Details on the detection of *TP53* co-mutations and the three different *TP53* classifications

Assay methods

Against the background of technological advances in recent years, *EGFR* exon 18–21 and *TP53* (exons 4–10) mutational analysis were performed by NGS-based methods. Alternatively, an amplicon-based NGS panel (Illumina platform) was used to detect mutations in 38-42 relevant genes, including *TP53*. Part of the samples were analyzed with a hybrid capture based target enrichment followed by massively parallel sequencing [Hybrid Capture NGS, NeoSelect, NEO New Oncology, IonTorrent (ThermoFisher Scientific)]. The library preparation for the samples was performed using the Agilent SureSelect XT Kit as per the manufacturers' recommendations (5,17).

TP53 mt+ were classified according to three different algorithms as previously described: (I) classification by Poeta *et al.* (14), (II) by an extended algorithm based on Poeta *et al.* (14) with additional parameters like structural prediction and GVDV biophysical analysis (25) and (III) based on exon 8 vs. non-exon 8 mutations (4).

In an effort to specify the functional significance of the respective mutations in further detail (14), we included additional parameters in order to modify differentiation into pathogenic vs. non-pathogenic *TP53* co-mutations (25). These mutations are likely to interfere with *TP53* function significantly. Also, if an Align-GVGD score of C65 was reached, mutations were classified as pathogenic. Specifically, DNA-contact-mutations R273C, R273G, R248Q were reclassified as pathogenic mutations, since functional impairment is likely (25). Mutation R280I is located within the LSH2- (loop-sheet-helix region 2), which is part of the DNA-binding core and was therefore re-categorized as pathogenic. Mutations H179R and C176S constitute Zn^{2+}-binding sites and were therefore also regarded as pathogenic upon review.

The third classification was recently proposed by the group of Canale *et al.* (3). The authors characterized a cohort of *EGFR* mt+ patients that in 30.1% of cases carried additional *TP53* mt+ and these were categorized based on exons. *TP53* mt+ within exon 8 were associated with significantly lower DCR, and shorter PFS and OS. In addition to that, we showed similar results for *TP53* exon 8 co-mutations in our *EGFR* mt+ NSCLC IV cohort treated with 1st or 2nd generation TKI's.

Detailed results on each treatment line with Osimertinib and the three different *TP53* classifications.

Results

Analysis and presentation

PFS

Table S1 Median PFS in months on Osimertinib in 2nd line therapy

	n	PFS	P value
EGFR exon status			0.684
del19	33	10	
L858R	15	11	
TP53 status			0.033
TP53 mt+	24	13	
*TP53*WT	27	9	
TP53 status according to Poeta *et al.* (14)			0.100
TP53 disruptive mt+	15	8	
TP53 non-disruptive mt+	9	11	
*TP53*WT	27	13	
TP53 status according to Roeper *et al.* (25)			0.079
TP53 pathogenic mt+	17	8	
TP53 non-pathogenic mt+	7	12	
*TP53*WT	27	13	
TP53 status according to Canale *et al.* (3)			0.052
TP53 exon 8	4	10	
TP53 non-exon 8	20	8	
*TP53*WT	27	13	

PFS, progression free survival; *EGFR*, epidermal growth factor receptor; del19, deletion 19; L858R, exon 21 L858R mutation; *TP53*, status tumor suppressor gene status; *TP53* mt+, tumor suppressor gene mutation; WT, wild-type; mt+, mutation.

https://dx.doi.org/10.21037/tlcr-21-754

Table S2 Median PFS in months on Osimertinib in 2nd and further line therapy

	n	PFS	P value
TP53 status according to Poeta *et al.* (14)			0.011
TP53 disruptive mt+	19	8	
TP53 non-disruptive mt+	13	11	
*TP53*WT	45	14	
TP53 status according to Roeper *et al.* (25)			0.030
TP53 pathogenic mt+	23	9	
TP53 non-pathogenic mt+	9	11	
*TP53*WT	45	14	
TP53 status according to Canale *et al.* (3)			0.017
TP53 exon 8	4	10	
TP53 non-exon 8	28	9	
*TP53*WT	45	14	

PFS, progression free survival; *TP53*, tumor suppressor gene status; mt+, mutation; *TP53* mt+, tumor suppressor gene mutation; WT, wild-type.

OS

Table S3 Median OS in months on Osimertinib in 2nd line therapy

	n	OS	P value
EGFR exon status			0.019
del19	33	24	
L858R	15	11	
TP53 status			0.135
TP53 mt+	24	16	
*TP53*WT	27	24	
TP53 status according to Poeta et al. (14)			0.287
TP53 disruptive mt+	15	21	
TP53 non-disruptive mt+	9	15	
*TP53*WT	27	24	
TP53 status according to Roeper et al. (25)			0.250
TP53 pathogenic mt+	17	21	
TP53 non-pathogenic mt+	7	15	
*TP53*WT	27	24	
TP53 status according to Canale et al. (3)			0.232
TP53 exon 8	4	27	
TP53 non-exon 8	20	15	
*TP53*WT	27	24	
*TP53*WT	27	13	

OS, overall survival; EGFR, epidermal growth factor receptor; del19, deletion 19; L858R, exon 21 L858R mutation; *TP53*, status tumor suppressor gene status; *TP53* mt+, tumor suppressor gene mutation; WT, wild-type; mt+, mutation.

https://dx.doi.org/10.21037/tlcr-21-754

Table S4 Median OS in months on Osimertinib in 2nd and further line therapy

	n	OS	P value
TP53 status according to Poeta *et al.* (14)			0.081
TP53 disruptive mt+	19	16	
TP53 non-disruptive mt+	13	15	
*TP53*WT	45	24	
TP53 status according to Roeper *et al.* (25)			0.032
TP53 pathogenic mt+	23	16	
TP53 non-pathogenic mt+	9	15	
*TP53*WT	45	24	
TP53 status according to Canale *et al.* (3)			0.054
TP53 exon 8	4	27	
TP53 non-exon 8	28	15	
*TP53*WT	45	24	

OS, overall survival; *TP53*, status tumor suppressor gene status; *TP53* mt+, tumor suppressor gene mutation; mt+, mutation; WT, wild-type.

References

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https://dx.doi.org/10.21037/tlcr-21-754