

## Curie-LC: mutation - Illumina HiSeq 2500, Targeted capture

### Molecular Methods Description:

PDX was analysed by targeted next generation sequencing (NGS) of 95 genes, chosen among the most frequently mutated genes in lung cancer (>1%) and including potential therapeutic targets (SAFIR Lung panel) (see table below).

NGS was performed on an Illumina HiSeq2500 sequencer and the genomic variants were annotated with COSMIC and 1000 genome databases.

### Analysis Description:

Reads were aligned using BWA allowing up to 4% of mismatches with the reference. Only those reads with a mapping quality higher than 20 were used for variant calling, performed with GATK unified genotyper.

NGS primers were selected based on their specificity on the human genome. Genomic alterations included single nucleotide variations of significantly mutated genes (ie, base substitutions and short insertions/deletions). Deleterious genomic alterations were defined as follows:

1. For oncogenes, only mutations driving to gain of function were considered (ie, hotspots missense mutations, in-frame insertions/deletions/splicing described as oncogenic),
2. For tumour suppressor genes, only mutations driving to loss of function were considered (ie, biallelic truncating alterations (nonsense mutations, frameshift insertions/deletions/splicing) or monoallelic truncating alterations associated with heterozygous deletion detected by copy number analysis). Variants with low allelic frequency (<5%) or low coverage (<100x) were excluded from the analysis.

Genomic variants were biologically validated by comparison with COSMIC, TumorPortal and cBioportal databases.

### Table:

AKT1	HotSpot	p.E17K	1 amplicon
AKT2	HotSpot	p.E17Klike	1 amplicon
AKT3	HotSpot	p.E17Klike	1 amplicon
ALK	HotSpot	ex 20,22,23,24,25	8 amplicons
ATM	FullCDS	96.40%	147 amplicons
BRAF	HotSpot	Ex 11&15	2 amplicons
BRCA1	FullCDS	92.80%	123 amplicons
BRCA2	FullCDS	99.10%	73 amplicons
DDR2	HotSpot	Ex6,9,14-16,18	7 amplicons
EGFR	HotSpot	Ex3,6,7,15,18,19,20,21	11 amplicons
ESR1	HotSpot	Ex4,5,7,8	4 amplicons
HER2 /ErbB2	HotSpot	Ex8, 17-22, 24	9 amplicons

HER3/ Erbb3	HotSpot	Ex2,3,6-8	6 amplicons
HER4 / Erbb4	HotSpot	Ex1	1 amplicon
FBXW7	HotSpot	Ex5,8-11	6 amplicons
FGFR1	HotSpot	ex4,7,12,14,15	5 amplicons
FGFR2	HotSpot	Ex3,7,9,12,14	17 amplicons
FGFR3	HotSpot	Ex7,9 (couv 52%),14,16,18	6 amplicons
FGFR4	HotSpot	Ex11,12,14,16	4 amplicons
HRAS	HotSpot	Ex2,3,4	4 amplicons
IDH1	HotSpot	Ex4	1 amplicon
IDH2	HotSpot	Ex4	2 amplicons
KDR	HotSpot	Ex7,8,23	3 amplicons
KEAP1	HotSpot	Ex2,3,4,6	8 amplicons
KIT	HotSpot	Ex8 à 14, 17, 18	15 amplicons
KRAS	HotSpot	Ex 2,3,4	3 amplicons
MAP2K1	HotSpot	Ex3,4,6,7,11	5 amplicons
MAP2K2	HotSpot	Ex2,4	2 amplicons
MAP2K4	FullCDS	99.30%	20 amplicons
MAP3K1	FullCDS	95.40%	58 amplicons
MET	HotSpot	Ex14 (+introns), 16,19-21	9 amplicons
MTOR	FullCDS	99.10%	113 amplicons
NF1	FullCDS	98.50%	137 amplicons
NFE2L2	HotSpot	Ex2	3 amplicons
NRAS	HotSpot	Ex 2,3,4	4 amplicons
PALB2	FullCDS	96.60%	44 amplicons
PDGFRA	HotSpot	Ex12,14,15,18	6 amplicons
PIK3CA	HotSpot	Ex2,5,8,10,14,21	11 amplicons
PIK3R1	HotSpot	Ex9-15	8 amplicons
POLE	HotSpot	Ex6,9,11,13,14,16,19,31,36	11 amplicons
PPP2R1A	HotSpot	Ex5,6	2 amplicons
PTEN	FullCDS	88.60%	19 amplicons
PTPN11	HotSpot	Ex3,13	2 amplicons
RAD51B	FullCDS	94.00%	22 amplicons
RAD51C	FullCDS	100%	22 amplicons
RET	HotSpot	Ex10,11,13,15,16	8 amplicons
ROS1	HotSpot	Ex 38	1 amplicon
STK11	FullCDS	94.30%	23 amplicons
TP53	FullCDS	97.70%	22 amplicons
TSC1	FullCDS	97.90%	49 amplicons

TSC2	FullCDS	97.20%	93 amplicons
VHL	FullCDS	97.80%	8 amplicons