Supplementary Information

RNA-based testing for multiplex detection of clinically relevant *MET* alterations in advanced non-small cell lung cancer. Cristina Aguado *et al*.

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CENTERS PARTICIPATING IN THE STUDY

Hospital	City
Hospital Clínic	Barcelona
Hospital Quirón Dexeus	Barcelona
Hospital General de Granollers	Granollers
Hospital de la Santa Creu i Sant Pau	Barcelona
Institut Català d'Oncologia	Hospitalet de Llobregat
Hospital General de Catalunya	Sant Cugat del Vallès
Hospital Sagrat Cor	Barcelona
Centro Médico Teknon	Barcelona
Clinica Universidad de Navarra /Center for Applied Medical Research (CIMA)	Pamplona
Clínica del Country	Bogotá

SUPPLEMENTARY TABLES

Table S1: MET amplification criteria used in clinical trials of MET inhibitors

Clinical trial	MET inhibitor	CRITERIA for MET amplification	References
NCT03911193 (CABinMET)	Cabozantinib	MET/CEP7 ≥2.2	[1]
NCT02435121	SAR125844	GCN >4 in ≥10% cells and MET/CEP7 ≥2	[2]
NCT00585195	Crizotinib	MET/CEP7 ≥1.8	[3]
NCT02648724	Sym015 humanized antibodies	MET/CEP7 >2.2	[4]
NCT02499614 (METROS)	Crizotinib	MET/CEP7 >2.2	[5]
NCT01324479	Capmatinib	MET/CEP7 >2 and GCN ≥5 (initial criteria) CGN ≥ 6 (final, published)	[6, 7]
NCT02414139 (GEOMETRY mono-1)	Capmatinib	GCN < 4, GCN ≥ 4 and < 6 and CGN ≥ 6 (initial criteria) GCN>10 (final, published)	[8]
CL1-49076-003	S49076	MET/CEP7 ≥ 2.0 GCN ≥ 6 ≥ 10% cells with ≥ 15 copies ≥ 50% cells with ≥ 5 copies	[9]

Table S2: Description of the NGS panels used in the study

NGS panel	Genes included
Oncomine™ Solid Tumour	Mutations: *AKT1, ALK, BRAF, CTNNB1, DDR2, EGFR, ERBB2/HER2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, MET, KRAS, PIK3CA, PTEN, NRAS, MAP2K1, NOTCH1, SMAD4, STK11, TP53*
GeneRead™ QIAact Lung DNA UMI	Mutations: *AKT1, ALK, BRAF, DDR2, EGFR, ERBB2/HER2, ESR1, FGFR1, KIT, KRAS, MAP2K1, MET, NRAS, NTRK1, PDGFRA, PIK3CA, PTEN, ROS1, RICTOR* Amplifications: *EGFR, FGFR1, ERBB2/HER2, MET, RICTOR*
GeneRead™ QIAact RNA Fusion UMI	Fusions: *BRAF, ALK, ROS1, RET, MET, NRG1, RAF, FGFR1, FGFR3, NTRK1, KRAS* Splicing variant: *MET*Δex14

Table S3: Description of the nCounter codeset used in the study and probes design for *MET* wild type and *MET*Δex14

Probes	Number
Gene Fusions Probes (*ALK, ROS1, RET, NTRK1*)	29 pairs
Imbalance Probes (*ALK, ROS1, RET, NTRK1*)	32 pairs
Housekeeping Genes Probes (*ACTB, PSMC4, MRPL19*)	3 pairs
MET Gene Probes (*MET*Δex14*, MET wild type**)	2 pairs

**MET*Δex14, accession number RCC_AS01_065.1, target sequence
TCCTGTGGCTGAAAAAGAGAAAGCAAATTAAAGATCAGTTTCCTAATTCATCTCAGAACGGTTCATGCCGA
CAAGTGCAGTATCCTCTGACAG
***MET* wild type;, accession number RCC_AS01_066.2, target sequence
CCTGTGGCTGAAAAAGAGAAAGCAAATTAAAGATCTGGGCAGTGAATTAGTTCGCTACGATGCAAGAGTAC
ACACTCCTCATTTGGATAGG

Table S4: Characteristics of the samples with valid results, *N* (%). Fusions were determined by nCounter, mutations by NGS

Characteristics	All samples (*N*=422)
Histological type	
Adenocarcinoma	299 (70.9)
Squamous cell carcinoma	45 (10.7)
Pleomorphic	6 (1.4)
Other	30 (7.1)
Unknown	42 (9.9)
UICC stage	
I-IIIA	59 (13.9)
IIIB-IV	286 (67.8)
Unknown	77 (18.3)
Collection time	
Basal	306 (72.5)
Progression	52 (12.3)
Unknown	64 (15.2)
Genetic alterations detected	
ALK fusion	58 (13.8)
ROS1 fusion	5 (1.2)
RET fusion	4 (0.9)
*MET*Δex14	13 (3.1)
EGFR mutations	32 (7.6)
KRAS mutations	65 (15.4)
BRAF mutations	12 (2.9)
Other alterations	21 (4.9)
None	212 (50.2)

Table S5: Results for *MET* exon 14 alteration splice-site regions and mutation types

Patient ID	*MET*Δex14 Intron	*MET*Δex14 Splice-site Region	*MET*Δex14 Mutation Type	*MET*Δex14 alteration
P1	13	Acceptor	Deletion	c.2942-6_2963delTTTAAGATCTG GGCAGTGAATTAGTTCG
P2	13	Acceptor	Deletion	c.2942-20_2942-9del
P3	13	Acceptor	Deletion	c.2942-52_2988delGGGGCCCATGAT AGCCGTCTTTAACAAGCTCTTTCTTTCTC TCTGTTTAAGATCTGGGCAGTGAATTAG TTCGCTACGATGCAAGAGTACACACTCC T
P4	14	Donor	Deletion	c.3082_3082+1delGG
P6	13	Acceptor	Deletion	c.2942-27_2942-16delAAGCTCTTTCTT

Table S6: Concordance of nCounter with RT-PCR and NGS for *METex*Δ14 status

Variable	***MET*Δex14 status**	
	***N* = 112**	***N* = 194**
Techniques compared	**nC *vs*. RT-PCR**	**nC *vs*. NGS**
Nº concordant samples	101	191
Nº discordant samples	11	3
Sensitivity	54.2% (CI = 35.1-72.1)	100% (CI = 56.6-100.0)
Specificity	100% (CI = 94.7-100.0)	98.4% (CI = 95.4-99.5)
Concordance	90.2% (CI = 83.3-94.4)	98.5% (CI = 95.6-99.5)
Cohen's kappa	0.650 (CI = 0.466-0.834)	0.762 (CI = 0.502-1.000)

Abbreviations: nC, nCounter; NGS, next generation sequencing; RT-PCR, reverse transcription polymerase chain reaction

Table S7: Concordance of the nCounter categorization using the cut-off for very high *MET* levels with IHC, FISH and NGS.

Variable	MET expression		*MET* amplification			
	***N* = 91**		***N* = 40**			***N* = *80***
Techniques compared	**nC *vs*. IHC (3+ in ≥ 50%)**	**nC *vs*. IHC (HS ≥ 220)**	**nC *vs*. FISH (r ≥ 2)**	**nC *vs*. FISH (GCN≥ 6)**	**nC *vs*. FISH (50%≥ 5 or 10%≥ 15)**	**nC *vs*. NGS**
Nº concordant samples	78	83	37	34	31	78
Nº discordant samples	13	8	3	6	9	2
Sensitivity	41.0% (CI = 23.3-61.3)	52.9% (CI = 31.0-73.8)	100% (CI = 64.6-100.0)	66.7% (CI = 88.3-100.0)	52.6% (CI = 31.7-72.7)	81.8% (CI = 52.3-94.9)
Specificity	100% (CI = 94.7-100.0)	100% (CI = 95.1-100.0)	90.9% (CI = 76.4-96.9)	92.9% (CI = 77.4-98.2)	100% (CI = 84.5-100.0)	100% (CI = 94.7-100.0)
Concordance	85.7% (CI = 77.1-91.5)	91.2% (CI = 83.6-95.5)	92.5% (CI = 80.1-97.4)	92.9% (CI = 77.4-98.0)	77.5% (CI = 62.5-87.7)	97.5% (CI = 91.3-99.3)
Cohen's kappa	0.513 (CI = 0.299-0.727)	0.647 (CI = 0.428-0.855)	0.778 (CI = 0.542-1.000)	0.625 (CI = 0.355-0.895)	0.538 (CI = 0.303-0.774)	0.886 (CI = 0.731-1.000)

Abbreviations: FISH, fluorescence in situ hybridization; HS, histoscore; IHC, immunohistochemistry; nC, nCounter; NGS, next generation sequencing

Table S8: Concordance of the nCounter categorization using the cut-off for moderately elevated *MET* levels with IHC, FISH and NGS.

Variable	MET expression		*MET* amplification			
	***N* = 91**		***N* = 40**			***N* = 80**
Techniques compared	**nC *vs.* IHC (3+ in ≥ 50%)**	**nC *vs.* IHC (HS ≥ 220)**	**nC *vs.* FISH (r ≥ 2)**	**nC *vs.* FISH (GCN≥ 6)**	**nC *vs.* FISH (50%≥ 5 or 10%≥ 15)**	**nC *vs.* NGS**
Nº concordant samples	79	86	20	25	28	66
Nº discordant samples	12	5	20	15	12	14
Sensitivity	63.6% (CI = 43.0-80.3)	88.2% (CI = 65.7-96.7)	100% (CI = 64.6-100)	100% (CI = 75.8-100)	89.5% (CI = 68.6-97.1)	90.9% (CI = 62.3-98.4)
Specificity	94.2% (CI = 86.0-97.7)	95,9% (CI = 88.7-98.6)	39.4% (CI = 24.7-56.3)	46,4% (CI = 29.5-64.2)	52.4% (CI = 32.4-71.7)	81.2% (CI = 70.4-88.7)
Concordance	86.8% (CI = 78.3-92.3)	94.5% (CI = 87.8-97.6)	50.0% (CI = 35.2-64.8)	62.5% (CI = 47.0-75.8)	70.0% (CI = 54.6-81.9)	82.5% (CI = 72.7-89.3)
Cohen's kappa	0.617 (CI = 0.421-0.812)	0.823 (CI = 0.673-0.973)	0.185 (CI = 0.04-0.331)	0.342 (CI = 0.144-0.540)	0.410 (CI = 0.153-0.668)	0.494 (CI = 0.281-0.708)

Abbreviations: FISH, fluorescence in situ hybridization; GCN, gene copy number**;** HS, histoscore; IHC, immunohistochemistry;; nC, nCounter; NGS, next generation sequencing; r, ratio *ME*

SUPPLEMENTARY FIGURES

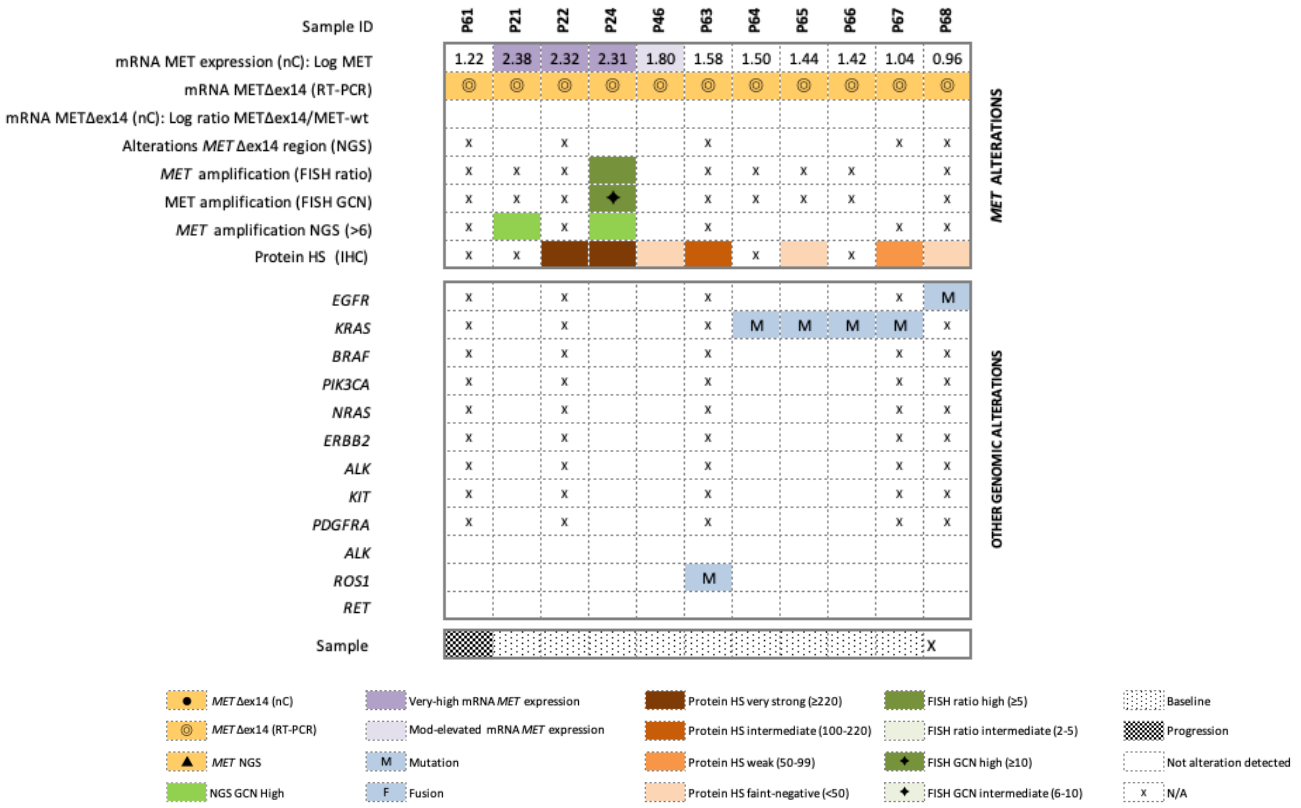

Figure S1: Heatmap displaying the molecular and clinical characteristics samples with *MET*Δex14 discordant results between RT-PCR and nCounter. Patient numbers are shown in the top row. Abbreviations: Mod, moderate; FISH, fluorescence in situ hybridization; NGS, next-generation sequencing; IHC, immunohistochemistry; nC, nCounter; HS, histoscore; RT-PCR, reverse transcription polymerase chain reaction; GCN, gene copy number; N/A non-available data.

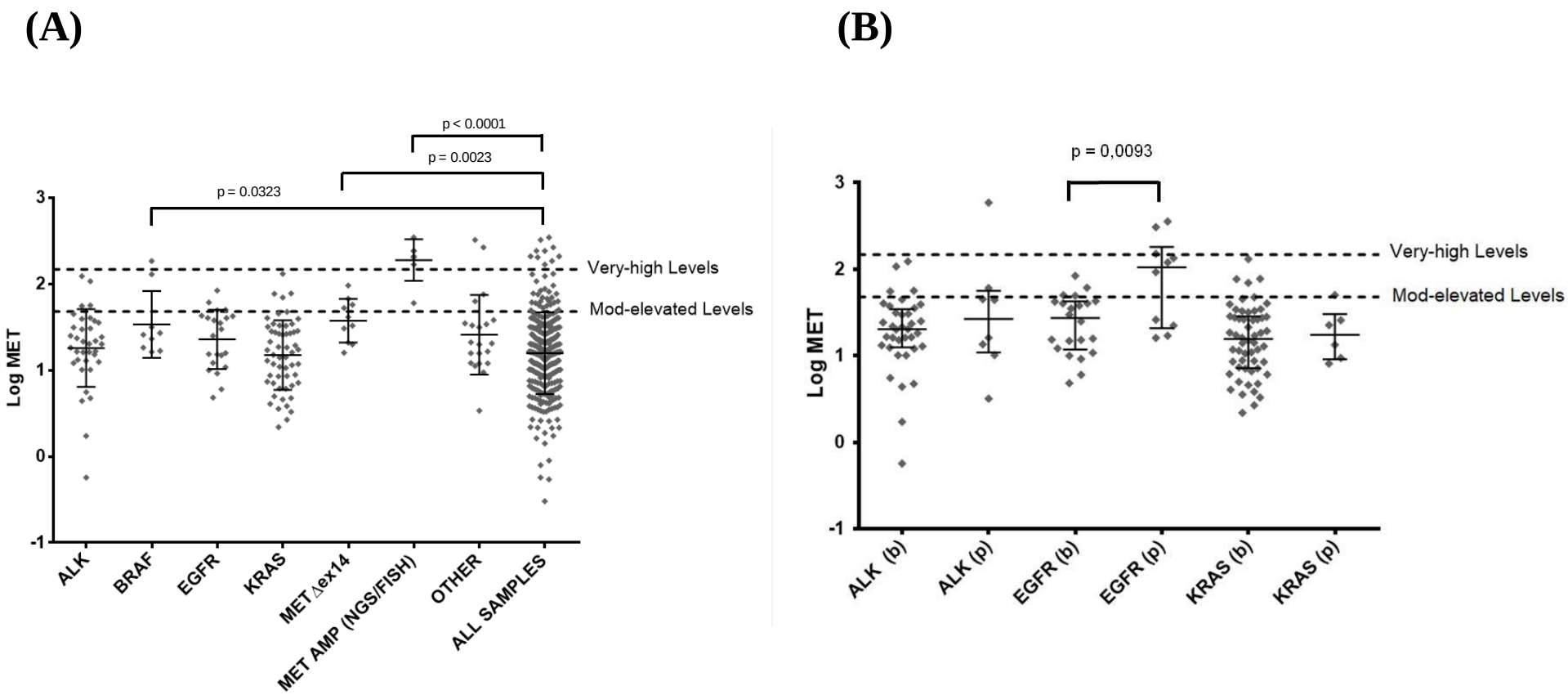

Figure S2: *MET* mRNA expression levels by nCounter in samples harboring different driver alterations. (A) *MET* mRNA expression levels at presentation. **(B)** Comparison of *MET* mRNA expression levels of basal samples (b) *vs.* samples at progression (p).

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