

# Next generation sequencing: Is it ready for prime time ? **PRO**

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# Outline

**Technology is robust. No need for large samples, no need for frozen samples**

Current system is not sustainable for hospitals

High throughput approaches identifies rare targetable gene alteration

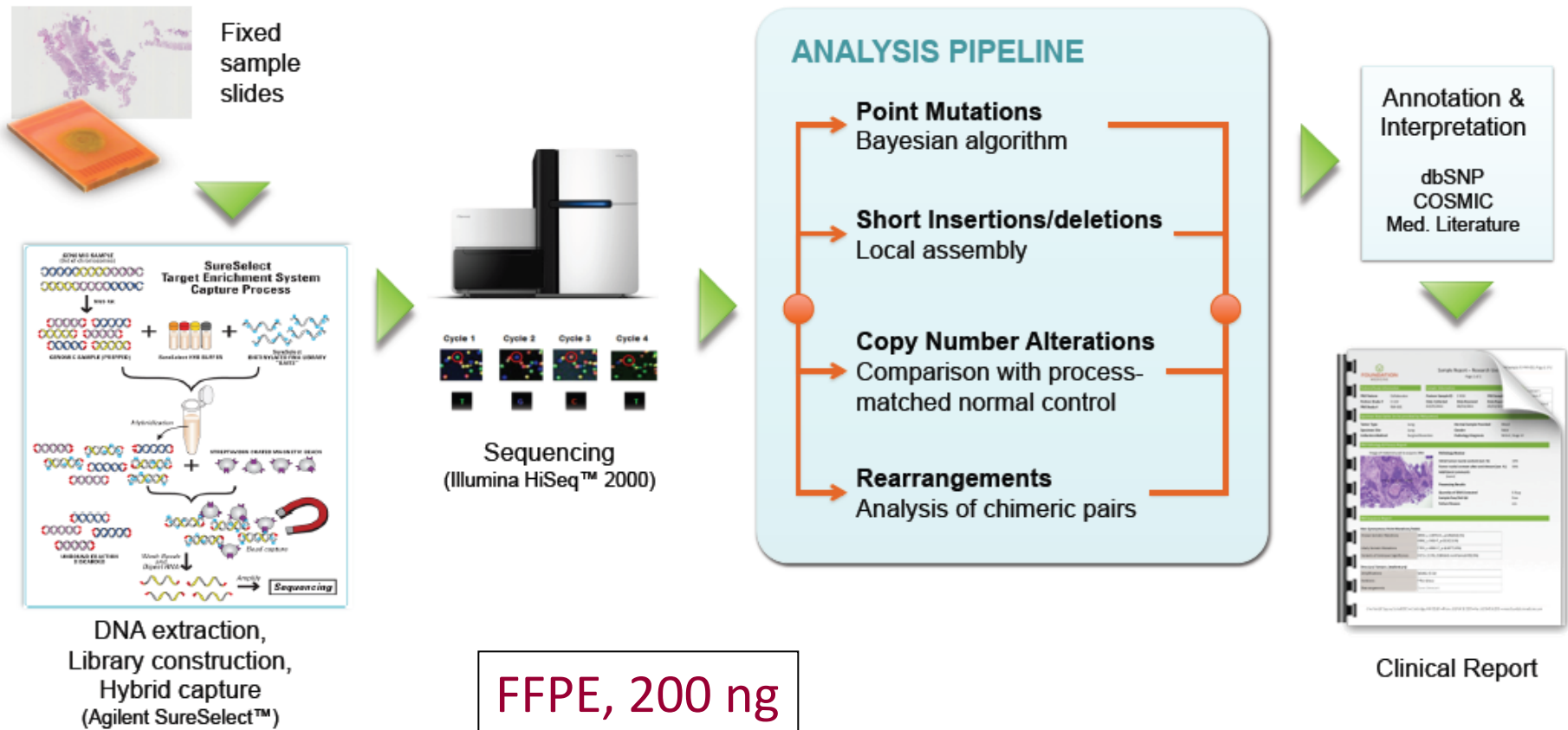
Patients deserve access to innovative drugs through NGS

NGS will allow capturing minority clones

NGS will capture ITH

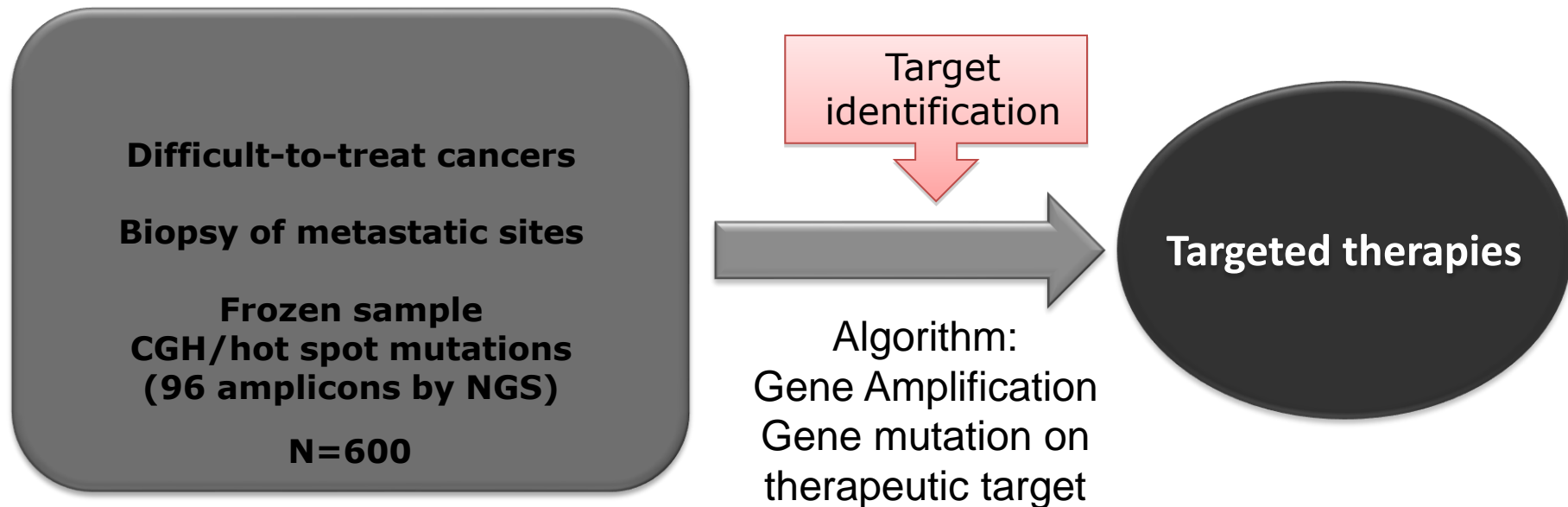
NGS will solve the problem of low % tumor cell in the sample

# NGS for personalized medicine: the foundation medicine workflow



CLIA certification, 1 000 tests done for the clinical use

# ... that is being used in Academic centers (MOSCATO trial)

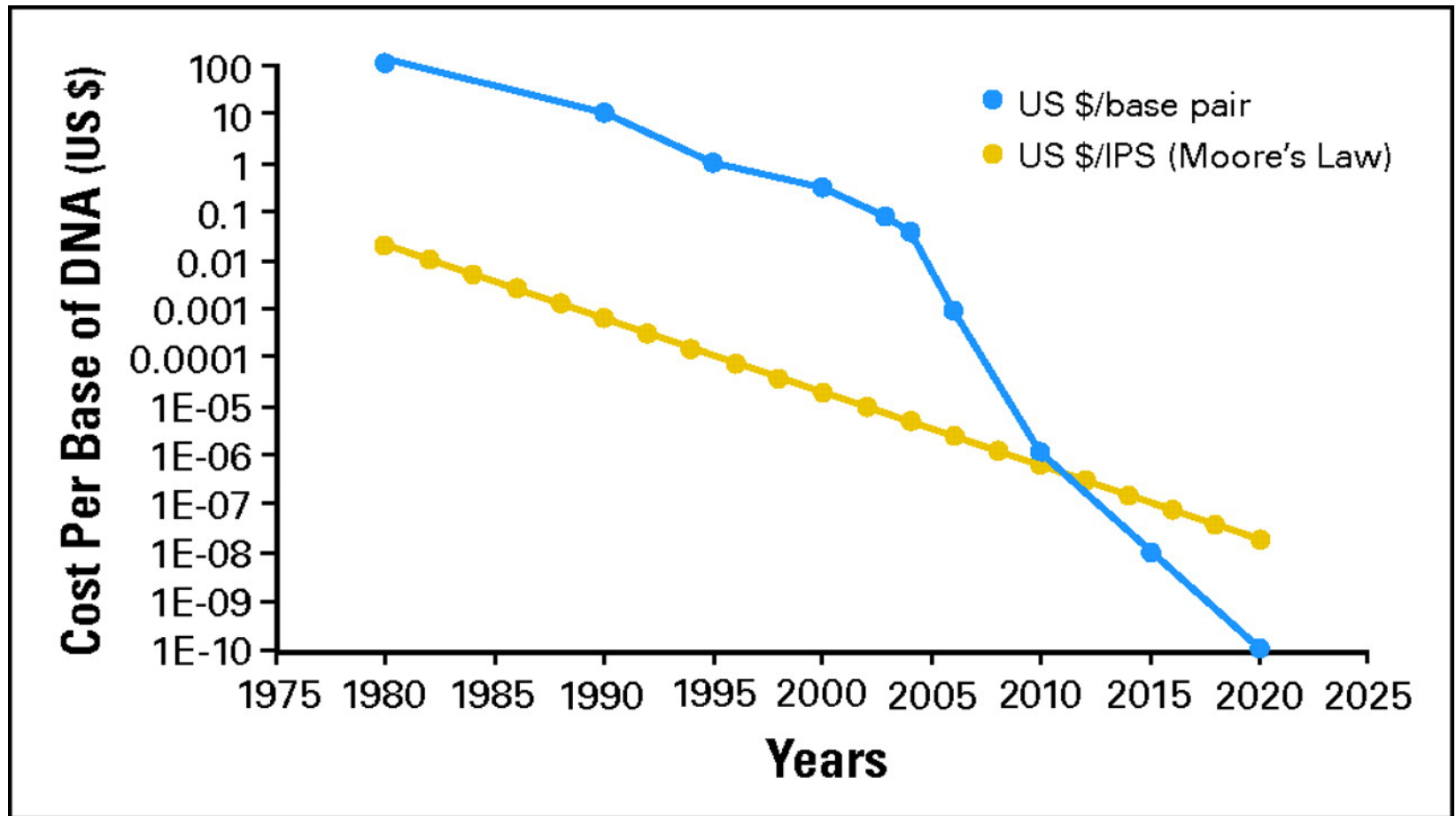


120 patients included until now

Turn-over: 15 days

Total cost: 1500 euros for NGS / CGH

# Cost is decreasing dramatically



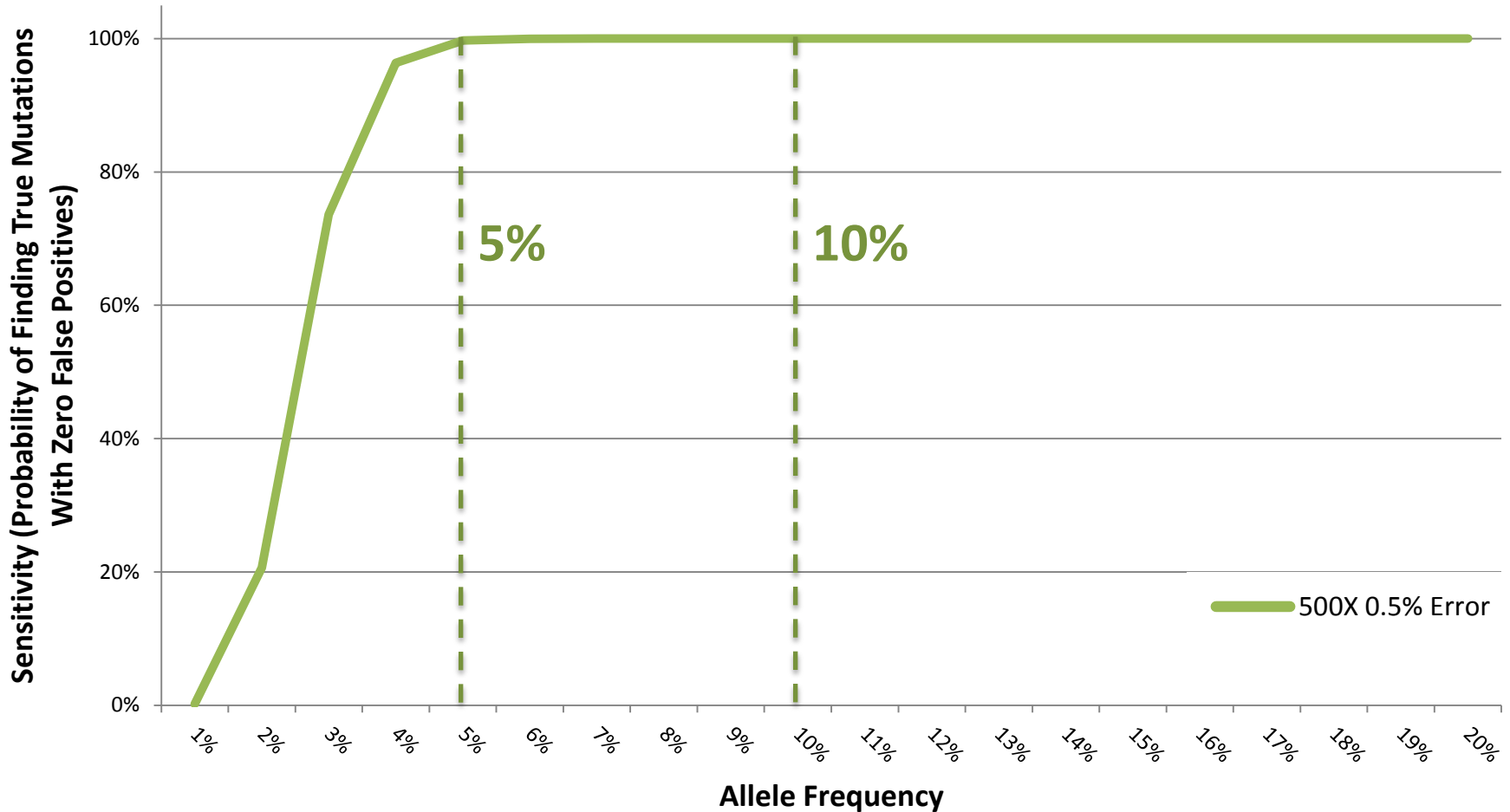
# is it accurate ?

Concordance with reference lab			
		<i>FMI NGS Test</i>	
		mutation positive	mutation negative
<i>Reference Lab</i>	mutation positive	15*	
	mutation negative		54

NGS accurately detect genomic alterations

# Increasing coverage to 500x allows for >99% sensitivity to detect mutant alleles >5% with no false positives

Sensitivity vs Allele Frequency at 500X Coverage (1Mb test)



*Deep coverage is necessary for clinical grade samples*

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Multiplex technologies are more robust than « cost-saving » approaches

Too many genes are rare and will NEVER be validated by single gene approach

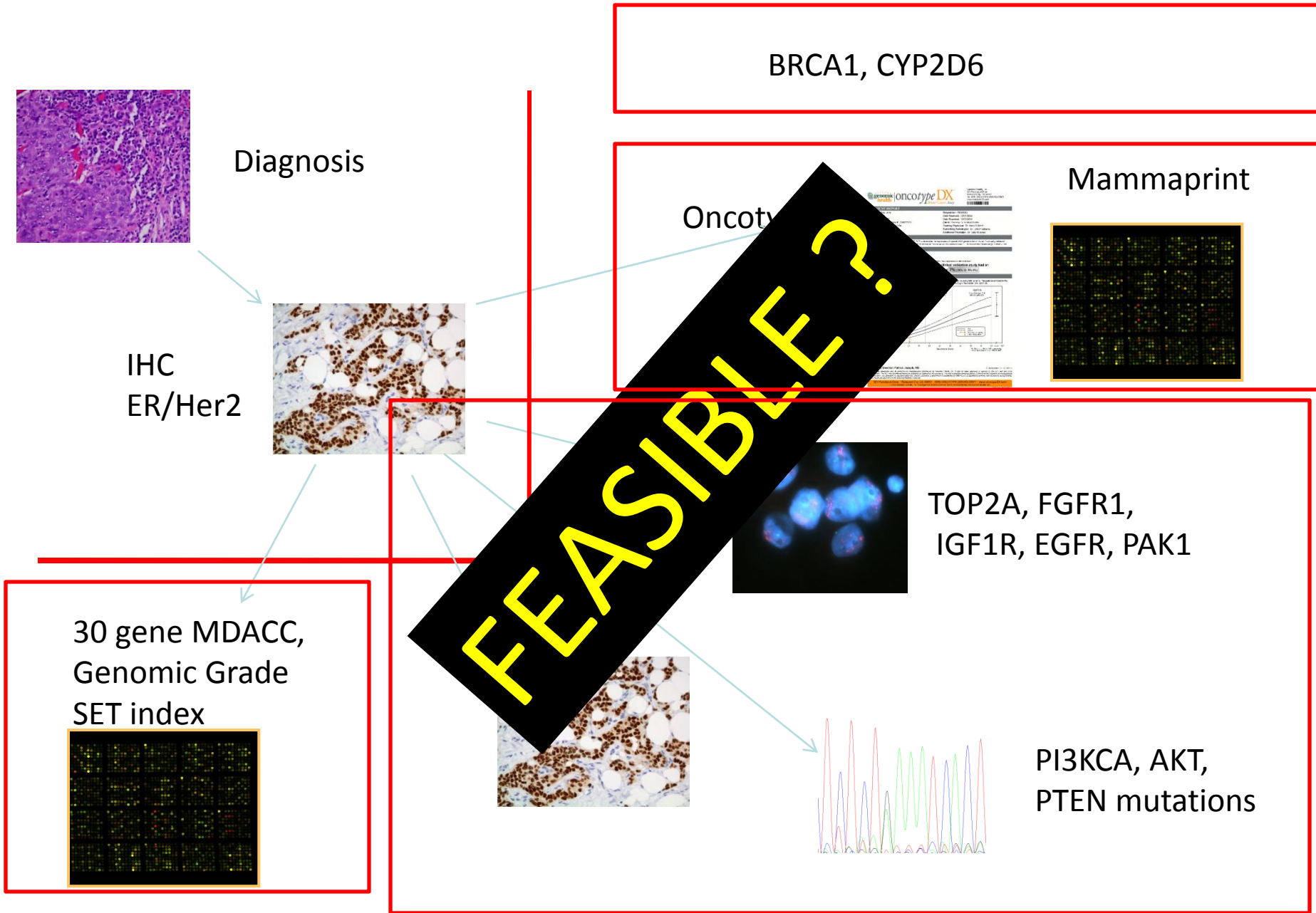
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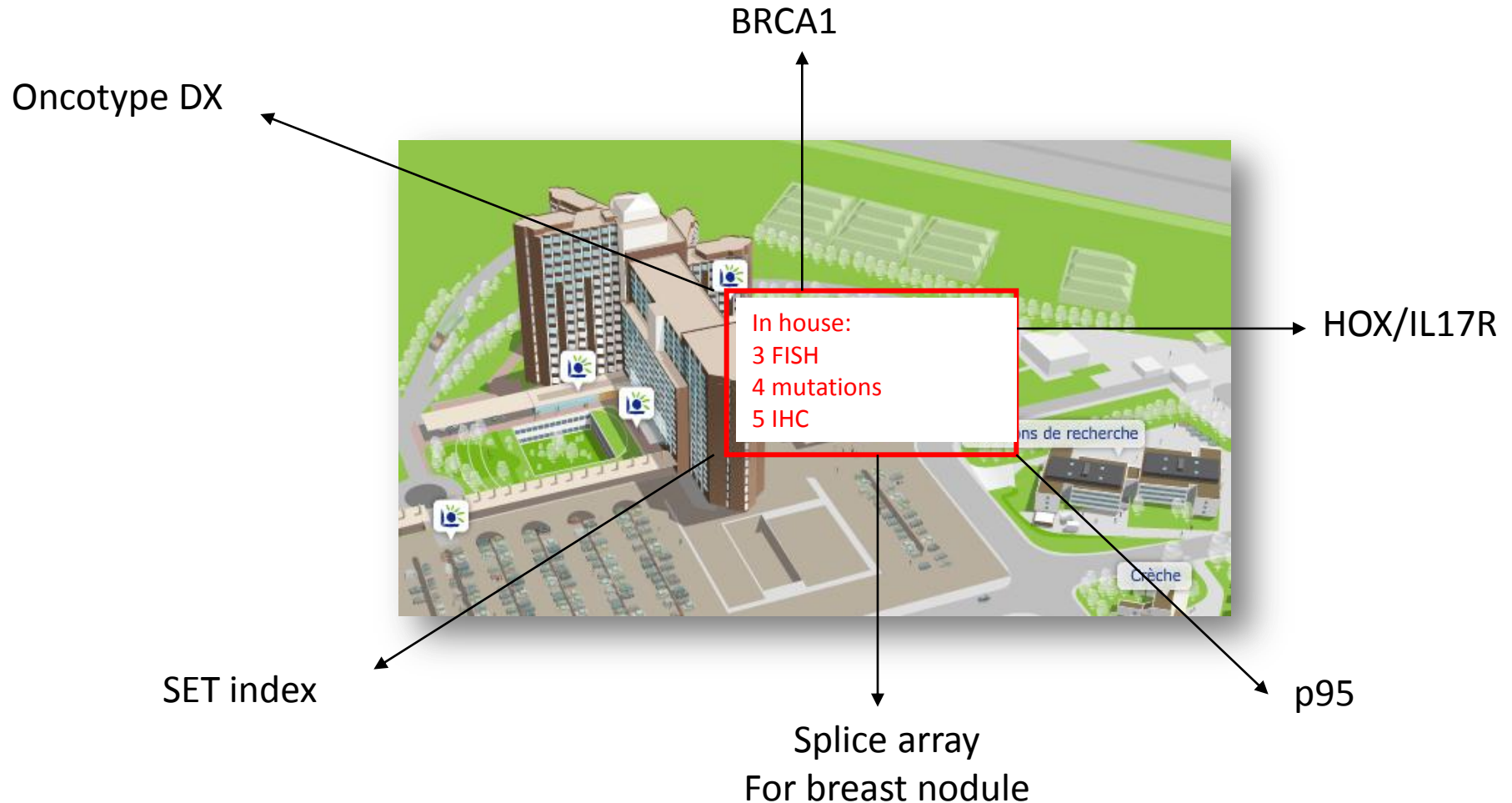
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# Biomarkers for breast cancer care in 2020



# The IGR's director nightmare: outsourcing + multiple tests house



# Where are we going ?

- Too many tests
- Not compatible with the hospital organisation
- Expensive !!!!! (3 FISH = 1 array CGH !!!)

Solution : multiplex technologies to allow a « all-in-one » tests, ie all tests done in a single technology

# **Point 1: why to implement NOW multiplex technologies ?**

Because the hospitals can not afford  
developing one assay for each predictor !!!

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# Her2 testing

**Table 2.** Concordance Between Central and Local Laboratories

Test at Local Laboratory	Specimens Confirmed by Central Testing* (No.)	Agreement With Central Laboratory		
		%	95% CI	Method
HercepTest	1,063	81.6	79.1% to 83.9%	HercepTest
Non-HercepTest	636	75.0	71.4% to 78.3%	HercepTest
FISH	813	88.1	85.6% to 90.2%	FISH

NOTE. HercepTest, DAKO, Carpinteria, CA.

Abbreviation: FISH, fluorescence in situ hybridization.

\*Testing using the same method at both laboratories was not possible for 23 specimens.

**High level of non reproducibility for protein-based assay**

# Next generation sequencing and high throughput DNA-based technologies

- Multiplicity of bioassays is not compatible with hospital organisation, is not cost-effective
- The recommended protein-based assays are not reliable
- Solution I: implement multiplex genomic approaches in daily practice:
  - « all-in-one » approach that avoids set-up one bioassay / gene
  - Highly reproducible approach
  - Not expensive

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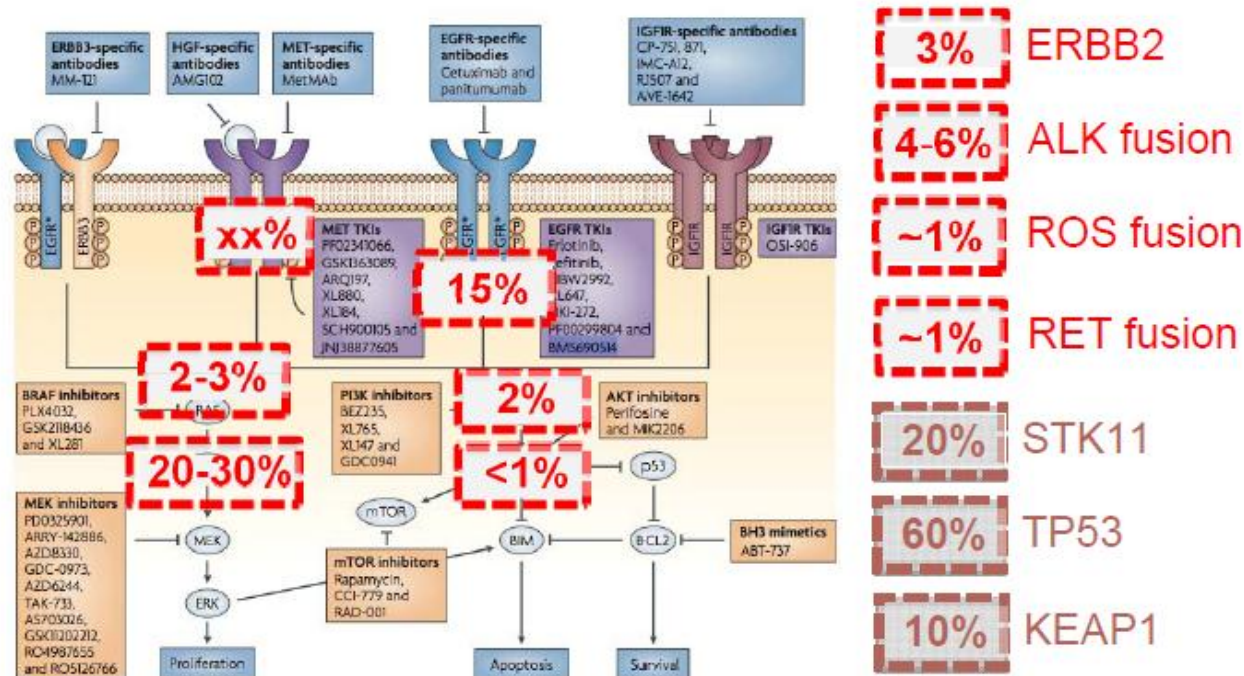
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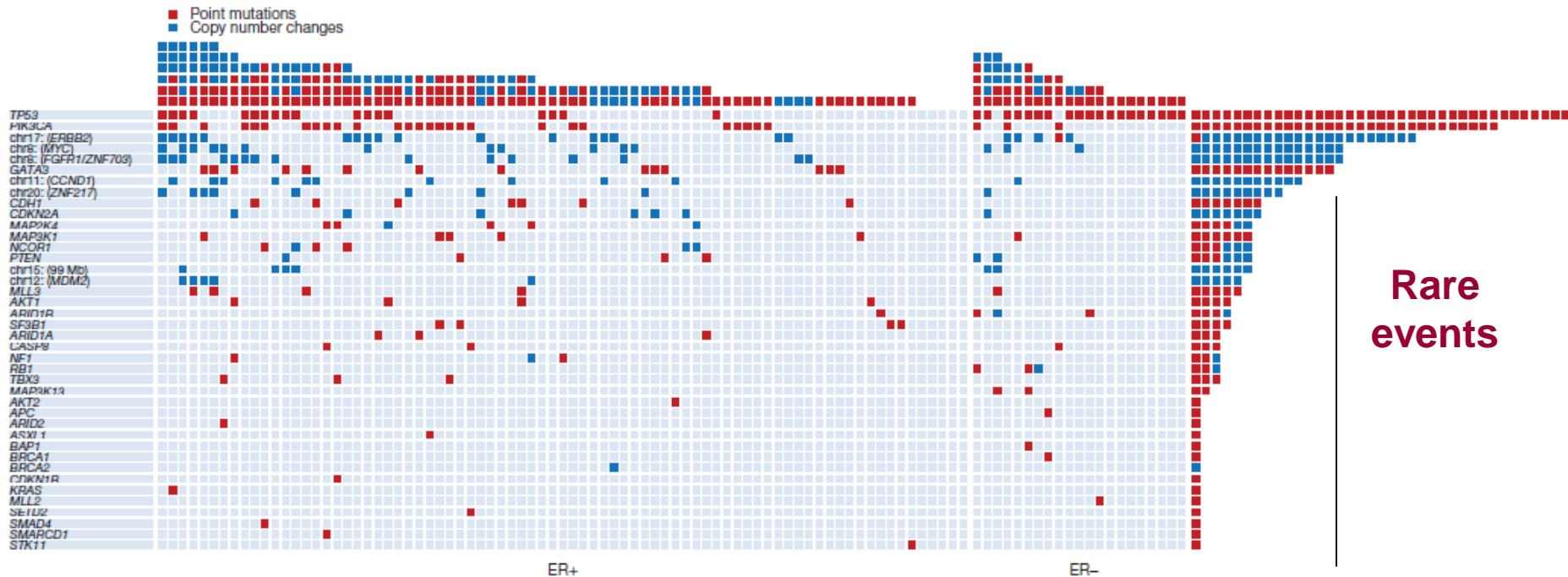
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# Cancer = multiple RARE genomic alterations

## The “targeted therapome” in lung adenocarcinoma



# Breast cancer genomic landscape

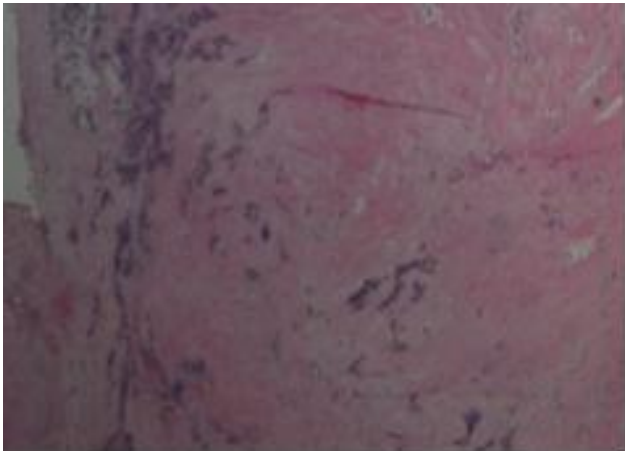


**Rare Events are relevant**

# Case Presentation

- 43 year old never-smoker
- Vysis-FISH test (approved companion diagnostic-EML4-ALK) was negative; EGFR PCR testing negative, begun on chemotherapy
- FMI test ordered based on clinical suspicion of a treatable oncogenic driver

# Genomic report



## About The Test:

FMI Test is a next-generation sequencing (NGS) based assay which identifies genomic alterations within 182 cancer-related genes.

## Lung Cancer

### Genomic Alterations

*ALK* – rearrangement, intron 19\*

Select Genes With No Actionable Alterations Detected

*EGFR*

*KRAS*

*BRAF*

### Therapies Associated With Clinical Benefit\*

There are no FDA approved therapies specific to the reported genomic alterations in lung cancer or other tumor types.

### Clinical Trials

No clinical trials were found for agents targeting the cancer pathways relevant to the alterations described in this report for this patient's tumor type. Please refer to [clinicaltrials.gov](http://clinicaltrials.gov) for other available trials.

\*To better define, testing of "ALK" by immunohistochemistry and RNA sequencing is recommended

## Genomic Alterations

GENE	ALTERATION(S) IDENTIFIED	INTERPRETATION
<i>ALK</i>	Rearrangement,	The ALK rearrangement involving intron 19 in this sample has not been previously reported in the literature. A different rearrangement involving ALK in lung cancer, EML4-ALK, has been found in ~5% of patients with non-small-cell lung cancer.

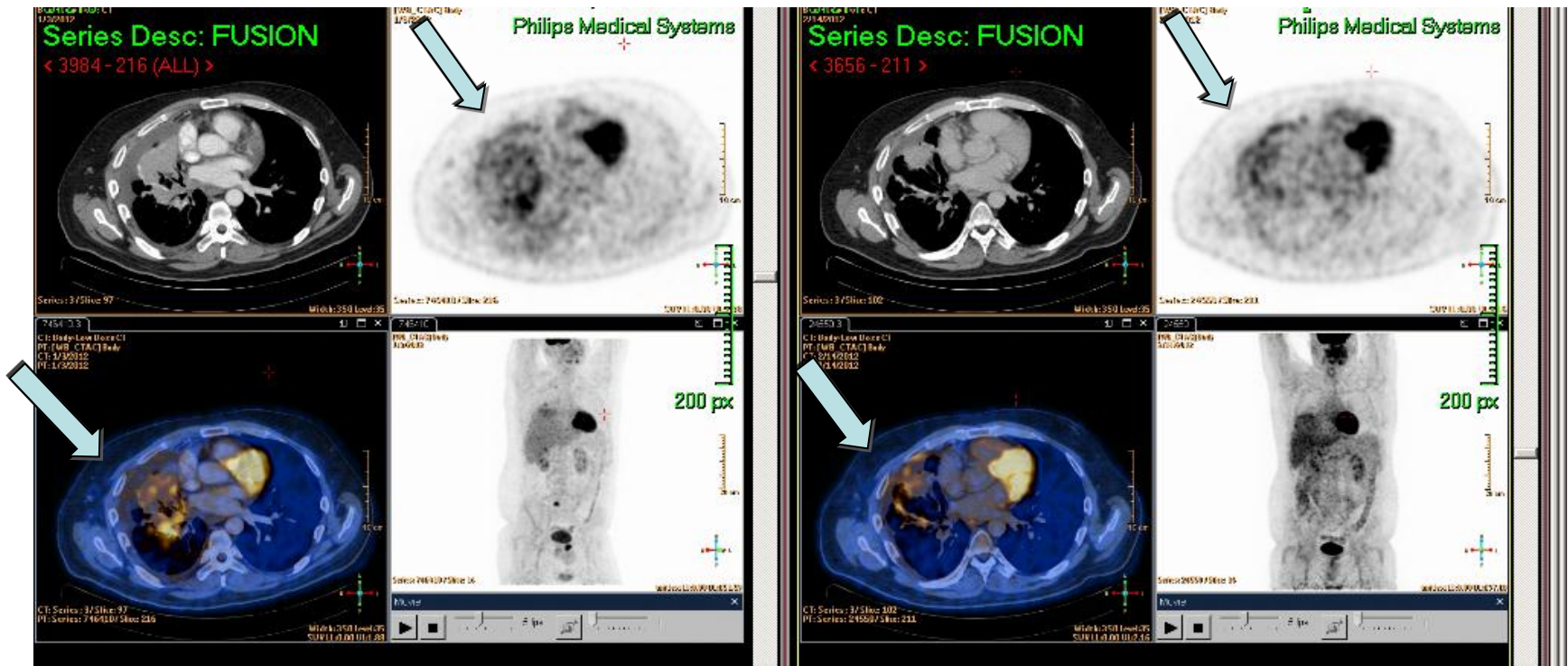
## **NGS outperforms approved companion diagnostic and changes treatment**

- **NGS reports a novel ALK fusion**
- **RNA seq confirms expression of the fusion transcript**
- **IHC (Cell Signaling Ab) is positive also suggesting the result is biologically relevant**
- **Patient begun on crizotinib**

# Response assessment after starting crizotinib

1/3/2012

2/14/2012



# Take home message

- NGS identifies a high number of RARE, targetable genomic alterations
- Evidence that some of these alterations are relevant
- Clinical trials testing drugs are not feasible in these almost unique alterations
- Drugs are available through phase I or compassionate access
- Solution II for practice: Deliver NGS in the context of molecular screening program with the aim of enriching phase I/II in patients presenting genomic alteration

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# Detection of low frequency clones

**Table 1.** *EGFR* Mutations Detected by Direct Sequencing, MALDI-TOF MS, and NGS in TKI-Naive and TKI-Treated Patients With NSCLC

Patient Population	Direct Sequencing		MALDI-TOF MS		<i>P</i> *	NGS Validation†			
						MALDI-TOF MS		NGS	
	No.	%	No.	%		No.	%	No.	%
TKI-naïve patients	107	100	107	100		38	100	38	100
<i>EGFR</i> wild type‡	67	62.6	59	55.1		19	50.0	19	50.0
<i>EGFR</i> -activating mutations§	40	37.4	48	44.9	.0196	19	50.0	19	50.0
<i>EGFR</i> -T790M	3	2.8	27¶	25.2	< .001	10	26.3	13	34.2
TKI-treated patients	88		88			16		16	
Pre-TKI	73¶	100	73¶	100		14	100	14	100
<i>EGFR</i> wild type‡	33	45.2	17	23.3		5	35.7	4	28.6
<i>EGFR</i> -activating mutations§	40	54.8	56	76.7	< .001	9	64.3	10	71.4
<i>EGFR</i> -T790M	2	2.7	23	31.5	< .001	1	7.1	2	14.3
Post-TKI	12	100	12	100		2	100	2	100
<i>EGFR</i> wild type‡	3	25.0	0	0.0		0	0.0	0	0.0
<i>EGFR</i> -activating mutations§	9	75.0	12	100		2	100	2	100
<i>EGFR</i> -T790M	4	33.3	10	83.3	.0143	2	100	2	100

Abbreviations: *EGFR*, epidermal growth factor receptor; MALDI-TOF MS, matrix-assisted laser desorption ionization–time of flight mass spectrometry; NGS, next-generation sequencing; NSCLC, non–small-cell lung cancer; TKI, tyrosine-kinase-inhibitor.

\*McNemar test.

†Fifty-four DNA samples (38 for TKI-naïve patients and 16 for TKI-treated patients) were available and qualified for NGS validation.

‡Patients without *EGFR* L858R or Del19 mutations.

§Patients with *EGFR* L858R or Del19 mutations.

¶Twelve T790M patients without *EGFR* L858R or Del19 mutations in MALDI-TOF MS analysis.

¶Three patients with *EGFR* mutations except L858R and Del19 were excluded from the analysis.

NGS and MALDI-TOF, but not SANGER sequencing can detect T790M in TKI-naïve pts

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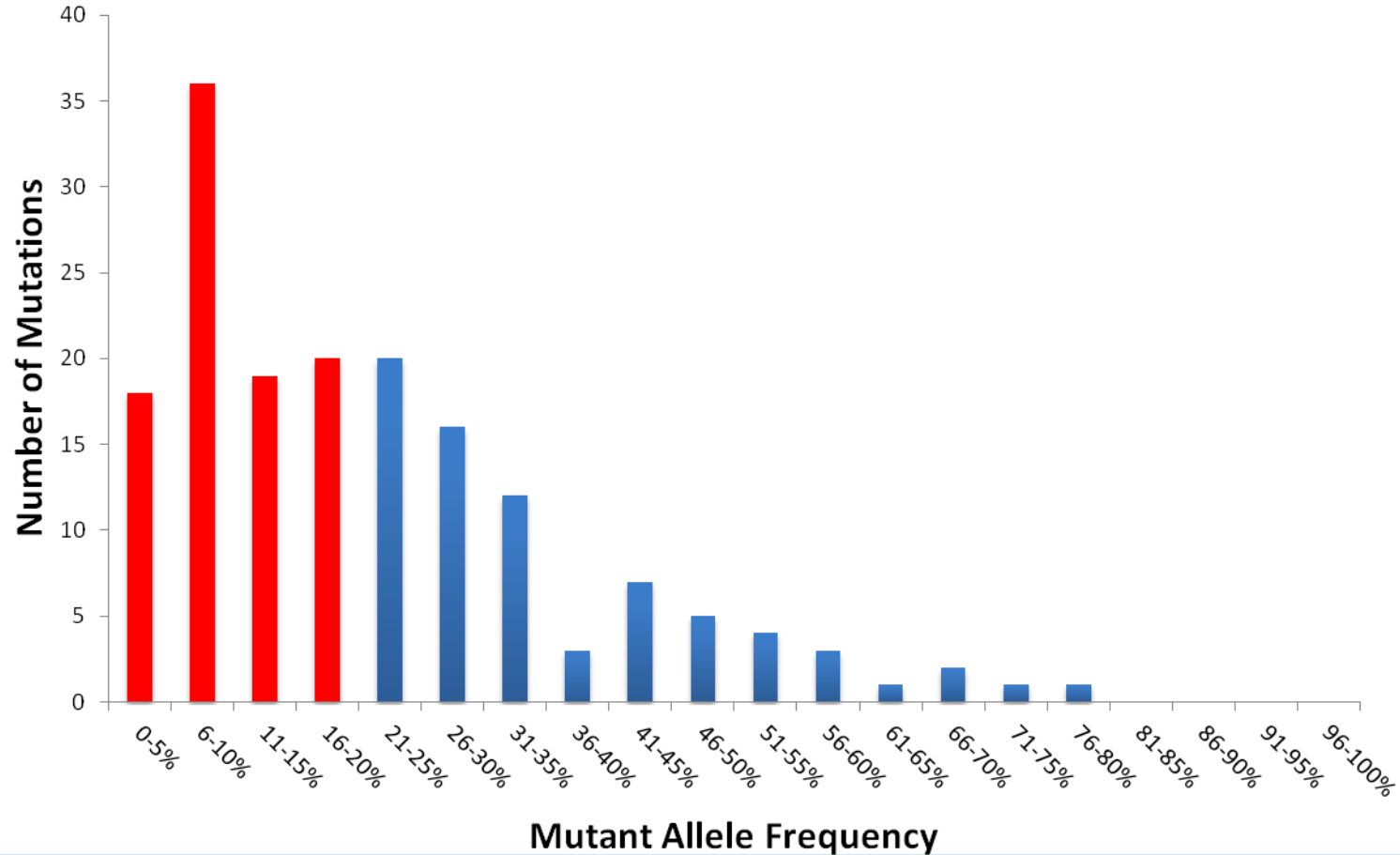
JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Pretreatment Epidermal Growth Factor Receptor (*EGFR*) T790M Mutation Predicts Shorter *EGFR* Tyrosine Kinase Inhibitor Response Duration in Patients With Non–Small-Cell Lung Cancer

Kang-Yi Su, Hsuan-Yu Chen, Ker-Chau Li, Min-Liang Kuo, James Chih-Hsin Yang, Wing-Kai Chan, Bing-Ching Ho, Gee-Chen Chang, Jin-Yuan Shih, Sung-Liang Yu, and Pan-Chyr Yang

# In depth NGS identifies low frequency mutations



Mutant Allele frequency spectrum of known mutations found in a series of clinical samples					
Fraction of mutations <5%	Fraction of mutations <10%	Fraction of mutations <20%	Fraction of mutations <25%	Fraction of mutations <50%	Fraction of mutations <100%
11%	32%	55%	67%	93%	100%

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# High throughput technologies should be used

- in daily practice: they are more robust, reproducible and easy to do as compared to single protein assays
- In the context of prospective cohorts (not clinical trials) : NGS will allow to detect a high number of rare, relevant genomic alterations. Treatment can be done in the context of phase I trials (MOSCATO program)
- Questions for clinical research include : medical usefulness of detecting low frequency clones