Multiple tests can lead to tissue exhaustion

To obtain adequate molecular analysis, patients may need to undergo several biopsies

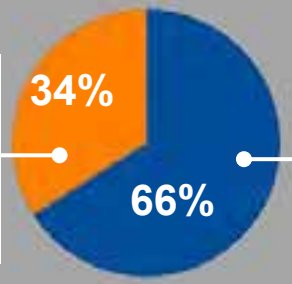
Results

47 patients that **tested negative** by **multiple** molecular tests*

- **31 patients** were **successfully profiled** using **CGP**
  - **84%** (26/31) of patients needed **≥ 2 biopsies to complete non-NGS and CGP testing**

Tissue exhaustion

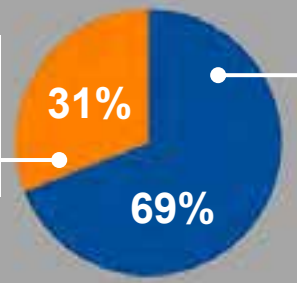
Multiple tests led to **tissue exhaustion** and repeat biopsy was not possible (16/47)



Tissue availability allowed **additional** testing (31/47)

Repeat biopsies

- **≥ 2 biopsies** were needed due to **tissue exhaustion** (8/26)

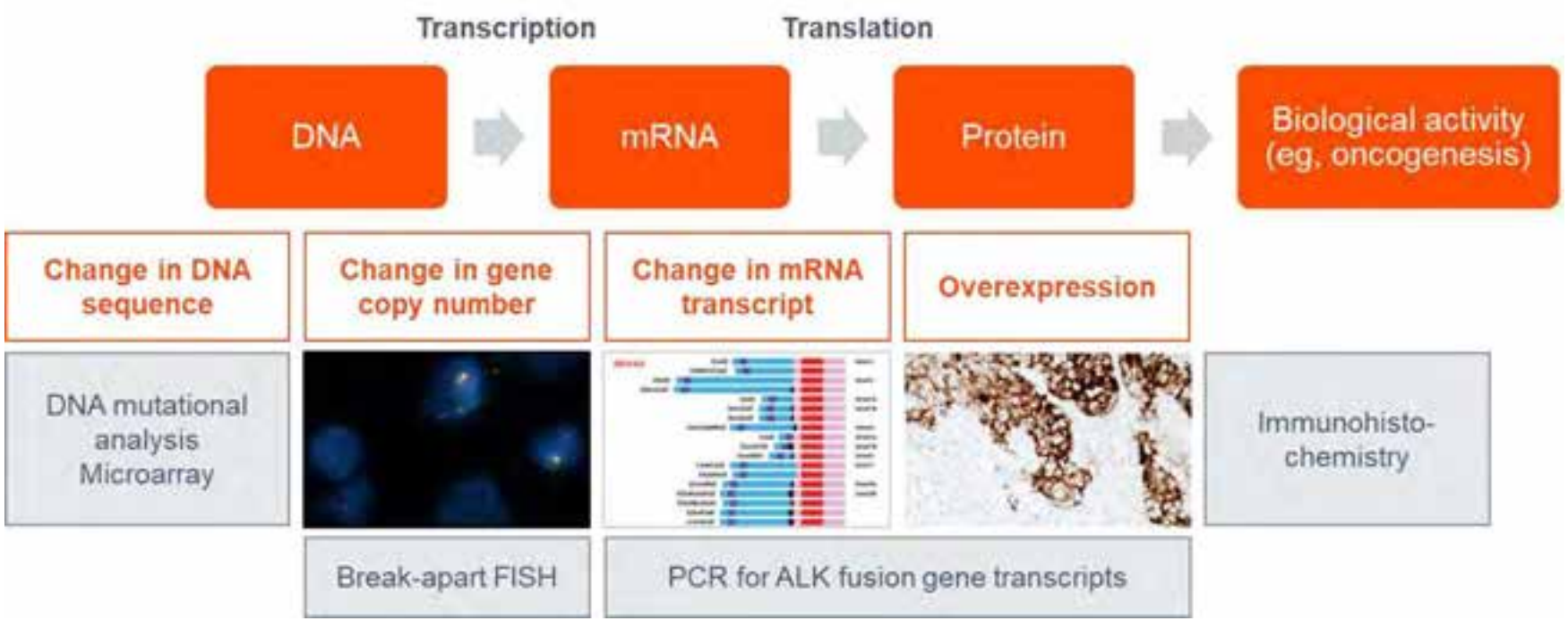


- **Multiple biopsies** were needed for **molecular analysis**
- **Sufficient tissue remained** for CGP test (18/26)

*Non-NGS methods tested for 11 genes known to drive lung cancer and included a mass spectrometry-based assay, sizing assays, and FISH break apart assays.
Drilon, A., *et al.* (2015) *Clin Cancer Res* 21(16):3631-9.

CGP: Comprehensive genomic profiling;
FISH: Fluorescence *in situ* hybridisation;
NGS: Next-generation sequencing.

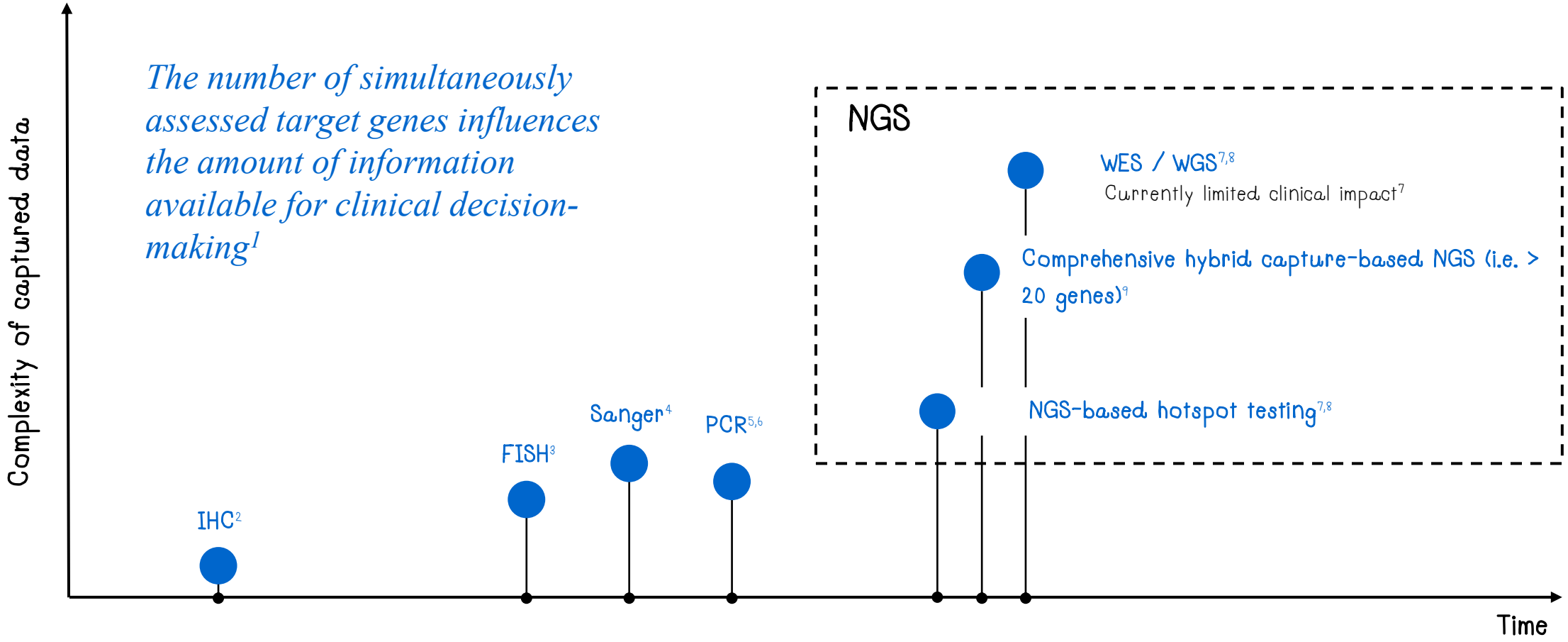
Next Generation Sequencing (NGS) help to overcome hurdle of single biomarker testing



Next Generation Sequencing

- Massively ***parallel sequencing*** (millions of parallel sequencing) reactions
- Advanced bioinformatics technologies
- Significantly improves turnaround time and cost-efficiency

Precision medicine was sparked by significant advancements in diagnostics

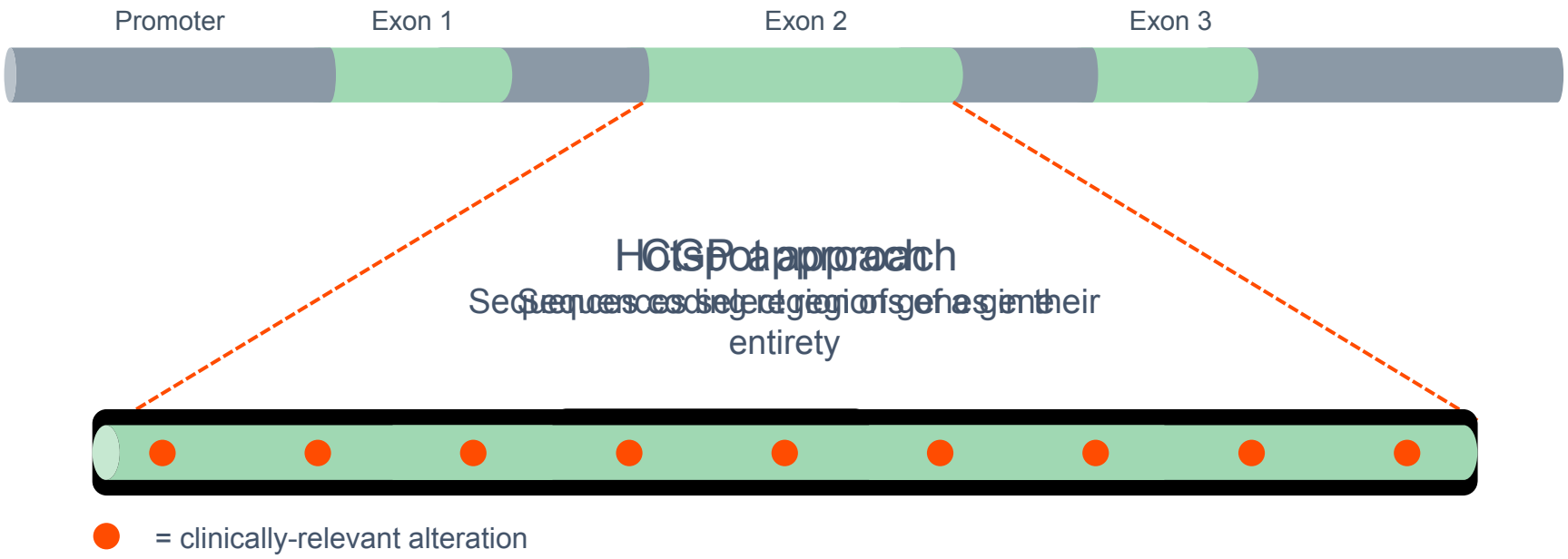


FISH: fluorescence *in situ* hybridisation; IHC: immunohistochemistry; NGS: next-generation sequencing; PCR: polymerase chain reaction; WES: whole exome sequencing; WGS: whole genome sequencing.

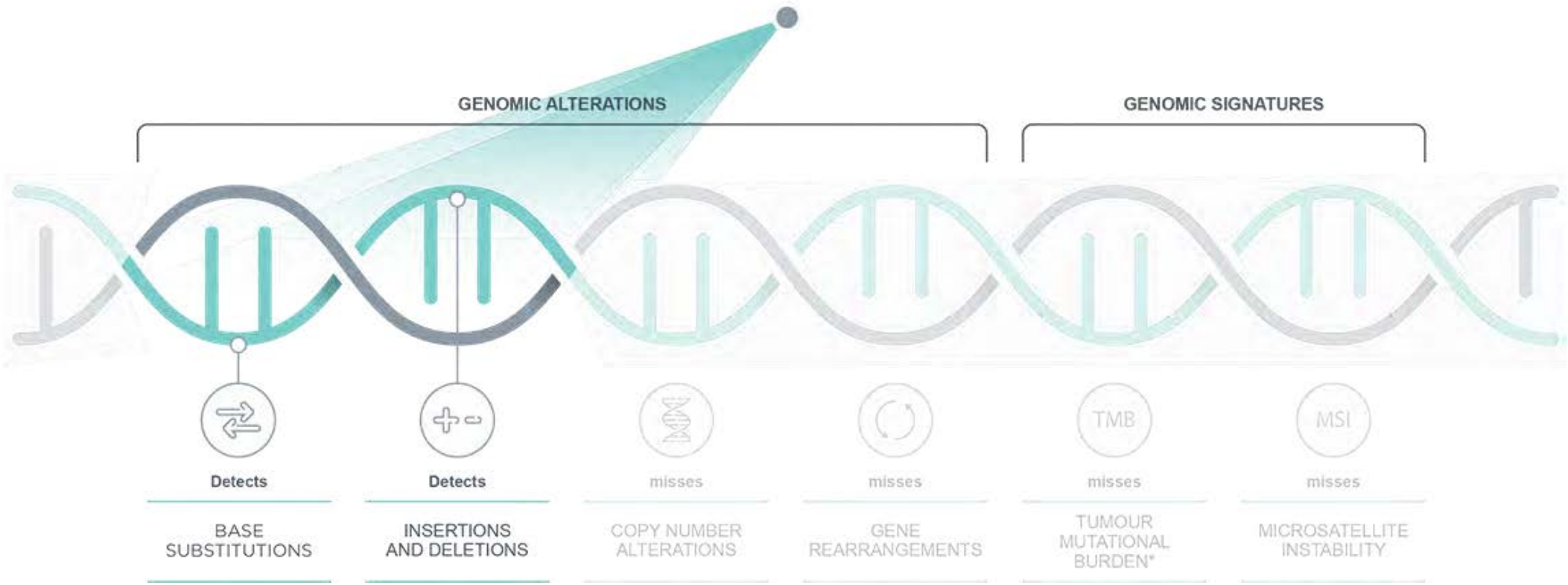
1. Russell, K., et al. (2014) *Front Pharmacol* 5:76; 2. de Matos, L.L., et al. (2010) *Biomark Insights* 5:9–20; 3. Huber, D., et al. (2018) *MNE* 1:15–24; 4. Stranneheim, H. and Lundeberg, J., (2012) *Biotechnol J* 7:1063–73; 5. Bernard, P.S. and Wittwer, C.T., (2002) *Clin Chem* 48:1178–85. 6. Kaunitz, J.D., (2015) *Dig Dis Sci* 60:22301; 7. Kulski, J.K., (2016) doi 10.5772/61964; 8. Dong, L., et al. (2015) *Curr Genomics* 16:253–63; 9. Frampton, G., et al. (2013) *Nat Biotech* 31:1023–31.

NGS techniques

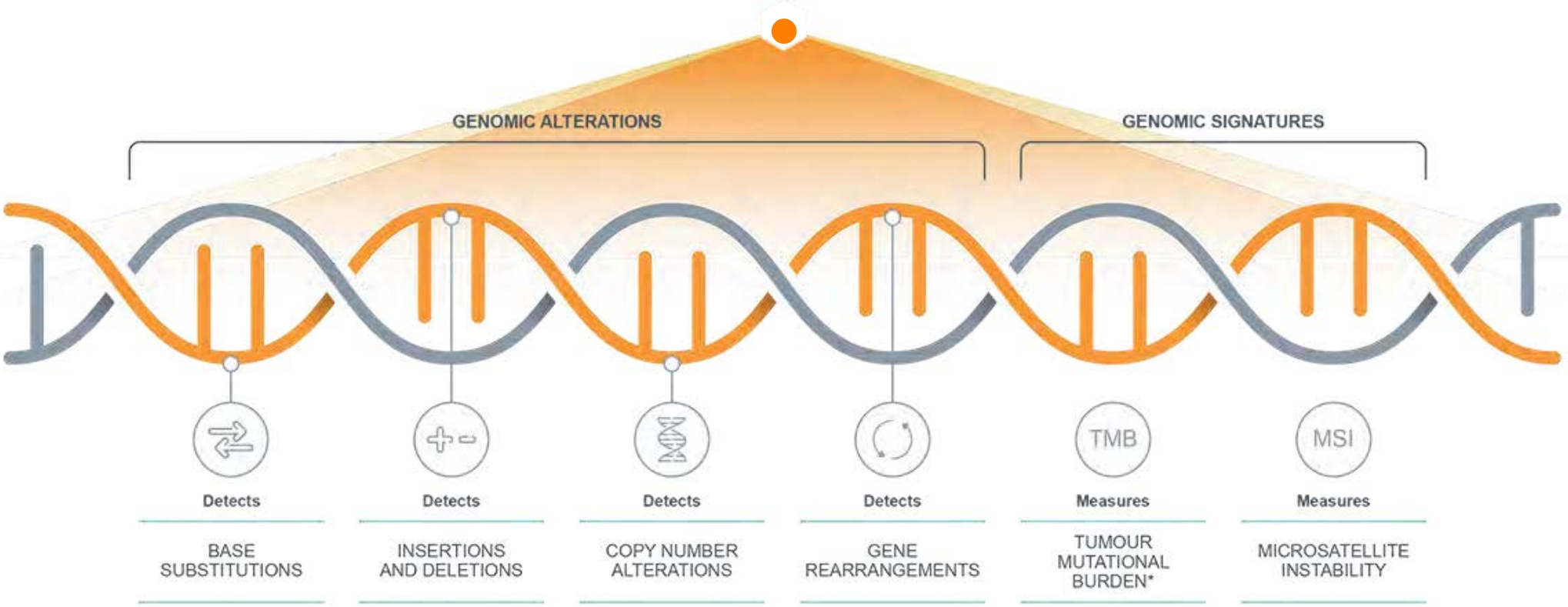
Comprehensive genomic profiling (CGP) provides extensive view of genomic alterations



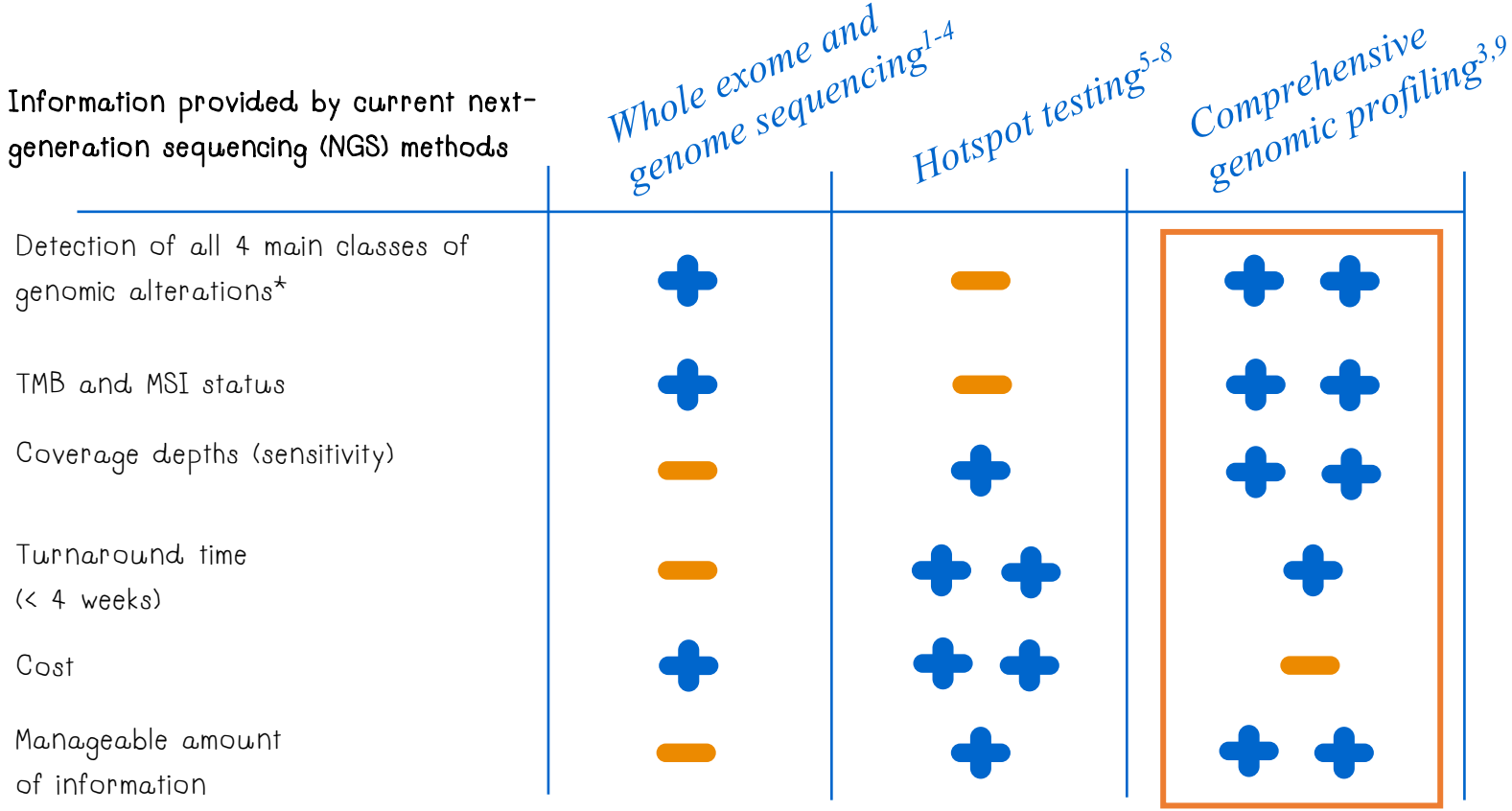
Multigene hotspot NGS tests can miss genomic alterations that are not initially selected



Broad analyses of tumour genome can identify clinically relevant alterations and potentially expands patients' treatment options



Comprehensive genomic profiling: An NGS method to efficiently inform clinical practice



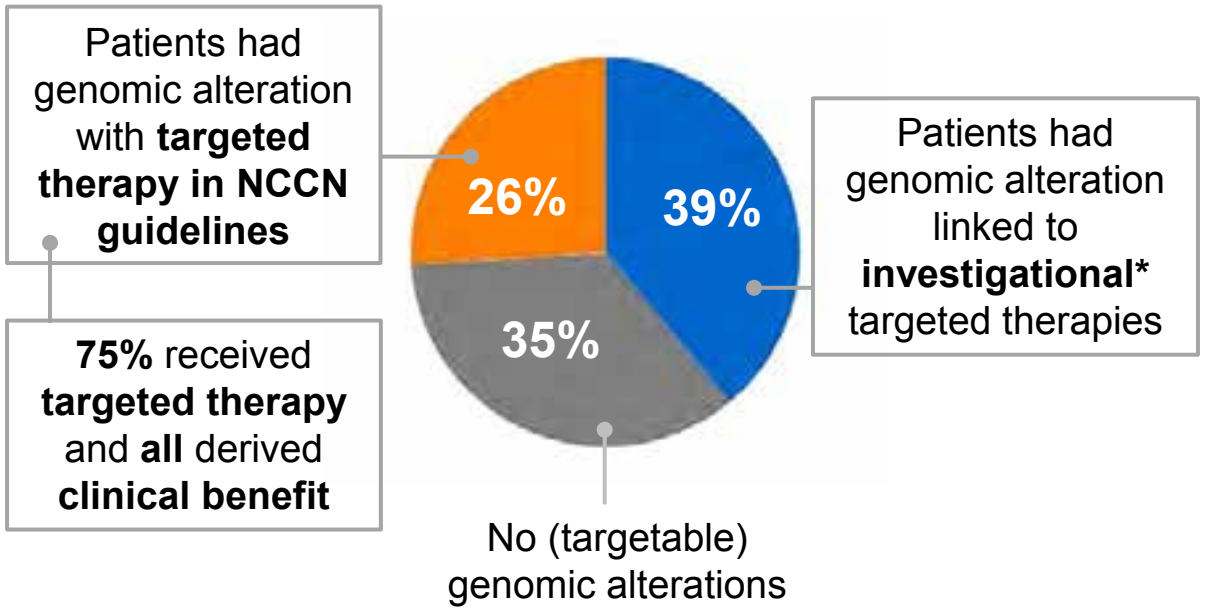
*The main classes of genomic alterations are copy number variations, insertions and deletions (Indels), rearrangement and base substitutions.
MSI: microsatellite instability; NGS: next-generation sequencing; TMB: tumour mutational burden.

1. Meldrum, C., et al. (2011) *Clin Biochem Rev* 2011; 32:177-95; 2. Serrati, S., et al. (2016) *Onco Targets Ther* 9:7355-65;
3. Borad, M.J. and LoRusso, P.M. (2017) *Mayo Clin Proc* 92:1583-91; 4. Stenzinger, A., et al. (2019) *Genes Chromosomes Cancer* 58:578-588;
5 Gray, P.N., et al. (2015) *Cancers* 7:1313-32; 6. Jennings, L.J., et. al. (2017) *J Mol Diagn* 19:341e365; 7. Dong, L., et al. (2015) *Curr Genomics* 16:253-63;
8. Buchhalter et al. (2019) *Int J Cancer* 144:848-58; 9. Frampton, G.M., et al. (2013) *Nat Biotechnol* 31:1023-31.

Comprehensive Genomic Profiling provides more treatment options for patients

Results

- **CGP** identified **clinically relevant genomic alterations** in **65%** (20/31) of patients, who previously tested negative by multiple non-NGS methods



Conclusions

- **CGP identified genomic alterations that would have not been detected by other methods. Therefore, open more possibility of personalized treatment for patients**
- Results support **1st-line profiling of lung adenocarcinomas** using **CGP** as a more **comprehensive** and **efficient** strategy compared to non-NGS testing

*Genomic alteration linked to targeted therapies in other tumour types, or a clinical trial.

Drilon, A., *et al.* (2015) *Clin Cancer Res* 21(16):3631-9.

CGP: Comprehensive genomic profiling;
NCCN: National Comprehensive Cancer Network;
NGS: Next-generation sequencing.

US FDA approved NGS test

- Memorial Sloan Kettering IMPACT (Internal service only)
- Foundation Medicine F1CDx (Commercially available)

Large panel

- Foundation Medicine CDxBRCA
- OncomineDX Target Test (NSCLC)
- Praxis Extended RAS Panel (Colo-rectal)

Disease-specific small panel

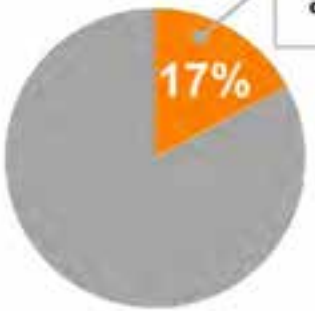
Hybrid capture CGP identified targetable mutations that were missed in the past

EGFR

400 NSCLC cases with ***EGFR* Δex19** identified by **CGP**

- Pathology reports were reviewed for **250** consecutive cases
- **71** had **previous *EGFR* hotspot test** results available

Hotspot EGFR test **missed *EGFR* Δex19** alteration identified by CGP (12/71 patients)



ALK

- **1,070** patient samples were profiled using **CGP**
- **47** were found to **harbour *ALK* rearrangements**, of which **31** had **prior FISH** results available

ALK break-apart **FISH assay missed *ALK* rearrangements** identified by CGP (11/31 patients)

9 patients received **crizotinib** and **7** had **confirmed responses**

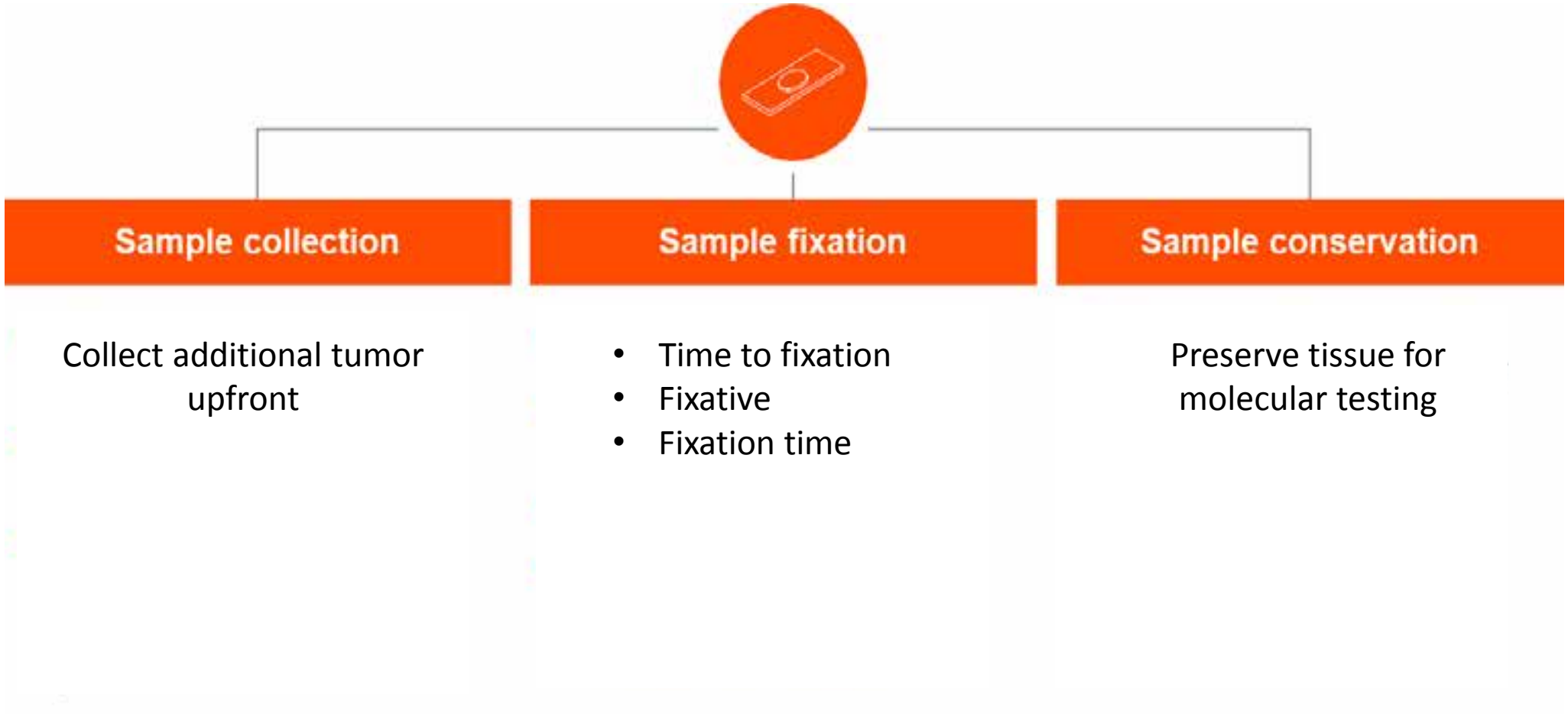
Substantial number of patients with important genomic alterations in NSCLC, EGFR and ALK, would have missed targeted therapy without comprehensive genomic profiling

Ali, S.M., *et al.* (2016) *The Oncologist* 21:762-770.
Schrock, A.B., *et al.* (2016) *Clin Cancer Res* 22(13):3281-5.

CGP: Comprehensive genomic profiling; *EGFR* Δex19: *EGFR* exon 19 deletions; NGS: Next-generation sequencing; NSCLC: Non-small cell lung cancer.

Recommendation for specimen preparation for CGP

Three critical factors for specimen preparation



Guidance for adequate specimen collection

Collect additional tumour upfront

- Perform multiple passes for all needle biopsies

For pulmonologists	For radiologists
After target acquisition is confirmed: • Perform at least 4 EBUS passes per target lesion • All subsequent passes should be placed in the cell block container • Use a 21-22 g needle	• Acquire 2+ (preferably 3-6) core needle biopsies • Use 18-20 g needles rather than or in addition to fine-needle aspirates

- Create cell blocks for all cytology specimens

Dietel M *et al. Thorax* 2016;71(2):177-84.

Recommendation for specimen selection for CGP

Block and Slide preparation

- Select the most appropriate tumour cell block to test[1]
- Maintain cleanliness of the tissue matrices, water bath and other instruments[2]
- Change or clean microtome between dissection[2]

Tumour purity

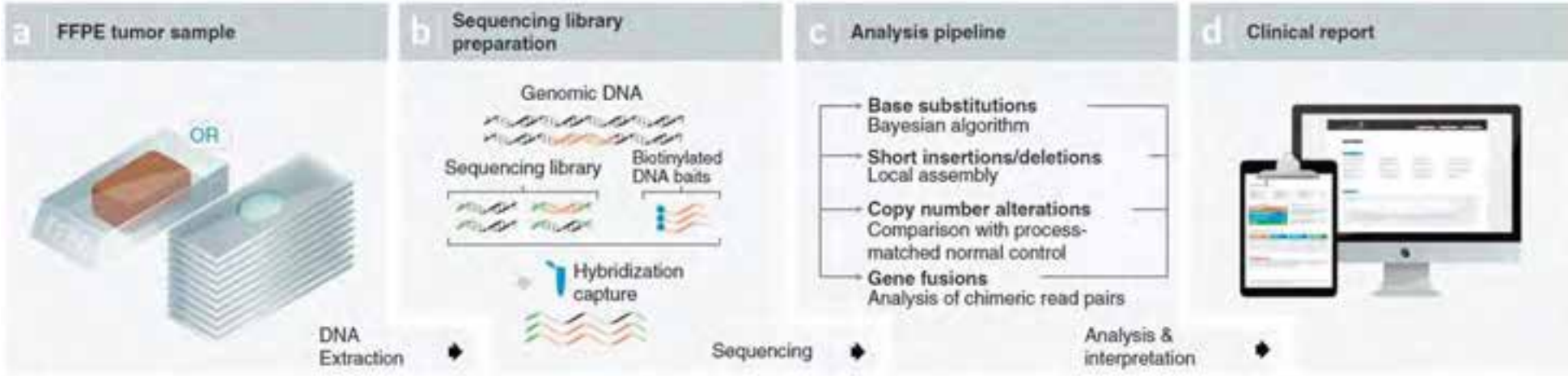
- Count only nucleated cells (exclude apoptotic cells) when assessing tumour cell percentage)[3]

% Tumour purity	Description
>30%	Optimal
>20%	Acceptable
10-20%	Suboptimal (often yields meaningful results)
<10%	Unacceptable

Remarks: Liver specimens require a tumour nuclei purity of at least 40%, as polyploidy is common in hepatocytes

1. Dietel M *et al. Thorax.* 2016;71(2):177-84. 2. Asor E *et al. PLoS ONE* 2017;12(3): e0173760. https://doi.org/10.1371/journal.pone.0173760. 3. FoundationOne® Specimen Preparation Instructions. 01.17-FMI-O01.

Alteration identification is not clinically useful unless it can be intelligently communicated



Sample requirements

- Surface area: ≥25 mm^2
- Sample volume: ≥1 mm^3
- Nucleated cellularity: ≥80% or ≥30,000 cells
- Tumor content: ≥20%

Fraction of patients with tissue insufficient for analysis: 10–15%

Laboratory process highlights

- Requires ≥50 ng of dsDNA (quantified by PicoGreen)
- Fragmentation by sonication (Covaris) and 'with-bead' library construction
- Hybridization capture with biotinylated DNA oligonucleotides
- 49 × 49 paired-end sequencing on the Illumina HiSeq platform to >500× average unique coverage, with >100× at >99% of exons

Analysis methods highlights

- Sensitivity to variants present at any mutant allele frequency
- Detection of long (1–40 bp) indel variants using de Bruijn graph–based local assembly
- CGH-like analysis of read-depth for CNAs assessment

Reporting approach

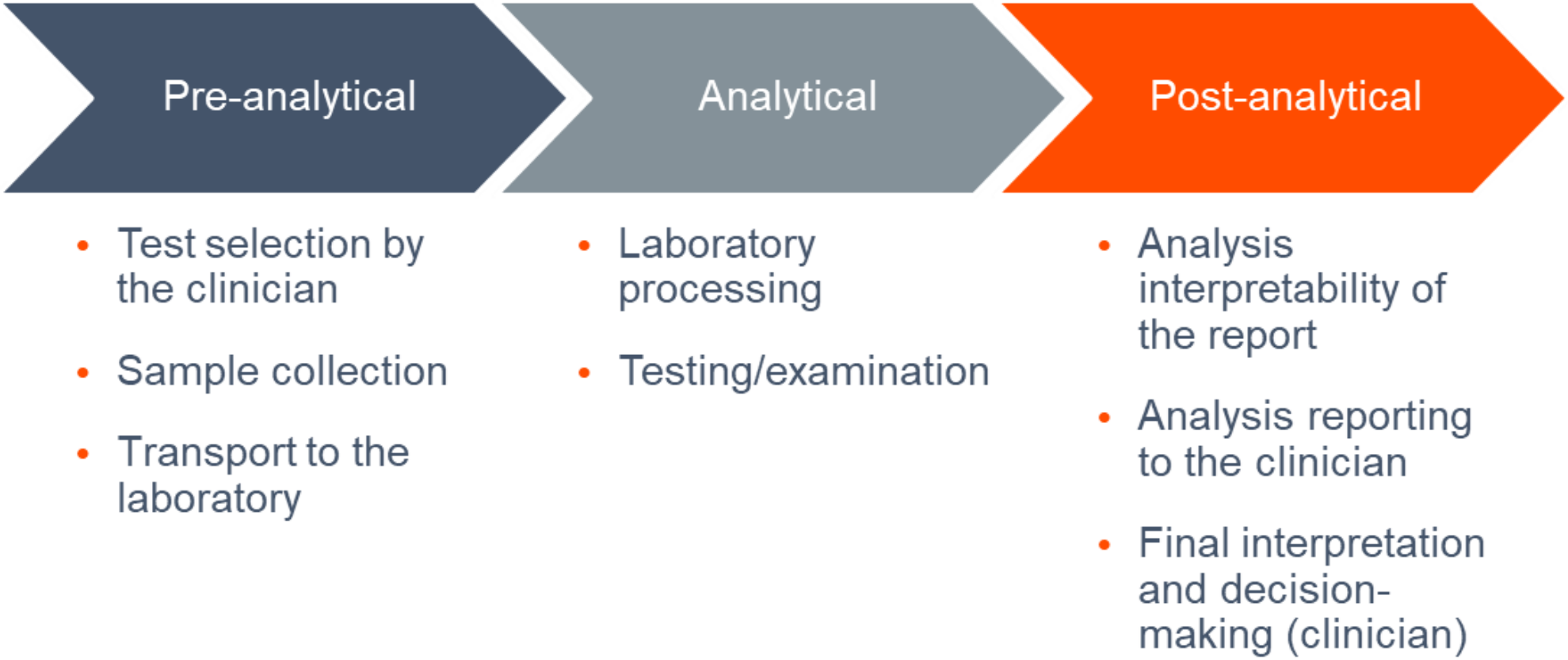
Interpretation without a matched normal

- Germline variants from 1000 Genomes Project (dbSNP135) removed
- Known driver alterations (COSMIC v62) highlighted as biologically significant

A concise summary of the biomedical literature and current clinical trials is provided for each highlighted alteration

Frampton, et al. Nature Biotech, 2013

Quality control in NGS testing



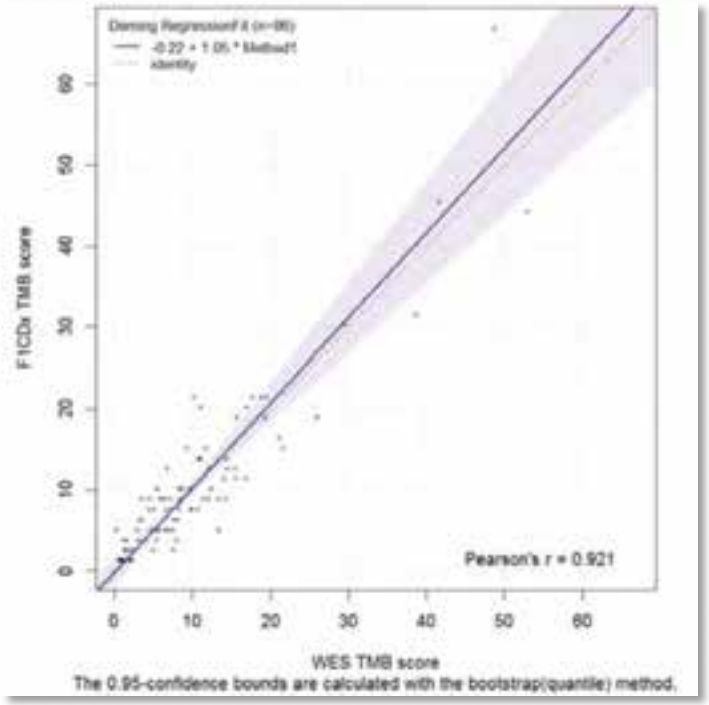
Hawkins R. *Ann Lab Med* 2012;32:5-16. Hewitt SM *et al. Clin Cancer Res* 2012;18(6);1524-30.

TMB from F1CDx is analytically validated for accuracy, precision and high sensitivity

Category	Target	Status	Achieved
Precision – Repeatability	>90%	PASSED	95.3% (95% CI; 92.2%-97.4%)
Precision – Reproducibility	>90%	PASSED	97.3% (95% CI; 95.7%-98.5%)
Limit of Detection	<20% tumor purity	PASSED	18.0% tumor purity with 95% probability of detection
Accuracy	>80%	PASSED	86.0% overall agreement (R^2=0.92)

Unpublished FMI validation data (Fabrizio et al, ESMO 2018 abstract)

Correlation of F1CDx TMB vs. WES (n=86)



MSI orthogonal platform concordance

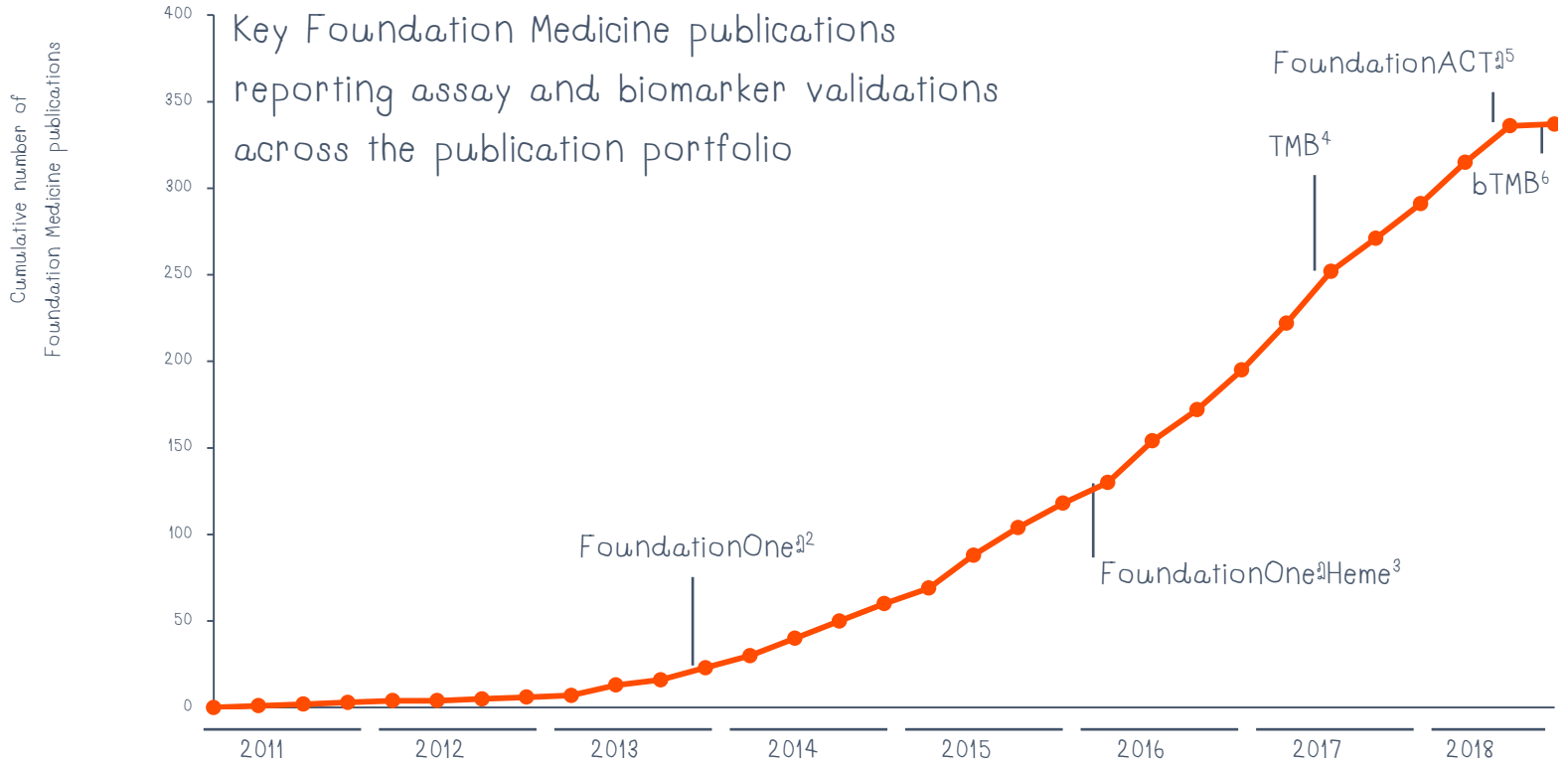
- Combined accuracy including both PCR and IHC comparison studies demonstrate 97% concordance (n = 69)
  - Sensitivity is 95% (18/19) and specificity is 98% (47/48)

		IHC		PCR		IHC/PCR combined	
		MSI-H	MSS	MSI-H	MSS	MSI-H	MSS
NGS	**MSI-H**	1	0	17	1	18	1
	MSS	0	29	1	18	1	47
	MSI-ambiguous	0	0	1	1	1	1

Foundation Medicine has (co-)authored over 337 publications between 2011 and 2018[1]

Publications **covering almost all tumour types**, some rare, include:

- Assay validations
- Biomarker validations
- Publications supporting the clinical validity and utility of CGP
- Case reports
- Review articles

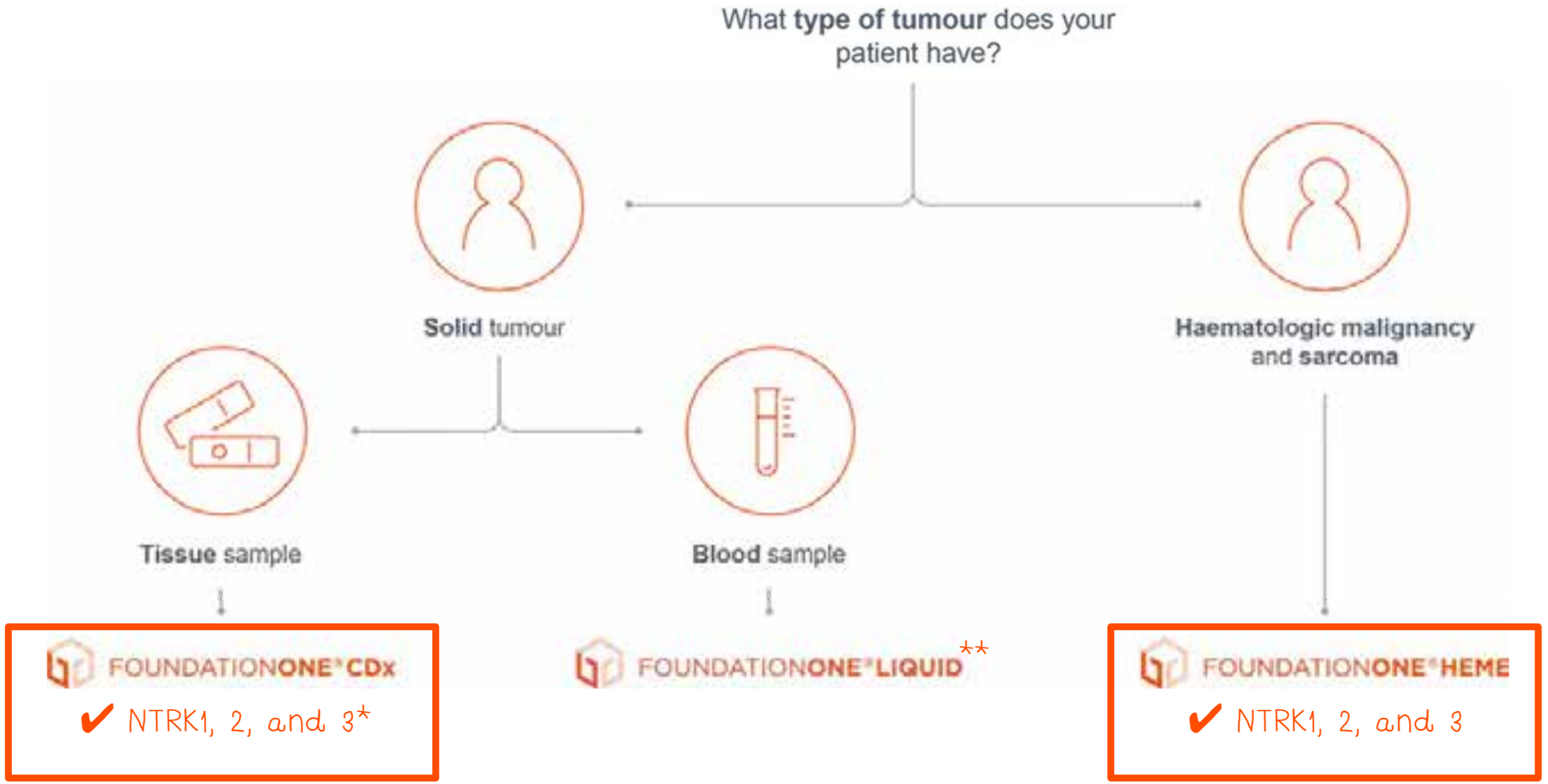


bTMB: : blood-based tumour mutational burden; CGP: comprehensive genomic profiling; Q: quarter; TMB: tumour mutational burden.

1. Foundation Medicine data on file; 2. Frampton, G.M., et al. (2013) *Nat Biotechnol* 31:1023-31; 3. He, J., et al. (2016) *Blood* 127:3004-14; 4. Chalmers, Z.R., et al. (2017) *Genome Med* 9:34; 5. Clark, T.A., et al. *J Mol Diagn* 20:686-702; 6. Gandara, D.R., et al. (2018) *Nat Med* doi: 10.1038/s41591-018-0134-3. [Epub ahead of print].

A high-quality portfolio of comprehensive genomic profiling services

Use F1CDx for solid tumour and F1Heme for hematological malignancy and sarcoma



* FoundationOne CDx detects NTRK3 fusion through the coverage of ETV6, the most common fusion partner of NTRK3.
** NTRK genes are not currently included in F1L and planned to be added to the new version, F1L CDx.
NTRK coverage information is based on the technical specification of each testing

FMI evidence detecting NTRK fusions

Evidence based on F1CDx & F1H

- The Foundation Medicine test was one of three major laboratory tests used to **screen patients with NTRK fusions for Loxo's phase I and II trials**[1]
- Foundation Medicine is also used to **screen patients with NTRK fusions for Roche`s STARTRK-2 trial**[10].
- Foundation Medicine has **co-authored seven peer-reviewed publications on NTRK fusions since 2013**, including papers on NTRK fusions in solid tumours (lung, gastrointestinal, pediatric, colorectal, and breast cancers) and sarcomas in adult and pediatric patients[2-8].

1.Supplement to: Drilon A. et al., N Engl J Med 2018;378:731-9: 2. Vaishnavi, et al., Nat Med. 2013 Nov;19(11):1469-1472. doi: 10.1038/nm.3352. Epub 2013 Oct 27: 3. Wong, et al., J Natl Cancer Inst. 2015 Nov 12;108(1): 4. Doebele et al.: Cancer Discov. 2015 Oct;5(10):1049-57. doi: 10.1158/2159-8290.CD-15-0443. Epub 2015 Jul 27: 5. Shi et al., J Transl Med. 2016 Dec 14;14(1):339: 6. Pavlick et al.: Pediatr Blood Cancer. 2017 Aug;64(8): 7. Landman et al., Clin Breast Cancer. 2018 Jun;18(3):e267-e270: 8. Pietrantonio et al., J Natl Cancer Inst. 2017 Dec 1;109(12): 10. www.clinicaltrials.gov (NCT02568267)

Conclusions

Comprehensive genomic profiling (CGP) offers broad vision of molecular targets across cancer-related genes that maybe missed by conventional testing

Standardization and validation are critical factors to ensure the precision of CGP panels.
FoundationONE CDx is the only commercialized CGP approved by US FDA.

NTRK1/2/3 fusions can be detected by FoundationONE CDx and FoundationONE HEME and are used for screening in several clinical trials and publications.

Amount and quality of specimen are important for successful molecular analysis

Outline

Current treatment from “one-size fits-all” to personalized oncology

Changing paradigms in molecular testing of tumors: Choosing the right start for the best outcomes

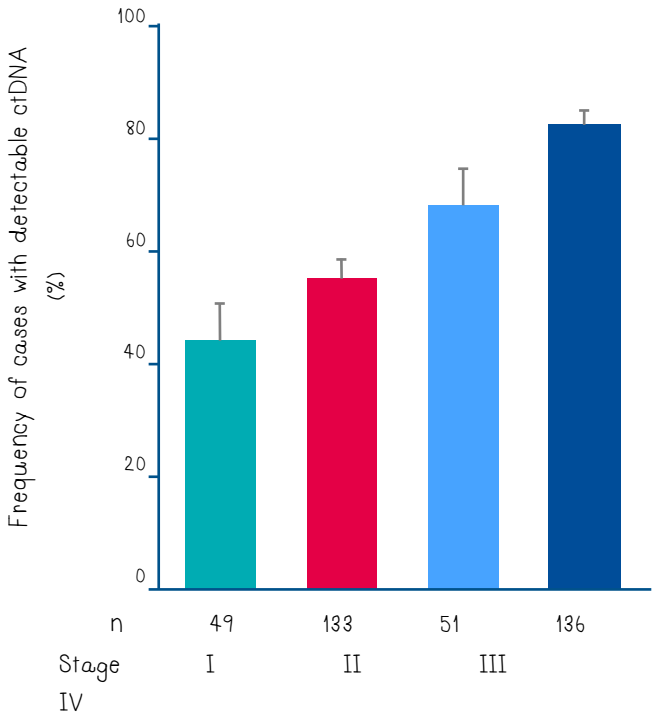
Taking diagnostics to the next level: Liquid biopsy

Liquid biopsy opens up the opportunity for CGP without the need for a tissue sample

ctDNA

- Detectable in patients with various tumour types and stages[1,2]
- Shed from primary tumours, circulating tumour cells and metastases[2,3]
- Reflects tumour heterogeneity[4]

ctDNA concentration increases with disease stage[2]



Several factors influence the amount of ctDNA released into the blood

- Tumour grade, histology and vascularity[5,6]
- Physiological clearance and degradation[7–9]
- Rate of release and cell status[6,7]
- Time of blood draw and therapy[5,10,11]

1. Shu, et al. Sci Rep 2017; 2. Bettegowda, et al. Sci Transl Med 2014; 3. Bidard, et al. Sci Transl Med 2013
4. Merker, et al. J Clin Oncol 2018; 5. Hinrichsen, et al. J Lab Med 2016; 6. Diaz and Bardelli. J Clin Oncol 2014
7. Siravegna, et al. Nat Rev Clin Oncol 2017; 8. Forte, et al. Cancer Biol Med 2016; 9. Leung, et al. Clin Chem 2016
10. Diehl, et al. Nat Med 2008; 11. Tie, et al. Ann Oncol 2015

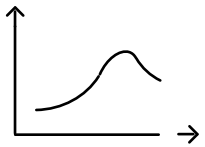
ctDNA, circulating tumour DNA

Liquid biopsies may add clinical value

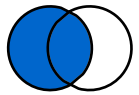
a patient has **insufficient**, inadequate or exhausted solid tissue[1–3]

direct biopsy is **difficult** or poses a high risk[1,4–5]

Liquid biopsy



disease progression / recurrence or resistance mutations are suspected[6,7]



complementary information to prior or future tissue testing is needed[5,8]

1. Francis, G. & Stein, S. (2015) *Int J Mol Sci* 16:14122–42; 2. Chouaid, C., et al. (2014) *Lung Cancer* 86:170–3; 3. Bardelli, A., et al. (2017) *Cell* 31:172–9;
4. Bidard, F., et al. (2013) *Sci Transl Med* 5:207ps14.; 5. De Mattos-Arruda, L., et al. (2015) *Nat Comm* 6:8839; 6. Siravegna, G., et al. (2015) *Nat Med* 21:795–801;
7. Luo, W., et al. (2018) *Am J Transl Res* 10(12): 3911–3923; 8. Krishnamurthy, N., et al. (2017) *J Clin Med* 6:3.

FMI has developed and analytically validated a highly specific and sensitive blood-based TMB (bTMB) assay

Average values across both bTMB cut-offs (established against an orthogonally validated TMB platform)[1]

95 % Positive predictive agreement

96 % Average precision

17 % Coefficient of variation

100 % Negative predictive agreement

Positive predictive value

Analytically validated for accuracy and precision with only

1 % of tumour content in ≥ 20 ng of cfDNA

The bTMB assay interrogates SNVs in 394 genes from cfDNA in plasma and reports a score based on the number of high-confidence SNVs identified[2]

bTMB: blood-based tumour mutational burden; cfDNA: cell-free DNA; CGP: comprehensive genomic profiling; MSAF: maximum somatic allele frequency; NSCLC: non-small cell lung cancer; SNV: single nucleotide variant.

1. Fabrizio, D.A., et al. (2017) *Ann Oncol* 28(suppl 5):v22-42;
2. Gandara, D.R., et al. (2017) *Ann Oncol* 28(suppl 5):v460-96.