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Supplementary Materials

Clinical and Molecular Features of Epidermal Growth Factor Receptor (EGFR) Mutation Positive Non-Small-Cell Lung Cancer (NSCLC) Patients Treated with Tyrosine Kinase Inhibitors (TKIs): Predictive and Prognostic Role of Co-Mutations

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Supplementary materials

List of rare and double mutations (8/106):

Exon 18 G719A (n:3), Exon 18 G719S (n:1), Exon 19 L747P (n:1), Exon 21 L861Q (n:1), Exon 18 E709K + Exon 21 L858R (n:1), Exon 18 G719S + Exon 20 S768I (n:1)

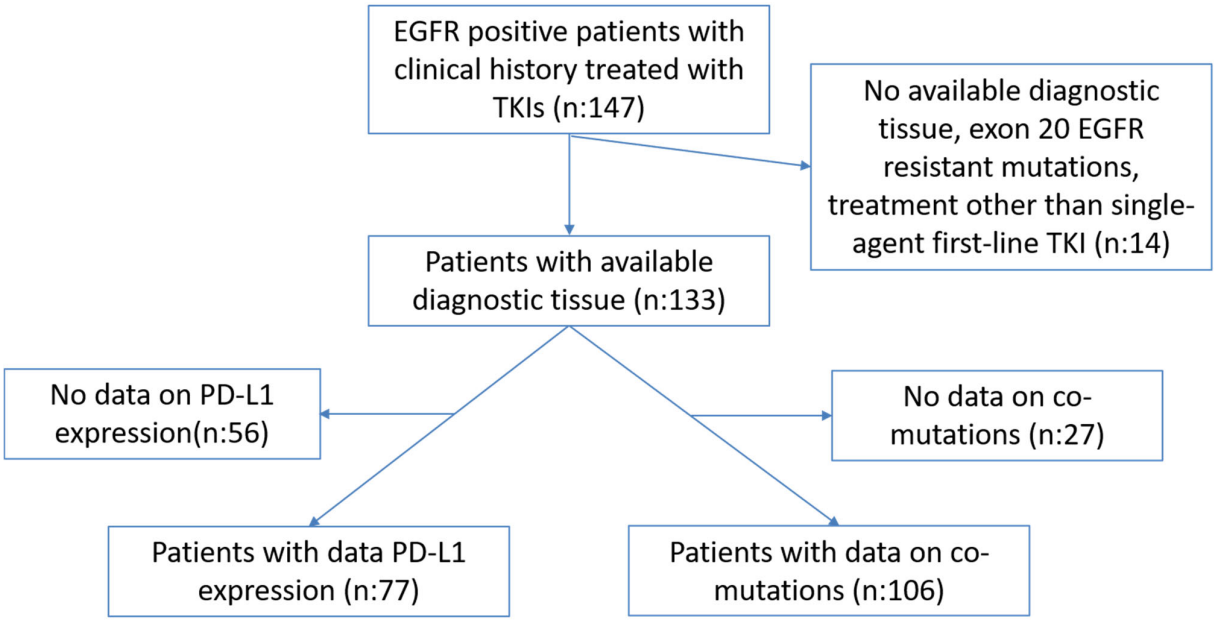
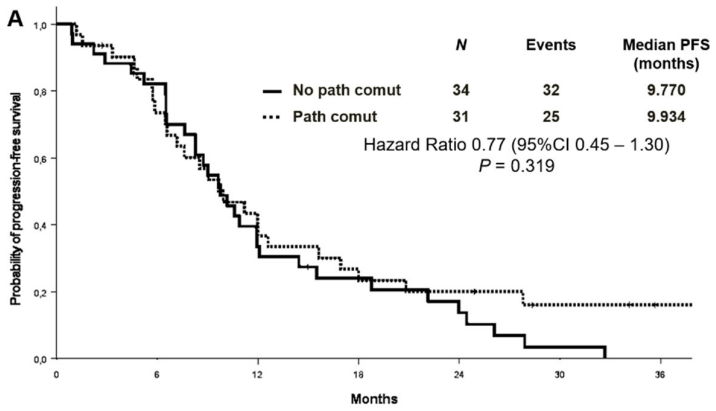


Figure S1. Consort diagram. EGFR: Epidermal Growth Factor Receptor; TKI: Tyrosine Kinase Inhibitor; PD-L1: Programmed Death-Ligand 1.



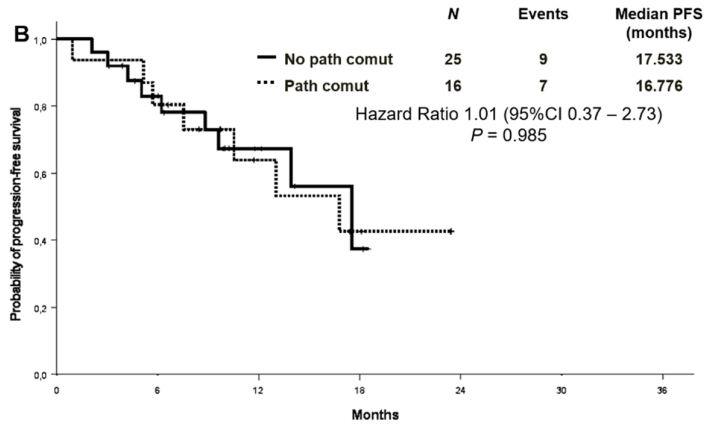


Figure S2. Progression-free survival according to the type of EGFR TKI in patients with and without concomitant mutaTable 3. Overall survival according to the type of EGFR TKI in patients with and without concomitant mutations: first/second generation (A) and third generation (B). EGFR: Epidermal Growth Factor Receptor. TKI: Tyrosine Kinase Inhibitor.

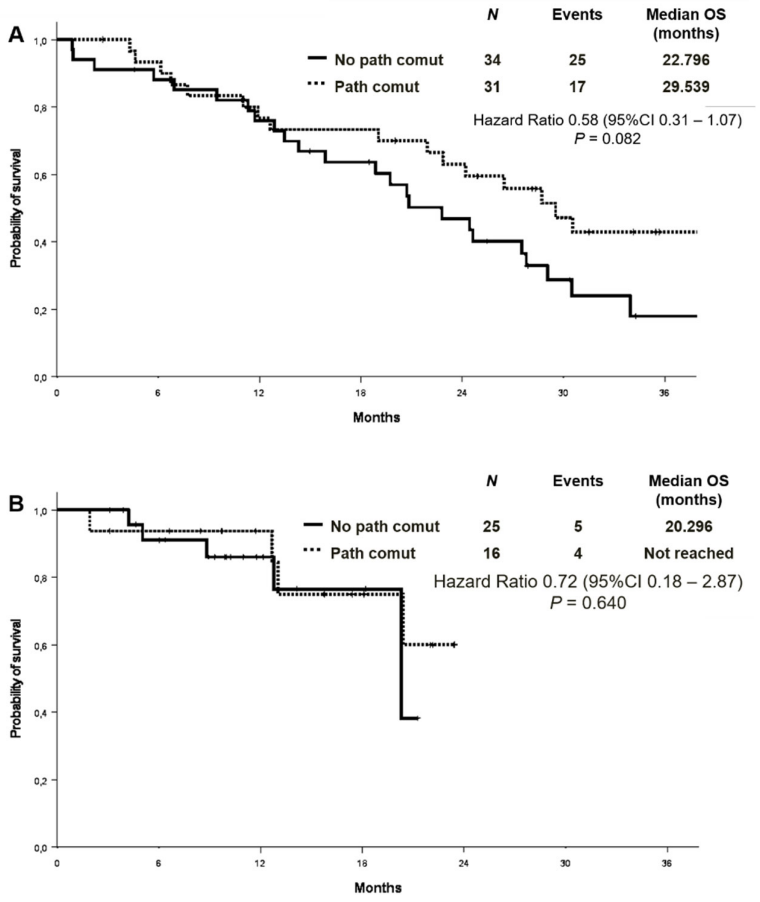
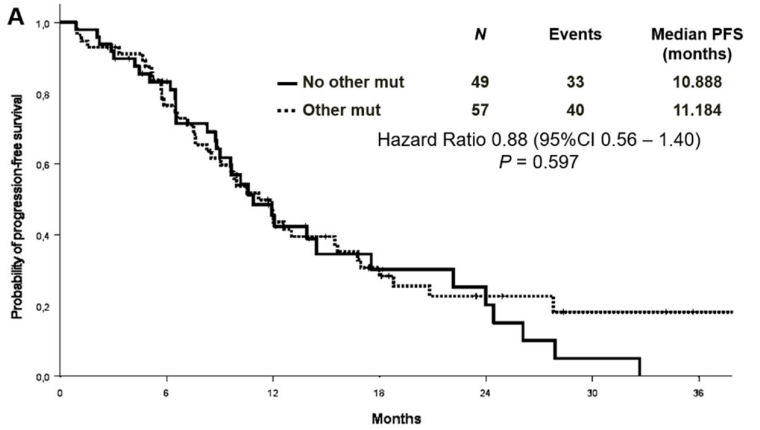


Figure S3. Overall survival according to the type of EGFR TKI in patients with and without concomitant mutations: first/second generation (A) and third generation (B). EGFR: Epidermal Growth Factor Receptor. TKI: Tyrosine Kinase Inhibitor.



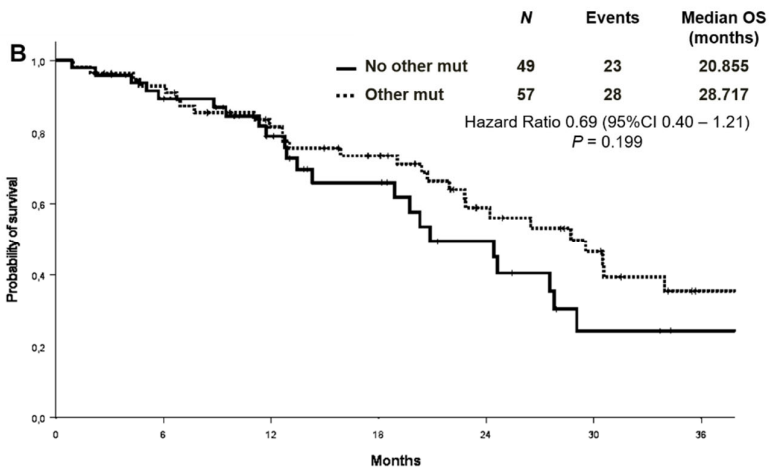


Figure S4. Progression-free survival (A) and overall survival (B) according to co-mutational status, considering all co-mutations (pathogenic and non-pathogenic/ of unknown significance).

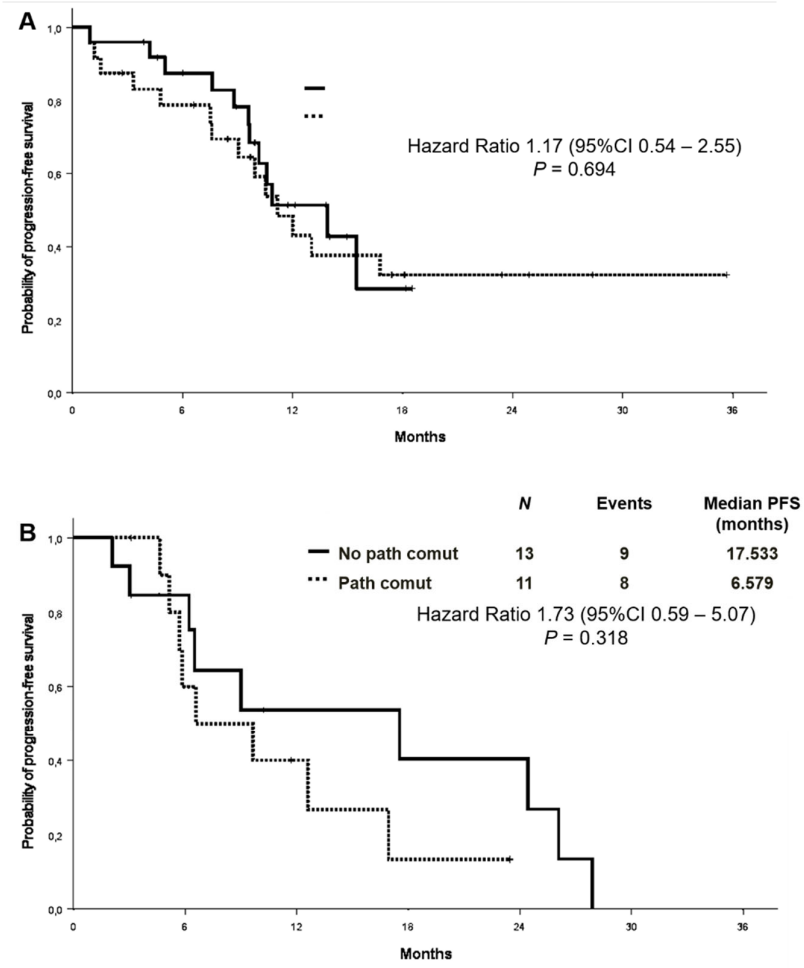


Figure S5. Progression-free survival in PD-L1 positive and negative patients by co-mutational status. PD-L1: Programmed death-ligand 1.

Table S1. Studies evaluating co-mutations in advanced NSCLC patients with activating EGFR mutations. EGFR: epidermal growth factor receptor; NSCLC: non-small cell lung cancer; TKI: tyrosine kinase inhibitors; NGS: next-generation sequencing; HR: hazard ratio; CI: confidence interval; CNV: copy number variation; PFS: progression-free survival; OS: overall survival; ORR: objective response rate; IHC: immunohistochemistry; FISH: fluorescence *in situ* hybridization.

Author, year	Type of study, Country	Number of advanced patients analysed	Type of first-line treatment (number treated)	Type of test	Most frequent concurrent alterations	Outcomes
Barnet, 2017[3]	Single-centre, retrospective, Australia	62	1st and 2nd generation TKIs (n: 62)	19 oncogenes, MassArray (OncoCarta v1.0 panel)	*EGFR* (8%) and *PIK3CA* (3.2%) mutations	Significantly shorter mPFS in patients with co-mutations (5.7 vs 12.3 months; p=0.02) and lower ORR (38% vs 89%, p<0.001)
Hu, 2017[4]	Single-centre, retrospective, China	320 (stage IIIB and IV)	1st or 2nd generation TKIs (n: 320)	PCR for *HER2*, *KRAS*, *NRAS*, *BRAF*, *PIK3CA* mutations and for *ALK*, *ROS1* and *RET* rearrangements	*PIK3CA* (2.8%) and *KRAS* (0.9%) mutations, *ALK* (1.9%), *RET* (0.8%) and *ROS1* (0.8%) rearrangements	Concomitant mutations are associated with significantly shorter PFS; no difference in OS were observed
Labbé, 2017[5]	Single-centre, retrospective, Canada, focused on *TP53* only	60	1st generation TKIs (n: 60)	*TP53* Sanger sequencing or NGS	*TP53* 56% (missense: 17%)	Significantly shorter mPFS in patients with *TP53* missense mutations (HR 1.91, 95% CI 1.01-3.60, p=0.04)
VanderLaan, 2017[6]	Single-centre, retrospective, United States	20	1st or 2nd generation EGFR TKIs (n: 16)	SNaPshot-NGS or JAX-Cancer Treatment Profile	*TP53* (50%), *PIK3CA* (10%), *PTEN* (5%) mutations	No significant differences between patients with and without co-mutations
Hong, 2018[7]	Single-centre, retrospective, China	58	1st generation TKIs (n: 58)	49 cancer-related genes with Ion Pi Sequencing 200 kit v2 (Thermo Fisher Scientific)	*TP53* (41.4%), *EGFR* T790M (13.8%), *KRAS* (6.9%), *PIK3CA* (5.2%) mutations	Concomitant mutations were associated with lower ORR, and shorter PFS and OS

Jakobsen, 2018[8]	Single-centre, retrospective, Denmark	18 (+ 5 with stage IIIA/B)	1st generation TKIs (n: 23)	22 lung/colon-cancer associated genes (Ion AmpliSeq Colon—Lung Cancer Research Panel v2 on Ion Torrent, Thermo Fisher Scientific); *MET* IHC and FISH, and *ALK* IHC were also performed	*TP53* (67%), *CTNNB1* (13%). 5 and 4 samples had *MET* overexpression and amplification, respectively.	No differences in terms of PFS or OS between patients with or without co-alterations
Yu, 2018[9]	Single-centre, retrospective, United States	200	1st or 2nd generation EGFR TKIs (n: 200)	NGS: MSK Impact, version 1, 2 or 3	*TP53* (60%), *PIK3CA* (12%), *CTNNB1* (9%), *RB1* (10%) mutations, and *EGFR* (22%), *TTF1*(15%), *MDM2 (12%), CDK4* (10%) and *FOXA1* (10%) amplifications	Shorter time to progression on TKI associated with *ERBB2* (HR 2.42, p=0.018) and *MET* (HR 3.65, p=0.029) amplifications, and *TP53* mutations (HR 1.68, p=0.006)
Kim, 2019[10]	Single-centre, retrospective, Korea	75	1st or 2nd generation EGFR TKIs (n: 75)	NGS: CancerSCAN panel, version 1 or 2	*TP53* (57.3%), *CTNNB1* (9.3%), *PIK3CA* (8%), *RB1*(6.7%) mutations	*TP53* mutations independently associated with worse PFS (HR 2.02, 95% CI 1.04-3.93; p=0.038)
Chang, 2019[11]	Single-centre, retrospective, Taiwan	33	1st and 2nd generation TKIs (n: 33)	ACTonco®+ panel using Ion Proton sequencer with Ion PI chip (Life Technologies)	*TP53* (32%) mutations, *CDK4* (26%) and *CDKN2A* (23%) alterations (mainly CNV gain or loss)	FGFR3 mutations and CDKN2A CNV loss associated with shorter PFS; patients with any concomitant mutations have worse OS (24.1 vs 40.8 momths; p=0.029)
Chen, 2019[12]	Singe-centre, retrospective, China	71 (selected according to PFS ≤6 months or ≥24 months)	1st generation TKIs (n: 71)	416 cancer-relevant genes (Illumina Hiseq 4000 NGS platforms)	*TP53* (51%), *MAP2K2* (15%), *NKX2-1* (15%), *CTNNB1* (15%), *RB1* (12%) mutations, *EGFR* amplification (18%).	*TP53* missense and *PIK3CA* missense mutations more frequent in the short PFS group as well as co-occurring driver mutations (*ALK* rearrangement, *MET* amplification, *BRAF V600E* mutation). No difference in *TP53* mutation rate between short and long PFS group.

Rachiglio, 2019[13]	Multicentre, retrospective, Italy	133	1st and 2nd generation TKIs (n: 133)	22 cancer-related genes panel (Ion AmpliSeq Colon—Lung Cancer Panel on Ion Torrent, Thermo Fisher Scientific)	*TP53* (17.3%), *KRAS* (14%), *PIK3CA* (9%), *EGFR* T790M (6.8%) mutations	Concomitant mutations (but not *TP53*) are associated with significantly shorter PFS on EGFR TKIS
Cheng, 2020[14]	Single-centre, retrospective, China	175 (stage IIIA or higher) 160 with EGFR mutations	1st, 2nd, 3rd generation TKIs +/- chemotherapy or anti-angiogenics (n: 110, 1st; n: 35, 2nd; n: 15, 3rd)	520 or 168 cancer-related gene panel based on NextSeq 500 (Illumina technology)	*TP53* (57%), *PIK3CA* (6%), *PMS2* (6%), *DMT3A* (6%), *APC* (6%), *MYC* (6%) mutations. 6.9% other driver alterations (*ERBB2* and *MET* amplifications, *ERBB2*, *BRAF* and *KRAS* mutations)	*TP53* mutation, *ERBB2* and *FGF19* amplifications associated with significantly worse OS upon treatment with 1st but not 2nd generation TKIs; CNV associated with significantly shorter PFS on 3rd generation TKIs
Christopoulos, 2020[15]	Multi-centre, retrospective, Germany	261	1st and 2nd generation TKIs (n: 219)	38-42 genes custom panel (NGS, ThermoFisher Scientific)	*TP53* (44%), *CTNNB1* (4.6%)	*TP53* mutations independently associated with PFS and OS

Table S2. Co-mutational status by EGFR mutation type. EGFR: Epidermal Growth Factor Receptor.

	Patients without concomitant pathogenic mutations (n=59)	Patients with concomitant pathogenic mutations (n=47)	All patients (n=106)
Main mutation			
Exon 19	33 (55.9%)	33 (70.2%)	66
L858R	23 (39.0%)	10 (21.3%)	33
Other	3 (5.1%)	4 (8.5%)	7
		Chi square p=0.138	

Table S3. Site of progression according to co-mutational status.

	Patients without concomitant pathologic mutations (n=34)	Patients with concomitant pathologic mutations (n=30)	Chi square
Lung	18/34 (52.9%)	13/30 (43.3%)	p=0.443
Pleural	10/34 (29.4%)	5/30 (16.7%)	p=0.230
CNS	12/34 (35.3%)	12/30 (40.0%)	p=0.698
Liver	7/34 (20.6%)	4/30 (13.3%)	p=0.443
Bone	9/34 (26.5%)	1/30 (3.3%)	**p=0.011**
Adrenal	1/34 (2.9%)	2/30 (6.7%)	p=0.482
Nodes	4/34 (11.8%)	1/30 (3.3%)	p=0.210

Table S4. Distribution of PD-L1 expression in patients with and without concomitant mutations. PD-L1: Programmed death-ligand 1.

	Patients without concomitant pathologic mutations (n=38)	Patients with concomitant pathologic mutations (n=35)	Wilcoxon – Mann-Whitney
Median	0	0	p=0.916
Range	0 - 75	0 - 95	

Table S5. Distribution of PD-L1 expression levels in patients with and without co-mutations. PD-L1: Programmed death-ligand 1.

	Patients without concomitant pathologic mutations (n=38)	Patients with concomitant pathologic mutations (n=35)	Chi square
PD-L1 0%	25 (65.8%)	24 (68.6%)	p=0.483
PD-L1 1-49%	11 (28.9%)	7 (20.0%)	
PD-L1 >=50%	2 (5.3%)	4 (11.4%)	