Disclosure belangen Anke van den Berg

Belangenverstrengelingen	Geen
Voor bijeenkomst mogelijk relevante relaties met bedrijven	Geen
Onderzoeksgeld	KWF

All-in-one RNA-based test to detect mutations and fusion genes in lung cancer

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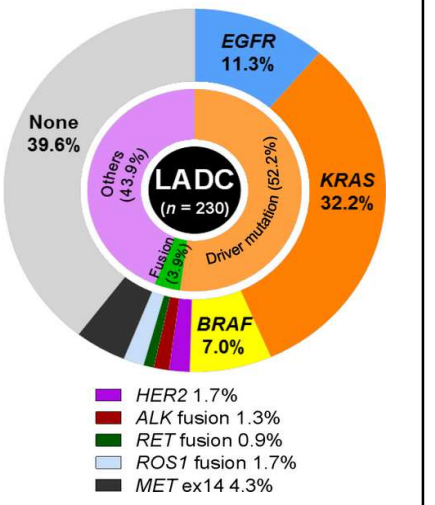
Broad spectrum of genomic abberations relevant for therapy

- Activating mutations
- Resistant mutations
- Fusion genes
- Exon skipping
- Amplifications



Saito et al., 2016, Cancer Science

Molecular diagnostic tests

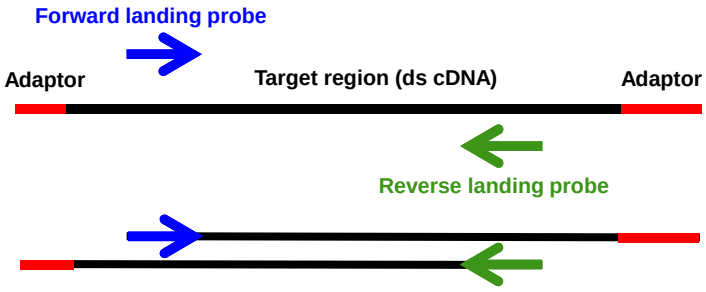
- Therapy decision making
  - ALK protein: Immuno
  - EGFR, KRAS, BRAF, MET, ERBB2, ALK mutations: Hot spot NGS
  - ALK, ROS1, RET breaks: FISH
  - MET, ERBB2, FGFR1 amplifications: FISH
  - Liquid biopsy: EGFR hotspot, e.g. T790M, L858R
- Can we detect all aberrations in one single assay?

RNA-based assay

- Why RNA-based?
  - Simultaneous detection of driver gene mutations, exon skipping and fusion genes
  - Amplifications? (by looking at overexpression)
- Single primer extension technology: SPET

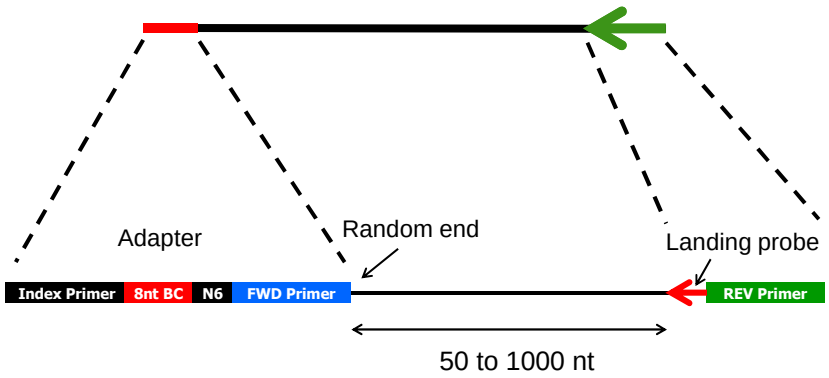
RNA-based SPET assay

- One end is defined by a gene specific landing probe !
- The second end is variable and defined by the adapter



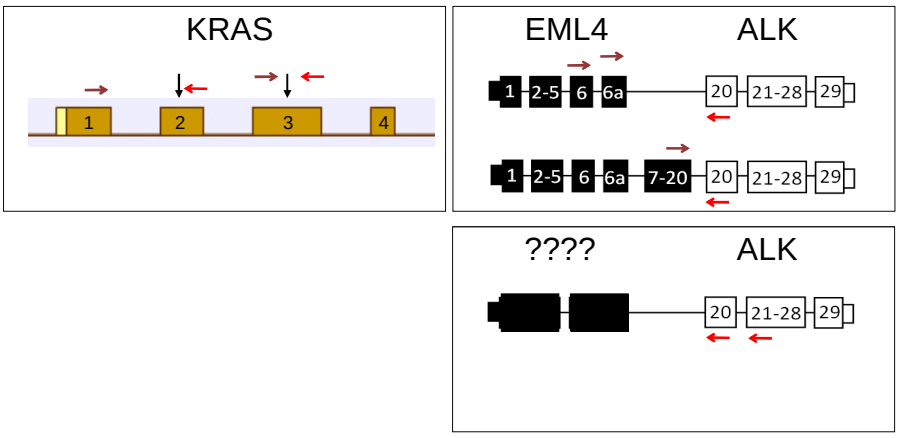
Single primer extension technology

- Target region is defined by the landing probe
- Molecular barcoding: unique reads



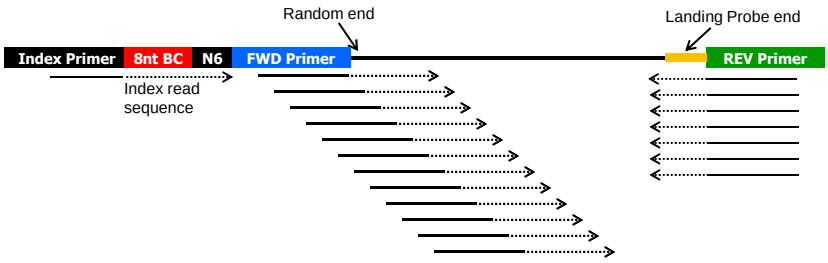
All-in-one RNA-based assay

- Landing probe design

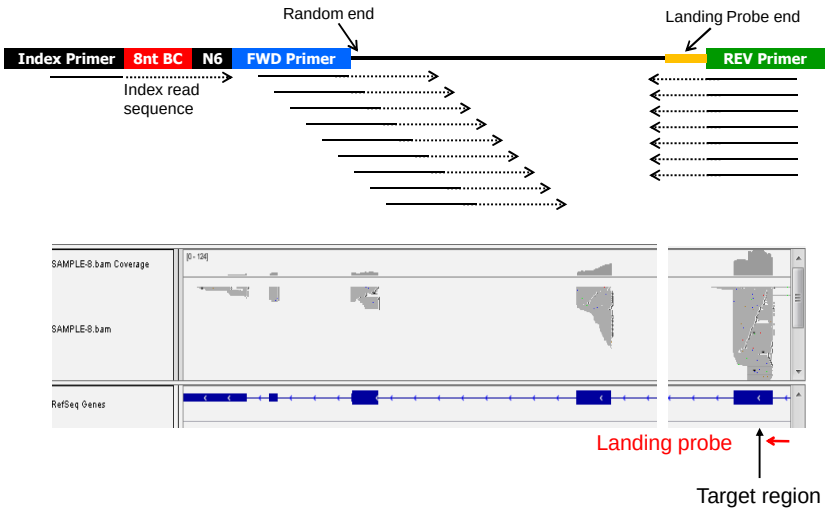


Single Primer Extension Technology

- Sequencing
  - Index read
  - Paired end: ~100 nt each



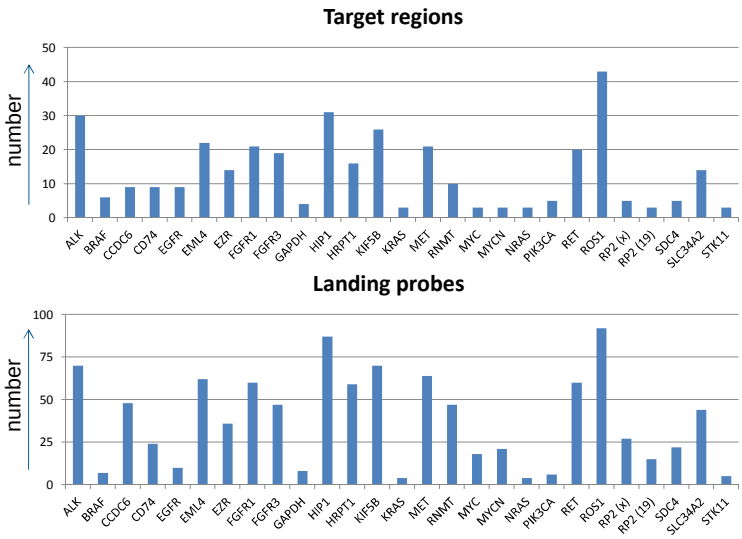
Read intervals up to 800 nucleotides



Genes included in 1st SPET design

- Mutation hotspots:
  - ALK, BRAF, EGFR, PIK3CA, KRAS, NRAS, MET
- Fusion genes:
  - ALK, RET, ROS1 (and translocation partners, not required)
- Exon skipping:
  - MET
- Housekeeping genes

1st SPET design

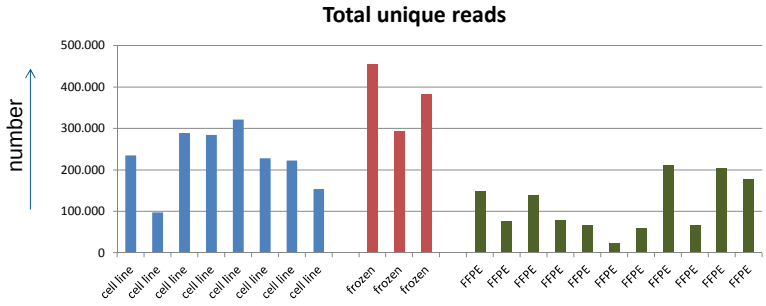


Selected samples

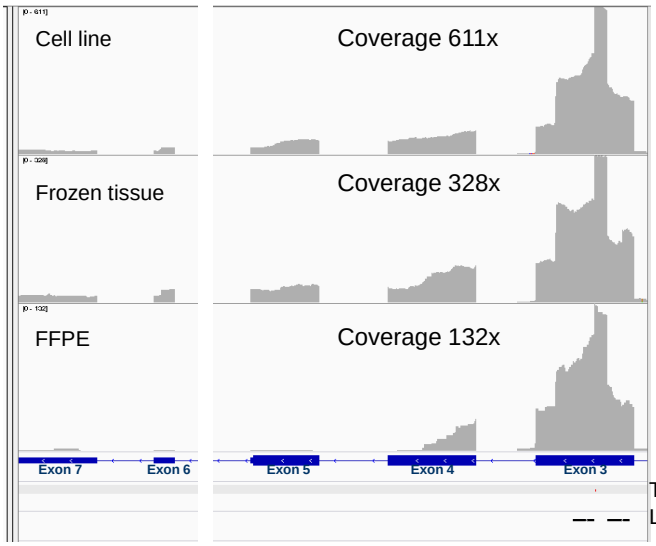
- Samples with known aberrations at DNA level as determined in MD on tumor cell enriched tissue samples
  - 7 Lung cancer cell lines
  - 2 Frozen lung cancer tissue samples (total tissue)
  - 1 Frozen normal lung tissue sample
  - 11 FFPE lung cancer tissue samples (total tissue)

Total unique reads

- FFPE tissue: 23.351 – 204.840
- Frozen tissue / cell lines: 97.845 – 455.287



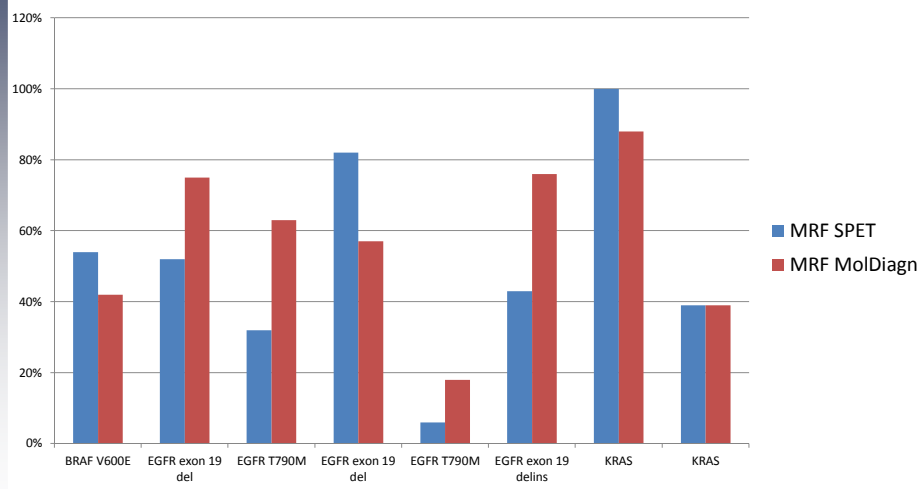
Coverage and read intervals



Validation: 14 / 18 mutations

Sample type	Gene	Mutation(s)	Validated	Cause
cell line	EGFR	p.(T790M); p.(L858R)	yes / yes	
cell line	EGFR	p.(E746_A750del)	yes	
cell line	KRAS	p.(G12S)	yes	
cell line	NRAS	p.(Q61K)	yes	
cell line	PIK3CA	p.(H1047R)	yes	
FFPE	BRAF	p.(V600E)	yes	
FFPE	EGFR	p.(E746_A750del); p.(T790M)	yes / yes	
FFPE	EGFR	p.(E745_A750del); p.(T790M)	yes / yes	
FFPE	KRAS	p.(G12A)	yes	
FFPE	KRAS	p.(Q61H)	yes	
FFPE	EGFR	p.(L747_P753delinsS); p.(T790M)	yes / no	low unique read counts and low coverage
FFPE	EGFR & PIK3CA	EGFR p.(L858R); PIK3CA p.(E542K)	no / no	low unique read counts and low coverage
Frozen	KRAS	p.G12D	no	FFPE vs Frozen?

Mutant read frequency



Validation: 3 /4 fusion genes

Sample type	Gene	Validated	Remark
cell line	ALK	yes	EML4-ALK
FFPE	ALK	yes	KIF5B-ALK
FFPE	ROS1	?	Data analysis ongoing
Frozen	ALK	yes	EML4-ALK

Control samples

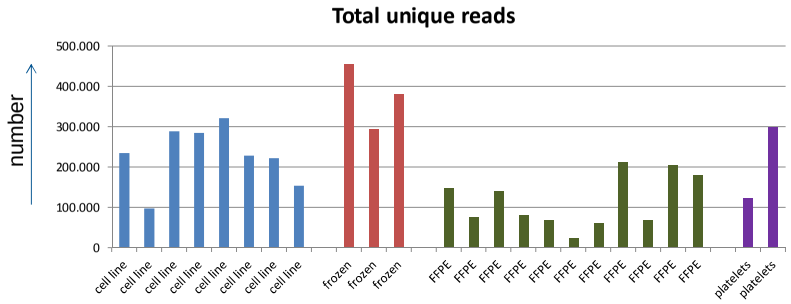
- One normal sample: No aberrations
- One tumor without known aberrations: No mutations / fusion genes identified

Conclusions

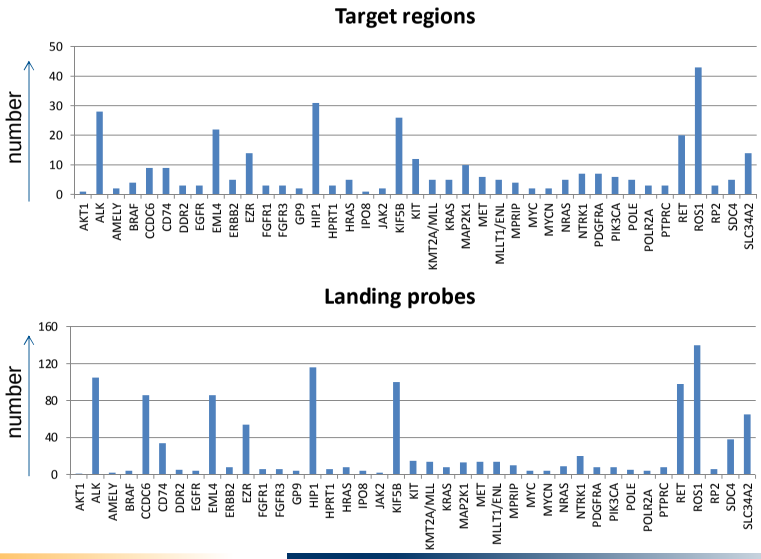
- RNA-based SPET assay can be used for detection of mutations and fusion genes
- MET exon 14 skipping not included yet
- Detection of overexpression as a measure of amplification (?) in progress
  - How to normalize?

Future: platelet derived RNA

- RNA yield is sufficient
- Quality is suitable for SPET
- Enough tumor-derived RNA?



Future: optimized SPET design



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