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Universitair Medisch Centrum Rotterdam

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RET fusion gene detection in NSCLC: how (not) to FISH?

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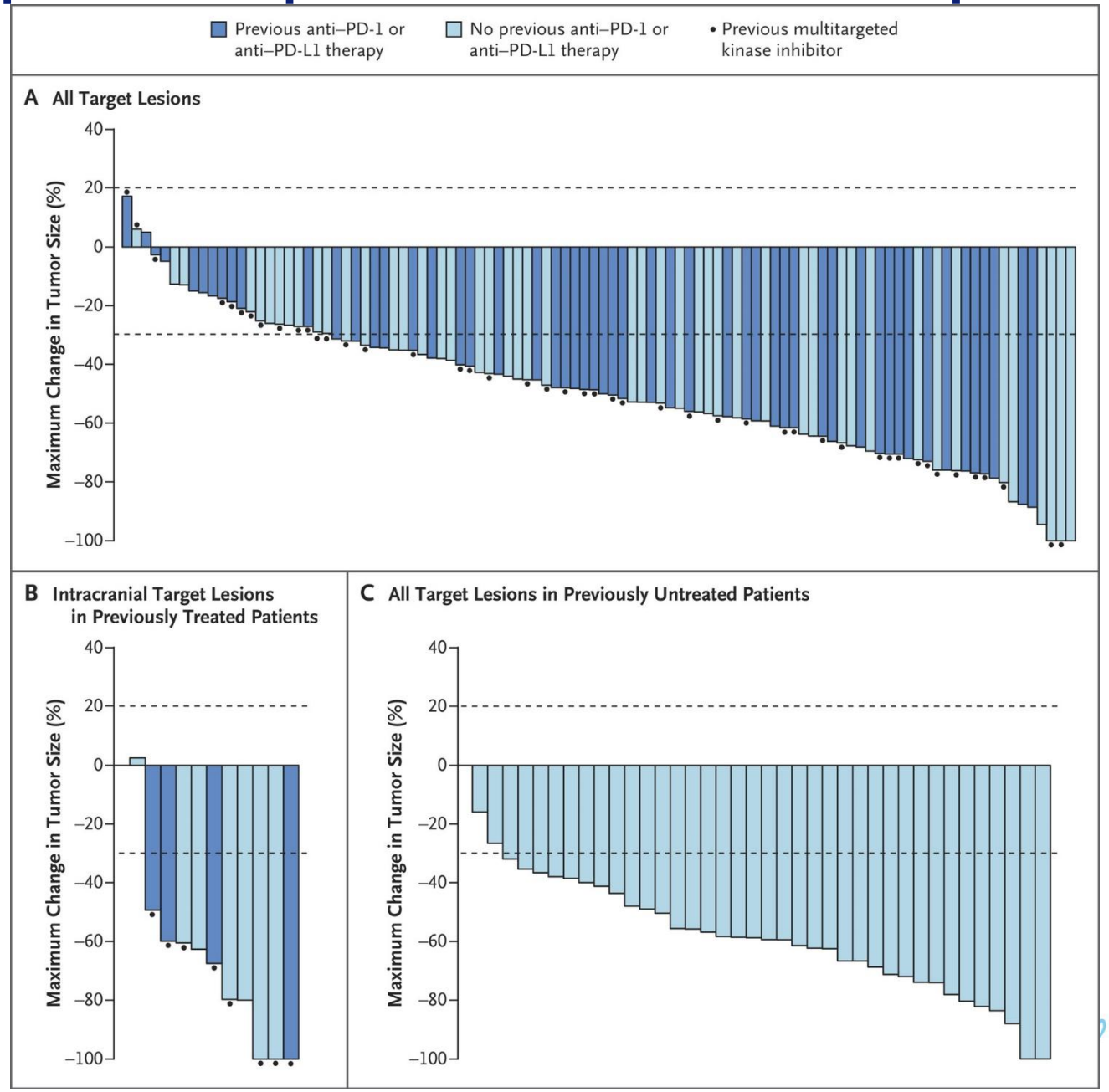
Honorarium: AstraZeneca, Lilly, Pfizer

RET-targeted diagnostics

- *RET* fusion genes are oncogenic drivers in NSCLC (adenocarcinoma)
- Predominantly found in younger NSCLC patients without smoking history
- Prevalence of *RET* fusion genes in NSCLC: 1-2%
- Multikinase RET inhibitors: objective response rates (ORR) of 30%
- Selective RET kinase inhibitors, e.g pralsetinib (BLU-667) and selpercatinib (LOXO-292): ORR of 64-85%

→FDA approval for NSCLC, EMA soon expected

Waterfall plots of selpercatinib-treated NSCLC patients



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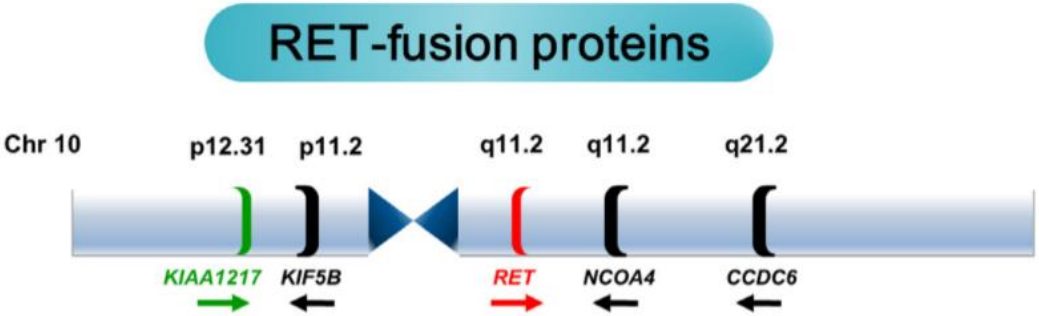
RET fusion genes in cancer



Thyroid

- **CCDC6-RET**
- **NCOA4-RET**
- **TRIM33-RET**
- ERC1-RET
- GOLGA5-RET
- HOOK3-RET
- KTN1-RET
- PCM1-RET
- PRKAR1A-RET
- TRIM24-RET
- TRIM27-RET
- AKAP13-RET
- FKBP15-RET
- SPECC1L-RET
- TBL1XR1-RET

10-20% in PTC

Lung

- KIF5B-RET
- CCDC6-RET
- NCOA4-RET
- TRIM33-RET
- CUX1-RET
- KIAA1468-RET
- KIAA1217-RET

1-2% in NSCLC

Others

Colon
- CCDC6-RET
- NCOA4-RET

Skin
- GOLGA5-RET

CML
- BRC-RET
- FGFR1OP-RET

Breast
- ERC1-RET

Low rates

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From ESMO recommendations 2021 on the standard methods to detect *RET* fusions

Table 1. Summary of main features, strengths and weaknesses of all available techniques to detect *RET* rearrangements

Method	Sensitivity	Specificity	Detection of partner	Detection of expression
IHC	Moderate[a]	Moderate[b]	No	Yes
FISH	High	High	No/Yes[c]	No
RT-PCR	Moderate/high[d]	High	Yes/No[e]	Yes
DNA-seq NGS	Moderate[f]	High/moderate[g]	Yes	No
RNA-seq NGS	High	High	Yes	Yes[h]

→ FISH is the currently used standard for the detection of *RET* fusions, with good sensitivity and specificity

BUT

In routine practice often discordant results between FISH and RNA NGS results

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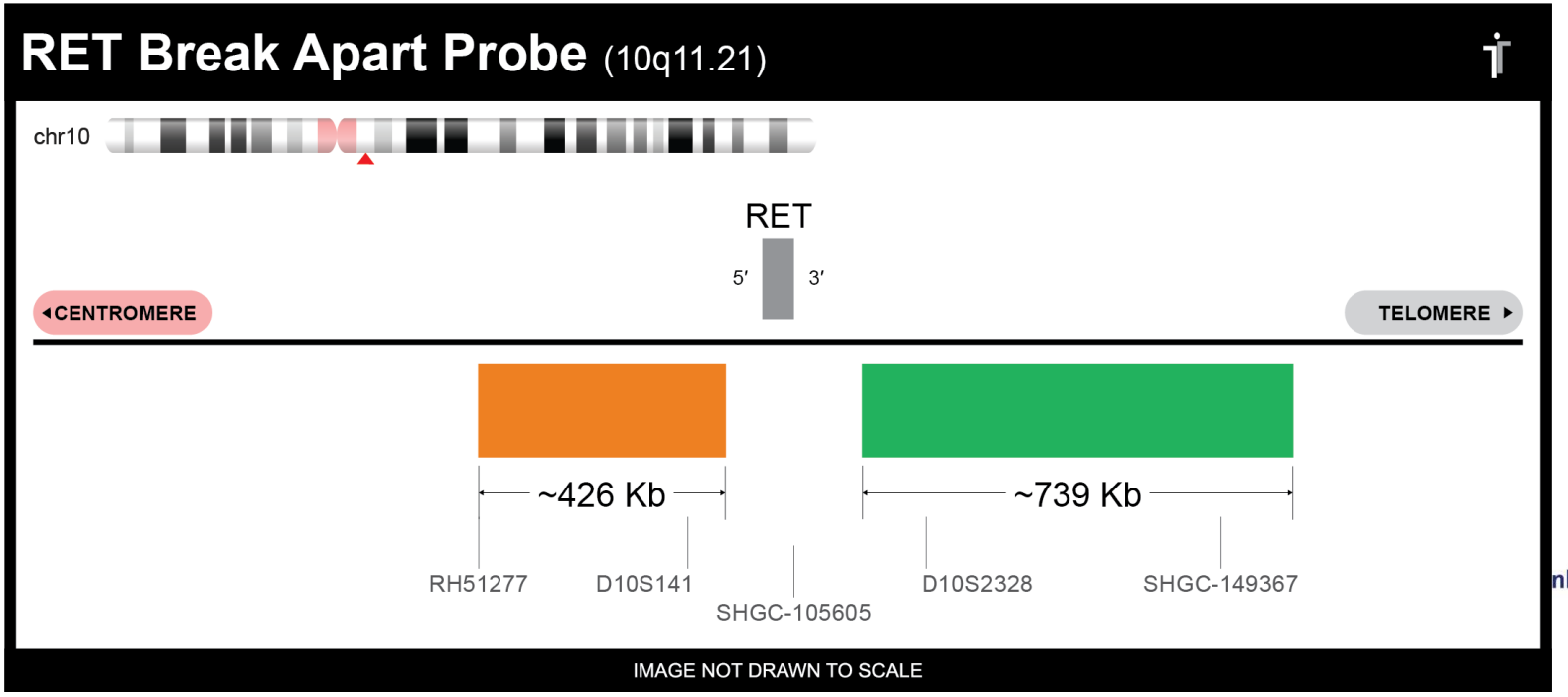
Estimation of sensitivity and specificity of *RET* FISH analysis of NSCLC

RET FISH analyis

Scoring system according to lab-specific routine practice

- 15% or more of cells with a split signal of at least more than 1 signal diameter between the 5’ and 3’ signals
- single 3’ signal
- complex pattern: any pattern except usual split or single 3’patterns
- Single 5’ signals: clinically negative, because of lost kinase domain



Patient cohort: 4873 NSCLC patients

- Case selection from January 2016 to November 2019

- Routine screening for *RET* fusions according to local logistics

  FISH: 2858; by RNA NGS: 2015

- Collaborating institutes:

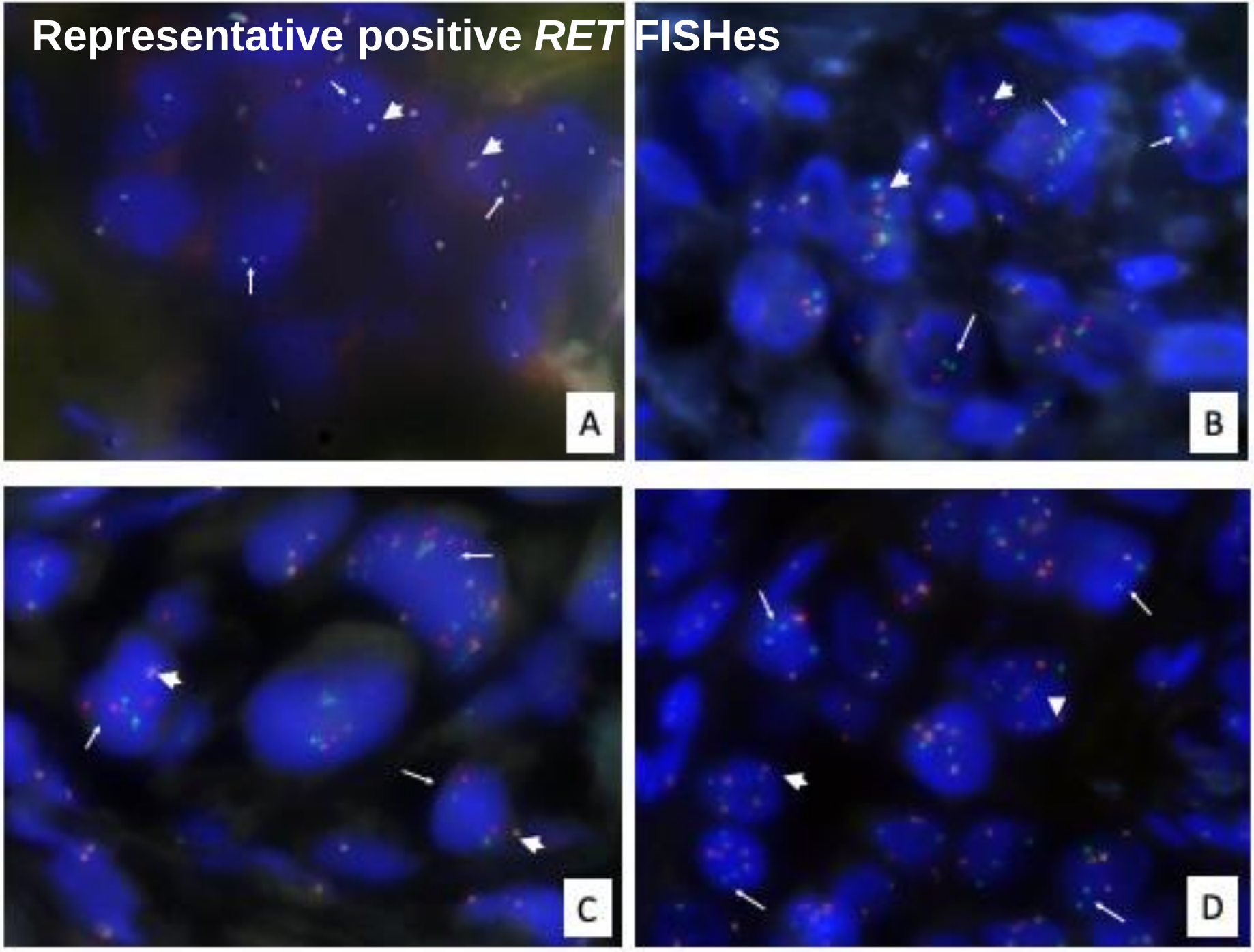
  ***RET* FISH**: Amsterdam UMC, Erasmus MC, Maastricht UMC+, and Netherlands Cancer Institute, the Netherlands

  RNA NGS: St. James Hospital, Dublin, Ireland and Leiden University Medical Center, the Netherlands

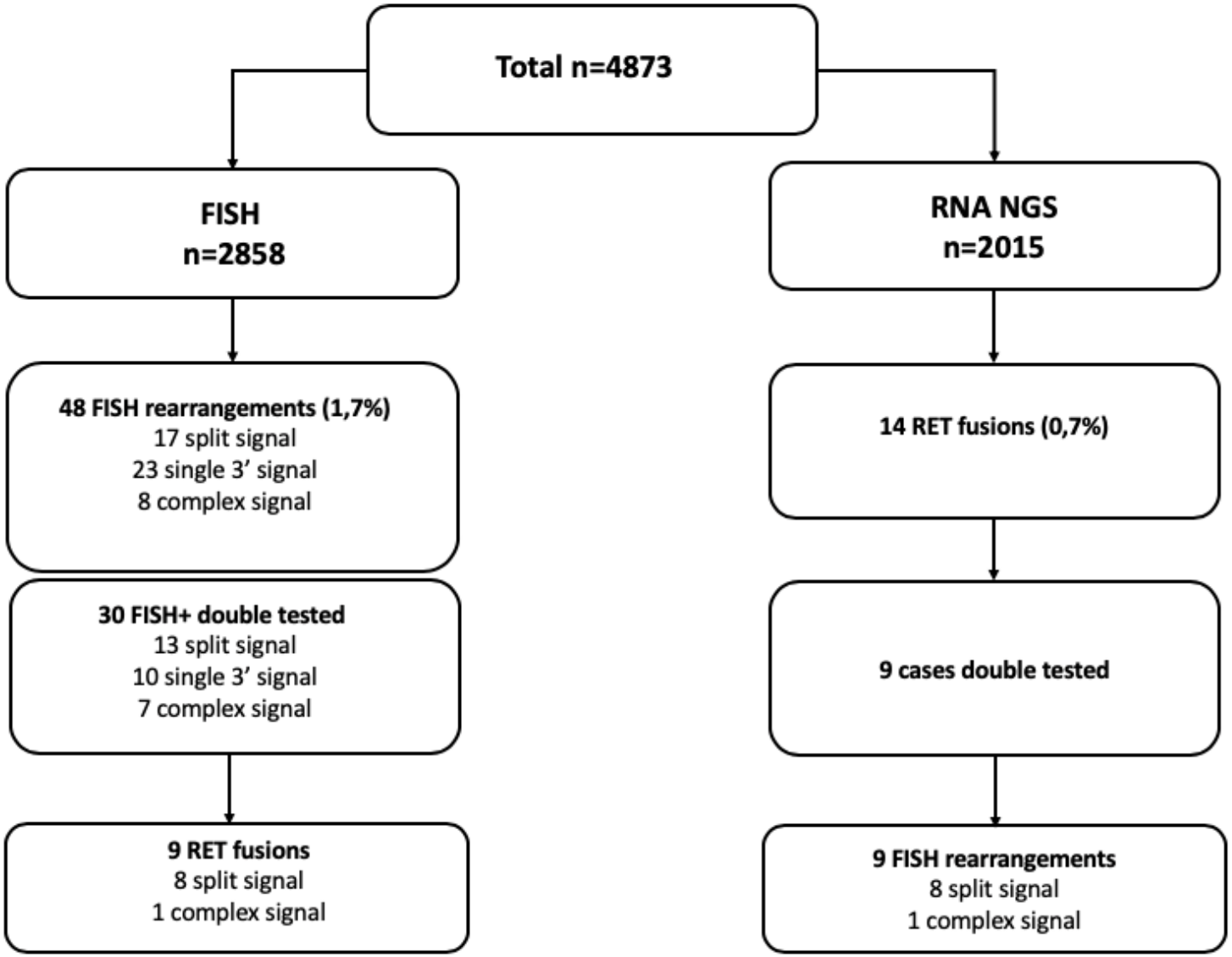
Study set up

- Comparison of data from routine FISH and RNA NGS analysis according to lab-specific procedures
- Four different *RET* break apart FISH assays: Vysis, SureFISH, Kreatech, and Cytocell Aquarius
- Three different RNA NGS methods: Archer, Oncomine, and Asuragen Quantidex
- Cross validation if possible

Representative positive *RET* FISHes



Yield


Total n=4873
FISH
n=2858
RNA NGS
n=2015
48 FISH rearrangements (1,7%)
17 split signal
23 single 3' signal
8 complex signal
14 RET fusions (0,7%)
30 FISH+ double tested
13 split signal
10 single 3' signal
7 complex signal
9 cases double tested
9 RET fusions
8 split signal
1 complex signal
9 FISH rearrangements
8 split signal
1 complex signal

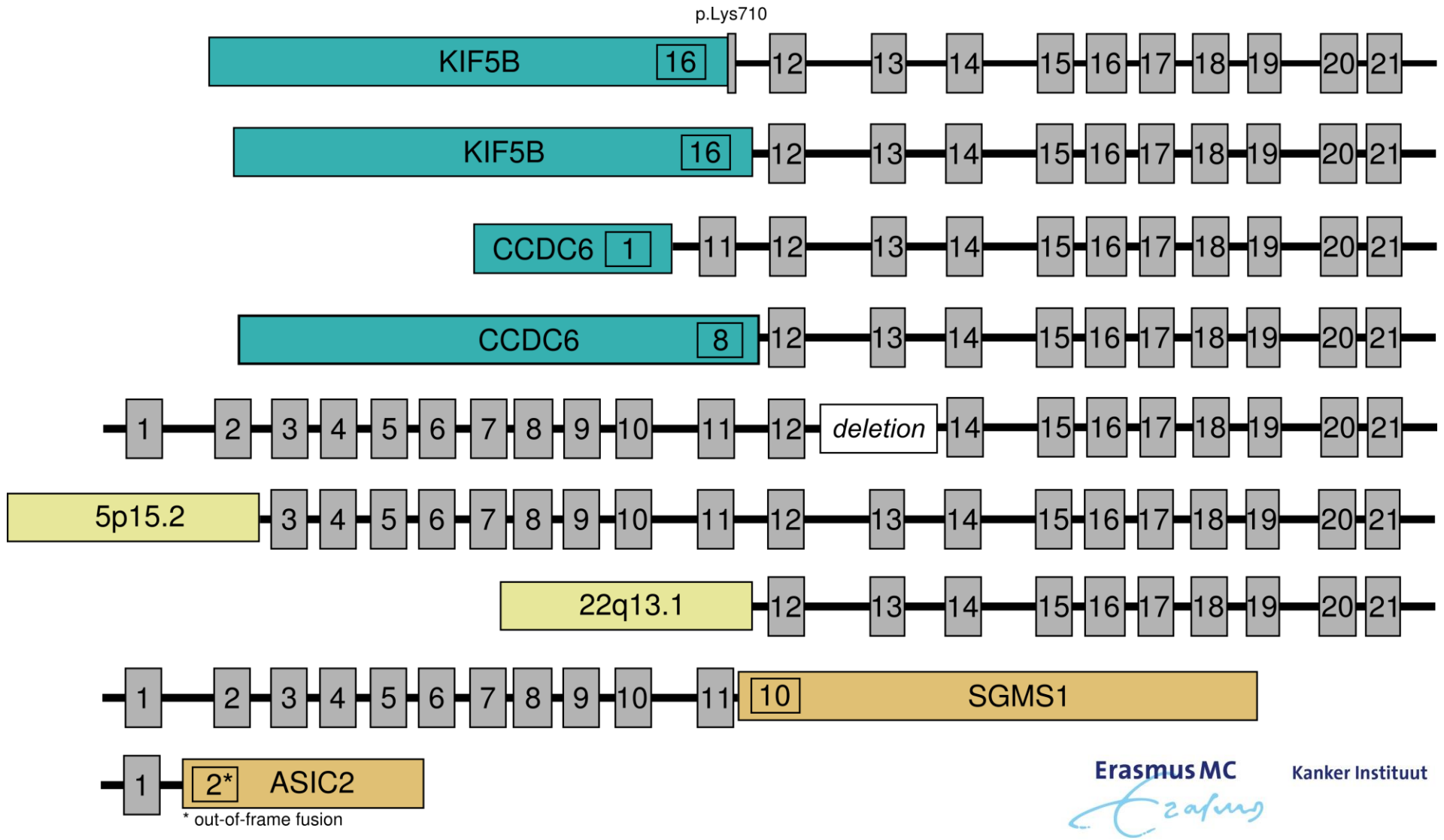
Conclusions 1

- *RET* FISH results in 70% of cases in false-positive results
- All RNA NGS positive cases could be detected by RET FISH

WGS cohort – Hartwig medical foundation

- WGS data from 520 NSCLC samples
- Stage IV lung cancer patients in all lines of screening and therapy
- Analysis of genomic loci of *RET*, *ALK*, *ROS1*

WGS analysis of *RET* locus for functional fusions (4) and disruptive events (5)



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WGS results and conclusion

- Functional fusions compared to all disruptive events per locus:

  - *RET* (4/9, 44%)

  - *ALK* (27/34, 79%)

  - *ROS1* (9/12, 75%)

- In NSCLC the *RET* locus shows more rearrangements not resulting in functional gene fusions than shown for the *ALK* and *ROS1* loci

- WGS provides a possible explanation that aberrations observed by *RET* FISH often could not be substantiated by RNA NGS

Final conclusions

- *RET* FISH analysis is a sensitive but highly unspecific screening method for *RET* fusions in lung cancer

- *RET* screening by FISH always requires confirmation by an orthogonal technique, due to frequently occurring *RET* rearrangements not resulting in functional fusions in NSCLC

- Ranodic et al (2021), *J Thorac Oncol*, accepted for publication

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Case	FISH	FISH pattern	RNA NGS
1	Kreatech and Vysis	Split	KIF5B(ex15): RET(ex12)
2	Kreatech and Vysis	Split	KIF5B(ex15): RET(ex12)
3	Kreatech and Vysis	Split	KIF5B(ex15): RET(ex12)
4	Kreatech and Vysis	Split	CCDC6(ex1):RET(ex12)
5	Sure FISH	Split	CCDC6(ex1):RET(ex12)
6	Sure FISH	Split	KIF5B(ex15): RET(ex12)
7	Vysis	Split	KIF5B(ex15): RET(ex12)
8	Vysis	Split	KIF5B(ex15): RET(ex12)
9	Kreatech and Vysis	Split	KIF5B (ex15):RET(ex11)
10	Vysis	Split	KIF5B(ex15): RET(ex12)
11	Sure FISH	Split	KIF5B(ex15): RET(ex12)
12	Sure FISH	Split	CCDC6(ex1):RET(ex12)
13	Cytocell Aquarius	Split	KIF5B(ex15): RET(ex12)
14	Sure FISH and Cytocell Aquarius	Split	KIF5B(ex15): RET(ex12)
15	Cytocell Aquarius	Split	CCDC6(ex1):RET(ex12)
16	Cytocell Aquarius	Split	KIF5B(ex15): RET(ex12)
17	Cytocell Aquarius	Split	No fusion
18	Cytocell Aquarius	Split	No fusion
19	Cytocell Aquarius	Split	No fusion
20	Kreatech	Split	No fusion
21	*Sure FISH and Cytocell Aquarius*	*Split*	*No fusion*
22	Cytocell Aquarius	Single 3'	No fusion
23	Kreatech	Single 3'	No fusion
24	Sure FISH	Single 3'	No fusion
25	*Sure FISH and Cytocell Aquarius*	*Single 3'*	*No fusion*
26	*Sure FISH, Kreatech and Vysis*	*Single 3'*	*No fusion*
27	Sure FISH, Kreatech and Vysis	Single 3'	No fusion
28	Sure FISH, Kreatech and Vysis	Single 3'	No fusion
29	Vysis	Single 3'	No fusion
30	Vysis	Single 3'	No fusion
31	Vysis	Single 3'	No fusion
32	Sure FISH and Vysis	Complex	KIF5B(ex15): RET(ex12)
33	Sure FISH	Complex	KIF5B(ex15): RET(ex12)
34	Kreatech	Complex	No fusion
35	Kreatech	Complex	No fusion
36	Kreatech and Vysis	Complex	No fusion
37	*Sure FISH, Kreatech and Vysis*	*Complex*	*No fusion*
38	Vysis	Complex	No fusion
39	Vysis	Complex	No fusion

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