTB

mutation ciblée	n	%
KRAS mut	183	51
STK11 mut	49	14
BRAF mut	19	5
PIK3CA mut	13	4
EGFR amp	12	3
NF1 mut	11	3
FGFR1 amp	10	3
HER2 mut	7	2
IGF1R amp	5	1
PIK3CA amp	5	1
EGFR amp	4	1
FGF4 amp	3	1
AKT2 amp	3	1
PTEN del	3	1
EGFR mut	3	1
NRAS mut	3	1
RICTOR amp	2	1
FGFR3 amp	2	1
HER3 mut	2	1
FRS2 amp	2	1
VEGFA amp	2	1

Retrait ciblage ampli FGFR
(décision SC Juin 2018)

Décisions thérapeutique



Programme MAPPYACTS

MAPPYACTS

A multicentric, prospective proof-of-concept study **MoleculAr Profiling for Pediatric and Young Adult Cancer Treatment Stratification**

Primary objective:

- To screen the maximum of relapsed or refractory pediatric patients
- To provide them with their individual molecular genetic tumor profile and
- Orient them towards matched innovative targeted agents

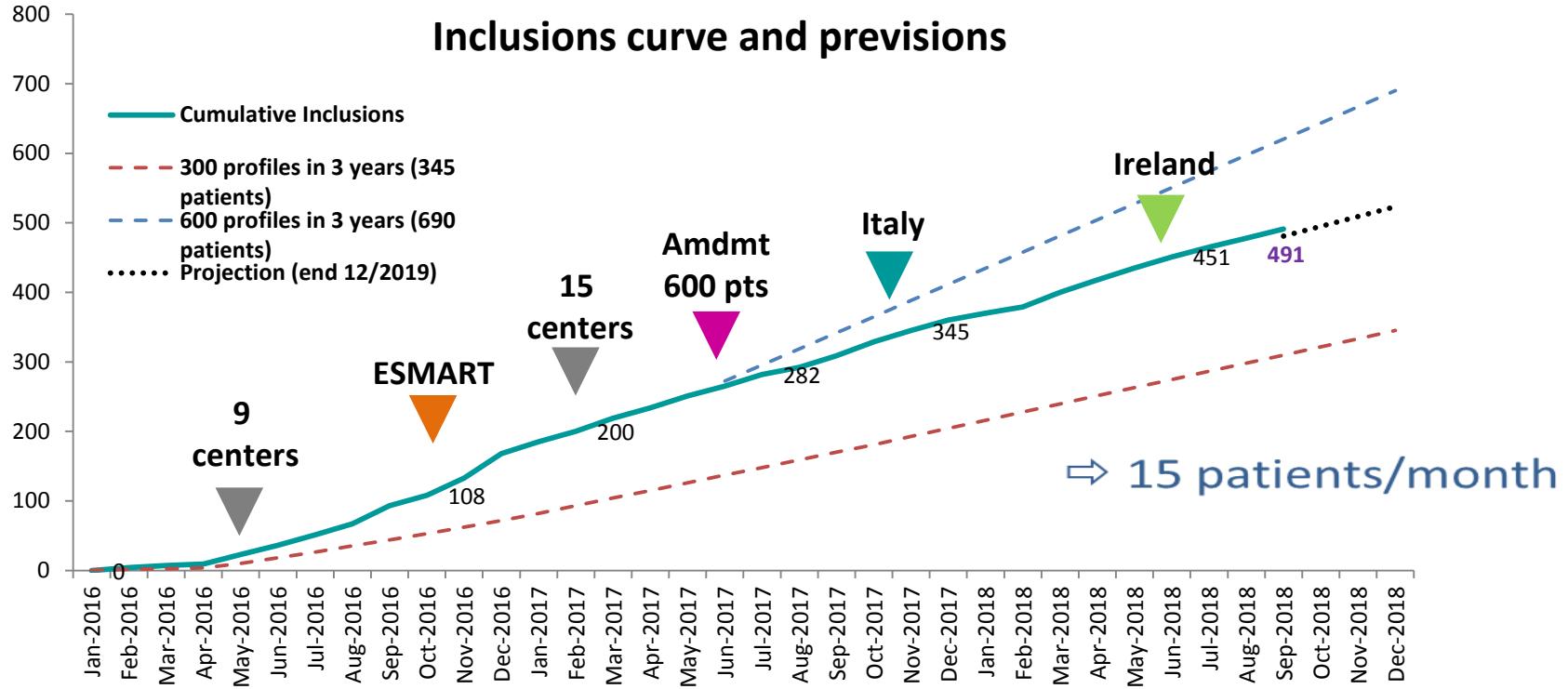
Main inclusion criteria:

- Informed Consent prior to sample acquisition intervention
- Patients with confirmed **solid tumor or leukemia** which is **recurrent or refractory to standard treatment and eligible for an early clinical trial**
- In case of solid tumor, lesion must be accessible for biopsy or surgical resection or cytological puncture
- Age: **≥ 6 months at relapse and ≤ 18 years at initial diagnosis**
- Performance Status: **≥ 70% / Life expectancy ≥ 3 months**
- Adequate organ function

MAPPYACTS

- Estimated Enrollment : 700 participants
- Study Start Date : December 2015
- Estimated Primary Completion Date : December 2020
- Primary Outcome Measures :
 - The percentage of patients with recurrent or refractory pediatric solid tumor or leukemia that could be **attributed to treatment with matched targeted agents**

MAPPYACTS - Inclusions

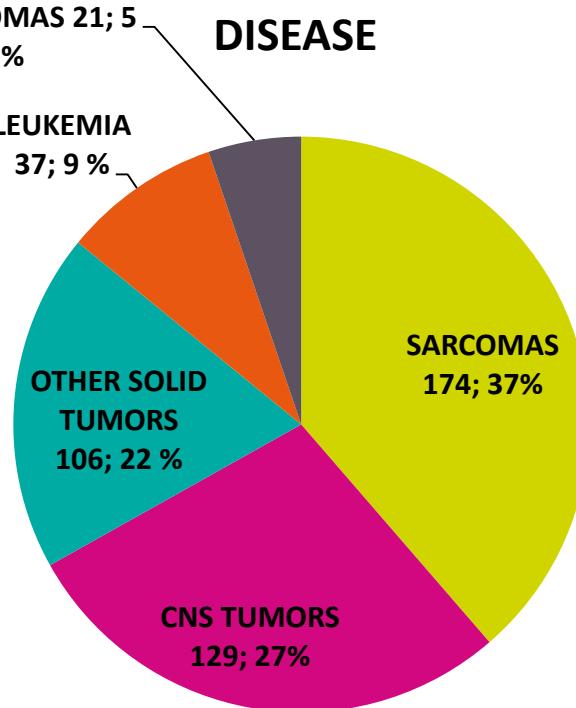


- February 2016 – 30 September 2018:
- 490 patients included in 14 French and 1 Italian centers

MAPPYACTS – Study Population

- Median age: 12 years (range: 1- 33)
- Delay initial diagnosis to registration: 1.8 years (0.1 - 22.6)
- Gender: 291 male (62%)

LEUKEMIA	n
B-ALL	16
T-ALL	10
AML	10
Other	1

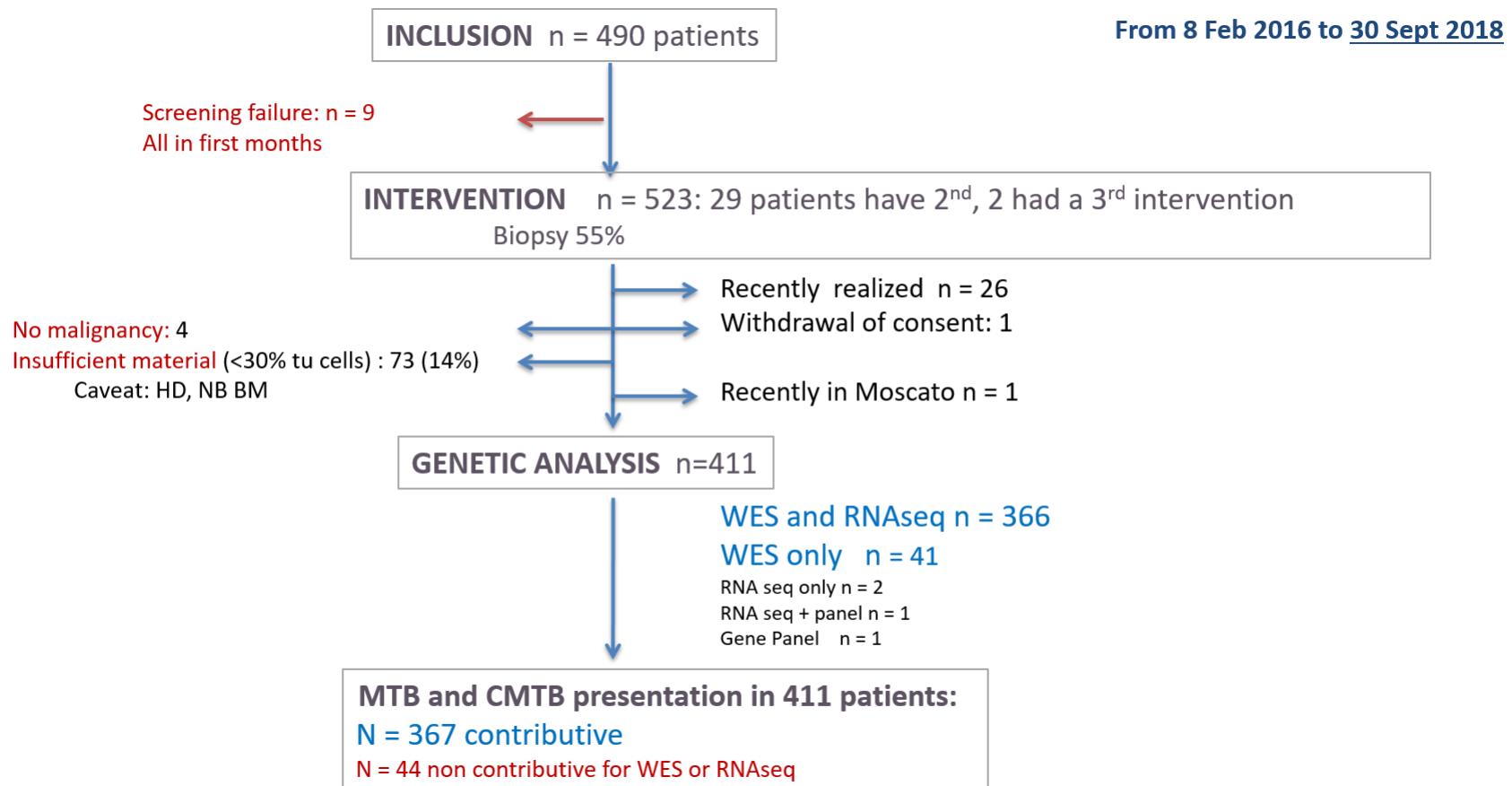


SARCOMAS	n
Osteosarcoma	44
Rhabdomyosarcoma	47
Ewing sarcoma	43
Other sarcoma	40

OTHER SOLID TUMORS	n
Neuroblastoma	54
Nephroblastoma	16
Carcinoma	15
Other	21

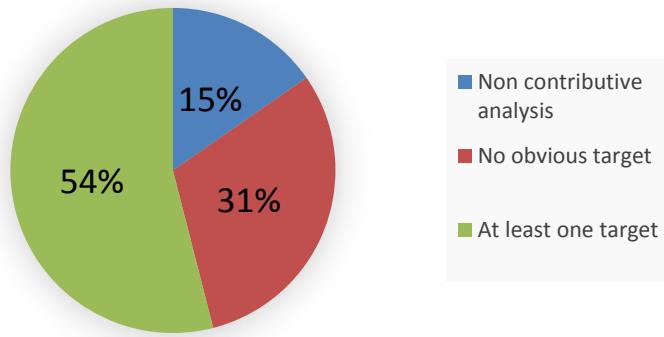
CNS TUMORS	n
High grade glioma	36
Embryonal tumor	10
Medulloblastoma	31
Low grade glioma	16
Ependymoma	26
Other	10

MAPPYACTS – Sample Analysis



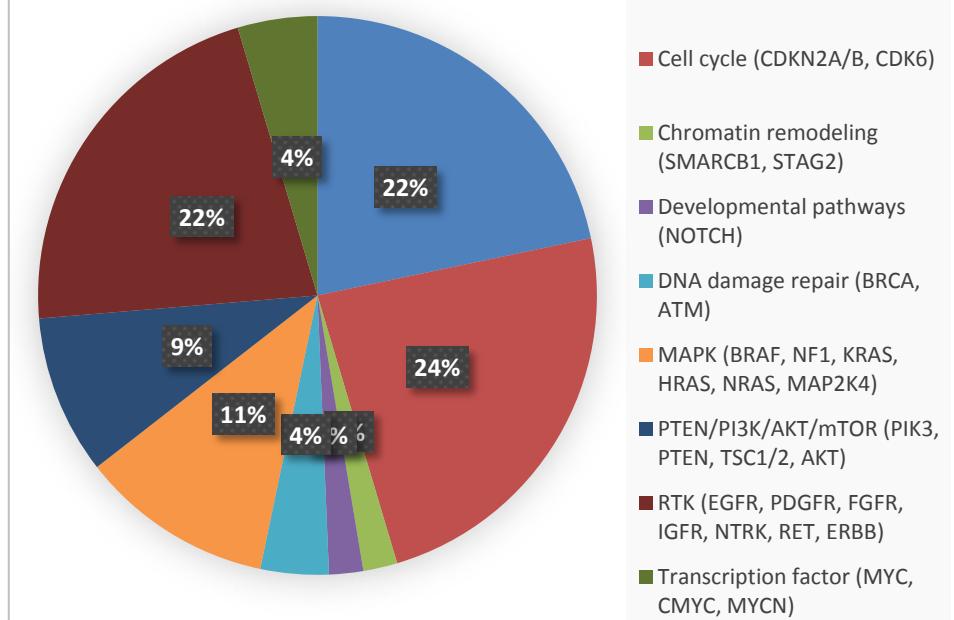
Actionable Molecular Alterations

Sarcomas

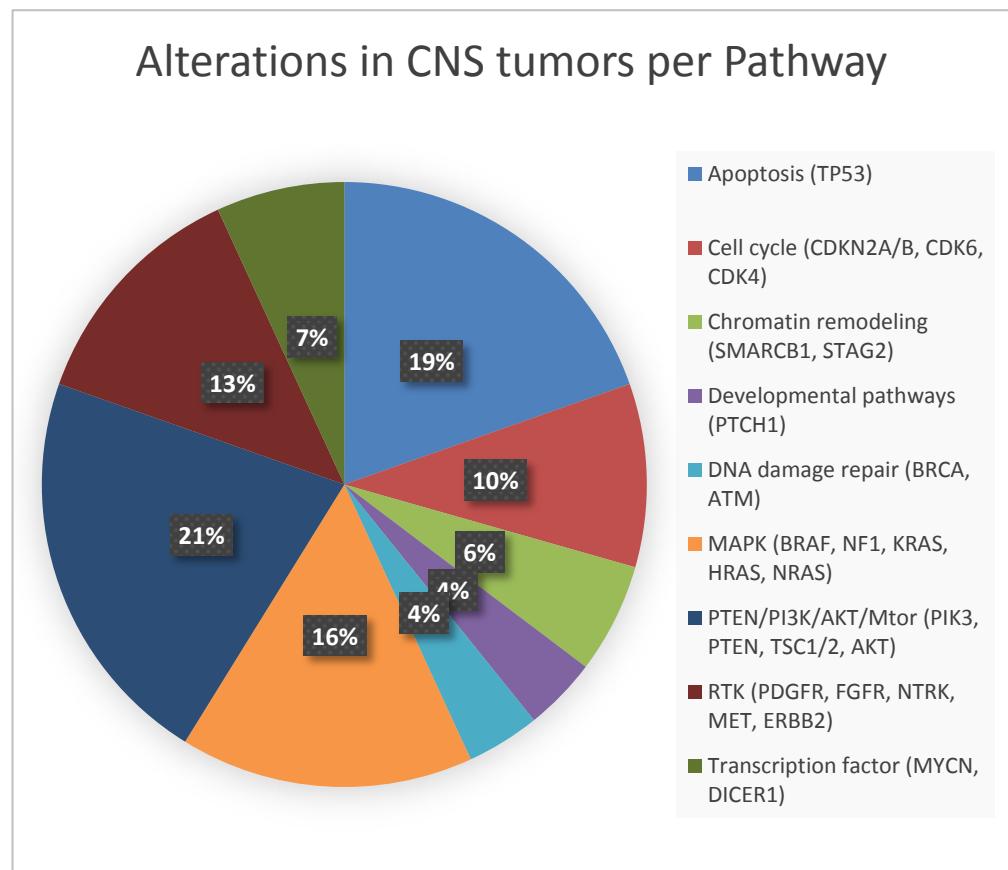
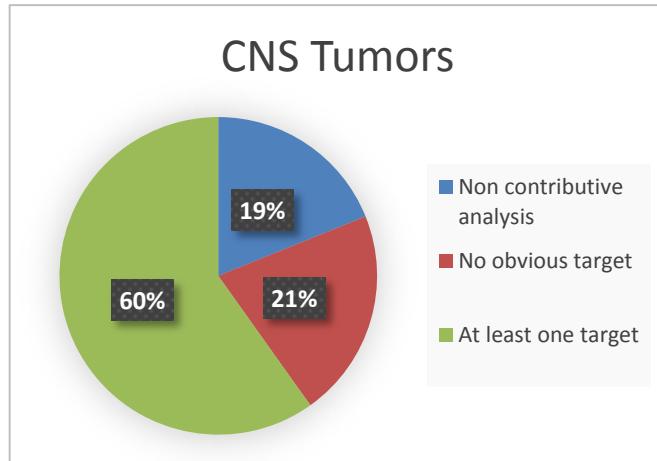


SARCOMAS	n
Osteosarcoma	44
Rhabdomyosarcoma	47
Ewing sarcoma	43
Other sarcoma	40

Alterations in Sarcoma per Pathways



Actionable Molecular Alterations



CNS TUMORS	n
High grade glioma	36
Embryonal tumor	10
Medulloblastoma	31
Low grade glioma	16
Ependymoma	26
Other	10

MAPPYACTS CMTB Report

Ready for routine use: 16 (5%)

NPM1/ALK , KIAA1549-BRAF, ETV6-NTRK3, KANK2/NTRK2, CCDC6-RET fusions; BRAF p.V600E, PTCH1

Investigational: ~40%

CDK4 ampli, CDKN2A/B del, PI3KCA, PTEN loss, FGFR ampli/mut, MYC ampli, ATR, ATM mut, SMARCA1...

Hypothetical target: ~ 20%

Histone mut, CNA gains, TP53 mut,

Resistance mutations:

SMO p.I408V, NTRK3 p.G623R

Oncogenic without level of evidence:

TP53 mut?, VUS, subclonal events

Oncogenic not targetable:

EWS/FLI1, PAX/FOXO1

Programme MATCH-R

- **A Prospective Trial to Study the Evolution of Clonal Architecture of Tumors From Patients Treated With Molecular Targeted Agents (MATCH-R)**
- Despite the impact of targeted therapies and immunotherapies in cancer treatment, the patients experience tumor progression owing to drug resistance.
- Goal : understand the molecular mechanisms underpinning primary or acquired resistance to targeted therapies and immunotherapies.

MATCH-R

Estimated Enrollment : 600 participants

Study Start Date : December 2014

Estimated Study Completion Date :December 2022

INCLUSION

- Patients with **unresectable or metastatic cancer**
- **5 cohorts** open :

Cohort 1: Single post drug biopsy in patients with drug's acquired resistance
(PD after a PR/CR or a SD for at least 6 months)

Cohorts 2-5: pre and post drug biopsies for selected populations:

prostate

bladder

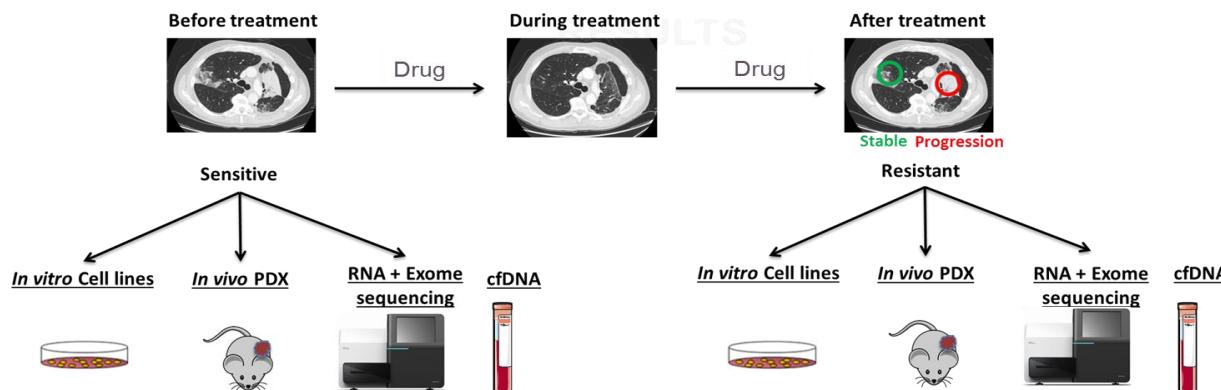
immunotherapy in lung cancer

targets (EGFR, ALK)

MATCH-R

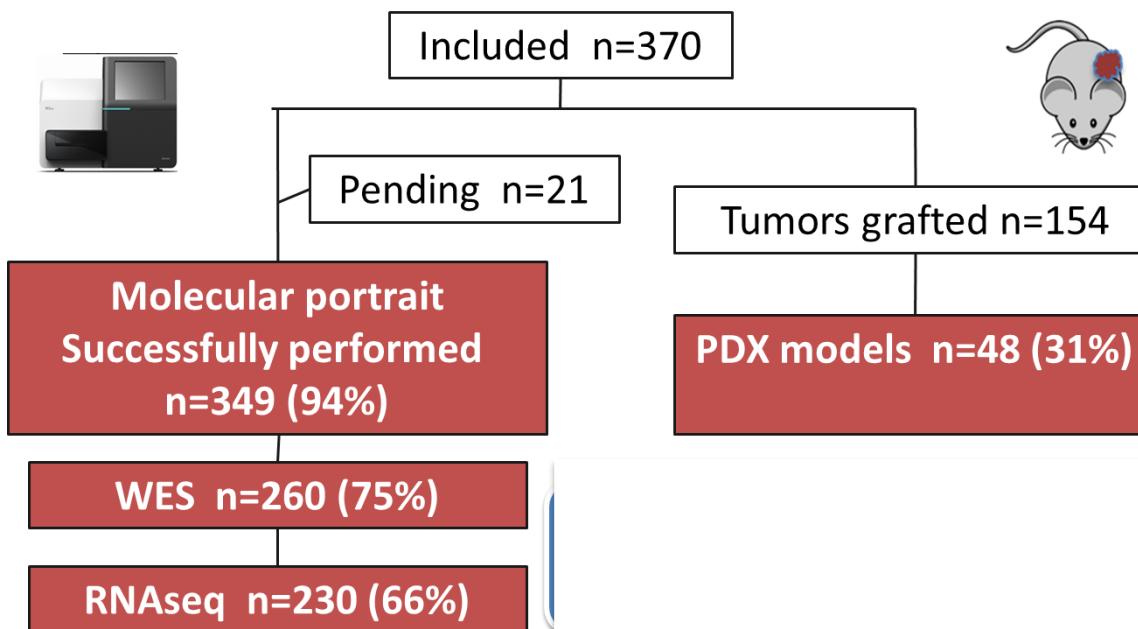
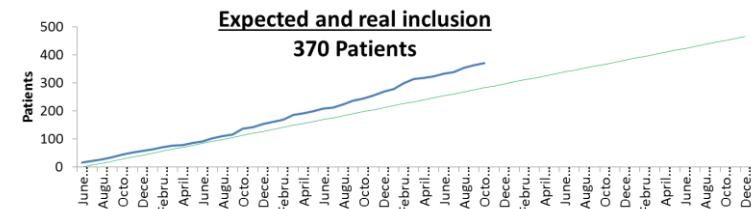
ANALYSIS

- Tissue biopsy: targeted NGS, SNP array, WES and RNAseq + PDXs
- Liquid biopsies mandatory
- Complete clinical characteristics records

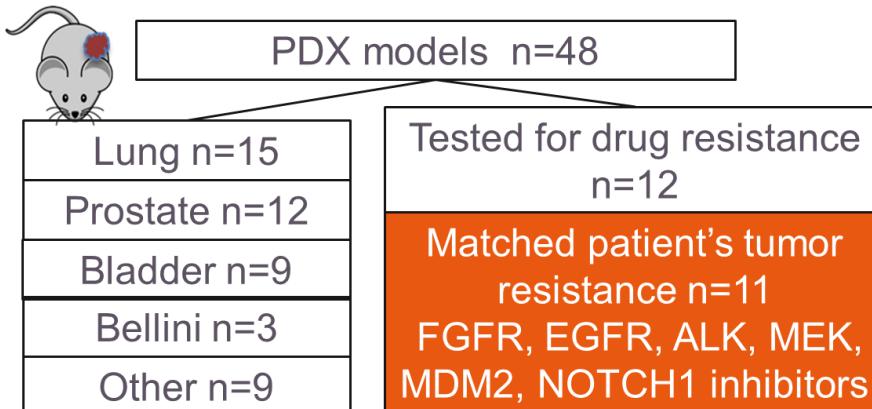
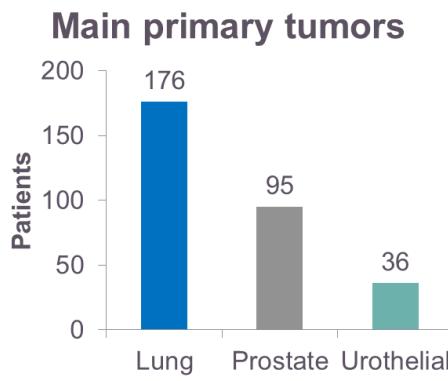


MATCH-R

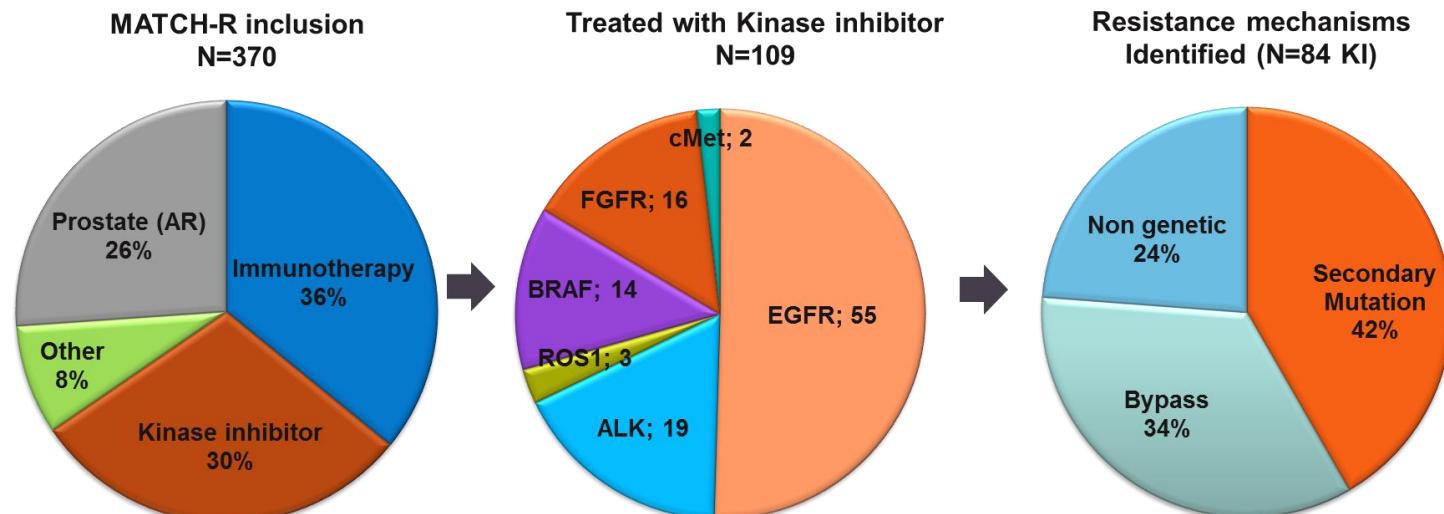
- June 29th, 2015 to October 10th, 2018 → 370 patients included
- Analysis failed in 82 (24%) patients, are pending in 21
- There were no grade 5 toxicities
- Tumor cells content was ≤10% in 33 patients (9%), median was 50% (0-95), 50% for the liver (n=52), nodes (n=47), or lung (n=101) biopsies, 40% for prostate (n=54) biopsies



MATCH-R



Resistance Mechanisms



MATCH-R successfully delivers molecular characterizations (94%).
Resistance mechanisms are identified in 77% of the cases.

This study generated PDXs models (success rate 31%) that mimic the patient's tumor resistance.

This ongoing trial will be amended to better suit potential future collaboration.

Quelques cas cliniques illustrent l'impact de cette organisation

Cholangiocarcinome intra-hépatique métastatique

- Femme, 30 ans, sans antécédents, hépatalgies

→ Cholangiocarcinome intra-hépatique métastatique

- GEMOX : réponse initiale puis progression à 8 mois
- FOLFIRI : progression
- 2 HGA sanguin : bas
- Screening AcSé : pas d'altération ROS1, ALK, MET, BRAF (V6600E)
- Portrait moléculaire tumoral : **translocation impliquant FGFR2 et CCAR1** (cell division cycle and apoptosis regulator 1)

→ Inhibiteur spécifique de FGFR

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

Mosaique M002 TAH00200 page 1/2

Analyse Exome - RNASEq (Pf Integrages)
 Laboratoire de Recherche Translatoire GUSTAVE ROUSSY

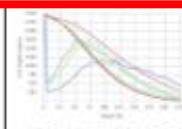
Information Patient:
 NCRN : Précis : CHOLESTER Herdige (RE BOPSE)
 Date de Référence : 28/04/2011 MRP : 2013-05002 EX

Information Pathologique:
 NP Histologiques : 14H00200
 Diagnose Clinique : cholangiocarcinome
 Site de prélèvement : tissu Date de biopsie : 11/03/2014
 Date Histologique : 30% de cellules cancéreuses
 Auteurs : Dr P. Weil ; Dr S. Abecassis
 Concentration : 10 ng/µL
 NCP : 1605002014

Conclusion :

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

Figure et Tableaux:



Sample	AvgExp	% of exp	% of 300X	% of 200X	Mean depth
M002-T1	10 genes	5.21	50.93	50.77	54.31
M002-T1-ACN	10 genes	3.7	50.17	50.0	53.00

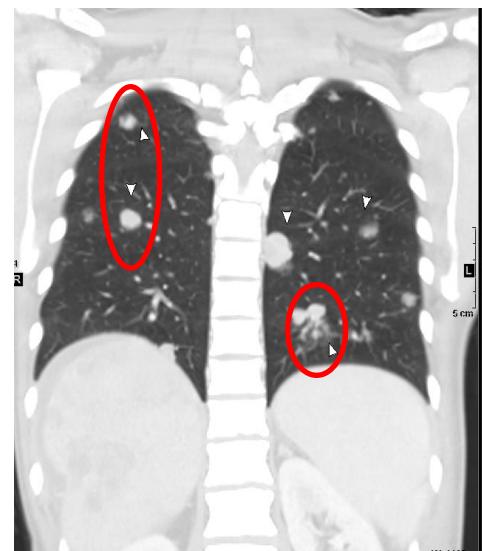
Sample	AvgExp	% of exp	% of 300X	% of 200X	Mean depth
M002-T1	14 2.24	54.57	50.5	54.75	
M002-T1-ACN	14 1.83	50.42	50.00	53.3	

SNV detection statistics		(Cut Off 10000, FDR < 0.05 and Q < 0.05)						
Sample	Number ^a	#germline ^b	Full SNV	Indel SNV ^c	Intronexon total	Intronexon not in CS	Intronexon total in CS	Intronexon not in CS in CS
M002-M005-T1-ACN	76	307	5200030001 (53.1%)	550 (1%)	475 (1%)	30137 (56.5%)	2081 (37.5%)	

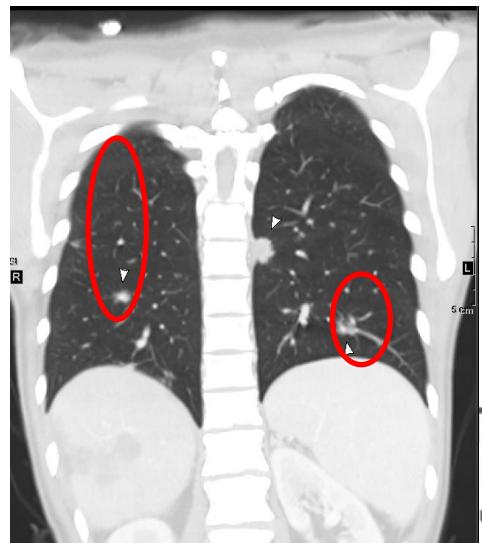
Indel detection statistics		(Cut Off 10000, FDR < 0.05 and Q < 0.05)					
Sample	Number ^a	#germline ^b	Indel total	Indel SNV ^c	Indel CS	Indel total in CS	Indel not in CS in CS
M002-M005-T1-ACN	7	30	1300030001 (54 %)	520 (20 %)	50 (5 %)		

Total detection statistics		(Cut Off 10000, FDR < 0.05 and Q < 0.05)					
Sample	Number ^a	#germline ^b	Total total	Total SNV ^c	Total CS	Total total in CS	Total not in CS in CS
M002-T1-ACN	2	0	5200030001	577 (42%)	50 (5%)	30137 (56.5%)	2081 (37.5%)

Mosaique M002 TAH00200 page 2/2



Evaluation at 2 months



Cholangiocarcinome intra-hépatique métastatique

- Intra hepatic Cholangiocarcinoma
- 65 y/o female
- Medical History
 - June 2016: diagnosis of an Intra hepatic cholangiocarcinoma with hepatic metastases
 - July 2016 – Sept 2016: Folfirinox (x 6 cycles). Best response = PD
 - Oct 2016 – Dec 2016: Gemcitabine (2 month). Best response = PD
 - 10 Jan 2017: Tumor biopsy for molecular profile
 - **07 Feb 2017: double NF1 mutation**
 - 11 Feb 2017: C1D1 Trametinib

Information Prélèvement :

N° Histologique : 17H00338

N° Bloc : K02

Données Cliniques : cholangiocarcinome

Site de prélèvement : FOIE

Date de Biopsie: 10/01/2017

Ctrole Histologique :

20% de cellules tumorales

évaluation : Dr J-Y.Scoazec

Conservation : Congelé

Concentration : 18,1 ng/µL
reçu le 16/01/2017**Information Analyse :** Séquençage NGSeqCan : panel Mosc3 -74 genes

Gene	RefSeq	Exons	Panel	Gene	RefSeq	Exons	Panel
ABL1	NM_007313..	4 à 7	CHP2	FGFR4	NM_002011..	2 to 18	SAF02
AKT1	NM_005163..	3&6	SAF02 + CHP2	FLT1	NM_002019..	1 to 30	SAF02
AKT2	NM_001626..	3	SAF02	FLT3	NM_004119..	11-14-16-20	CHP2
AKT3	NM_005465..	3	SAF02	GNA11	NM_002067..	5	CHP2
ALK	NM_004304..	20 to 26	SAF02 + CHP2	GNAQ	NM_002072..	588	CHP2
APC	NM_000038..	16 (partiel)	CHP2	GNAS	NM_005165..	889	CHP2
ATM	NM_000053..3-3436-39-50		CHP2	HNF1A	NM_005455..	384	CHP2
BRAF	NM_004333..	11&15	SAF02 + CHP2	HRAS	NM_005343..	2 to 4	SAF02 + CHP2
BRCA1	NM_007294..	2 to 23	SAF02	IDH1	NM_005896..	4	CHP2
BRCA2	NM_000059..	2 to 27	SAF02	IDH2	NM_002168..	4	CHP2
CDH1	NM_004360..	3-8-9	CHP2	INPP4B	NM_003866..	5 to 27	SAF02
CDKN2A	NM_000774..	2	CHP2	JAK2	NM_004972..	14	CHP2
CSF1R	NM_005211..	7&22	CHP2	JAK3	NM_002125..	4-13-16	CHP2
CTNNB1	NM_NM	3	SAF02 + CHP2	KDR	NM_002253..	1 to 30	SAF02 + CHP2
DOR2	NM_001047..	4 to 19	SAF02	KEAP1	NM_203500..	2 to 6	SAF02
EGFR	NM_005228..7-12-15-180..		SAF02 + CHP2	KIT	NM_000222..;i11-13-15-17	SAF02 + CHP2	
ERBB2	NM_004448..	8-19&21	SAF02 + CHP2	KRAS	NM_003360..	2 to 4	SAF02 + CHP2
ERBB3	NM_001982..	1 to 28	SAF02	MAP2K1	NM_002755..	283	SAF02
ERBB4	NM_005235..3 to 9-15&23		SAF02 + CHP2	MAP2K4	NM_003010..	1 to 11	SAF02
EZH2	NM_004456..	16	CHP2	MAP3K1	NM_005921..	1 to 20	SAF02
FBXW7	NM_033632..	2 to 12	SAF02	MET	NM_0011275();e1..11-14-16	SAF02 + CHP2	
FGFR1	NM_023110..A-7-12-148..15		SAF02 + CHP2	MLL1	NM_000249..	12	CHP2
FGFR2	NM_000141..	7-9-12&14	SAF02 + CHP2	MLL3	NM_170606..	8-9&43	SAF02
FGFR3	NM_000142..7-9-14-16&18		SAF02 + CHP2	MPL	NM_005373..	1	CHP2
				MTOR	NM_004958..	1 to 58	SAF02
				VHL	NM_000551..	1 to 3	SAF02 + CHP2

Données sur l'analyses PGM

Reference du Run :

Nom du Run d'analyse : Run - R_2017_01_18_08_47_59_user_S5-00631-44-RT05516-RT11013_batch622_20170117

Barcode name : IonXpress_038

Analyse réalisée par :

Sample name : M2229_K02

G.FAUCHER

Analysis setting :

Variant Caller Version : Generic - S5/S5XL (520/530) - Somatic - Low Stringency

Targeted regions ref.: Mosc3_Design_CHP2_plusSaf02_v2_20140320 BED

Hotspot regions ref.: Mosc3_hotspots_CHP2_plusSaf0220131203 BED

Configuratio : Mosc3_Design_CHP2_plusSaf02_v2_20140320_effective BED

Couverture par Amplicon :

Nb amplicons : 1,425

% Amplicon "on target" : 96.45%

Moyenne Couverture : 1,013

Uniformité de couverture : 97.33%

%age à 100X : 98.60%

%age à 500X : 80.00%

Couverture par Base 'HotSpot' :

Nb Bases : 138,164

% base "on target" : 91.93%

Moyenne Couverture : 1,15

Uniformité de couverture : 97.85%

%age à 100X : 99.03%

%age à 500X : 82.11%

Variations de séquence observées:

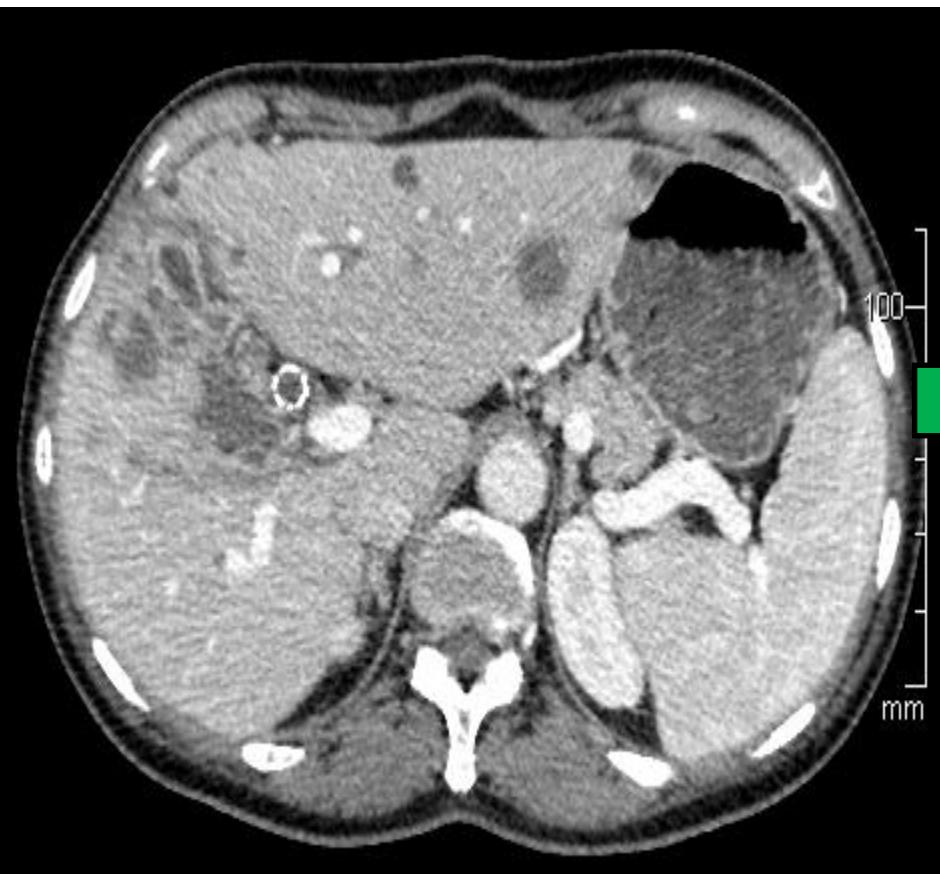
Position Chr	Gene	Nucléotide	Fréquence	Couverture	Prot.	RefSeq	Réf.	Remarques
chr17:29653198	NF1	c.5198delT	37%	ref.499X-Var297X	p.Leu1733fs	NM_00104249	2,2	/ Variant pathogène
chr17:29592315	NF1	c.4793_4798delCTTCCA	35%	ref.409X-Var:218X	p.Thr1598_Ser1599del	NM_00104249	2,2	/ Variant pathogène

Conclusion :

Présence d'une double mutation oncogénique du gène NF1. Elles sont probablement bi-allélique au vu de la fréquence allélique et du mécanisme d'inactivation.

Validé le : 26/01/2017

02.Feb.17



01.Jun.17



Stable Disease (-20%)

Precision medicine for patients with advanced biliary tract cancers: an effective strategy within the prospective MOSCATO trial

Loic Verlingue, David Malka, Adrien Allorant, Christophe Massard, Charles Ferté, Ludovic Lacroix, Etienne Rouleau, Nathalie Auger, Julia Delahousse, Maud Ngo, Claudio Nicotra, Thierry De Baere, Lambros Tselikas, Bakar Ba, Jean-Yves Scoazec, Stefan Michiels, Valérie Boige, Michel Ducreux, Jean-Charles Soria, Antoine Hollebecque
Gustave Roussy and Dug Development Department (DITEP), Gustave Roussy, Villejuif, France

Loic Verlingue et al. European Journal of Cancer 87 (2017)

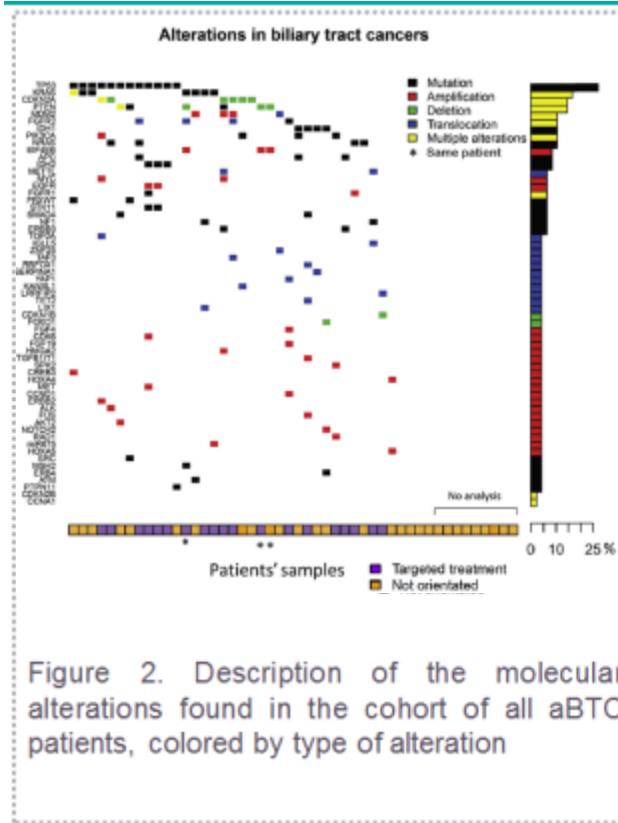


Figure 2. Description of the molecular alterations found in the cohort of all aBTC patients, colored by type of alteration

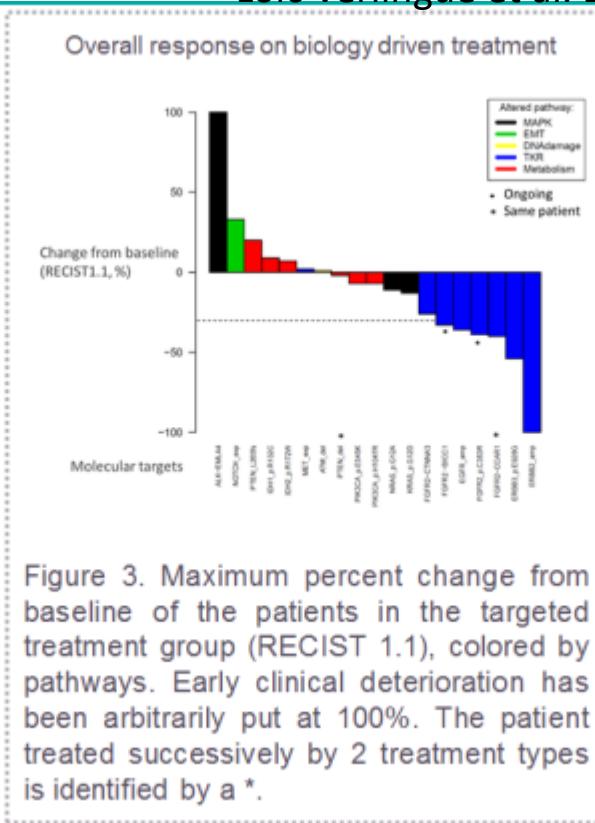


Figure 3. Maximum percent change from baseline of the patients in the targeted treatment group (RECIST 1.1), colored by pathways. Early clinical deterioration has been arbitrarily put at 100%. The patient treated successively by 2 treatment types is identified by a *.

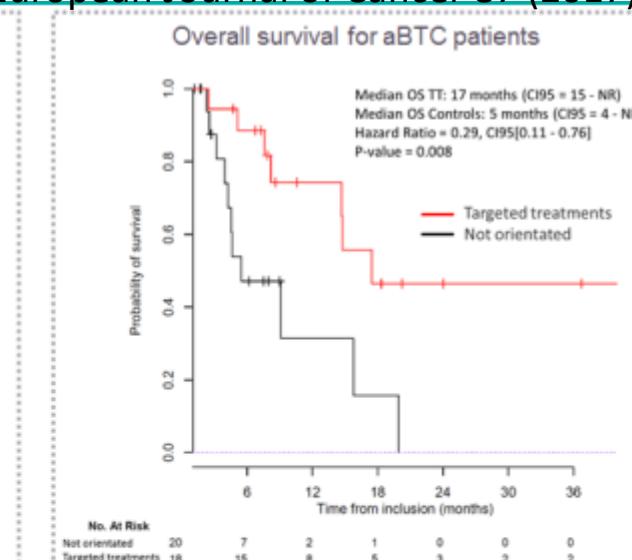


Figure 4. Kaplan-Meier curve of overall survival (No at risk: number of patients at risk, OS: Overall Survival, NR: Not Reached)

CONCLUSION

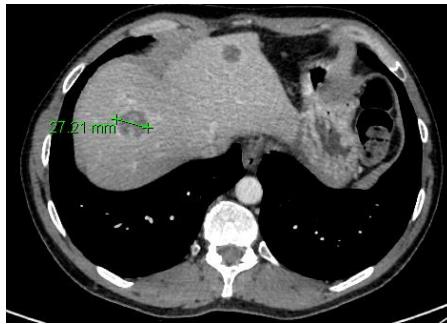
- We could orientate 23 patients out of 43 (53%) to matched MTAs and treat 18 patients (42%)
- We found convergent signs of clinical benefit for treatment matched to the biology of aBTCS:
 - ORR 33%, PFS ratio >1.3 for 50% of the patients
 - Lower risk for death (HR, 0.29; 95%CI, 0.11-0.76; p = 0.008).

Cancer du pancréas métastatique

- Mr S, 44 ans, cancer du pancréas métastatique
- Antécédents
 - Cancer colorectal et de prostate chez le père à 58 ans
 - Cancer chez le grand-père et la tante paternels
- DPC puis gemcitabine adjuvante
- 1^{er} bilan : ↑ CA19-9, métastases hépatiques et ganglionnaires
 - FOLFIRI-3 : RO puis PD
 - FOLFIRINOX : PD
 - Gemcitabine + nab-paclitaxel : PD
- **PS 0, adénomes sébacés multiples**
- PAL 147, RAS par ailleurs
- **Identification d'une mutation *MLH1* et d'une instabilité des microsatellites**

Observation

- Inclusion dans l'essai MK3475-158 (pembrolizumab, **cohorte dMMR**)
- Très bonne tolérance, RO prolongée



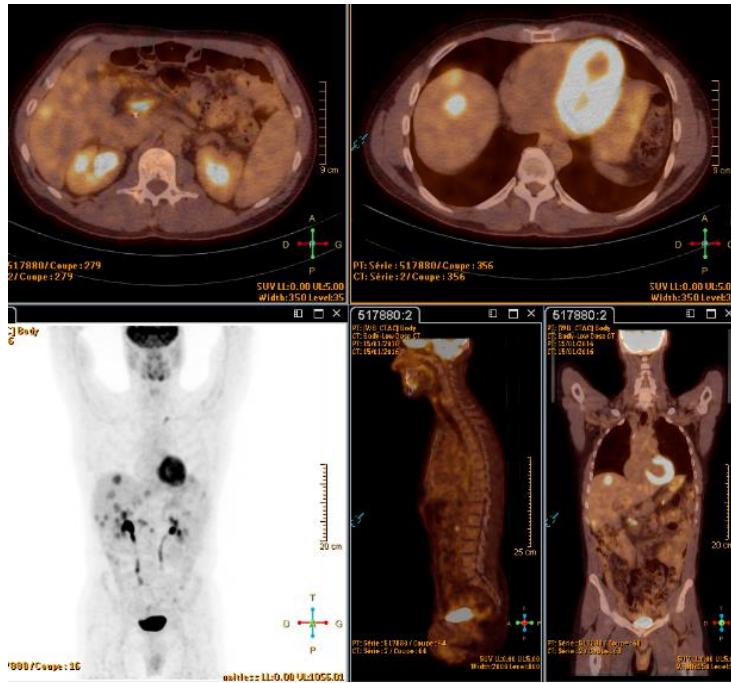
12/02/2016



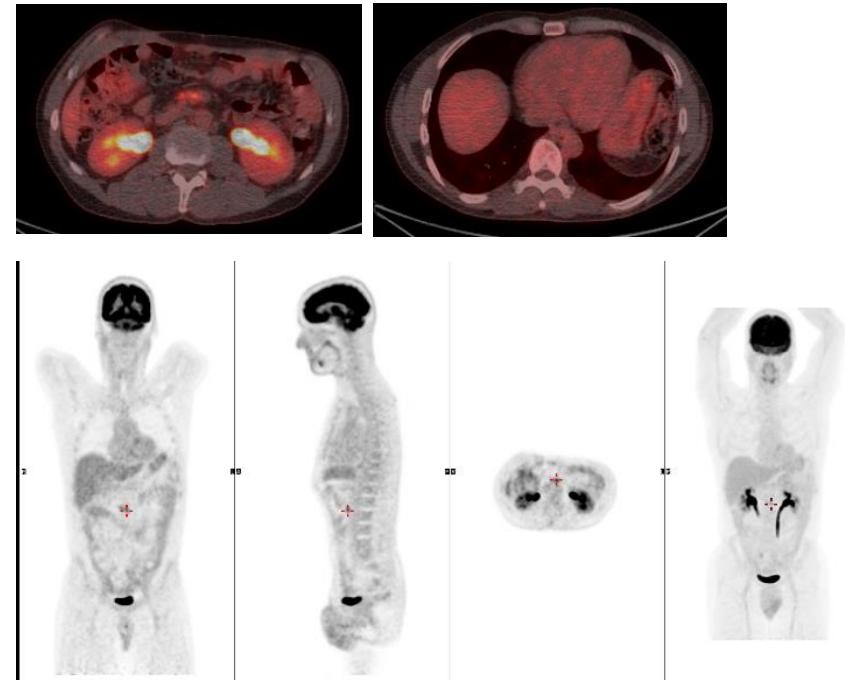
27/04/2018

Observation

15/01/2016



12/03/2018



- 35 cycles reçus → arrêt protocolaire à 2 ans, surveillance trimestrielle

Landscape of DNA Damage Response (DDR) Genes Alterations in Prospective MOSCATO and MATCH R Trials

Yolla El Dakdouki, Loïc Verlingue, Christophe Massard, Rastislav Bahleda, Antoine Hollebecque, Yohan Loriot, Linda Mahjoubi, Etienne Rouleau, Ludovic Lacroix, Nathalie Auger, Jean-Yves Scoazec, Eric Solaro, Aurélien Marabelle, Fabrice André, Jean-Charles Soria, Sophie Postel-Vinay, Gustave Roussy, Drug Development Department (DITEP), Villejuif, France



BACKGROUND

- DDR deficiency is a hallmark of cancer.
- DDR alterations occur regularly in solid tumors. It gained more attention with emergence of IO and DDR inhibitors such as PARP inhibitors.

AIM

- Describing molecular and clinical characteristics of patients (pts) harboring DDR gene alterations with special focus on mismatch repair (MMR) and response to immunotherapy (IO).

METHODS

- 1092 pts with metastatic solid tumors enrolled in two prospective trials MOSCATO (NCT01566019) and MATCHR (NCT02517892) had tumor biopsy between Dec. 2011 and Oct. 2016.

- Techniques of molecular profiling:
 - Targeted Next Generation Sequencing (TGS- N= 1090)
 - Comparative Genomic Hybridization array (CGH- N= 838)
 - Whole Exome Sequencing (WES- N= 304).

- Alterations in 46 genes searched:
 - Pathogenic Variants (PV): Variants causing protein truncation or known to be deleterious missense variants according to databases such as LOVD, BRCAShare and OncoKB.
 - Variants with Unknown Pathogenicity (VUP): Not reported missense variants

RESULTS

- 156 alterations in 107 pts (9.8%) and 30 DDR genes have been identified

Table 1. Patient Characteristics

	N (%)
Age at biopsy (years)	
Median	57
Range	4-88
Sex	
F	56 (52)
M	51 (48)
Nb of previous therapies	
Median	3
Range	1-8
Immunotherapy*	
All (N=107)	
Yes	33 (31)
No	72 (67)
NA	2 (2)
MMRd (N=27)	
Yes	14 (52)
No	13 (48)
PARP Inhibitors*	
Yes	5 (5)
No	102 (95)
Platinum CT in HR alterations*	
Yes	63 (71.5)
No	25 (28.5)

Figure 1. Distribution of pts according to primary tumors (Total N= 107)

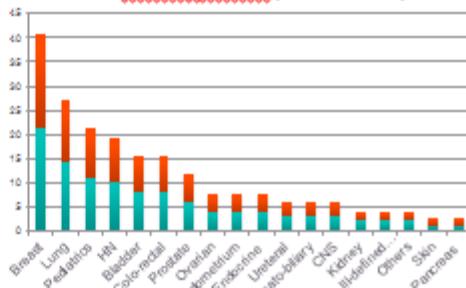


Table 2- Distribution of alterations according to DDR pathways (Bold) and most frequently altered genes

Pathway	Gene	PV	VUP	CGH	Total
HR	ATM	7	16	2	25
HR	BRCAG1	15	9	0	24
HR	BRCAG1	15	4	0	19
HR	ATR	2	6	1	9
HR	BRCA1	3	2	0	5
HR	PALB2	0	5	0	5
Total HR		47	63	6	116
POLE	POLE	2	5	0	7
MMR	MLH3	2	3	0	5
MMR	MSH2	2	2	0	4
MMR	MSH3	2	2	0	4
MMR	MSH3	1	2	1	4
POLE/ MMR		12	20	3	35
Total NER		1	0	1	2
Total BER		0	3	0	3
Total		60	86	10	156

Survival and Response to IO

Whole cohort

- From the time of biopsy, median OS of the cohort was 11 months (95% C.I. 9-13 months)
- 33/107 pts across 12 primary tumors (including bladder, breast, lung and colo-rectal tumors) have been treated with IO. Median PFS with IO was 6.5 months (95% C.I. 2.4-10.5 months) with ORR of 15.2 % (5/33): 2 CR, 3 PR and 7 SD as best response
- Median PFS in the 17/33 pts with PV was 11 months (95% C.I. 0.7-23 months) with ORR of 17.6% (3/17): 1 CR, 2 PR and 6 SD as best response

POLE/MMR aberrations

- The 27 pts with 35 MMR and POLE alterations across 11 different primary tumors including most frequently colorectal, GU and breast. Fourteen patients received immunotherapy.
- Median PFS was 8.5 months with IO and 8.2 months with conventional therapy. One CR, 1 PR and 4 SD were observed with IO, whereas 0 CR, 3 PR and 4 SD were observed with conventional therapy.

CONCLUSION

DDR genes alterations occur in almost 10% of locally advanced or metastatic solid tumors. Systematic analysis of DDR alterations and more specifically PV could allow customizing treatment of pts that specifically benefit from IO or DNA repair inhibitors through synthetic lethality. This has to be further evaluated in larger cohorts.

Nouveau programme PCM2.0

Nouveau Programme Precision Cancer Medicine

COMPRENDRE

PCM RECHERCHE

Résistance / sensibilité
Evolution Clonale
Diagnostics rares

Caractérisation complète:
WES, RNAseq, Immuno,
Single cell,.....

- Déclinaison par cohorte
- Valorisation :
 - biopsies multiples
 - auprès des industriels

ORIENTER VERS les INNOVATIONS

PCM GUIDE

Un portrait moléculaire
pour patients « avancés »
à Gustave Roussy
et inclusion dans les essais

Panel de gènes
sur FFPE et ctDNA

avec les comités
déploiement des
essais baskets

PCM RESEAU*

Un portrait moléculaire
pour patients « avancés »
dans réseau et inclusion
dans les essais de GR

Panel de gènes
sur ctDNA

avec les partenaires de GR
et avec les comités

ODIN

Organisation, structuration, exploitation des
données de génomique et d'immunologie

*réseau territorial en cours de création

RCP moléculaire institutionnelle

- Une RCP hebdomadaire commune
DMO/DITEP le mardi de 11 à 13h
- 2 modes d'entrées pour présentation dossier
- Tous les portraits faits à GR (PCM GUIDE ou MATCH) / externe
- Un CR est édité et envoyé au clinicien.

Conclusion

- Accessibilité de l'information génomique
- Développement des plateformes France Médecine Génomique
- Importance de circuits fiables d'analyse
- Importance d'un réseau pour proposer des traitements dédiés
- Coordination via les RCP moléculaires

Responsabilité génomique



Genomic Alterations Identified[†]

*ERBB3 R475W
FBXW7 R479Q
KRAS A146T
NF1 R2450* – subclonal*, R816*
ARID1A E896*, Q625* – subclonal*
BRAF D594G
CUL3 E246*
EPHA5 E701*
JAK1 K496N
KLHL6 S306L
LRP1B R3638W
MAP2K4 E107*
POLE P286R
RB1 E137*, E237*
RNF43 R337*
ROS1 E738D
TP53 R213**

Additional Findings[†]

*Microsatellite status MS-Stable
Tumor Mutation Burden TMB-High; 43 Muts/Mb*

PATIENT RESULTS

22 genomic findings

15 therapies associated with potential clinical benefit

0 therapies associated with lack of response

21 clinical trials

Séquence de traitement ?

Patient incluable ?

Quel essai clinique ?



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