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Molecular diagnostics in clinical practice: how to deal with the rapid developments in Precision Medicine ?

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Disclosures of Ed Schuuring

Consultant/Advisory Board:

AstraZeneca, Bayer, BMS, Roche, Pfizer, Novartis, Amgen, Lilly, BioCartis, Illumina, Astellas Pharma, Agena Biosciences, MSD/Merck, CC Diagnostics, Janssen Cilag (Johnson&Johnson), Diaceutics

Speaker's fee:

Bio-Rad, Abbott, Roche, Biocartis, Illumina, Pfizer, Astrazeneca, Agena Biosciences

Grants/Sponsoring:

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Stock/Royalties:

None

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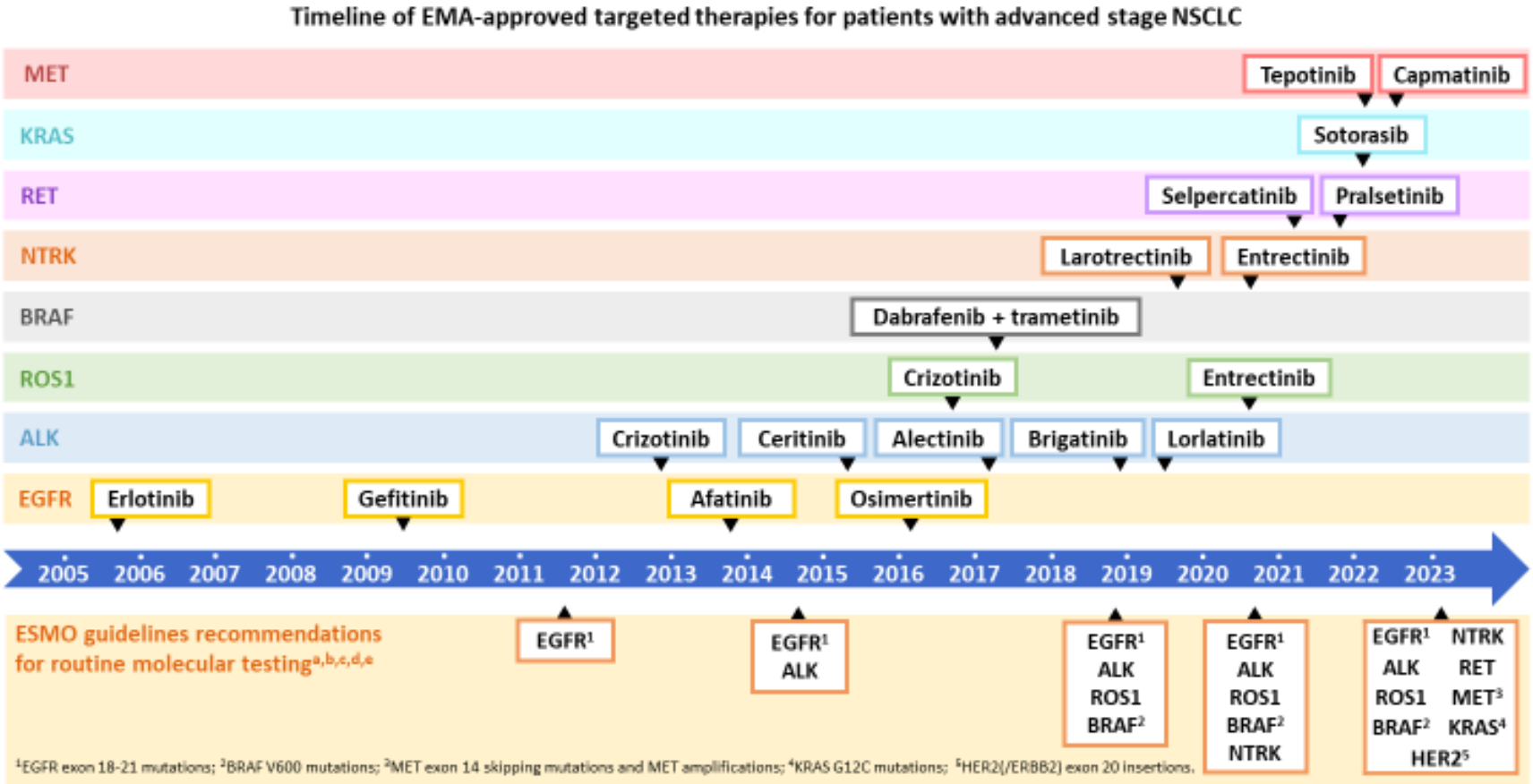
Transferred to UMCG-account

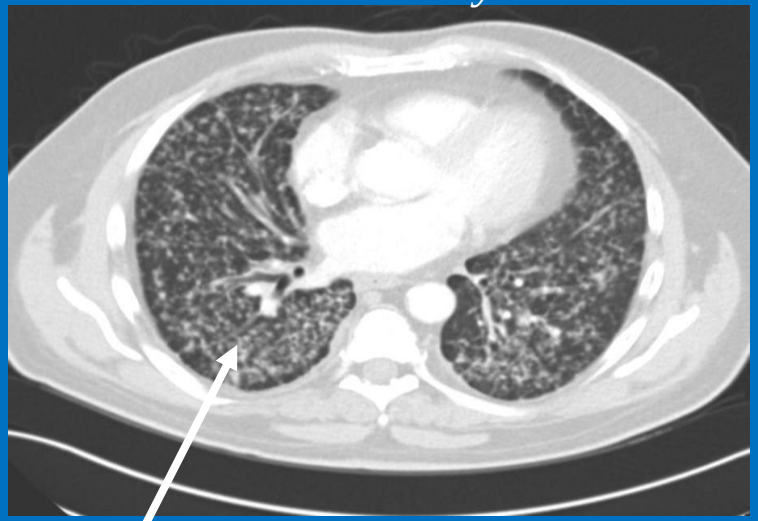


de Jager, Schuuring, unpublished data

The impressive effect of specific targeted therapy in patient with lung adenocarcimoma carrying a chemoresistant EGFR mutant

PRE-treatment

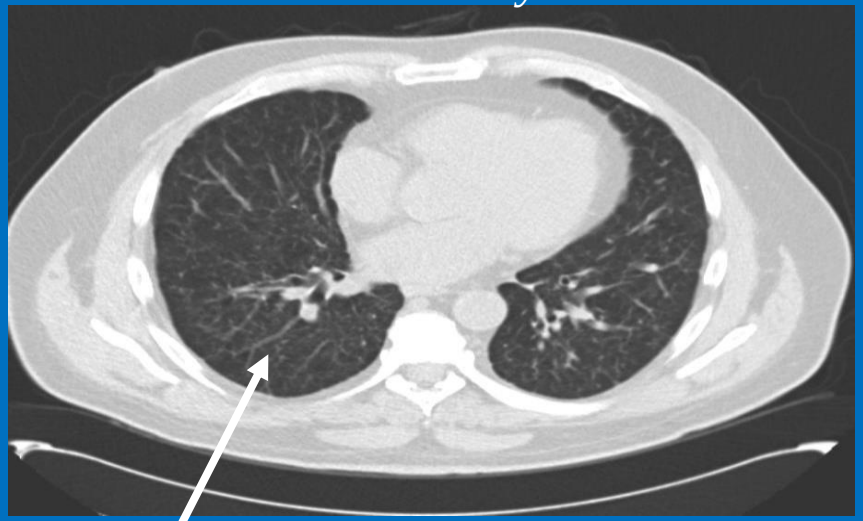
CT thorax 28 May 2010



Lung completed filled with tumor nodules

6 weeks after start treatment

CT thorax 6 July 2010



Lungs are “clean” (no nodules detectable)

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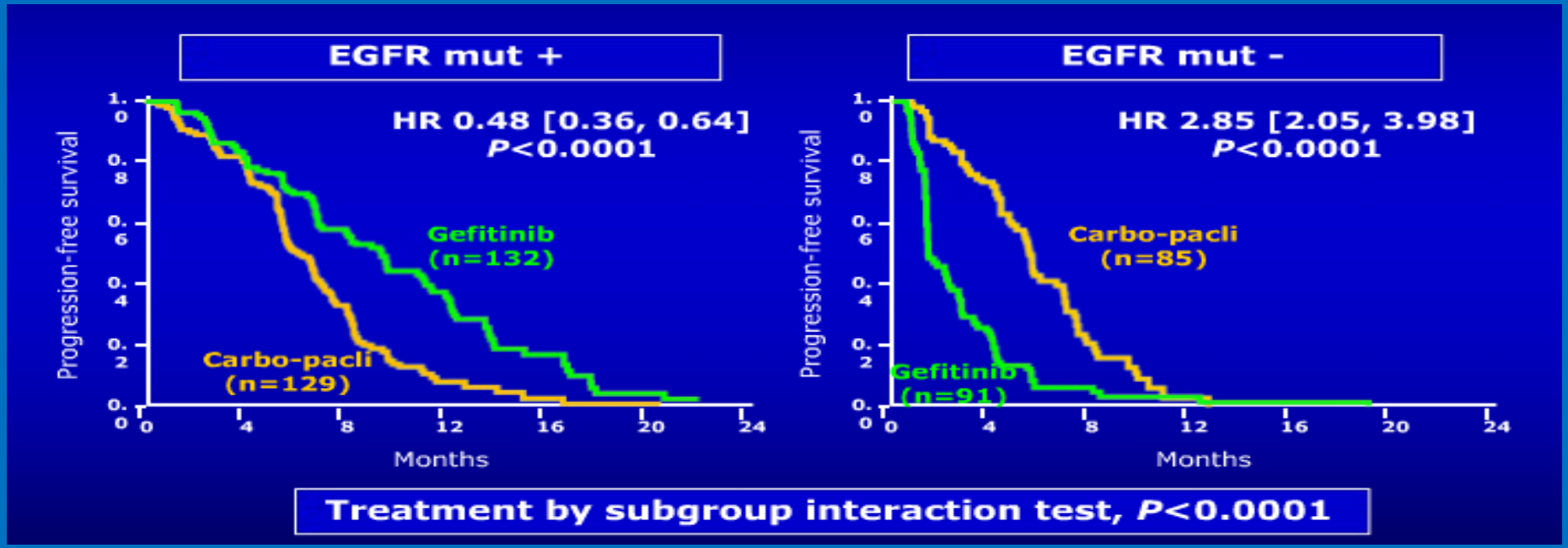
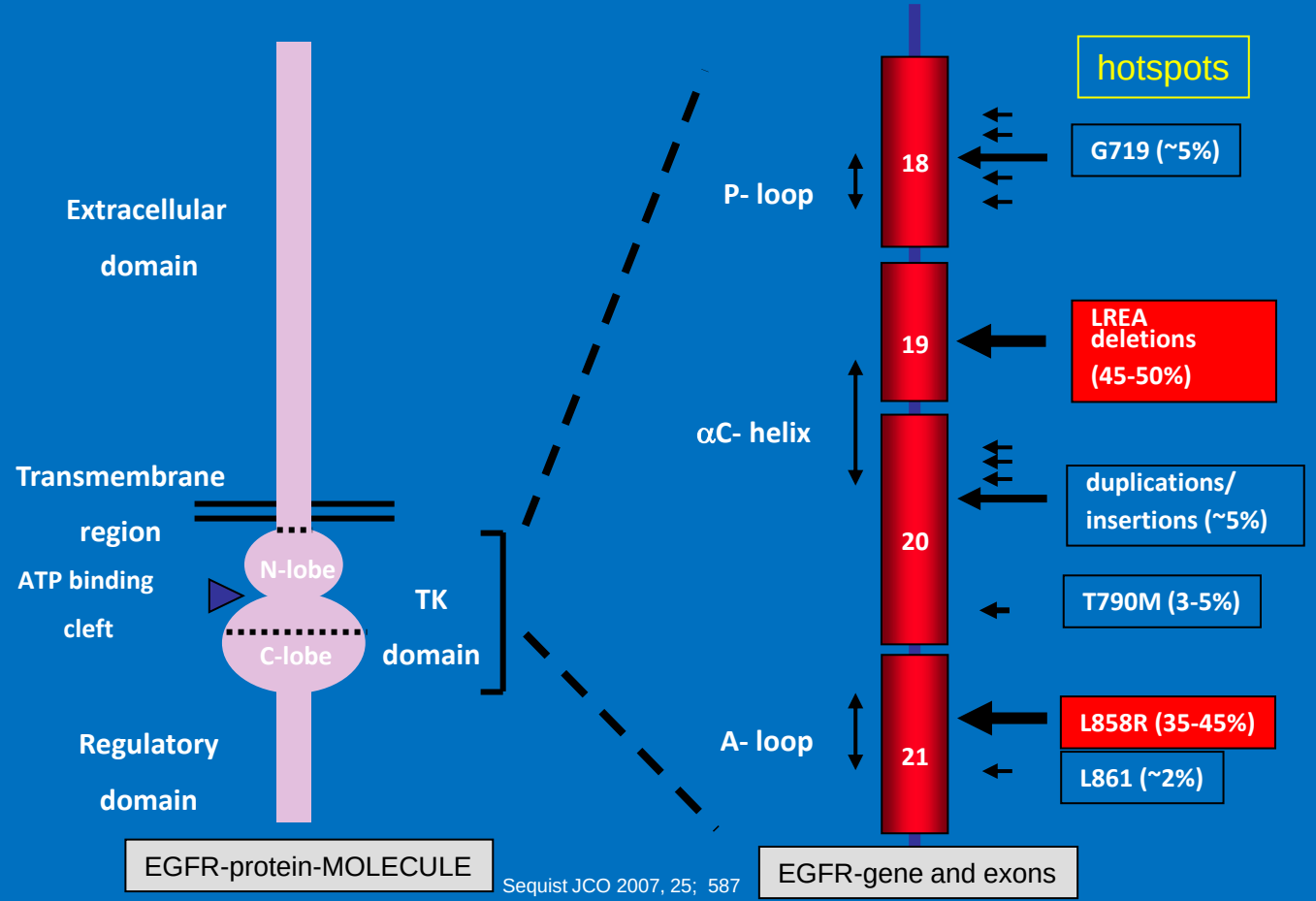
Disease-free-survival of NSCLC patients with/without EGFR-mutations treated with gefitinib or standard chemotherapy



Principal of targeted therapy:
> only treat patients that benefit from TKI
> appropriate molecular profiling is essential

Mok T et al, NEJM 2009

Detection of mutations in EGFR



Sequist JCO 2007, 25; 587

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Dutch landscape of *EGFR* variants in 925 (11.7%) NSCLC (2013/2015/2017)

Reported in PALGA (Dutch Pathology Registry)

Variant(s)	*n* (%) [a]
R108K	1 (0.11%)
C595F	1 (0.11%)
Q701K	1 (0.11%)
L703F	1 (0.11%)
R705S	1 (0.11%)
E709_T710delinsD	3 (0.32%)
E709A/G719A	4 (0.43%)
E709A/G719R	1 (0.11%)
E709A/G719S	1 (0.11%)
E709D	1 (0.11%)
E709K/G719S	1 (0.11%)
I715fs*	1 (0.11%)
G719_S720delinsAF	1 (0.11%)
G719A	18 (1.95%)
G719A/D761Y	1 (0.11%)
G719A + Exon 20 insertion, NOS	1 (0.11%)
G719A/L861Q	3 (0.32%)
G719A/R776H	1 (0.11%)
G719A/S768I	5 (0.54%)
G719A/V769M	1 (0.11%)
G719C	2 (0.22%)
G719C/S768I	6 (0.65%)
G719S	1 (0.11%)
G719S/L747S	1 (0.11%)
G719S/L861Q	1 (0.11%)
G719S/S768I	1 (0.11%)
G719X, NOS	2 (0.22%)
G719X, NOS/S768I	3 (0.32%)
G724A/S768I	1 (0.11%)
G724S/S768I	1 (0.11%)
c.2184 + 19G > A	1 (0.11%)
L730fs*1	1 (0.11%)
I740_K745dup	2 (0.22%)
[illegible]	1 (0.11%)
I744_P753delinsSNISG	1 (0.11%)
E746_A750del	210 (22.7%)
E746_A750del/G873E	1 (0.11%)
E746_A750del/K754Q	1 (0.11%)
E746_A750del/P848L	1 (0.11%)
E746_A750del/V765M	1 (0.11%)
E746_A750delinsEP	1 (0.11%)
E746_A750delinsIP	1 (0.11%)
E746_A750dup	1 (0.11%)
E746_K754delinsVSR	1 (0.11%)
E746_L747delinsIP	1 (0.11%)
E746_P753delinsANKE	1 (0.11%)
E746_P753delinsIS	1 (0.11%)
E746_P753delinsVS	2 (0.22%)
E746_S752delinsI	1 (0.11%)
E746_S752delinsV	20 (2.16%)
E746_T751del	2 (0.22%)
E746_T751delinsA	3 (0.32%)
E746_T751delinsAA	1 (0.11%)
E746_T751delinsI	2 (0.22%)
E746_T751delinsK	1 (0.11%)
E746_T751delinsL	1 (0.11%)
E746_T751delinsP	1 (0.11%)
E746_T751delinsS	1 (0.11%)
E746_T751delinsVP	1 (0.11%)
L747_A750delinsP	10 (1.08%)
L747_A750delinsP/V845L	1 (0.11%)
L747_A755delinsSKD	1 (0.11%)
L747_E749del	3 (0.32%)
L747_K754delinsATSPE	1 (0.11%)
L747_K754delinsG	1 (0.11%)
L747_K754delinsQPN	1 (0.11%)
L747_P753delinsQ	1 (0.11%)
L747_P753delinsS	25 (2.70%)
L747_S752del	7 (0.76%)
L747_S752del/K754R	1 (0.11%)
L747_S752del/L777Q	1 (0.11%)
L747_S752delinsQ	2 (0.22%)
L747_T751del	16 (1.73%)
L747_T751del/S768I	1 (0.11%)
L747_T751delinsP	6 (0.65%)
L747P	4 (0.43%)
E749_A755delinsD	1 (0.11%)
A750_E758delinsP	1 (0.11%)
T751_I759delinsN	1 (0.11%)
S752_I759del	4 (0.43%)
P753L	1 (0.11%)
K757R	1 (0.11%)
A763S	2 (0.22%)
A763_Y764insFQEA	1 (0.11%)
V765M	2 (0.22%)
A767_V769dup	16 (1.73%)
A767T	1 (0.11%)
S768_D770dup	9 (0.97%)
S768_V769delinsIL	4 (0.43%)
S768I	5 (0.54%)
S768I/L861Q	2 (0.22%)
S768I/V774M	2 (0.22%)
V769L	2 (0.22%)
V769M	2 (0.22%)
D770_H773dup	1 (0.11%)
D770_N771insG	3 (0.32%)
D770_N771insGF	1 (0.11%)
D770_N771insSVA	2 (0.22%)
D770_N771insT	1 (0.11%)
D770_N771insY	1 (0.11%)
D770_P772dup	1 (0.11%)
D770delinsEQPP	1 (0.11%)
D770delinsGY	3 (0.32%)
D770Y	1 (0.11%)
N771_H773dup	2 (0.22%)
N771_P772insH	1 (0.11%)
N771_P772insR	1 (0.11%)
N771delinsGF	1 (0.11%)
N771delinsGY	1 (0.11%)
N771delinsKG	1 (0.11%)
N771delinsTH	1 (0.11%)
N771L	1 (0.11%)
P772_C775dup	1 (0.11%)
P772_H773dup	3 (0.32%)
P772_H773insANP	1 (0.11%)
H773_V774dup	2 (0.22%)
H773_V774insAH	2 (0.22%)
H773delinsYNPY	1 (0.11%)
H773dup	5 (0.54%)
V774delinsHC	1 (0.11%)
V774M	3 (0.32%)
V774M/L861R	1 (0.11%)
C775F	1 (0.11%)
R776H	1 (0.11%)
R776L	1 (0.11%)
G779F	3 (0.32%)
G779S	1 (0.11%)
C781F	1 (0.11%)
Q787E	1 (0.11%)
T790M	1 (0.11%)
G796C	1 (0.11%)
L799M	1 (0.11%)
D830Y	1 (0.11%)
L832T	1 (0.11%)
L833V/H835L	2 (0.22%)
R836L	1 (0.11%)
R836R	4 (0.43%)
A840T	4 (0.43%)
P848L	5 (0.54%)
L858_K860delinsRTI	1 (0.11%)
L858R	274 (29.6%)
L858R/A871E	1 (0.11%)
L858R/A871G	1 (0.11%)
L858R/E709G	1 (0.11%)
L858R/L718M	1 (0.11%)
L858R/L747V	1 (0.11%)
L858R/R776H	2 (0.22%)
L858R/S768I	4 (0.43%)
L858R/V834L	1 (0.11%)
A859T	1 (0.11%)
L861Q	19 (2.05%)
L861R	3 (0.32%)
G863D	1 (0.11%)
A864T	1 (0.11%)
A864V	1 (0.11%)
E866K	1 (0.11%)
EGFR variant, NOS	4 (0.43%)
Exon 18 variant, NOS + Exon 20 variant, NOS	2 (0.22%)
Exon 19 deletion, NOS	52 (5.62%)
Exon 19 variant, NOS	3 (0.32%)
Exon 20 insertion, NOS	3 (0.32%)
Exon 20 SNV (silent)	1 (0.11%)
Exon 21 variant, NOS	3 (0.32%)

Koopman et al Cancers 2021;13:1341

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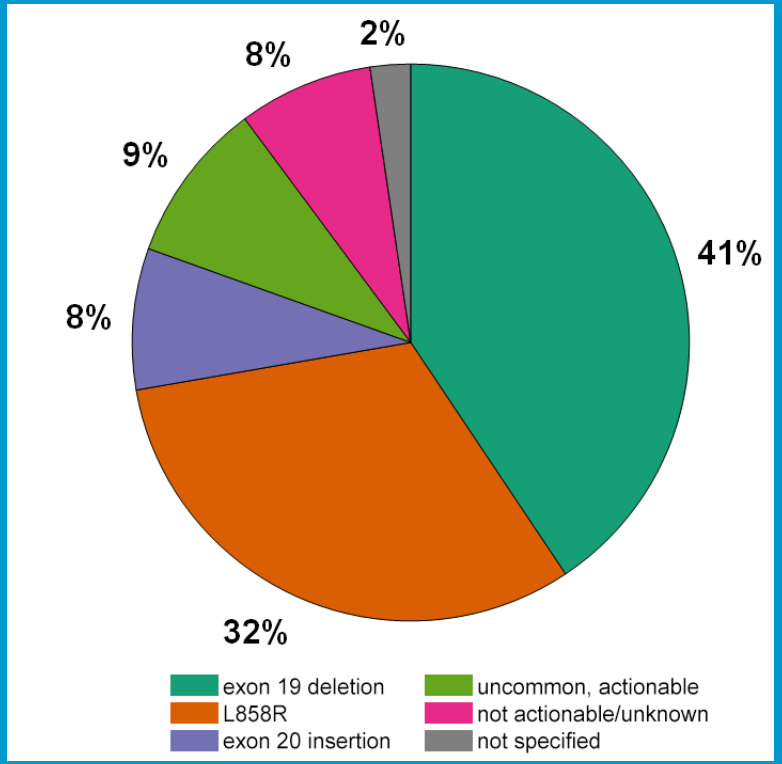
Dutch landscape of *EGFR* variants in NSCLC (2013/2015/2017)

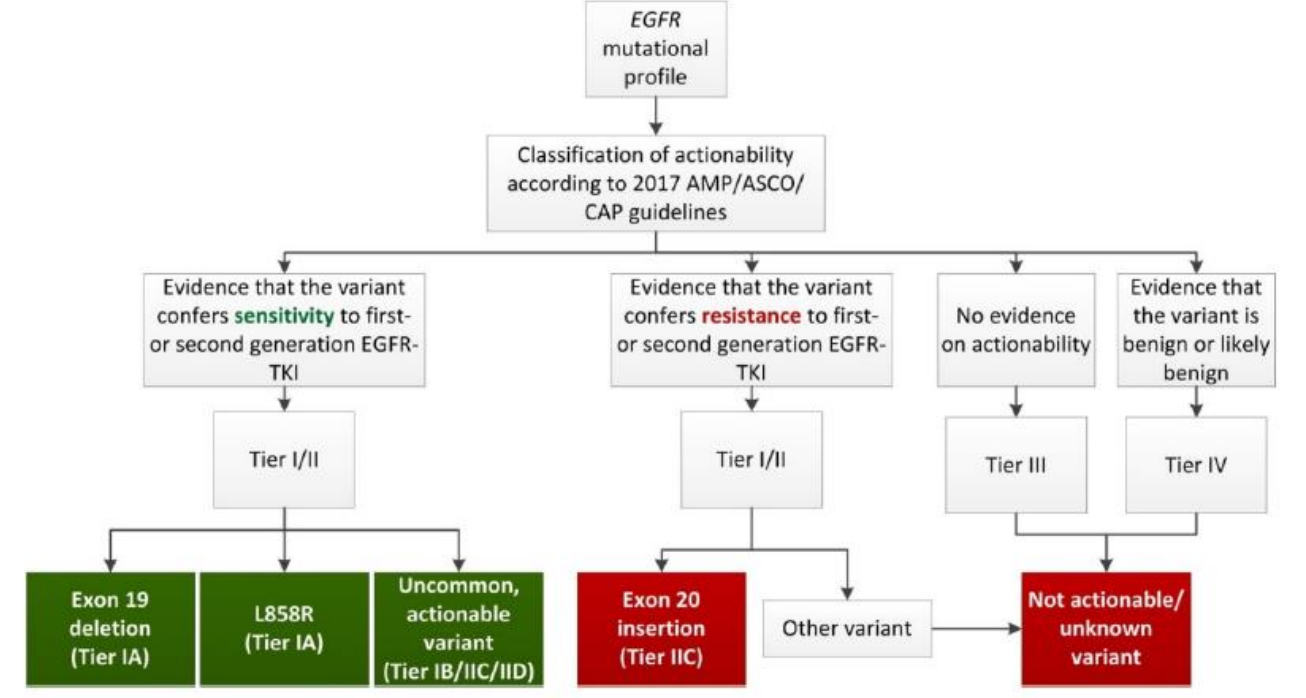
Reported in PALGA (Dutch Pathology Registry)



- *EGFR* mutation prevalence: 11.7% (925/7908)
- 19% uncommon, rare EGFR mutations
- Correct clinical interpretation and actionability

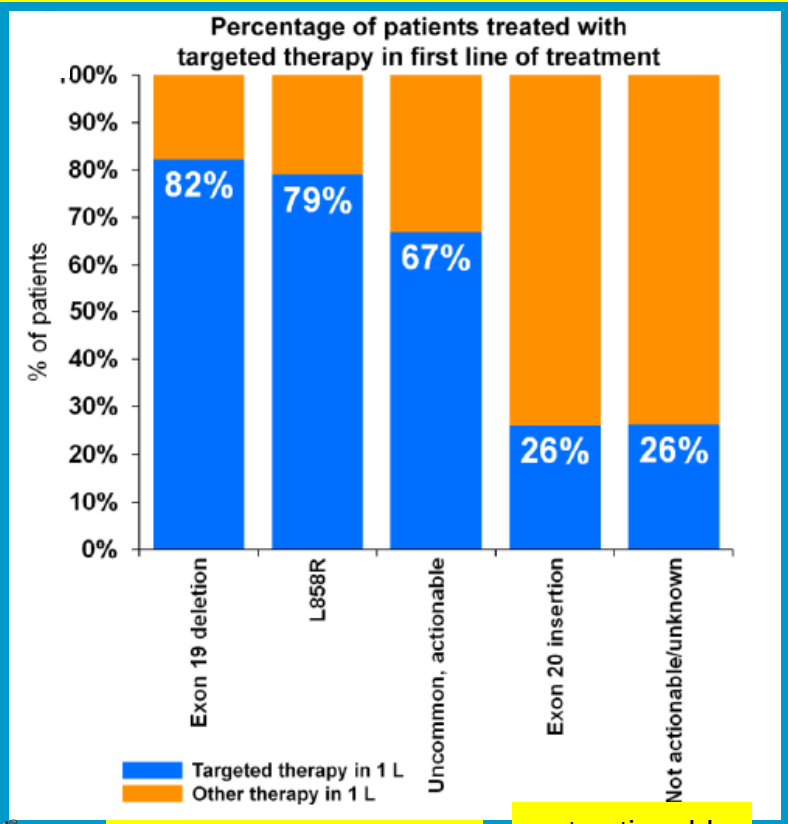
Koopman et al Cancers 2021;13:1341

Reclassification of actionability of all EGFR variants in 925 patients reported in 2013/2015/2017 in Dutch Pathology Registry



Koopman et al Cancers 2021;13:1341

Sensitivity of targeted therapy of all EGFR-variants reported in 2013/2015/2017 in Dutch Pathology Registry and Dutch Cancer Registry

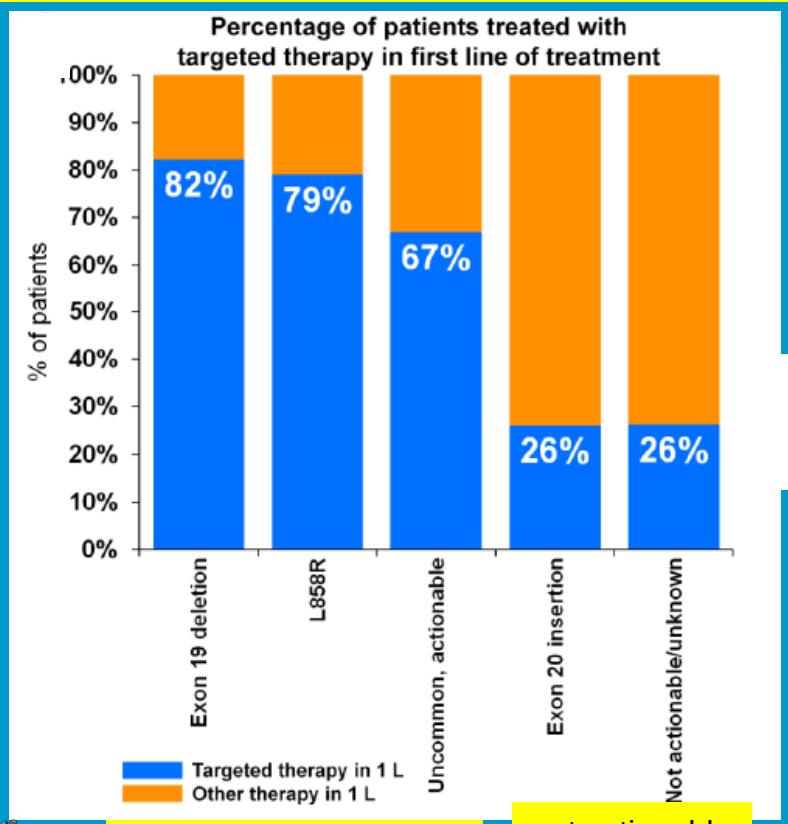


Actionable

not actionable
TKI-resistent

Koopman et al Cancers 2021;13:1341

Sensitivity of targeted therapy of all EGFR-variants reported in 2013/2015/2017 in Dutch Pathology Registry and Dutch Cancer Registry



Actionable

not actionable
TKI-resistent

Genotyping: proper classification of variants essential for treatment-decision-making

Koopman et al Cancers 2021;13:1341

Molecular diagnostics of lung cancer for treatment planning using gene-targeted therapy
international guidelines

Dutch Oncoline guideline for NSCLC (Jan 2020)

EGFR, ALK, ROS, HER2, BRAF, KRAS, RET, MET-skipping, NRG1, NTRK1/2/3 and PD-L1

CAP-IASLC-AMP guideline for NSCLC (Aug 2017) (Hanna JCO 2017)

EGFR, ALK, ROS and PD-L1 (recommendation HER2, BRAF and RET)

ASCO guirdeline for NSCLC (Kalemkerian JCO 2018)

EGFR, ALK, ROS, HER2, BRAF, MET and RET

NCCN guideline for NSCLC (June 2020)

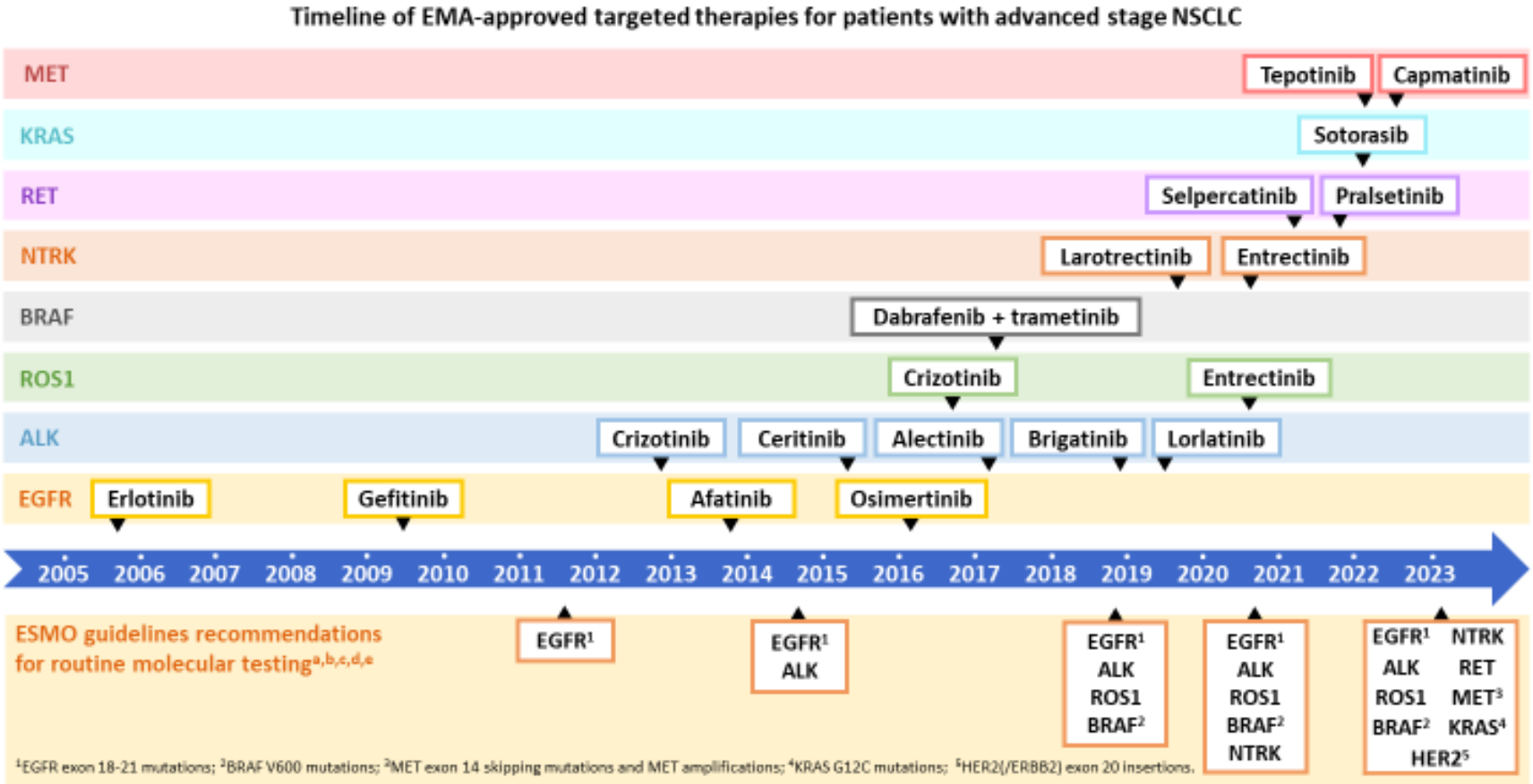
EGFR, ALK, ROS, BRAF, MET-skipping, RET and PD-L1 (recommended broad profiling: HER2, MET-amp, TMB, NTRK)

ESMO guideline for NSCLC (Sept 2019)

EGFR, ALK, ROS, BRAF and PD-L1 (with TMB); not routinely recommended RET, HER2, MET-skipping and NTRK)

ESMO guideline for NSCLC (Mosele Ann Oncol 2020)

Recommendations for the use of broad NGS for patients with metastatic cancers

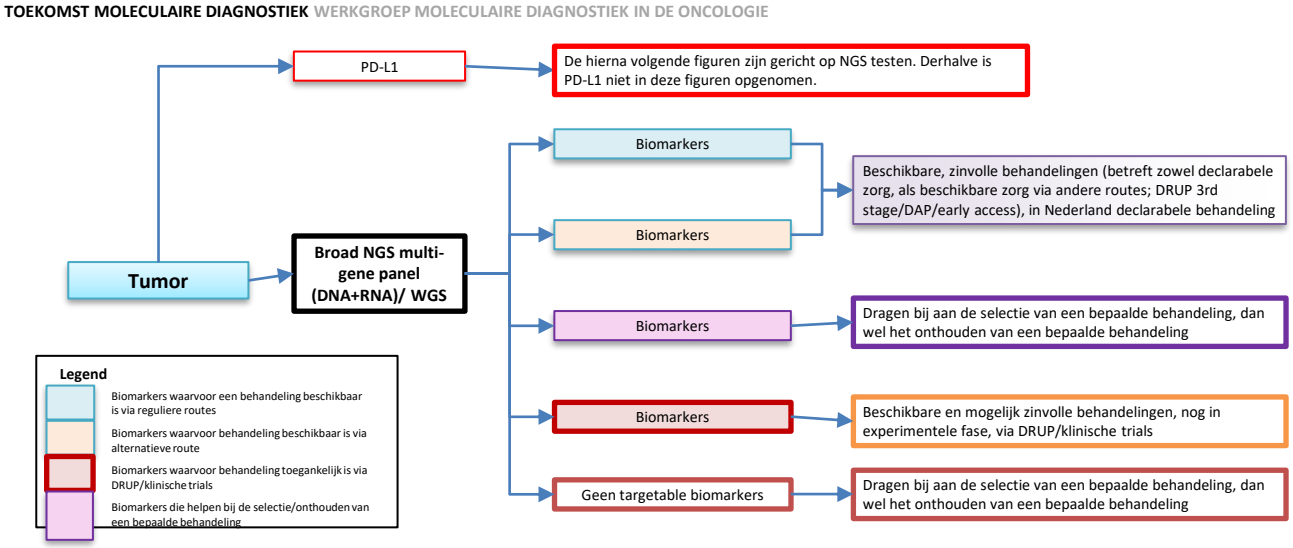


de Jager, Schuuring, unpublished data

National committee (ZIN): define “the list of minimal clinical required molecular tests”

Brief van de Minister aan de voorzitter van de 2de Kamer:

Datum 19 mei 2021
Betreft Advies Zorginstituut Nederland over moleculaire diagnostiek in de oncologie



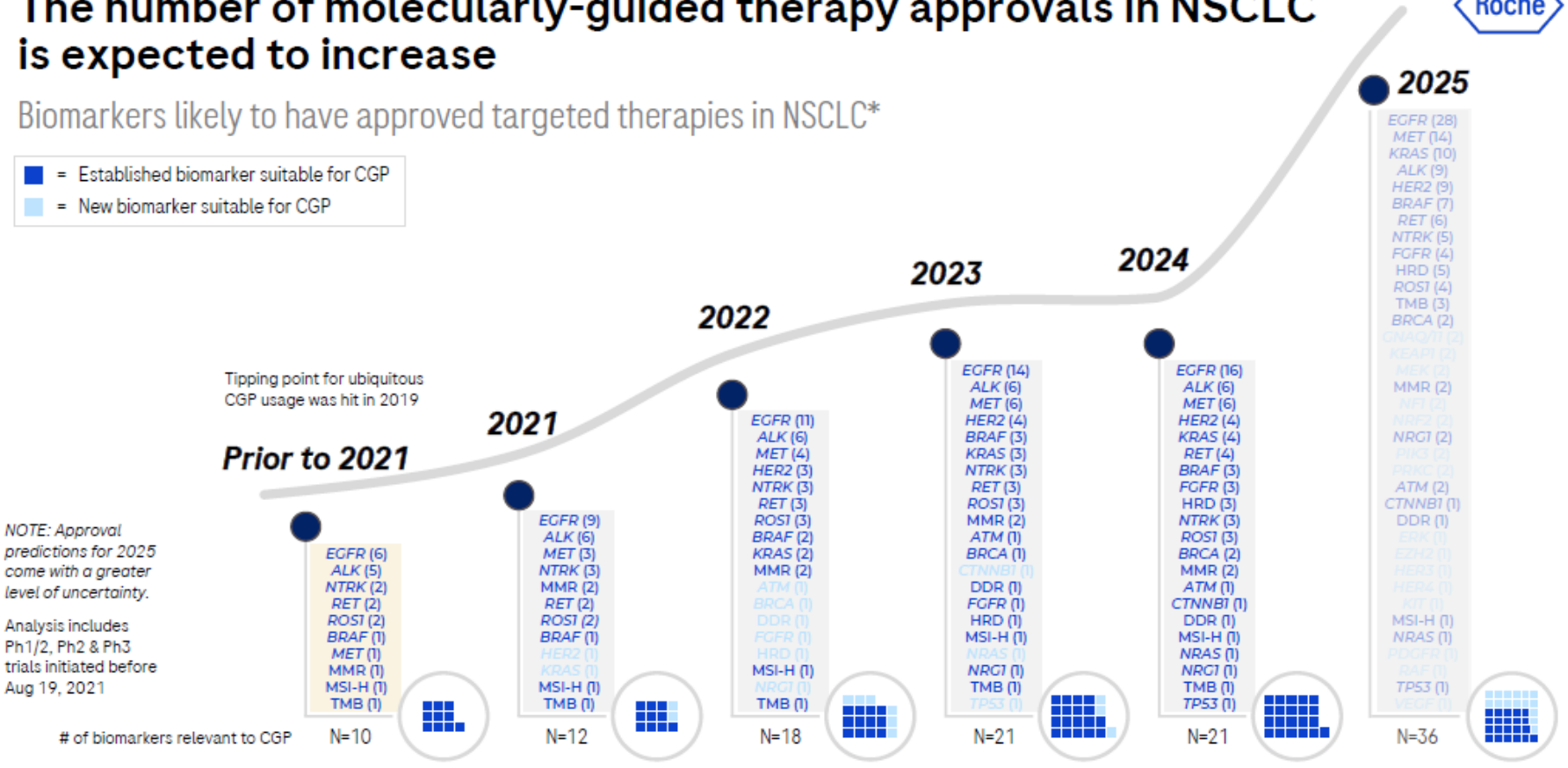
Landelijke commissie ZIN over Moleculaire Pathologie (advies naar Minister/VWS) (2019-2023)

Projectgroep 1 (Monkhorst, Bloemendaal, Gelderblom, Smit, Schuuring):

Lijst Minimaal Klinisch Noodzakelijk Moleculaire testen Nederland (list of minimal clinical required molecular tests)

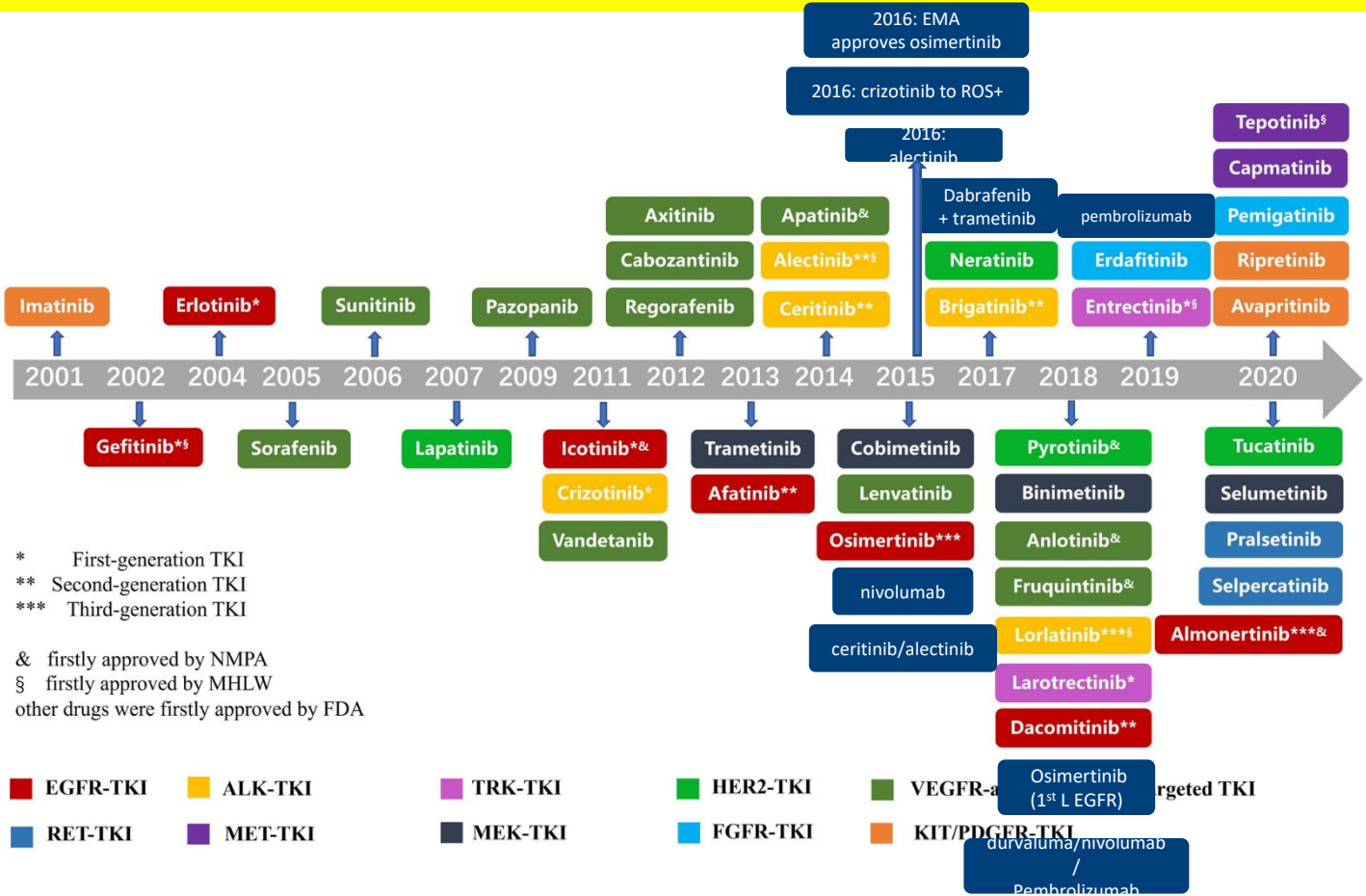
The number of molecularly-guided therapy approvals in NSCLC is expected to increase

Biomarkers likely to have approved targeted therapies in NSCLC*



*Multiple secondary sources used to cross validate information, including Trialtrove, clinicatrials.gov, EudraCT, ChiCTR; FDA approval timeline estimation based on Ph3 PCD + 8 months review; analysis based on current Phase 1/2, Phase 2 and Phase 3 trials with inclusion criteria requiring patient selection based on alterations to specific biomarkers; assumption made that all ongoing trials will lead to approval; "biomarker" defined as any biological molecule found in blood or tissues that has either prognostic or predictive significance in cancer treatment, and for which the effectiveness of a therapy in a patient population defined by the detection of this molecule or molecular aberration is currently being tested or has already been approved. CGP: comprehensive genomic profiling; DDR: DNA damage response; FDA: U.S. Food and Drug Administration; HRD: homologous recombination deficiency; MMR: mismatch repair; MSI-H: microsatellite instability-high; Ph: Phase; TMB: tumour mutational burden.

Tyrosine kinase inhibitors (solid tumors)



J Hematol Oncol 2020; 13:143

Molecular tumor profiling 2023 at MP UMCG Groningen:

predictive and diagnostic markers based on current guidelines and ongoing clinical trial

Lung cancer:	**PDL1, EGFR, ALK, ROS, RET, KRAS, BRAF, PIK3CA, MET, FGFR1, others**
Melanoma:	**BRAF (NRAS, cKIT) (plus new WHO-classification)**
Colon cancer:	**KRAS, NRAS, BRAF (PIK3CA), MSI, MHL1-methylations, MMR-IHC**
GIST:	**cKIT, PDGFRA, NRAS**
Breast cancer:	**HER2, BRCA1, PIK3CA, ESR1, AKT1, others**
Neuro-oncology:	**IDH1, 1p/19q del, MGMT-methylation, methylation profile**
Thyroid cancer:	**RET-mutations, RET-fusions, NTRK**
Ovarian cancer:	**BRCA1/2**
HNSCC:	**hrHPV, lrHPV, p16-IHC, HRAS**
Endometrium:	**POLE, p53, CTNBB1**
Malignant lymfoma:	**MYC/BCL2/6 translocation, mutation panel, MYD88, GCB/ABC expression profile, clonality testing**
Cervical cancer:	**hrHPV**
Prostate cancer:	**HRD, HRR**
Pancreas cancer:	**HRD, HRR**

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Molecular Oncological Pathology at UMCG: www.MolOncoPath.nl

- The list of new targeted drugs is growing
- Also drugs available via off-label, clinical trials (e.g. DRUP), compassionate use (with lower LoE)
- Options for targeted therapy in most malignancies increasing
- shift histology to molecular diagnostic and prognostic markers

Broad molecular testing is required

What do we need in 2023:

- Broader panels (>350 clinical relevant markers)
- Detection of SNVs, deletions, insertions, BUT also: fusions, TMB, MSI, signatures, CNV
- Per 2023: molecular test should be CE-IVD
- Applicable on FFPE (WGS not yet suitable for all patients)

Nation-wide implementation of broad NGS (and WGS) in molecular pathology for rational treatment decision making (2021-2025)

TSO500 genenpanel

ABL1	BRD4	CUX1	FAM175A	GATA6	IGF1	MAP3K13	NOTCH4	POLE	RPTOR	TAF1
ABL2	BRIP1	CXCR4	FAM46C	GEN1	IGF1R	MAP3K14	NPM1	PPARG	RUNX1	TBX3
ACVR1	BTG1	CYLD	FANCA	GID4	IGF2	MAP3K4	NRAS	PPM1D	RUNX1T1	TCEB1
ACVR1B	BTK	DAXX	FANCC	GLI1	IKBKE	MAPK1	NRG1	PPP2R1A	RYBP	TCF3
AKT1	C11orf30	DCUN1D1	FANCD2	GNA11	IKZF1	MAPK3	NSD1	PPP2R2A	SDHA	TCF7L2
AKT2	CALR	DDR2	FANCE	GNA13	IL10	MAX	NTRK1	PPP6C	SDHAF2	TERC
AKT3	CARD11	DDX41	FANCF	GNAQ	IL7R	MCL1	NTRK2	PRDM1	SDHB	pTERT
ALK	CASP8	DHX15	FANCG	GNAS	INHA	MDC1	NTRK3	PREX2	SDHC	TET1
ALOX12B	CBFB	DICER1	FANCI	GPR124	INHBA	MDM2	NUP93	PRKAR1A	SDHD	TET2
ANKRD11	CBL	DIS3	FANCL	GPS2	INPP4A	MDM4	NUTM1	PRKCI	SETBP1	TFE3
ANKRD26	CCND1	DNAJB1	FAS	GREM1	INPP4B	MED12	PAK1	PRKDC	SETD2	TFRC
APC	CCND2	DNMT1	FAT1	GRIN2A	INSR	MEF2B	PAK3	PRSS8	SF3B1	TGFBR1
AR	CCND3	DNMT3A	FBXW7	GRM3	IRF2	MEN1	PAK7	PTCH1	SH2B3	TGFBR2
ARAF	CCNE1	DNMT3B	FGF1	GSK3B	IRF4	MET	PALB2	PTEN	SH2D1A	TMEM127
ARFRP1	CD274	DOT1L	FGF10	H3F3A	IRS1	MGA	PARK2	PTPN11	SHQ1	TMPRSS2
ARID1A	CD276	E2F3	FGF14	H3F3B	IRS2	MITF	PARP1	PTPRD	SLIT2	TNFAIP3
ARID1B	CD74	EED	FGF19	H3F3C	JAK1	MLH1	PAX3	PTPRS	SLX4	TNFRSF14
ARID2	CD79A	EGFL7	FGF2	HGF	JAK2	MLL	PAX5	PTPRT	SMAD2	TOP1
ARID5B	CD79B	EGFR	FGF23	HIST1H1C	JAK3	MLLT3	PAX7	QKI	SMAD3	TOP2A
ASXL1	CDC73	EIF1AX	FGF3	HIST1H2BD	JUN	MPL	PAX8	RAB35	SMAD4	TP53
ASXL2	CDH1	EIF4A2	FGF4	HIST1H3A	KAT6A	MRE11A	PBRM1	RAC1	SMARCA4	TP63
ATM	CDK12	EIF4E	FGF5	HIST1H3B	KDM5A	MSH2	PDCD1	RAD21	SMARCB1	TRAF2
ATR	CDK4	EML4	FGF6	HIST1H3C	KDM5C	MSH3	PDCD1LG2	RAD50	SMARCD1	TRAF7
ATRX	CDK6	EP300	FGF7	HIST1H3D	KDM6A	MSH6	PDGFRA	RAD51	SMC1A	TSC1
AURKA	CDK8	EPCAM	FGF8	HIST1H3E	KDR	MST1	PDGFRB	RAD51B	SMC3	TSC2
AURKB	CDKN1A	EPHA3	FGF9	HIST1H3F	KEAP1	MST1R	PDK1	RAD51C	SMO	TSHR
AXIN1	CDKN1B	EPHA5	FGFR1	HIST1H3G	KEL	MTOR	PDPK1	RAD51D	SNCAIP	U2AF1
AXIN2	CDKN2A	EPHA7	FGFR2	HIST1H3H	KIF5B	MUTYH	PGR	RAD52	SOCS1	VEGFA
AXL	CDKN2B	EPHB1	FGFR3	HIST1H3I	KIT	MYB	PHF6	RAD54L	SOX10	VHL
B2M	CDKN2C	ERBB2	FGFR4	HIST1H3J	KLF4	MYC	PHOX2B	RAF1	SOX17	VTCN1
BAP1	CEBPA	ERBB3	FH	HIST2H3A	KLHL6	MYCL1	PIK3C2B	RANBP2	SOX2	WISP3
BARD1	CENPA	ERBB4	FLCN	HIST2H3C	KMT2B	MYCN	PIK3C2G	RARA	SOX9	WT1
BBC3	CHD2	ERCC1	FLI1	HIST2H3D	KMT2C	MYD88	PIK3C3	RASA1	SPEN	XIAP
BCL10	CHD4	ERCC2	FLT1	HIST3H3	KMT2D	MYOD1	PIK3CA	RB1	SPOP	XPO1
BCL2	CHEK1	ERCC3	FLT3	HLA-A	KRAS	NAB2	PIK3CB	RBM10	SPTA1	XRCC2
BCL2L1	CHEK2	ERCC4	FLT4	HLA-B	LAMP1	NBN	PIK3CD	RECQL4	SRC	YAP1
BCL2L11	CIC	ERCC5	FOXA1	HLA-C	LATS1	NCOA3	PIK3CG	REL	SRSF2	YES1
BCL2L2	CREBBP	ERG	FOXL2	HNF1A	LATS2	NCOR1	PIK3R1	RET	STAG1	ZBTB2
BCL6	CRKL	ERRFI1	FOXO1	HNRNPK	LMO1	NEGR1	PIK3R2	RFWD2	STAG2	ZBTB7A
BCOR	CRLF2	ESR1	FOXP1	HOXB13	LRP1B	NF1	PIK3R3	RHEB	STAT3	ZFHX3
BCORL1	CSF1R	ETS1	FRS2	HRAS	LYN	NF2	PIM1	RHOA	STAT4	ZNF217
BCR	CSF3R	ETV1	FUBP1	HSD3B1	LZTR1	NFE2L2	PLCG2	RICTOR	STAT5A	ZNF703
BIRC3	CSNK1A1	ETV4	FYN	HSP90AA1	MAGI2	NFKBIA	PLK2	RIT1	STAT5B	ZRSR2
BLM	CTCF	ETV5	GABRA6	ICOSLG	MALT1	NKX2-1	PMAIP1	RNF43	STK11	
BMPR1A	CTLA4	ETV6	GATA1	ID3	MAP2K1	NKX3-1	PMS1	ROS1	STK40	
BRAF	CTNNA1	EWSR1	GATA2	IDH1	MAP2K2	NOTCH1	PMS2	RPS6KA4	SUFU	
BRCA1	CTNNB1	EZH2	GATA3	IDH2	MAP2K4	NOTCH2	PNRC1	RPS6KB1	SUZ12	
BRCA2	CUL3	FAM123B	GATA4	IFNGR1	MAP3K1	NOTCH3	POLD1	RPS6KB2	SYK	

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Nation-wide implementation of broad NGS (and WGS) in molecular pathology for rational treatment decision making (2021-2025)

- Introduction of 523 gene NGS panel (**commercial Illumina TSO500**) for single nucleotide variants (SNV), copy number variants (CNV); tumour mutational burden (TMB) and microsatellite instability (MSI) for most clinical-relevant markers

- Definition of virtual subpanels dependent on clinical request, e.g.

Lung cancer:	14 genes
Lymphoma:	26 genes
Prostate cancer:	46 genes
Actionable target panel:	54 genes (with all targets for inclusion in DRUP)
Upcoming indications	(breast, ovary, endometrium, pancreas, others, …)

TSO500 gevalideerd in RadboudMC (JMD2020)
UMCG: nieuwe standaard
Validatie: MUMC, ErasmusMC, UMCU
CE-IVD-TSO500: 15th March 2022

TSO500 is combined with:
- RNA-based NGS test (Archer lung)
- PDL1-IHC

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PATH
Predictive Analysis for THerapy

Nation-wide implementation of broad NGS (and WGS) in molecular pathology for rational treatment decision making (202 25)

- Introduction of 523 gene NGS panel (**comme** ingle nucleotide variants (SNV), copy num nal burden (TMB) and microsatellite ins rs
- Definition of vir
  Lung c
  Lymph
  Prostat
  Actionat clusion in DRUP)
  Upcomin etrium, pancreas, others, …)

Broad NGS testing:

Ready for the future (easy extent the list of target)

Same panel for several indication > efficient workflow

> cost-effective, tissue-efficient, overall acceptable TAT

TS dMC (JMD2020)
UM ard
Vali C, ErasmusMC, UMCU
CE-I D-TSO500: 15th March 2022

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PAT
Predictive Analysis for THerapy

Some new challenges using broad NGS panels

- More rare and uncommon variants (appropriate calling and interpretation)
- Nation-wide implementation (to ensure equal access to targeted therapies for all cancer patients in the NL)
- Tumor tissue management (small biopsies, FFPE, fresh/frozen, liquid biopsies)
- Reporting molecular results (international annotations for professionals but useful treatment advice for physicians)
- Costs, cost-effectiveness, reimbursements
- Quality control, quality assurance

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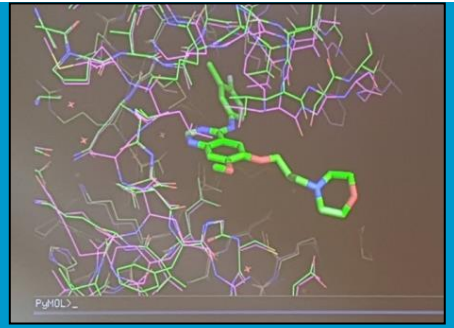
PATH
Predictive Analysis for THerapy

Variant calling, clinical interpretation and classification of actionability proper treatment decision making (example)

- Analysis and classification of the variant by Clinical Scientist in Molecular Pathology (CSMP):
- Reporting common variants in the Molecular Pathology Registry (available for treating physicians)
- Rare and uncommon variants submitted to Molecular Tumor Board for advice on treatment

Standard variant calling, clinical interpretation and classification for actionability

3D-modeling of mutated protein: drug binding to predict actionability



6 in vitro models to predict specific response

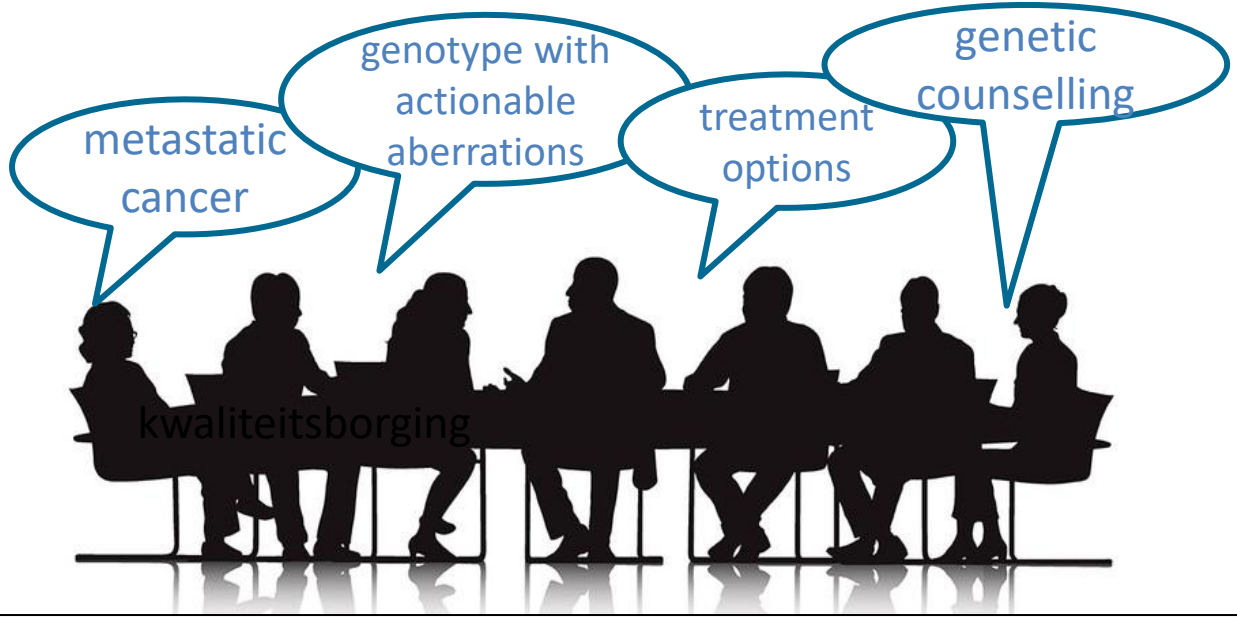
Characteristic	Fontana-V3 (2015)	Gainor-V1 (2016)	Gainor-V3 (2016)	Yoda-V3 (2018)	Horn-V1 (2019)	Horn-V3 (2019)
Cell type used	Ba/F3 cells	Ba/F3 cells	Ba/F3 cells	Ba/F3 cells	Ba/F3 cells	Ba/F3 cells
EML4-ALK variant tested	Variant 3 EML4 ex 6:ALK ex 20	Variant 1 EML4 ex 13:ALK ex 20	Variant 1 EML4 ex 13:ALK ex 20	Variant 3 EML4 ex 6:ALK ex 20	Variant 1 EML4 ex 13::ALK ex 20	Variant 3 EML4 ex 6 ::ALK ex 20
Mutational profiles tested	L1152R C1156Y L1196M G1202R S1206Y G1269A	C1156Y I1171N I1171S I1171T F1174C L1196M L1198F G1202del G1202R D1203N E1210K G1269A F1174C/D1203N D1203N/E1210K	L1196M G1202R L1196M/L1198F L1196M/G1202R L1198F/G1202R	L1196M G1202R L1196M/L1198F L1196M/G1202R L1198F/G1202R	G1123S L1152R C1156Y I1171T F1174C F1174V V1180L L1196M L1198F G1202del G1202R S1206Y E1210K F1245C G1269A	G1123S L1152R C1156Y I1171T F1174C F1174V V1180L L1196M L1198F G1202del G1202R D1203N S1206Y E1210K F1245C G1269A
ALK inhibitors tested	Alectinib ASP3026 Brigatinib Ceritinib Crizotinib	Alectinib Brigatinib Ceritinib Crizotinib Lorlatinib	Alectinib Brigatinib Ceritinib Crizotinib Lorlatinib	Alectinib Brigatinib Ceritinib Crizotinib Lorlatinib	Alectinib Brigatinib Ceritinib Crizotinib Ensartinib Lorlatinib	Alectinib Brigatinib Ceritinib Crizotinib Ensartinib Lorlatinib
Type of assay used	^{3}H incorporation	ALK phosphorylation	Cell viability	Cell viability	Cell viability	Cell viability
Reported value	Ratio IC_{50} mt/WT	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}
Threshold for sensitivity	IC_{50} Ratio < 2	IC_{50} < 50 nmol/L	IC_{50} < 50 nmol/L	IC_{50} < 50 nmol/L	Gradual scale	Gradual scale
PMID	25727400	27432227	29650534	29650534	31461411	31461411

Koopman, Clinical Lung Cancer 2021, Jul 3; doi: 10.1016/j.cllc.2021.06.011

Tumor-related molecular findings discussed in our MTB
(some examples)

- Patients with rare or uncommon variants (frequency <1%)
- Patients with pathogenic/actionable variant without standard-of-care treatment options
- Patients with known targetable variant in combination with other oncogenic/pathogenic variants
- Patients with rare treatment-resistant mechanism
- Patients with variants that are reported targetable in other cancers
- Patients with variants (potentially) also known as germline mutation
- Patients with any uncommon findings
- To discuss additional testing for inclusion in clinical studies

Molecular tumour board



Clinical Scientist in Molecular Pathology, Medical oncologists; Pulmonologists; Pathologists; Clinical Geneticists; Geneticist experts in molecular diagnostics and targeted therapy, and active in an NVALT-designated centre for targeted treatment.

- Treatment decision making for uncommon/rare variants
- MTB is complementary to multidisciplinary tumor board teams
- Organisation: centralized MTB; regional networking with local MDT teams

https://richtlijnendatabase.nl/richtlijn/niet_kleincellig_longcarcinoom/algemeen.html (In Dutch only)

Koopman et al. *JCO Precision Oncology* 2020;4:393-410; Koopman et al. *The Oncologist* 2020;25:1-12

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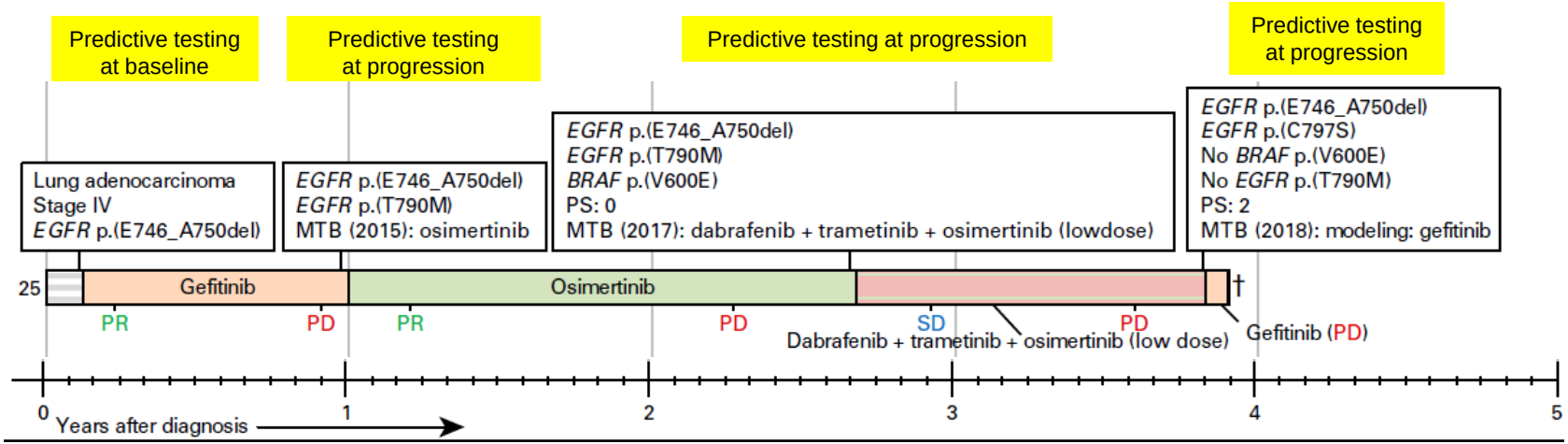
Federatie Medisch Specialisten

PATH
Predictieve Analyse voor THerapie

New class of predictive markers in Precision Medicine:

EGFR/ROS/ALK/ICI-treatment-resistent alterations targetable with second-line TKI



Molecular Tumor Board UMCG 2019: Koopman, van Kempen, Schuuring JCO PO 2020

MTBs are hosted in all tertiary cancer referral centers in the Netherlands

1 umcg

2 Antoni van Leeuwenhoek Nederlands Kanker Instituut

3 Amsterdam UMC Universitair Medische Centra

4 LUMC Leids Universitair Medisch Centrum

5 UMC Utrecht

6 Erasmus MC University Medical Center Rotterdam

7 Radboudumc

8 Maastricht UMC+

Ovarian Renal GIST Prostate Glioma Endometrium Melanoma Sarcoma CRC

NSCLC

Koopman et al. 2021: Multicenter Comparison of Molecular Tumor Boards in the Netherlands: Definition, Methods and Targeted Therapy Recommendations. The Oncologist. DOI: 10.1002/onco.13580

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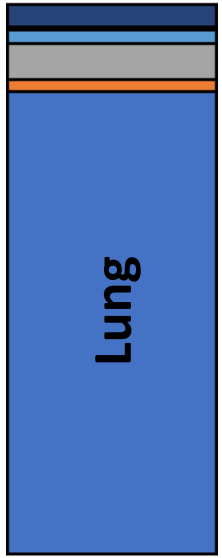
PATH

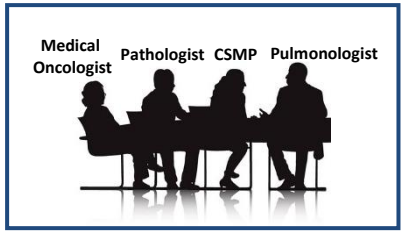
Molecular Tumor Boards: today and near-future

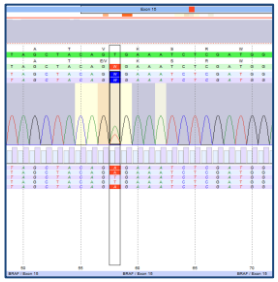
MTBs today ...

Mutual educational platform for various professional

2020

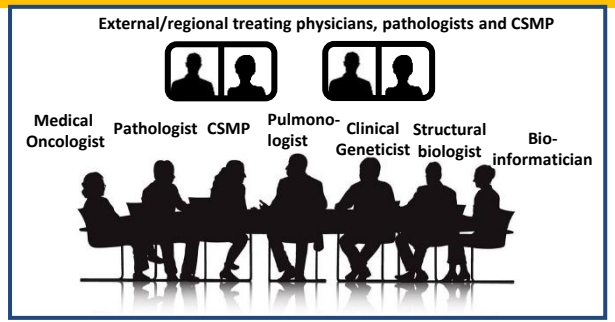


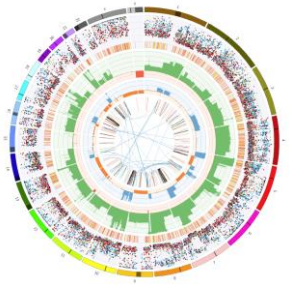




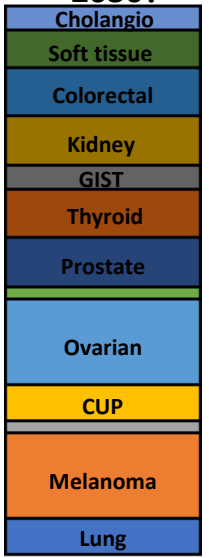
MTBs in near future ?

MTB is expertisegroup required for professional guidelines and reimbursement of complex MD-based health care





2030?



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cieBOD – commissie ter BeOordeling Diagnostiek (jan 2022)

No national direction on implementation of new diagnostics

No link between registration of new oncolytics/indication and **the associated diagnostics**, resulting in delays in the implementation and appropriate use of both registration and diagnostics

No point of contact for CieBOM, cieBAG, insurrance companies, regulatory bodies, health care agencies (ZIN) and others, resulting in a lack of direction

Current national onco-guidelines:

No advice regarding molecular diagnostic algorithms/techniques
Incorporation of new developments have great delay (guideline are not uptodate)

This leads to unnecessary heterogeneity in molecular diagnostic algorithms in the Netherlands

National advisory body with regard to method, execution and implementation of oncological diagnostics (installed nov 2021)

Group of experts from the scientific societies, the NVVP, NVALT, NVMO, VKGL, NVKC, NVKG, mandated by various professional societies with an emphasis on **scientific content/ expertise**

The cieBOD assesses independently of its own professional interest or (other) commercial interest

NEDERLANDSE VERENIGING VOOR PATHOLOGIE

https://pathology.nl/ciebod/

cieBOD – commissie ter BeOordeling Diagnostiek (jan 2022)

DOEL: Toetsing op basis van **stand van wetenschap en praktijk met advies**

OBJECTIVE: Assessment based on state of science and practice with advice

Effectiveness (effectiviteit): which tests are suitable? Which should not be used? Sensitivity/specificity

Position determination (plaatsbepaling):
Diagnostic landscape: List of minimal clinical required molecular targets
Future-proof: be up to date quickly (broad panels?)
More efficient organization
Efficient tissue-management
Cost efficiency

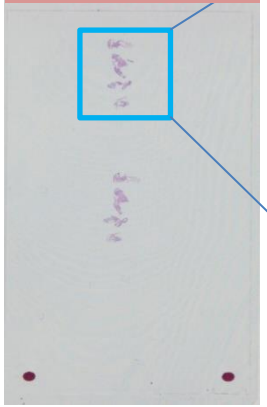
Eerste cieBOD-advies (jan 2023): RET-fusie detectie bij niet-kleincellig longcarcinoom

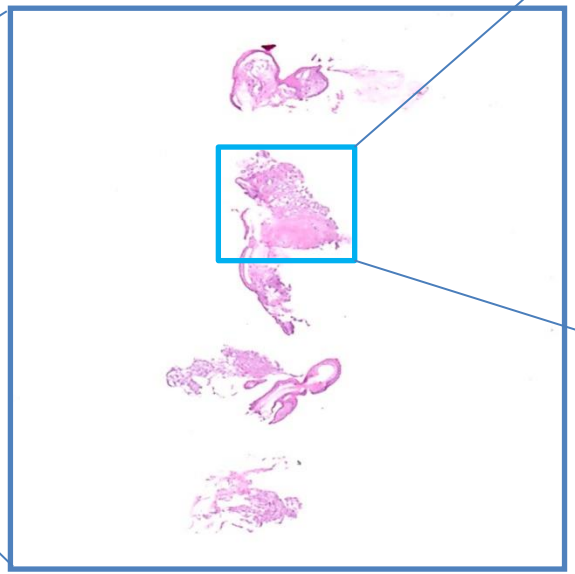
NEDERLANDSE VERENIGING VOOR PATHOLOGIE

https://pathology.nl/ciebod/

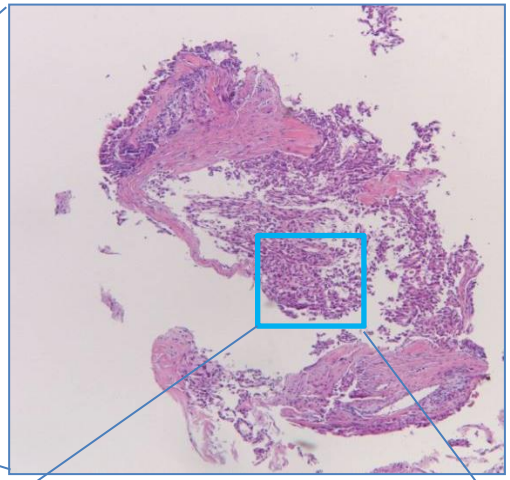
Diagnostic predictive testing
Today's tissue-management lung cancer (2023)

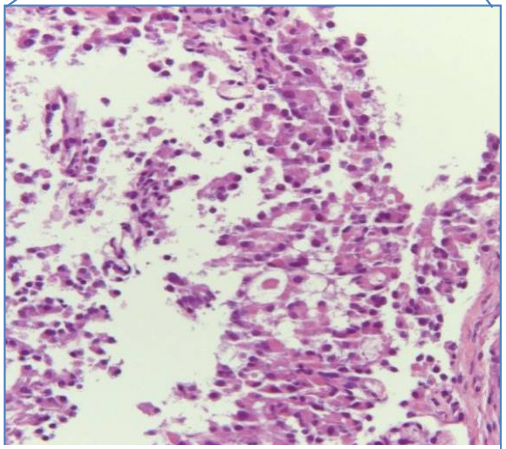
PA-T-nr:
xx-xxxx





5% neoplastic cells

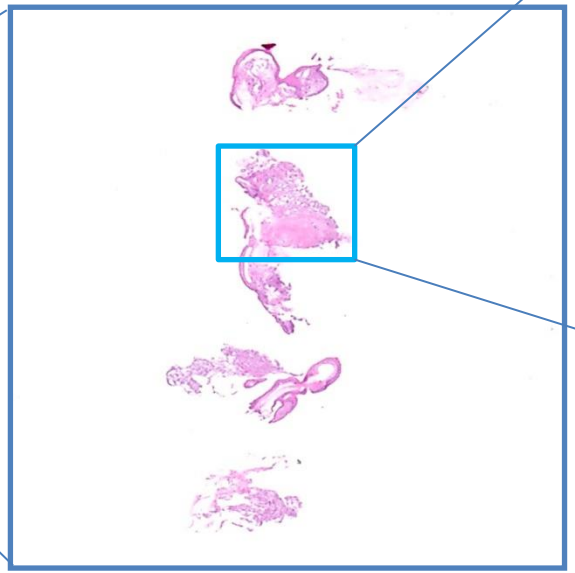




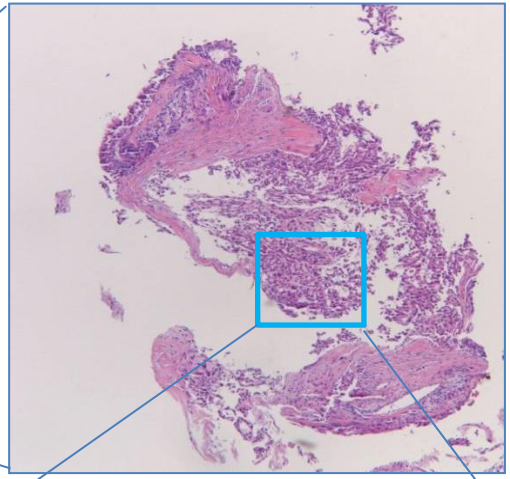
>50% neoplastic cells

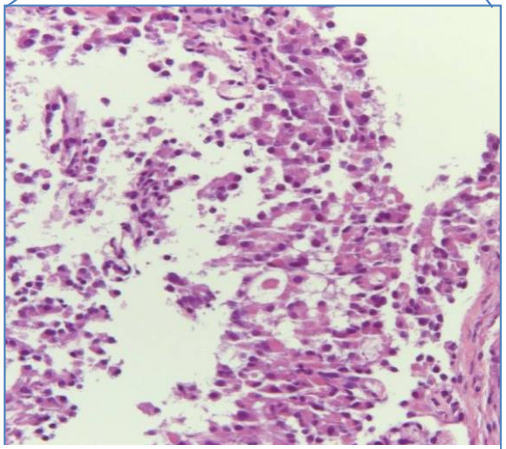
This example represents a typical biopsy in our clinical practice (lung cancer)

Diagnostic predictive testing
Today's tissue-management lung cancer (2023)



5% neoplastic cells





>50% neoplastic cells

This example represents a typical biopsy in our clinical practice (lung cancer)

In NL in **25-30%** of clinical lung cancer samples no molecular predictive testing could be performed !!!

- Not enough tumour DNA - No neoplastic cells (<1%) - MD is not requested

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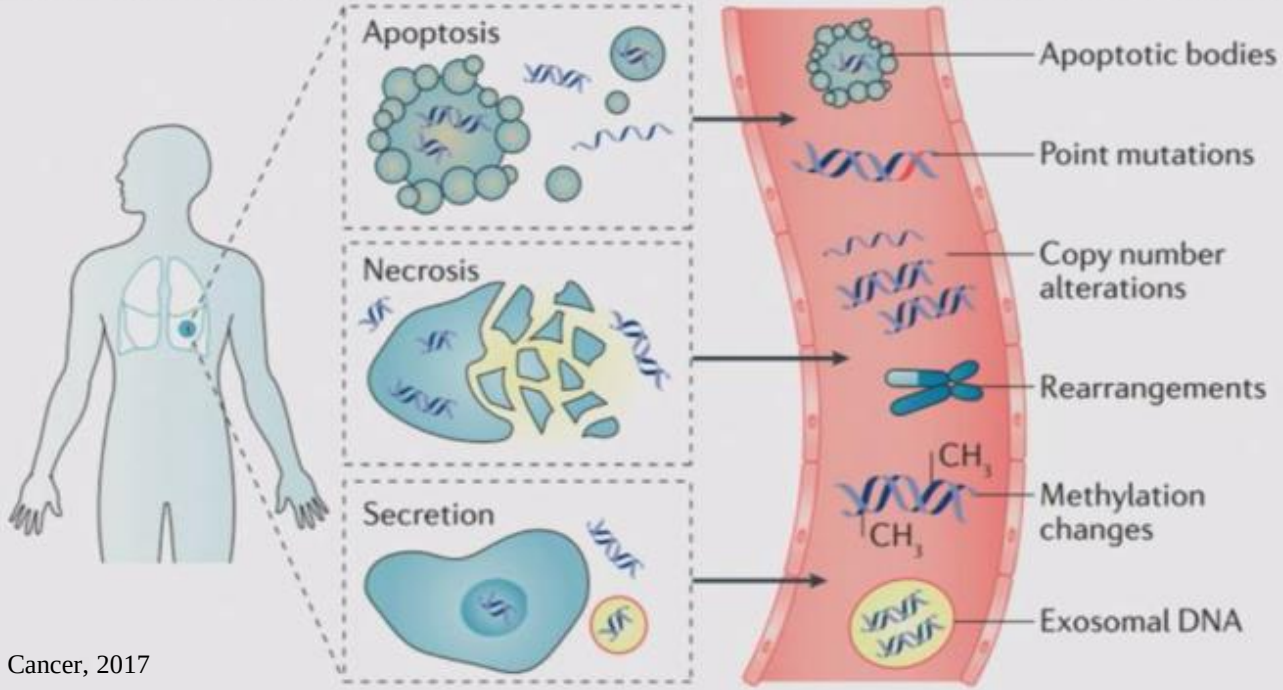
Liquid biopsies in clinical practice: circulating tumor DNA (ctDNA) is shedded in the bloodstream

Mandel P, Métais P. Les acides nucléiques du plasma sanguin chez l'homme. C R Séances Soc Biol. 1948;142:241–3.



Wan JCM et al, Nat Rev Cancer, 2017

Routine EDTA-tube after centrifugation

Detection of mutations in circulating tumor DNA in cell free plasma finding the “abnormal” hay in the haystack

Image from www.palaisdetokyo.com

Different molecular assays are required compared to tissue-based molecular profiling

Fraction of tumor DNA in tissue biopsy: >10%
Fraction of prenatal DNA in plasma of mother: ~15%
Fraction of tumor DNA in total circulating cell-free DNA in plasma: <0.1-1%

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Liquid Biopsy: circulating tumor DNA in cel-free plasma

very promising diagnostic tool for prediction and monitoring

- None-invasive (easy blood-drawn)
- Simple logistics (routine)
- Cheap

- No one-fits-all-ctDNA assay; guided by the clinical application
- Most appropriate test depends on reimbursement, QC, experience, ...

Approaches and opportunities:

Standardization/harmonisation of technical/logistic issues
By collaboration at Dutch (COIN) and European (ELBS) level

THE EUROPEAN LIQUID BIOPSY SOCIETY

CANCER ID

www.cfDNA/COIN

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Take-home message

For proper treatment response molecular target-testing AND accurate genotyping of targets is required

Precision Medicine is very dynamic (new drugs/treatment): the pressure on diagnostics is high

We need to be ready for the future, cost-efficient, tissue-management, efficient workflows, high quality

SOLUTION: a complete molecular profile of tumors using **broad target-panel/WGS** for optimal treatment decision making

Essential role for MTB, cieBOD, central-testing/regional networking, PATH/COIN

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Molecular Pathologie UMCG-team

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Annelies ten Caat
Erik Nijboer
Paskal Modderman-van Norel
Rianne Pelgrim
Inge Platteel
Martin Schipper
Jantine Sietzema
Tom Artz
Marleen Smits
Frank Scherpen
Klaas Kooistra

Clinical scientists in Molecular Pathology (KMBP)
Elise van der Logt (PAF, UMCG)
Arja ter Elst
Anke van den Berg
Ed Schuuring (hoofd/MP)
Leon van Kempen
Joost Kluiver

Pathologists:
Wim Timens
Stefan Willems
Arjan Diepstra (moleculaire patholoog)
Arjen Cleven

Molecular Tumor Board Groningen (in dutch)
Ed Schuuring, KMBP
Arja ter Elst, KMBP
Anke van den Berg, KMBP
Wim Timens, lungpatholoog
Harry Groen, pulmonologist
Jeroen Hiltermann, pulmonologist
Anthonie van der Wekken, pulmonologist
Geke Hospers, medisch oncologist
Lucy Hijmering-Kappelle, pulmonologist
Birgitta Hiddinga, pulmonologist
Hilde Jalving, medisch oncoloog
Michiel van der Kruchten, medical oncologist
Sjoukje Oosting, medisch oncoloog
Mathew Glover, drug-design (GRI of Pharmacy)
Joost Kluiver, KMBP

Jose van Gaal, pathologist Isala
Jos Stigt, pulmonologist Isala

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Info: www.moloncopath.nl

PAT
Predictieve Analyse voor THerapie

Molecular diagnostics of NSCLC and available targetable drugs in the Netherlands (2023)

Next generation sequencing: SNV, indels and CNV

EGFR	(12%)	Erlotinib, Gefitinib, Afatinib, Osimertinib
BRAF	(5.2%)	Dabrafinib
MET	(2.6%)	Crizotinib, Capmatinib, Tepotinib
ERBB2	(1.7%)	Trastuzumab, Afatinib
KRAS	(40%)	p.G12D-specific inihibitor BI-2852, pG12C-specific inhibitor AMG 510 /sotorasib
ALK	(1.2%)	Brigatinib, Lorlatinib, Crizotinib, Ceritinib, Alectinib

Gene fusion events

ALK	(2.4%)	Crizotinib, Ceritinib, Alectinib
ROS1	(0.8%)	Crizotinib, Ceritinib
RET	(0.5%)	Selpercatinib, Pralsetinib/Blu-667
NTRK1	(<0.1%)	Entrectinib, Larotrectinib
NRG1	(<0.1%)	Afatinib
BRAF	(<0.1%)	MEKi (preclinical), RAFi (preclinical)

Protein expression profiles

ALK	(4%)	Crizotinib, Ceritinib, Alectinib
ROS1	(2%)	Crizotinib, Ceritinib
PD-L1	(var)	Pembrolizumab, Nivolumab, Atezolizumab

Koopman et al Cancers 2021; 13: 3641
Koopman et al Clin Lung Cancer 2022; 23: e104
Koopman el al Diagnostics 2022; 12: 668
Garcia et al Lung Cancer 2022; 167: 1
Steeghs, PATH-consortium, Lung Cancer 2022; 167: 87

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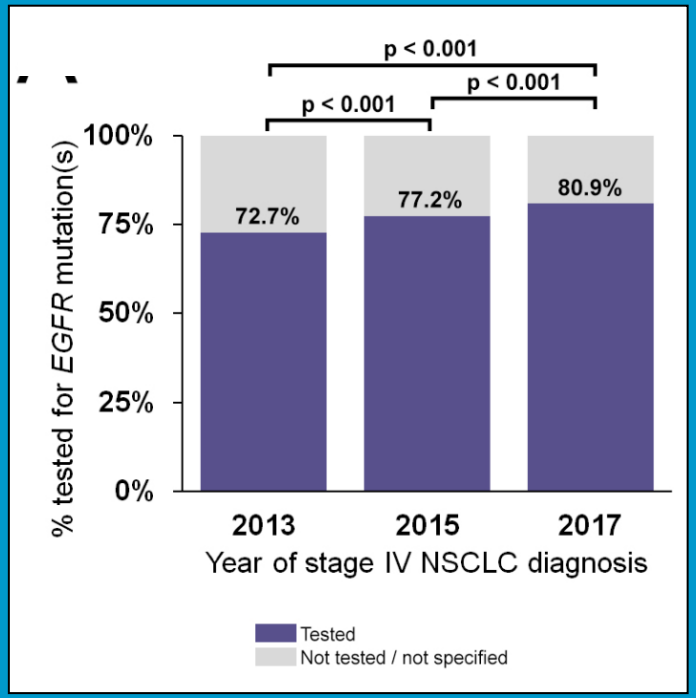
NOT all patients with advanced-stage lung cancer are tested for predictive tumor mutations in the NL (reported in PALGA - Dutch Pathology Registry)

EGFR-mutation analysis



Koopman et al Cancers 2021;13:1341

KRAS-mutation analysis

2013: 70.1%
2015: 78.5%
2017: 82.0%

Garcia et al Lung Cancer 2022: 176:1

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cieBOD – commissie ter BeOordeling Diagnostiek (jan 2022)

DOEL: Toetsing op basis van **stand van wetenschap en praktijk met advies:**

- **Effectiviteit:** welke testen zijn geschikt ? Sensitiviteit/specificiteit
- **Plaatsbepaling:**
  - Diagnostische landschap: Lijst minimaal klinisch noodzakelijke targets
  - Toekomstbestendigheid: snel up to date zijn (brede panels ?)
  - efficientere organisatie
  - Weefselefficientie
  - Kostenefficientie

Eerste cieBOD-advies (jan 2023): RET-fusie detectie bij niet-kleincellig longcarcinoom

https://pathology.nl/ciebod/

cieBOD – commissie ter BeOordeling Diagnostiek (jan 2022)

- **Geen landelijke regie** op implementatie nieuwe diagnostiek
- **Geen link tussen registratie** nieuwe oncolytica /indicatie **en de gerelateerde diagnostiek** waardoor vertraging optreedt in de implementatie en gepast gebruik van beiden
- Geen aanspreekpunt voor CieBOM, verzekeraars, ZIN e.a. met daardoor gebrek aan regie

- **Richtlijnen:**
  - **geen advies** met betrekking tot moleculair diagnostische algoritmes/ technieken
  - Nieuwe ontwikkelingen worden met grote vertraging opgenomen

- **Hierdoor onnodige heterogeniteit in moleculair diagnostische algoritmes** in Nederland

- **Landelijke adviesorgaan** mbt methode, uitvoering en implementatie van oncologische diagnostiek
  - **Groep van experts** vanuit de wetenschappelijke verenigingen, de **NVVP, NVALT, NVMO, VKGL, NVKC, NVKG, gemandateerd** vanuit verschillende beroepsverenigingen met een nadruk op **wetenschappelijke inhoud/ expertise**

  - De cieBOD beoordeelt onafhankelijk van eigen beroepsbelang of (ander) commercieel belang.

https://pathology.nl/ciebod/