Genomic Landscape of Primary Resistance to Osimertinib among Hispanic patients with *EGFR*-Mutant Non-Small Cell Lung Cancer (NSCLC): Results of an observational longitudinal cohort study

Diego F. Chamorro[1,2,¶], Andrés F. Cardona[3,4,*,¶], July Rodríguez[1,2,¶], Alejandro Ruiz-Patiño[1,2,¶], Oscar Arrieta[5,¶], Darwin A. Moreno-Pérez[1,2], Leonardo Rojas[5], Zyanya Lucia Zatarain-Barrón[5], Dora V. Ardila[1,2], Lucia Viola[6], Gonzalo Recondo[7], Juan B. Blaquier[7], Claudio Martín[8], Luis Raez[9], Suraj Samtani[10], Camila Ordóñez-Reyes[1,2], Juan Esteban Garcia-Robledo[11], Luis Corrales[12], Carolina Sotelo[1,2], Luisa Ricaurte[13], Mauricio Cuello[14], Sergio Mejía[15], Elvira Jaller[1,2], Carlos Vargas[1,2], Hernán Carranza[1,2], Jorge Otero[1,2], Pilar Archila[1,2], Maritza Bermudez[1,2], Tatiana Gamez[1,2], Alessandro Russo[16], Umberto Malapelle[17], Diego de Miguel Perez[18], Vladmir C. Cordeiro de Lima[19], Helano Freitas[19], Erick Saldahna[19], Christian Rolfo[18], Rafael Rosell[20]; on behalf of *CLICaP*[j]

[1]Foundation for Clinical and Applied Cancer Research – FICMAC, Bogotá, Colombia
[2]Molecular Oncology and Biology Systems Research Group (Fox-G), Universidad el Bosque, Bogotá, Colombia
[3]Direction of Research, Science, and Education, Luis Carlos Sarmiento Angulo Cancer Treatment and Research Center (CTIC), Bogotá, Colombia
[4]Thoracic Oncology Unit, Luis Carlos Sarmiento Angulo Cancer Treatment and Research Center (CTIC), Bogotá, Colombia
[5]Thoracic Oncology Unit and Personalized Oncology Laboratory, National Cancer Institute (INCan), México City, México
[6]Thoracic Oncology Unit, Fundación Neumológica Colombiana – FNC, Bogotá, Colombia
[7]Thoracic Oncology Unit, Centro de Educación Médica e Investigaciones Clinicas (CEMIC), Buenos Aires, Argentina
[8]Thoracic Oncology Unit, Alexander Fleming Institute, Buenos Aires, Argentina
[9]Thoracic Oncology Program, Memorial Cancer Institute, Florida Atlantic University (FAU), Miami, Florida, US
[10]Medical Oncology Department, Bradford Hill Institute, Santiago, Chile
[11]Division of Hematology/Oncology, Mayo Clinic, Scottsdale, United States
[12]Thoracic Oncology Unit, Centro de Investigación y Manejo del Cáncer – CIMCA, San José, Costa Rica
[13]Pathology Department, Mayo Clinic, Rochester, Minnesota, United States
[14]Medical Oncology Department, Hospital de Clínicas, Universidad de la Republica – UdeLAR, Montevideo, Uruguay

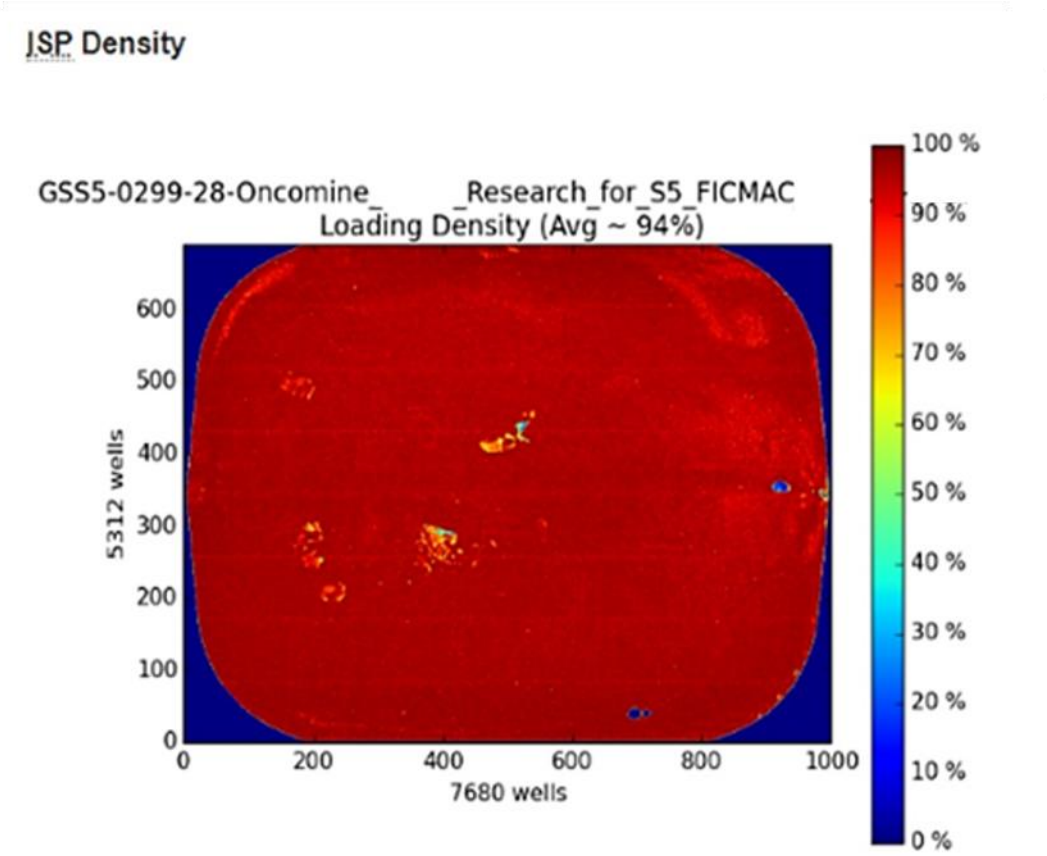
[15]Toracic Oncology Unit, Oncology Department, Cancer Institute, Clínica de las Américas, Medellín, Colombia
[16]Medical Oncology Department, Azienda Ospedaliera Papardo, Messina, Sicilia, Italy
[17]Predictive Molecular Pathology Laboratory, Department of Public Health, University Federico II of Naples, Naples, Italy
[18]Thoracic Oncology Center, Tisch Cáncer Center, Mount Sinai Hospital System & Icahn School of Medicine, Mount Sinai, New York, NY, USA
[19] Thoracic Oncology Unit, A.C. Camargo Cancer Center, Sao Paulo, Brazil
[20]Cancer Biology and Precision Medicine Program, Germans Trias i Pujol Research Institute (IGTP) / Dr. Rosell Oncology Institute (IOR) Quirón-Dexeus University Institute, Barcelona, Spain

[¶]These authors contributed equally to the study.
[ʃ]Latin American Consortium for the Investigation of Lung Cancer

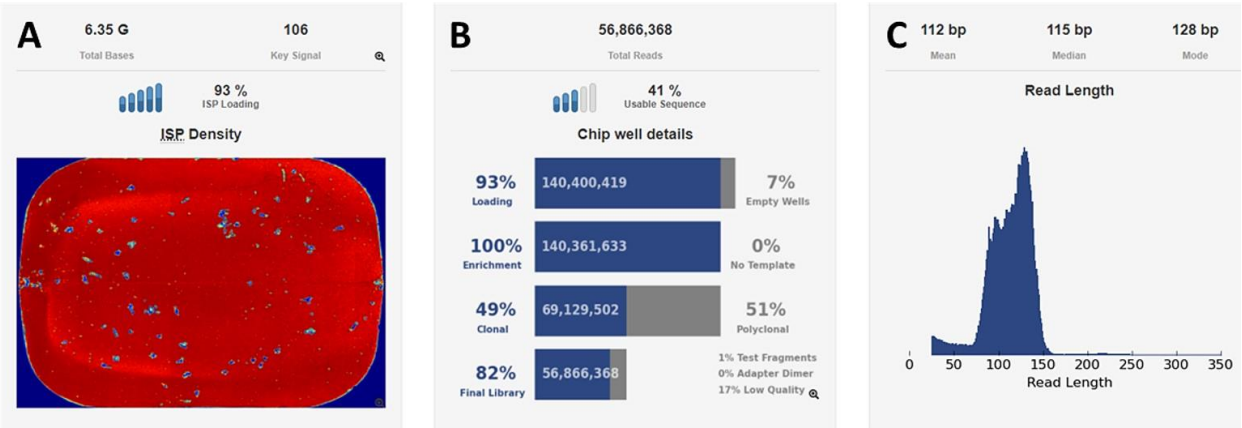
***Correspondence:** Andrés F. Cardona, MD MSc PhD MBA.
Direction of Research, Science, and Education, Luis Carlos Sarmiento Angulo Cancer Treatment and Research Center (CTIC), Bogotá, Colombia
Address: Calle 168 # 14, 110221
Phone: +57 6190052
e-mail: acardona@fctic.org
Twitter: @AndresFCardonaZ

Supplementary material

Supplementary Data 1. Density analysis refers to the homogeneity of the samples in the sequencing chip; for this assembly, the Ion 540™ chip was used. As can be seen in the graph, there are different percentages of homogeneity represented in colors. The lowest rate is represented by the color blue (0 to 10%) and the highest by the color red (90 to 100%).



Supplementary Data 2. In the SD3A, it is observed that the run density was 93%, so the quality metric was adequate for the analysis. In SD3B, the percentage of usable sequences was 41%. Additionally, 82% was obtained for the final library. In the SD3C graph, it is evident that the length of most of the read fragments was between 100 and 150 bp with a mean of 112 bp (data analysis carried out using the S5 torrent server). With the results obtained, it was possible to conclude that, although the integrity of the DNA was moderate, the use of the UDG enzyme and the purification of DNA fragments helped considerably to obtain better quality metrics.



Supplementary Table 1. Genes and alterations included in the analysis.

Hotspot and CNVs	Full-Coding DNA Sequence (CDS)	RNA Fusions
A1CF	ABRAXAS1	AKT2
ABCB1	ACVR1B	ALK
ABL1	ACVR2A	AR
ABL2	ADAMTS2	AXL
ACVR1	ADAMTS12	BRAF
AKT1	AMER1	BRCA1
AKT2	APC	BRCA2
AKT3	ARHGAP35	CDKN2A
ALK	ARID1A	EGFR
AR	ARID1B	ERB84
ARAF	ARID2	ERBB2
ATP1A1	ARID5B	ERG
AURKA	ASXL1	ESR1
AURKB	ASXL2	ETV1
AURKC	ATM	ETV4
AXL	ATR	ETV5
BCL2	ATRX	FGFR1
BCL2L12	AXIN1	FGFR2
BCL6	AXIN2	FGFR3
BMP5	B2M	FGR
BRAF	BAP1	FLT3
BTK	BARD1	JAK2
CACNA1D	BCOR	KRAS
CARD11	BLM	MDM4
CBL	BMPR2	MET
CCND1	BRCA1	MYB
CCND2	BRCA2	MYBL1
CCND3	BRIP1	NF1
CCNE1	CASP8	NOTCH1
CD79B	CBFB	
CDK4	CD274	
CDK6	CD276	

CHD4	CDC73	
CSF1R	CDH1	
CSMD3	CDH10	
CTNNB1	CDK12	
CTNND2	CDKN1A	
CUL1	CDKN1B	
CYSLTR2	CDKN2A	
DDR1	CDKN2B	
DDR2	CDKN2C	
DGCR8	CHEK1	
DROSHA	CHEK2	
E2F1	CIITA	
EGFR	CIC	
EIF1AX	CREBBP	
EMSY	CSMD3	
EPAS1	CTCF	
ERBB2	CTLA4	
ERBB3	CUL3	
ERBB4	CUL4A	
ESR1	CUL4B	
EZH2	CYLD	
FAM135B	CYP2C9	
FGF19	CYP2D6	
FGF23	DAXX	
FGF3	DDX3X	
FGF4	DICER1	
FGF7	DNMT3A	
FGF9	DOCK3	
FGFR1	DPYD	
FGFR2	DSC1	
FGFR3	DSC3	
FGFR4	ELF3	
FLT3	ENO1	
FLT4	EP300	
FOXA1	EPCAM	
FOXL2	EPHA2	

FOXO1	ERAP1	
FYN	ERAP2	
GATA2	ERCC2	
GLI1	ERCC4	
GLI3	ERCC5	
GNA11	ERRFI1	
GNAQ	ETV6	
GNAS	FANCA	
H3F3A	FANCC	
H3F3B	FANCD2	
HIF1A	FANCE	
HIST1H1E	FANCF	
HIST1H2BD	FANCG	
HIST1H3B	FANCI	
HRAS	FANCL	
IDH1	FANCM	
IDH2	FAT1	
IGF1R	FBXW7	
IKBKB	FUBP1	
IL6ST	GATA3	
IL7R	GNA13	
IRF4	GPS2	
IRS4	HDAC2	
KCNJ5	HDAC9	
KDR	HLA-A	
KIR3DL1	HLA-B	
KIT	HNF1A	
KLF4	ID3	
KLF5	INPP4B	
KNSTRN	JAK1	
KRAS	JAK2	
MAGOH	JAK3	
MAP2K1	KDM5C	
MAP2K2	KDM6A	
MAPK1	KEAP1	
MAX	KMT2A	

MCL1	KMT2B	
MDM2	KMT2C	
MDM4	KMT2D	
MECOM	LARP4B	
MED12	LATS1	
MEF2B	LATS2	
MET	MAP2K4	
MITF	MAP2K7	
MPL	MAP3K1	
MTOR	MAP3K4	
MYC	MAPK8	
MYCL	MEN1	
MYCN	MGA	
MYD88	MLH1	
MYOD1	MLH3	
NFE2L2	MRE11	
NRAS	MSH2	
NSD2	MSH3	
NT5C2	MSH6	
NTRK1	MTAP	
NTRK2	MTUS2	
NTRK3	MUTYH	
NUP93	NBN	
PAK5	NCOR1	
PAX5	NF1	
PCBP1	NF2	
PDGFRA	NOTCH1	
PDGFRB	NOTCH2	
PIK3C2B	NOTCH3	
PIK3CA	NOTCH4	
PIK3CB	PALB2	
PIK3CD	PARP1	
PIK3CG	PARP2	
PIK3R2	PARP3	
PIM1	PARP4	
PLCG1	PBRM1	

PPP2R1A	PDCD1	
PPP6C	PDCD1LG2	
PRKACA	PDIA3	
PTPN11	PGD	
PTPRD	PHF6	
PXDNL	PIK3R1	
RAC1	PMS1	
RAF1	PMS2	
RARA	POLD1	
RET	POLE	
RGS7	POT1	
RHEB	PPM1D	
RHOA	PPP2R2A	
RICTOR	PRDM1	
RIT1	PRDM9	
ROS1	PRKAR1A	
RPL10	PSMB9	
RPS6KB1	PSMB10	
RPTOR	PTCH1	
SETBP1	PTEN	
SF3B1	PTPRT	
SIX1	RAD50	
SIX2	RAD51	
SLCO1B3	RAD51B	
SMC1A	RAD51C	
SMO	RAD51D	
SNCAIP	RAD52	
SOS1	RAD54L	
SOX2	RASA1	
SPOP	RASA2	
SRC	RB1	
SRSF2	RBM10	
STAT1	RECQL4	
STAT3	RNASEH2A	
STAT5B	RNASEH2B	
STAT6	RNASEH2C	

TAF1	RNF43	
TERT	RPA1	
TGFBR1	RPL22	
TOP1	RPL5	
TPMT	RUNX1	
TRRAP	RUNX1T1	
TSHR	SDHA	
U2AF1	SDHB	
USP8	SDHC	
WAS	SDHD	
XPO1	SETD2	
YAP1	SLX4	
YES1	SMAD2	
ZNF217	SMAD4	
ZNF429	SMARCA4	
	SMARCB1	
	SOCS1	
	SOX9	

Supplementary Table 2. Genetic variants detected at diagnosis and after progression to Osimertinib in Cohorts A and B.

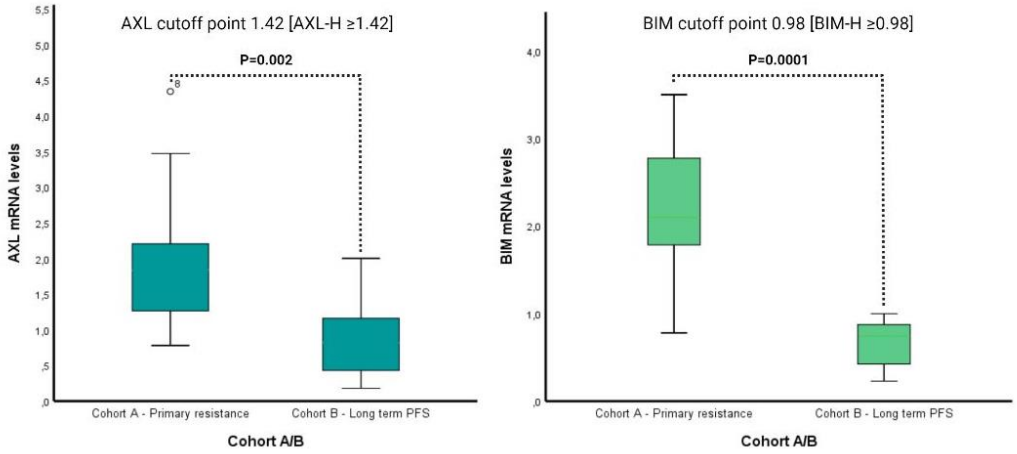
Genomic alteration identified at diagnosis	
Variant	**N (%)**
TP53	22
p.V173fs	3 (13.7)
p.A159P	1 (4.5)
p.C277F	1 (4.5)
p.D281E	2 (9.2)
p.D281V	1 (4.5)
p.D281V	1 (4.5)
p.E286G	1 (4.5)
p.G245V	2 (9.2)
p.R337L	1 (4.5)
p.H168L	2 (9.2)
p.R175H	1 (4.5)
p.R280T	1 (4.5)
p.T155I	2 (9.2)
p.V272G	1 (4.5)
p.Y205C	1 (4.5)
p.Y220C	1 (4.5)
ARID1A	6
p.E2032K	3 (50.2)
p.Q1357L	1 (16.6)
p.Q754*	1 (16.6)
p.E1760*	1 (16.6)
SETD2	5
p.F1651fs	3 (60.0)
p.E1518*	1 (20.0)
p.R1063*	1 (20.0)
BRAF	4
Amp	3 (75.0)
p.G466V	1 (25.0)
SMARCA4	3
p.R381*	3 (100.0)
RBM10	3

p.Q155*	1 (33.3)
p.Q192_splice	1 (33.3)
p.E559*	1 (33.3)
CDKN2A	2
p.V51 splice	1 (50.0)
p.H83Y	1 (50.0)
FGFR2	2
p.S137W	1 (50.0)
Amp	1 (50.0)
KRAS	2
Amp	2 (100.0)
NOTCH1	2
p.Q1584*	1 (50.0)
p.Q2459*	1 (50.0)
PDGFR	2
Amp	2 (100.0)
RB1	2
p.E398L	1 (50.0)
p.Y756C	1 (50.0)
NRAS	1
p.A146T	1 (100.0)
RB1	1
p.Y756C	1 (100.0)
MGA	1
p.R702*	1 (100.0)
NF1	1
p.Q1189*	1 (100.0)
KIT	1
p.V556D	1 (100.0)
TSC1	1
p.Q527*	1 (100.0)
FANCA	1
p.E1196*	1 (100.0)
FGFR3	1
p.R248C	1 (100.0)
SMAD4	1

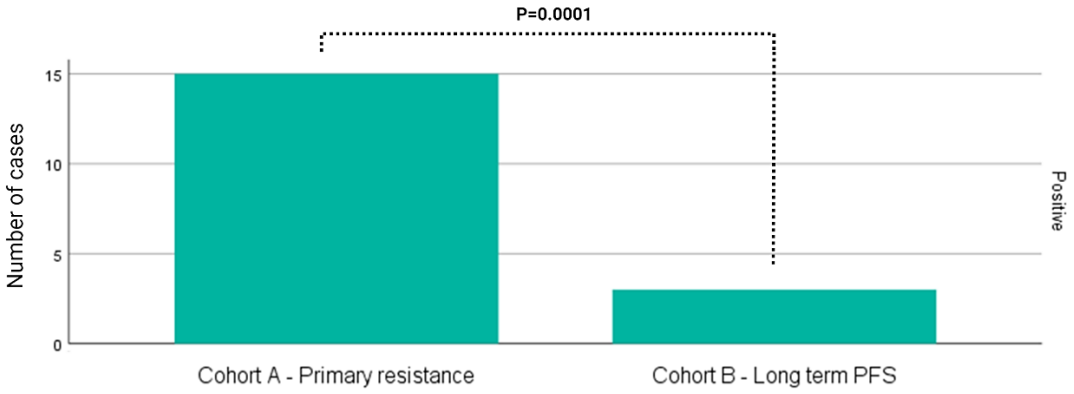
p.R361	1 (100.0)
U2AF1	1
p.S34F	1 (100.0)
AKT1	1
p.D323Y	1 (100.0)
DMNT3A	1
p.W314*	1 (100.0)
KEAP1	1
p.D479G	1 (100.0)
MTOR	1
p.E2014K	1 (100.0)
Genomic alteration identified at the time of progression to Osimertinib	
Variant	**N (%)**
TP53	16
p.E286G	1 (6.2)
p.G245V	2 (12.6)
p.R337	1 (6.2)
p.H168L	1 (6.2)
p.R175H	1 (6.2)
p.R280T	1 (6.2)
p.T155I	2 (12.6)
p.V173fs	2 (12.6)
p.Y205C	1 (6.2)
p.A159P	1 (6.2)
p.C277	1 (6.2)
p.Q192*	1 (6.2)
p.G245S	1 (6.2)
BRAF	8
Amp	6 (75.0)
p.G466V	2 (25.0)
EGFR	7
Amp	5 (71.5)
p.C719S	2 (28.5)
KRAS	7
Amp	2 (28.6)

p.G12C	2 (28.6)
p.G12D	3 (42.8)
MET	5
Amp	5 (100.0)
RB1	5
p.Y756C	4 (80.0)
p.E398L	1 (20.0)
NF1	3
p.Q1189*	2 (66.6)
p.E2357*	1 (33.3)
PI3K	3
p.E545K	1 (33.3)
p.Q546K	2 (66.6)
NRAS	2
p.A146T	1 (50.0)
p.Q61L	1 (50.0)
GNAS	2
p.R201S	1 (50.0)
p.R205T	1 (50.0)
HER2	1
Amp	1 (100.0)
SETD2	1
p.E1518*	1 (100.0)
ARID1A	1
p.Q754*	1 (100.0)
CTNNB1	1
p.G34R	1 (100.0)
MTOR	1
p.E2014K	1 (100.0)
PDGFRa	1
Amp	1 (100.0)
RT1	1
p.M90I	1 (100.0)

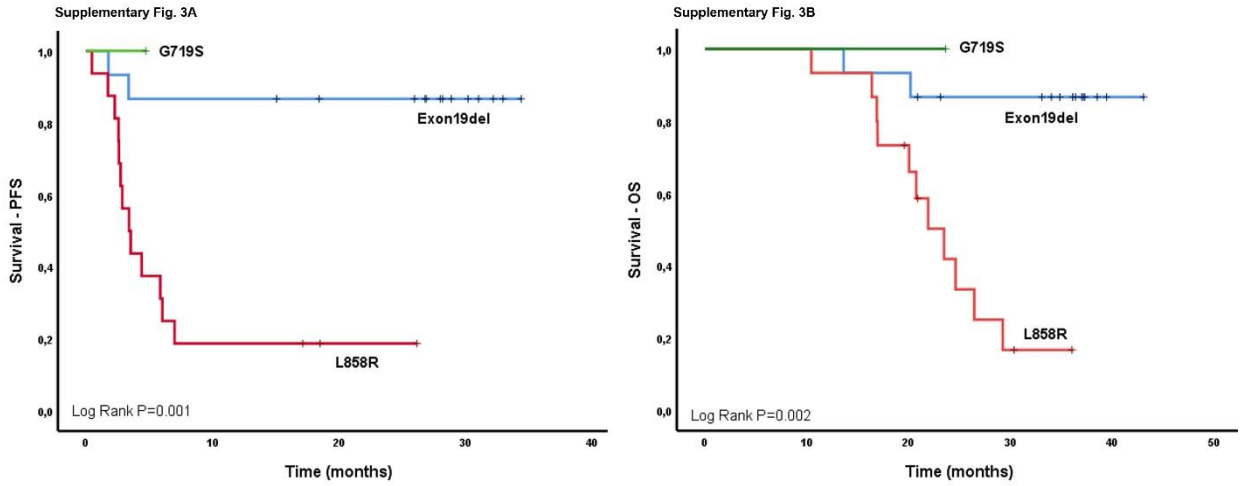
Supplementary Figure 1. Distribution of mRNA levels for *AXL* and *BIM* in Cohorts A and B.



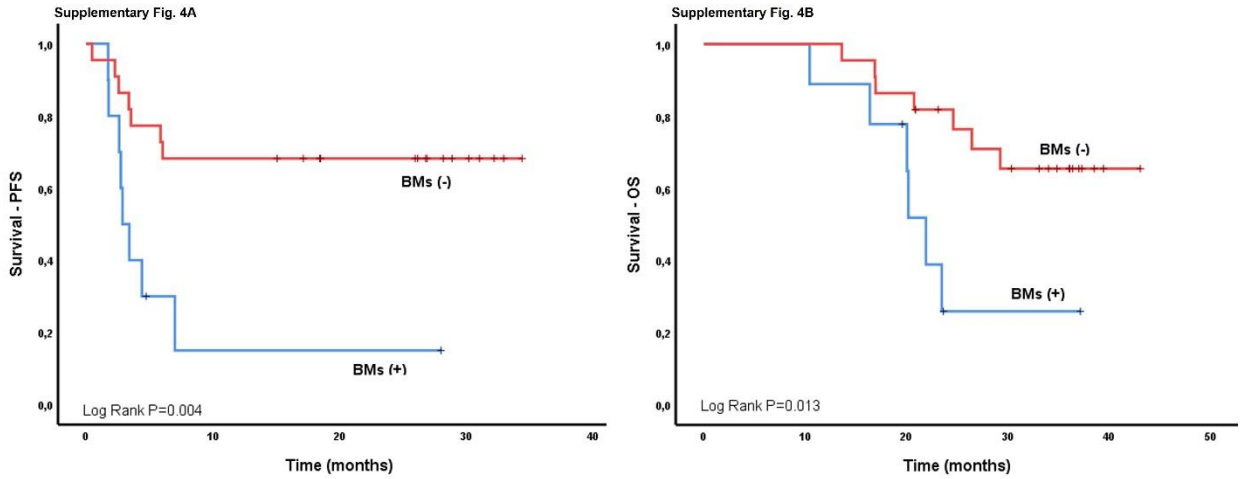
Supplementary Figure 2. Detection of *EGFR cf*DNA by droplet digital PCR EGFR multiplex assays performed at week 8 after starting treatment with Osimertinib.



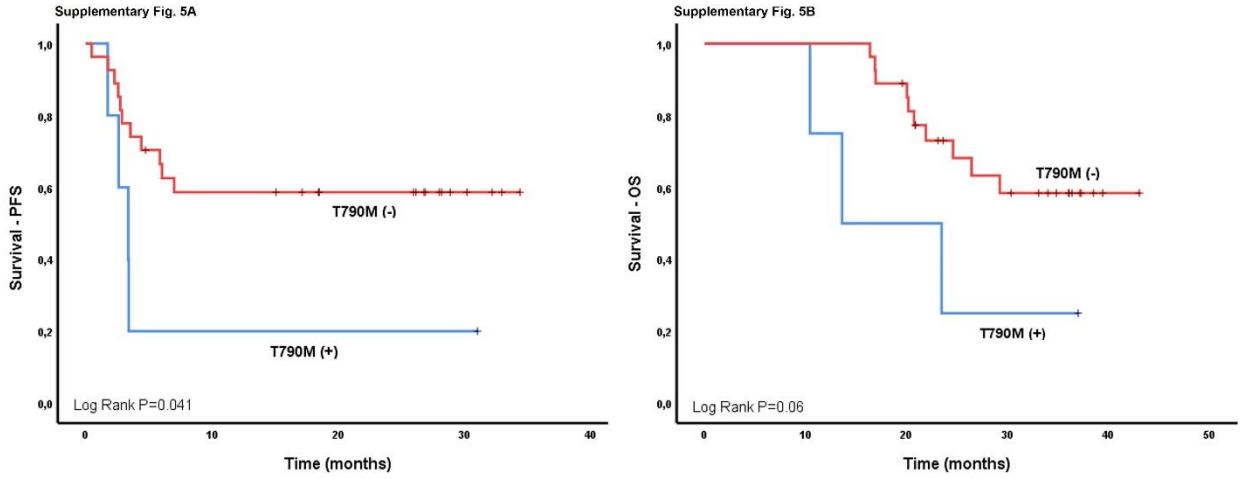
Supplementary Figure 3. (A) PFS for Osimertinib according to the type of sensitizing mutation. As mentioned, patients in cohort A had a higher number of alterations in exon 21 compared with those in cohort B. **(B)** OS discriminated by the type of mutation in *EGFR* (immature data for the calculation of medians).



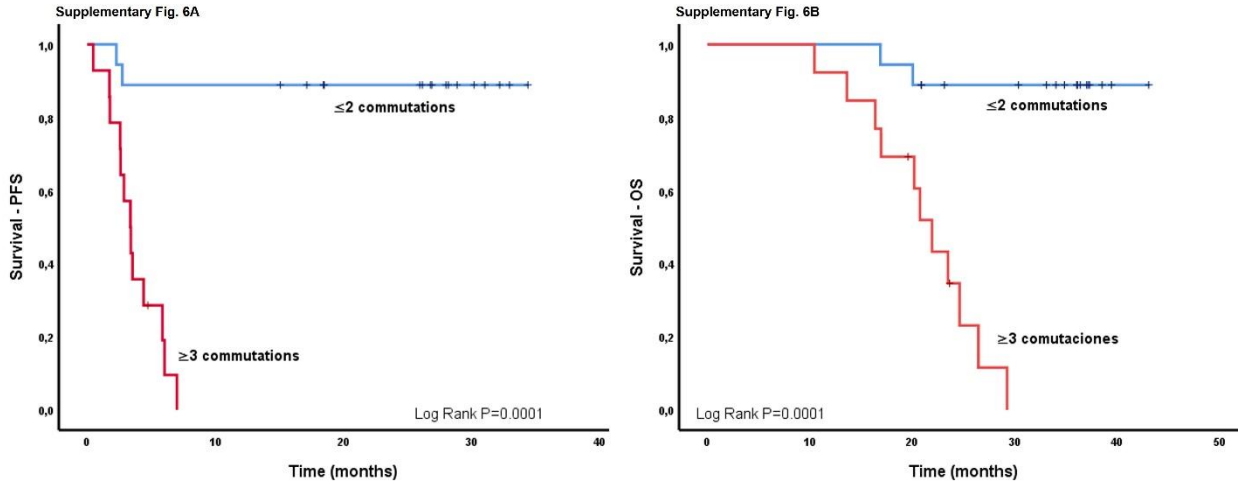
Supplementary Figure 4. (A) PFS for Osimertinib according to presence of brain metastases at the time of diagnosis. **(B)** OS in patients with and without brain metastases at diagnosis was 21.0 months (95%CI 19.4-24.3) and 35.0 months (95%CI 31.0-40.2), respectively (P=0.013).



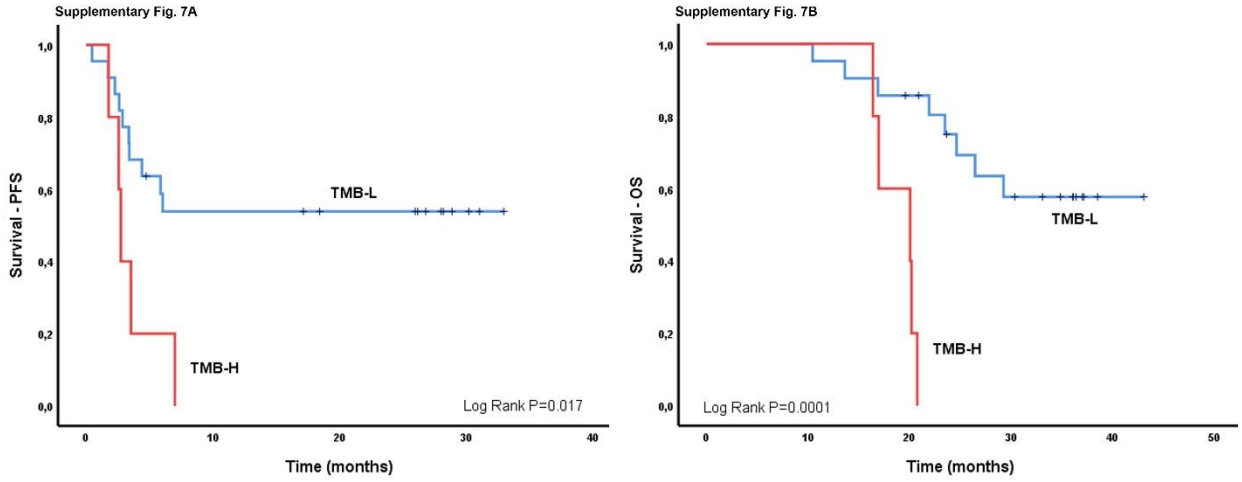
Supplementary Figure 5. (A) PFS for Osimertinib according to the presence of T790M mutation at baseline, a finding that was dominant in Cohort A. The PFS for Osimertinib in those with baseline T790M was 8.4 months (95%CI 1.7-5.0) versus 21.6 months (95 %CI 15.8-27.4; P=0.041) among those without T790M mutation. **(B)** OS according to the presence (21.1 months, 95%CI 10.9-31.2) or absence (34.2 months, 95%CI 29.8-38.5) of the baseline T790M mutation (P=0.06).



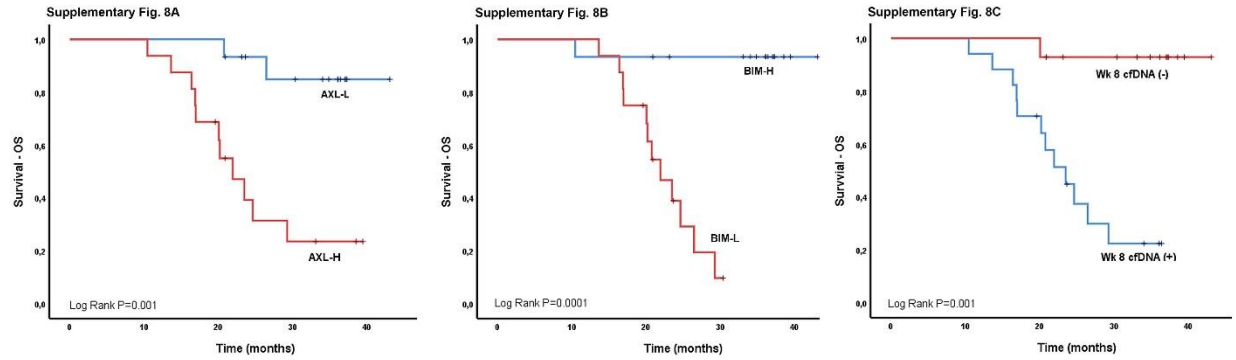
Supplementary Figure 6. (A) PFS for Osimertinib according to the number of co-mutations. Those with ≤2 co-mutations had a PFS of 30.8 months (95% CI 26-2-35.4) versus the group with ≥3 co-mutations (3.3 months, 95% CI 2.3-4.3; P=0.0001). **(B)** OS discriminated for patients with ≤2 co-mutations (40.3 months, 95%CI 36.7-43.9) and ≥3 co-mutations (21.1 months, 95%CI 17.9-24.2; P=0.0001).



Supplementary Figure 7. (A) PFS for Osimertinib according to TMB. Patients with TMB-H had a PFS of 2.7 months (95%CI 2.3-3.0) versus 19.2 months (95%CI 12.0-25.5) for those with TMB-L (P=0.017). **(B)** The median OS according to TMB was 33.3 months (95%CI 28.7-38.9) for those with TMB-L and 18.8 months (95%CI 17.0-20.6) for those with TMB-H (P=0.0001).



Supplementary Figure 8. (A) According to mRNA expression for *AXL*, OS is 40.1 months (95%CI 36.4-43.8) for patients with *AXL*-L and 24.4 months (95%CI 19.6-29.3; P=0.001) for those in the *AXL*-H group. **(B)** According to *BIM* mRNA expression, OS was 40.8 months (95%CI 36.7-45.0) for the *BIM*-H group and 22.4 months for the *BIM*-L (95%CI 19.9-24.9; P=0.0001). **(C)** OS for patients with (24.0 months, 95%CI 20.1-28.0) and without (41.4 months, 95%CI 38.3-44.5) detectable cfDNA at week eight after starting Osimertinib (P=0.001).



Supplementary Figure 9. Representative case (case No. 13 of Cohort A) of primary resistance to osimertinib, including genomic variations throughout the history of the disease