Plasma analysis performed in VISION trial

Circulating tumor DNA (ctDNA) was isolated and tested from freshly collected plasma samples. Cell free DNA (cfDNA) is extracted from a routine blood draw (custom collection kit with two 10 mL Streck tubes). 5.0–30 ng of ctDNA were extracted from plasma, enriched for targeted regions and undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing was conducted using the Illumina platform (HiSeq 2500) and hg19 as the reference genome. Multi-analyte algorithms and bioinformatics were used to reconstruct the progenitor cfDNA fragment sequences. Quantitative reporting of single nucleotide variants mutant allele fraction (VAF) and gene copy numbers were reported. Genes included in Guardant360® test gene-panel (n=73) are the indicated below:

- AKT1, ALK, APC, AR, ARAF ARID1A, ATM, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCNE1, CDH1, CDK4, CDK6, CDKN2A, CTNNB1, DDR2, EGFR, ERBB2, ESR1, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, GATA3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, APK1, MAPK3, MET, MLH1, MPL, MTOR, MYC, NF1, NFE2L2, NOTCH1, NPM1, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, PTEN, PTPN11, RAF1, RB1, RET, RHEB, RHOA, RIT1, ROS1, SMAD4, SMO, STK11, TERT, TP53, TSC1 and VHL

Plasma analysis performed in MAGIC trial

Plasma samples were collected at baseline, i.e., at the time of first administration of systemic treatment and in two other time-points, as previously described by Zulato *et al.* (40). cfDNA was extracted from 3 to 5 mL of plasma using the QIAamp® circulating nucleic acid Kit (QIAGEN, Hilden, Germany). 2 to 25 ng of cfDNA were used for library preparation, following the protocol of Myriapod NGS-IL 56G (Diatech Pharmacogenetics Srl.) assay. The panel covers mutational hotspots of 56 tumor-associated genes. Sequencing was performed with the Illumina MiSeq Sequencer in paired-end mode (2×151 cycles). Myriapod NGS Data Analysis Software was used for variant calling. Single nucleotide variants mutant allele fraction (VAF) and deletions/insertions were reported. Genes included in the Myriapod® NGS 56G Onco panel panel (n=56) are indicated below:

- ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, DDR2, DNMT3A, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, FOXL2, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MAP2K1, MET, MLH1, MPL, MSH6, NOTCH, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, STK11, SMAD4, SMARCB1, SMO, SRC, TP53, TSC1 and VHL

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Table S1 *KRAS*, *TP53* and *STK11* gene alterations detected in study population

KRAS		*TP53*		*STK11*	
Code	Alteration	Code	Alteration	Code	Alteration
V10	Q61R	V10	R280G	V104	E256*
V54	G12A		P190R	V136	H168L
V69	G12A		P177L	V162	Splice site SNV
V79	G12A		c.920-2del	V169	K178*
V90	*C80F (VUS)*	V12	Y220B	V184	Splice site SNV
V96	G12V		P278A	V190	D277fs
V98	G12C	V25	T81fs	V197	E293*
V104	G12C		V274G	V199	E223*
V124	G12D		P250L	M54	G251V
V155	Q61L	V26	K120R		
V157	G12R		H214R		
V187	V14I		S392fs		
V189	G12F		R248Q		
V197	G12C		V216M		
V199	G12R	V30	Y220C		
V206	G12C	V59	C238Y		
V209	G12D	V61	I162F		
V212	G13E	V67	R337C		
V233	G12C	V79	R306*		
M129	G12C	V80	S160fs		
M289	G12V	V81	H179R		
M315	G13D	V90	F270L		
		V91	*R270T (VUS)*		
		V94	*I50T (VUS)*		
		V96	H168L		
		V104	P301fs		
		V108	R158L		
			M246V		
			L257P		
		V125	R158L		
		V126	R175H		
		V136	R273C		
		V142	H179R		
		V150	S240R		
		V155	W91*		
		V163	I195F		
		V169	V143fs		
		V183	H193N		
		V184	R249M		
		V190	C275Y		
		V196	E180K		
		V197	E171fs		
		V204	*V157F (neutral)*		
		V206	L369fs		
		V216	L111P		
		V220	V216L		
			L257Q		
			S241Y		
		V233	A159_ P177delinsK		
		V237	R337P		
		V240	Splice site SNV		
		M125	C277F		
		M153	E258*		
		M240	V172D		
		M247	A159P		
		M263	M246V		
			R158L		
		M83	C275F		
			V274F (neutral)		
		M54	R333fs		
		M110	R248Q		
		M143	R273L		
		M191	A159P		
			L130H		
		M243	R181P		
		M276	E298*		
		M304	E343*		
		M315	K164E		
		M270	M246L		
		M320	Y163C		

*, Nonsense mutation: a point mutation that results in a premature stop codon. SNV, single nucleotide variant; Fs, frame shift. In *italics* gene alterations not considered for analysis, either being variants of unknown significance (VUS), or non-pathogenic variants.

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Table S2 Other most common gene alterations detected in study population

Gene	Code	Alteration
EGFR	V18	N771_H773dup
	V26	*Q812K (VUS)*
	V30	P644L
	V79	Amplification 2+
	V155	E114K
	V190	*S380T (VUS)*
	V196	E709_T710delinsD Amplification 1+
	V199	*C307S (VUS)*
	V201	*G652delinsER (VUS)*
	M153	*T785= (VUS)*
	V158	A767_V769dup
	V132	Amplification 1+
	V220	Amplification 3+
MET	V61	Exon 14 skipping mutation
	V183	Exon 14 skipping mutation Amplification 1+
	V204	Exon 14 skipping mutation Amplification 1+
	V31	*D1039N (VUS)*
	V132	E91* E967* Amplification 2+
	V220	E34K (neutral)
	V240	R469*
	M269	S178= (neutral)
	V26	Amplification 1+
	V59	Amplification 2+
NF1	V30	L2209*
	V80	*H2457R (VUS)*
	V126	*D1269Y (VUS)*
	V137	*F694L (VUS)*
	V183	E524Q R1534*
	V187	L1543P
	V233	*V2175I (VUS)*
	V237	*P2246_R2247insL (VUS)*
	V240	*Q1360E (VUS)*

Table S2 (*continued*)

Gene	Code	Alteration
PIK3CA	V26	E545K
	V67	Amplification 1+
	V97	E545K
	V124	H1047R
	V196	E545K
	V201	E542K
	V216	Amplification 2+
	V233	T1025S
	M153	*I391M (neutral)*
	M240	*I391M (neutral)*
	M304	E545K
ARID1A	V13	*Y454fs (VUS)*
	V81	*Q564* (VUS)*
	V136	*H782D (VUS)* *Q1402H (VUS)*
	V157	*Q1095fs (VUS)*
	V196	*F1245L (VUS)*
	V212	*M1564fs (VUS)*
CCNE1	V79	Amplification 3+
	V81	Amplification 1+
	V108	Amplification 1+
	V142	Amplification 1+
	V158	Q277*
	V184	Amplification 1+
	V197	Amplification 1+
BRCA2	V125	*T2350S (neutral)*
	V130	*T2031A (VUS)*
	V136	*R2888H (neutral)*
	V137	*L3215fs (VUS)*
	V199	D1386Y
RB1	V81	R262Q
	V96	*I244K (VUS)*
	V199	*S795S (VUS)* R798fs
	V206	C706F
	V240	Splice site SNV

Table S2 (*continued*)

Gene	Code	Alteration
CDKN2A	V136	L130Q
	V197	D108N
	V201	R80*
	V240	R80*
	143	A118*
PTEN	V26	H123Y
	V94	R130G
	V240	S287*
	M143	D92Y
	M270	P96S
BRAF	V61	Amplification 1+
	V155	I326V
	V183	Amplification 1+
	M83	L597V
	V187	V600E
APC	V30	R332*
	V80	*Y1135C (VUS)*
	V190	*I2083M (VUS)*
	V240	N32S
	M191	S1503*
MTOR	V18	*I1118V (VUS)*
	V184	R1286Q
	V240	*T416I (VUS)*
MYC	V125	Amplification 3+
	V132	Amplification 1+
	V190	Amplification 2+
	V197	Amplification 3+
FGFR1	V52	Amplification 3+
	V125	*E138K (VUS)*
	V216	Amplification 3+
AR	V59	*I184T (VUS)*
	V216	*P135T (VUS)*
	V237	R856C
DDR2	V26	*R611Q (VUS)*
	V31	*L735M (VUS)*
	V94	R752H

Table S2 (*continued*)

Gene	Code	Alteration
KIT	V81	*D327H (VUS)*
	V96	*E85K (neutral)*
	M269	*M541L (neutral)*
SMAD4	V61	R361C
	V130	*T349fs (VUS)*
	V183	E390*
PDGFRA	V136	*S91A (VUS)* *A90D (VUS)*
	V209	K964N
	M269	*V824= (neutral)*
IDH1	V184	R132H
	M216	*G105= (neutral)*
	M237	*G105= (neutral)*
CTNNB1	V104	S37C
	V201	S37F S37C
	M188	*S45F (VUS)*

*Nonsense mutation: a point mutation that results in a premature stop codon. SNV, single nucleotide variant; Fs, frame shift. In *italics* gene alterations not considered for analysis, either being variants of unknown significance (VUS), or non-pathogenic variants.

Gene amplification is expressed as:

- Low (1+): amplification magnitude is below the 50th percentile of amplifications;
- Medium (2+): amplification magnitude is between the 50th and 90th percentiles;
- High (3+): amplification magnitude is above the 90th percentile.

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Table S3 P values of chi-squared test performed between clinical features and gene alterations

	Smoker versus non-smoker	Adenocarcinoma versus other histotypes	PD-L1 TPS ≥1%	PD-L1 TPS ≥50%	Extrathoracic metastases	Liver metastases	Bone metastases
TP53	0.019	0.446	0.462	0.658	0.013	0.055	0.156
	*OR =4.020; P=0.023				*OR =1.703; P=0.010		
KRAS	0.104	0.321	0.005	0.016	0.882	0.529	0.253
			*OR =4.500; P=0.008	*OR =3.361; P=0.020			
STK11	0.163	0.095	0.559	0.630	0.340	0.705	0.796
EGFR	0.246	0.162	0.506	0.219	0.537	0.053	0.289
MET	0.004	0.335	0.937	0.302	0.242	0.565	0.655
	*OR =NC						
NF1	0.735	0.091	0.835	0.918	0.625	0.637	0.800
PIK3CA	0.841	0.511	0.654	0.520	0.459	0.738	0.943
ARID1A	0.234	0.007	0.908	0.322	0.462	0.023	0.471
						*OR =6.000; P=0.040	
CCNE1	0.926	0.093	0.465	0.934	0.462	1.000	0.471
BRCA2	0.408	0.642	0.612	0.954	0.507	0.431	0.888
RB1	0.280	0.330	0.572	0.686	0.200	0.837	0.603
CDKN2A	0.919	0.031	0.192	0.548	0.048	0.377	0.616
					*OR =NC		
PTEN	0.900	0.907	0.210	0.119	0.276	0.363	0.637
BRAF	0.087	0.276	0.068	0.011	0.361	0.639	0.354
				*OR =NC			
APC	0.280	0.897	0.028	0.116	0.719	0.837	0.626
			*OR =NC				
MTOR	0.408	0.345	0.612	0.229	0.609	0.431	0.253
MYC	0.713	0.175	0.264	0.424	0.929	0.360	0.184
FGFR1	0.486	0.061	0.504	0.954	0.609	0.431	0.888
AR	0.486	0.642	0.612	0.954	0.066	0.431	0.253
DDR2	0.486	0.345	0.092	0.229	0.066	0.431	0.253
KIT	0.411	0.069	0.518	0.978	0.596	0.424	0.166
SMAD4	0.026	0.345	0.504	0.187	0.507	0.431	0.888
	*OR =0.099; P=0.065						
PDGFRA	0.411	0.069	0.518	0.978	0.520	0.434	0.912
IDH1	0.414	0.352	0.610	0.967	0.072	0.437	0.260
CTNN1	0.705	0.276	0.053	0.166	0.947	0.363	0.354

*, for significant associations, logistic regression test was tried and performed, in order to calculate odds ratio (OR). NC, not calculable.

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Table S4 Univariate and multivariate analysis of overall survival performed in the control group

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Clinical variables						
Gender (female)	0.768	0.413–1.427	0.403	–	–	–
Age	1.006	0.979–1.033	0.679	–	–	–
Smoking status	1.913	0.876–4.177	0.103	–	–	–
ECOG PS	2.310	1.222–4.365	0.010	1.531	0.795–2.946	0.203
Weight loss	4.133	2.169–7.875	<0.001	3.499	1.766–6.933	<0.001
Number of metastases	1.703	1.268–2.286	<0.001	1.246	0.870–1.837	0.218
Extrathoracic metastasis	1.958	1.035–3.701	0.039	0.665	0.274–1.615	0.368
Liver metastasis	2.021	0.885–4.619	0.095	–	–	–
Bone metastasis	3.181	1.689–5.991	<0.001	3.179	1.366-7.399	0.007
Molecular variables						
TP53 alteration	1.498	1.105–2.029	0.009	1.422	1.045–1.936	0.025
KRAS alteration	2.999	1.525–5.899	0.001	2.701	1.362–5.356	0.004
STK11 alteration	1.166	0.922–1.475	0.199	–	–	–
KRAS/*STK11* co-mutation	2.180	0.510–9.326	0.293	-	–	–
STK11/*TP53* co-mutation	2.051	0.628–6.698	0.234	–	–	–
KRAS/*STK11*/*TP53* co-mutation	2.411	0.324–17.913	0.390	–	–	–

CI, confidence interval; HR, hazard ratio.

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