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THE EVOLVING ROLE OF LIQUID BIOPSY

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EDUCATIONAL OBJECTIVES

Upon completion of this activity, the learner will:

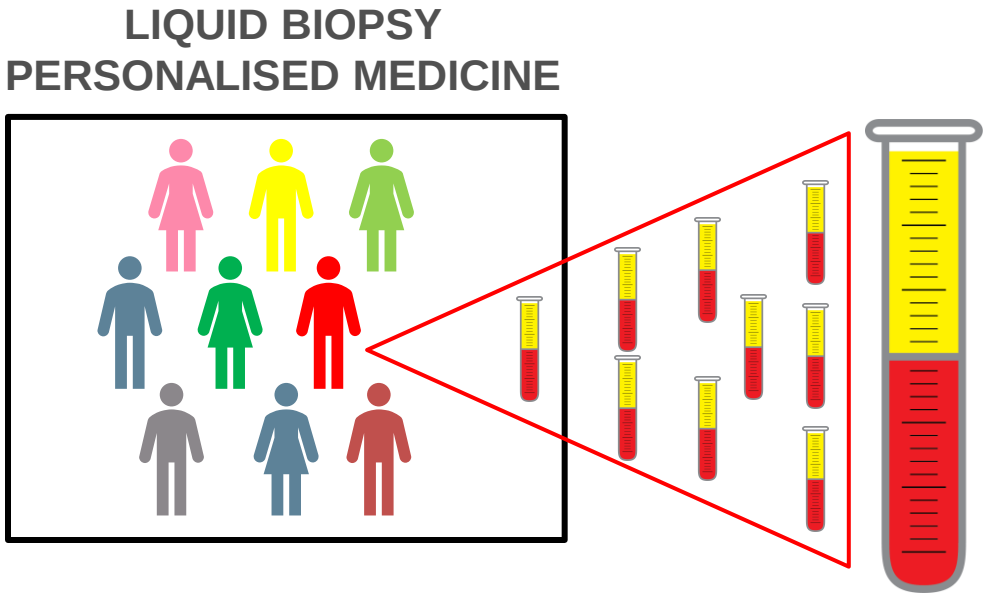
- Know the latest developments and practical recommendations on the role of liquid biopsy in precision oncology, and how to apply this across the patient journey

CLINICAL TAKEAWAYS

- Liquid biopsies offer a less invasive alternative to traditional tissue biopsies in patients with solid tumours, to examine molecular features
- The role of liquid biopsy should be seen as a complementary testing method to tissue-based assays and the information derived should be reviewed in combination with tissue results
- The clinical application of liquid biopsies ranges from screening and diagnosis, treatment guidance, monitoring minimal residual disease to assessing chemotherapy resistance

WHAT IS A LIQUID BIOPSY?

- A liquid biopsy can be derived from the blood and other body fluids, e.g., saliva, ascites fluid, urine, cerebrospinal and pleural fluid



Enrichment and detection from plasma/serum of:

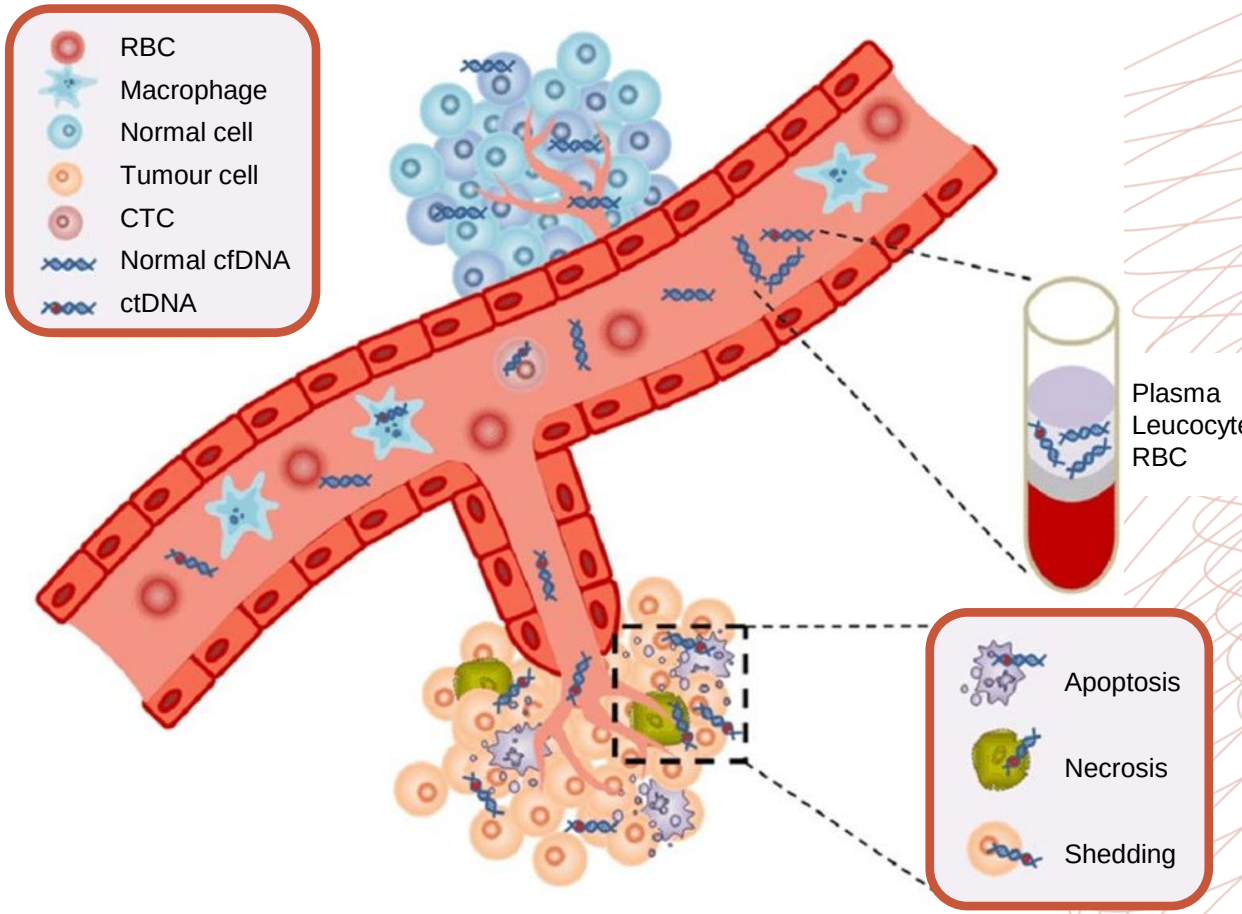
- Circulating tumour cells
- Circulating tumour nucleic acids
  (cell-free DNA, microRNA, mRNA, lncRNA)
- Circulating tumour microvesicles/exosomes

lncRNA, long non-coding RNA; mRNA, messenger RNA

Raimondi L, et al. Oncotarget. 2017;8:100831-100851; Nikanjam M, et al. J Hematol Oncol. 2022;15:131

WHAT IS CELL-FREE DNA (cfDNA) AND CIRCULATING TUMOUR DNA (ctDNA)

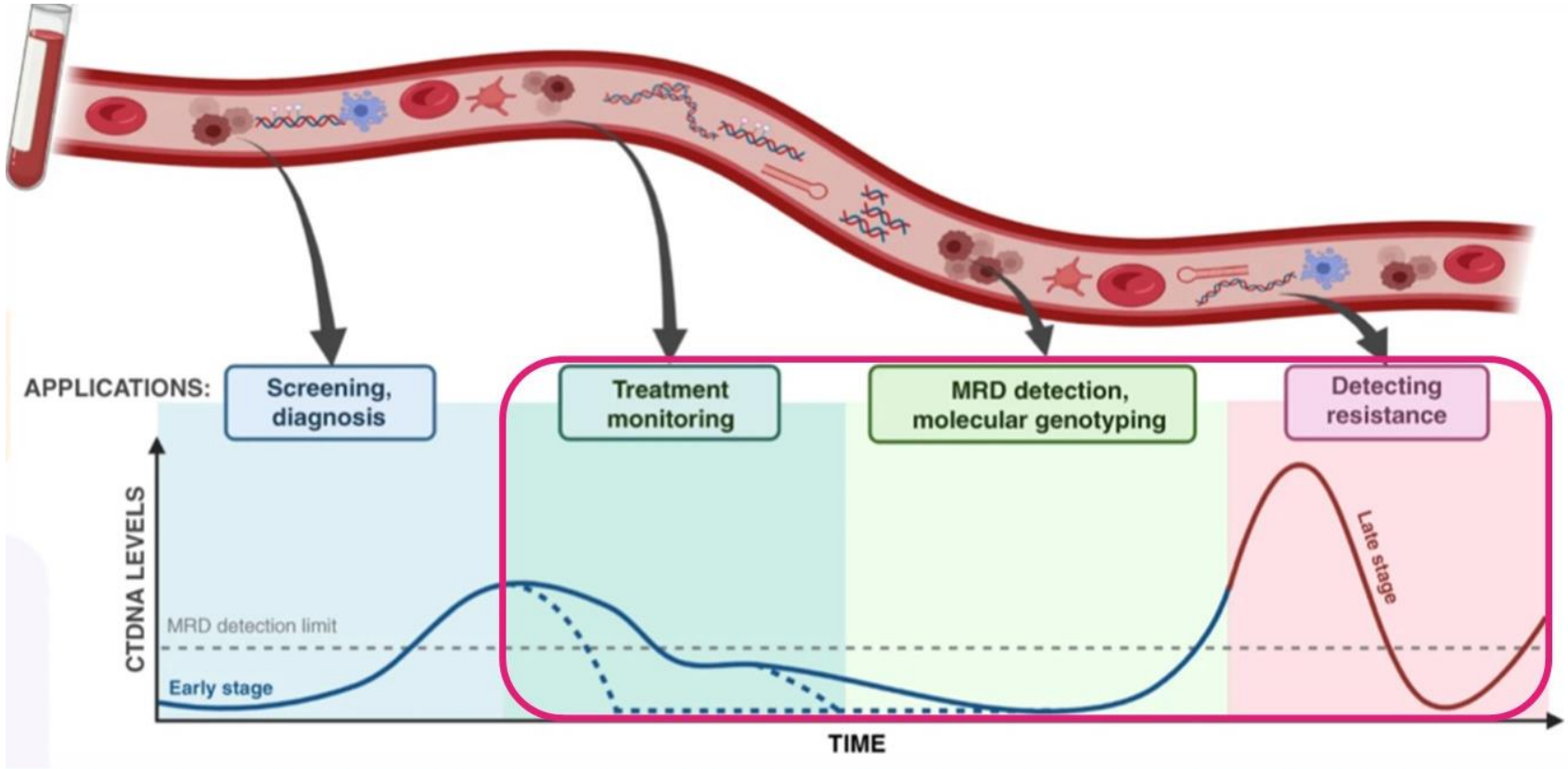
- cfDNA
  - dsDNA fragments associated with histones in circulation that have been released by cells
- Circulating tumour DNA (ctDNA)
  - cfDNA derived from cancer cells
  - Characterised by somatic, cancer-specific alterations, cancer-specific methylation patterns
- Most cfDNA is released by normal leukocytes
- Higher levels of ctDNA associated with:
  - Certain cancer subtypes
  - Higher burden of disease
  - Liver metastases



cfDNA, cell-free DNA, CTC, circulating tumour cell; ctDNA, circulating tumour DNA, dsDNA, double-stranded DNA; RBC, red blood cell

Hahn AW, et al. Kidney Cancer 3, 2019;7-13; Qi T, et al. Int J. Mol Sci. 2023; 24: 1503; Sanchez-Herrero E, et al. Front. Oncol. 2022; 12: 943253; Parsons HA. ASCO 2024

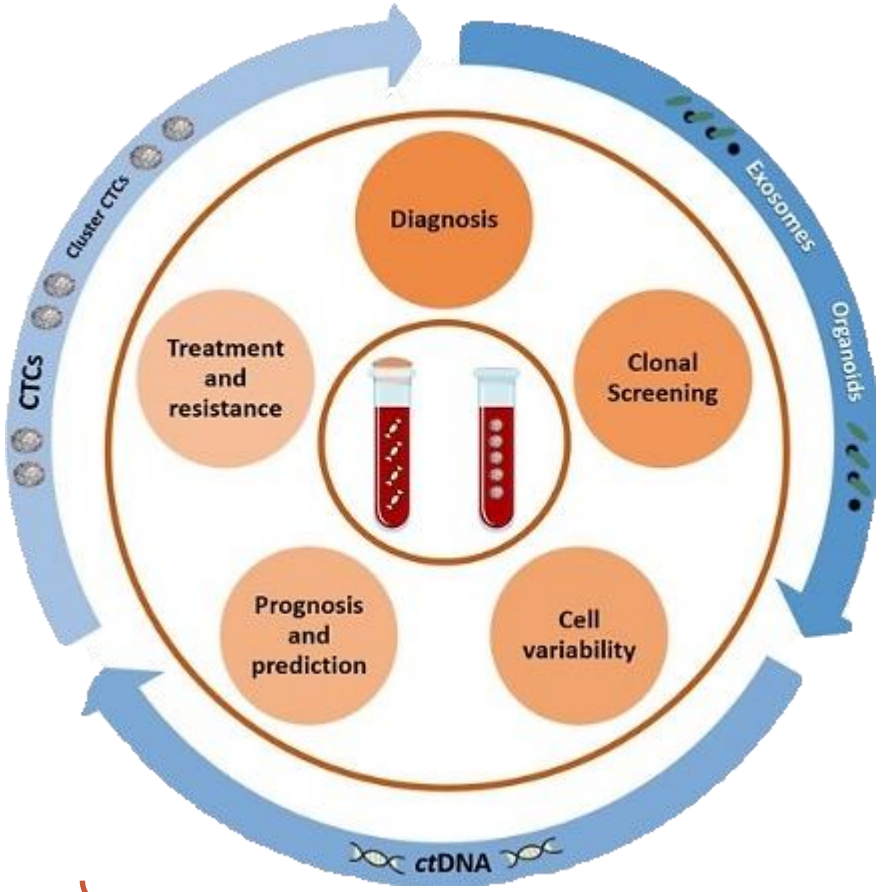
ctDNA MAY HAVE UTILITY ACROSS THE DISEASE SPECTRUM



CT(ct)DNA, circulating tumour DNA; MRD, minimal residual disease

García-Pardo M, et al. Br J Cancer. 2022;127:592-602

CHALLENGES AND OPPORTUNITIES OF cfDNA



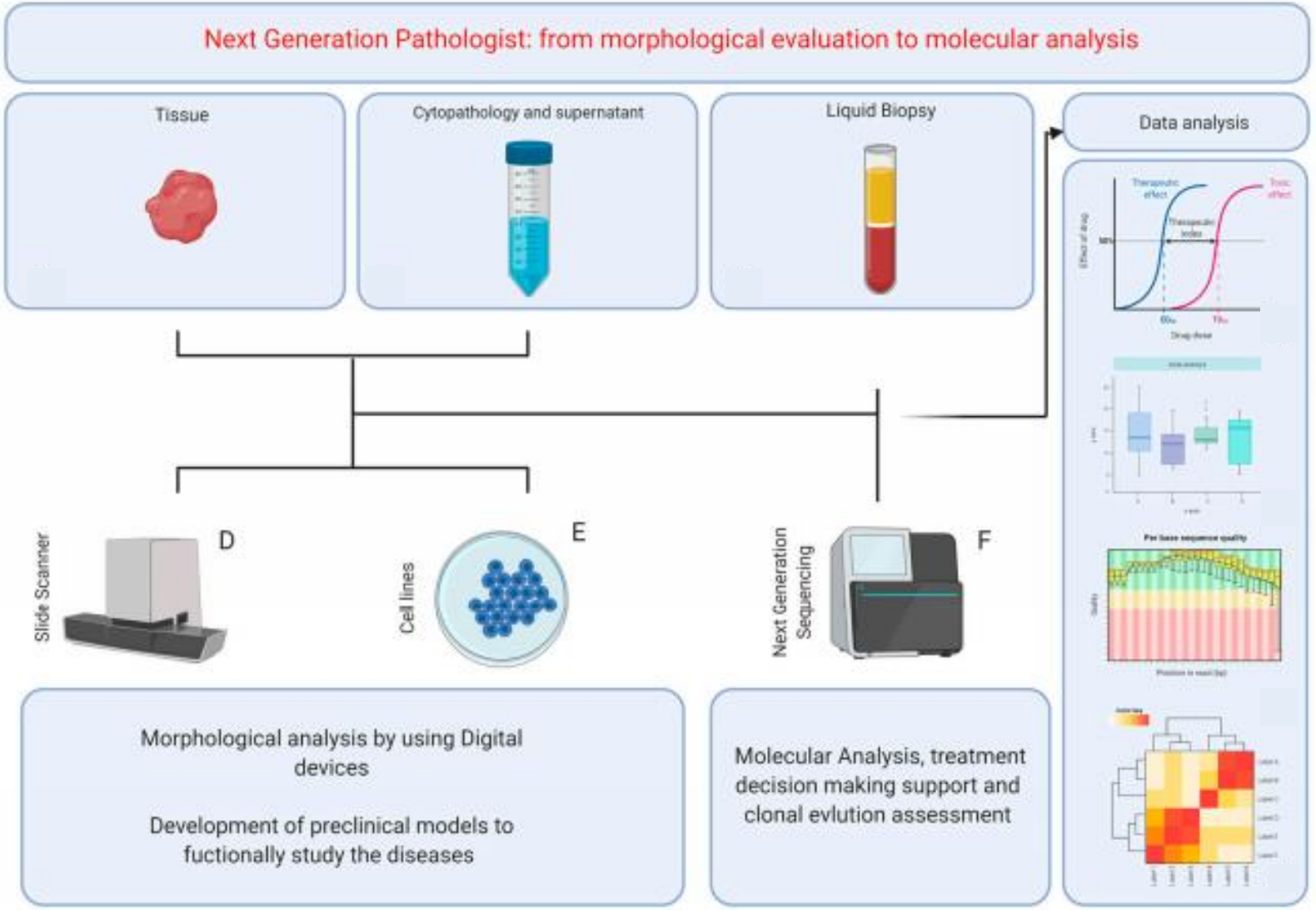
	Breast Cancer	Colorectal cancer	Lung Cancer
Targetable genomic alterations	• Gene amplifications • Gene expression • Genomic signatures	• Gene expression • Genomic signatures • Gene mutations	• Mutations (insertions, deletions, etc..) • Translocations • Gene amplifications • Gene expression • Genomic signatures
Uses	• BC CTCs based on prognosis • cfRNA (miRNAs) in HER2 BC • TEPs in BC diagnosis • *PIK3CA* mutations in ctDNA • *ESR1* mutations • CancerSEEK BC early diagnosis • cfDNA plasma levels and BC stages • BC intra/intertumoural heterogeneity	• Cancer screening • Residual disease evaluation • CRC CTCs prognosis score • Prognostic assessment in early CRC • *RAS* panel mutation status • Prognostic significance of miRNAs • Mechanism of acquired resistance to anti-EGFR drugs	• Drug selection (1L) • Resistance detection • Early disease detection in high-risk populations • bTMB measurement • CTCs and lung cancer prognosis • ctDNA detection in pleural and CSF • PD1/PDL1 expression in CTCs and exosomes • Alteration such as *EGFR, KRAS, BRAF , ALK, ROS1, RET fusions, MET* exon 14 skipping

Step forward to personalised treatment

1L, first line; ALK, anaplastic lymphoma kinase; BC, breast cancer; BRAF, b-Raf proto-oncogene; bTMB, blood-based tumour mutational burden; cfDNA/RNA, cell-free DNA/RNA; CRC, colorectal cancer; CSF, cerebrospinal fluid; CTC, circulating tumour cell; ctDNA, circulating tumour DNA; EGFR, epidermal growth factor; ESR1, estrogen receptor 1; HER2, human epidermal growth factor receptor 2; KRAS, Kirsten rat sarcoma viral oncogene homologue; MET, hepatocyte growth factor receptor; miRNA, micro-RNA; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; Ras, rat sarcoma; RET, rearranged during transfection; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase; TEP, tumour-educated platelets

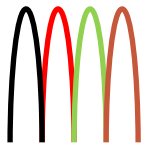
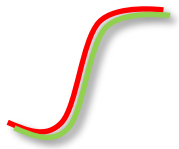
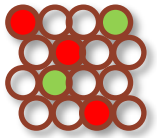
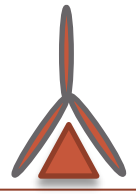
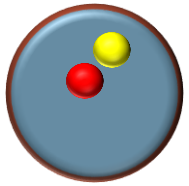
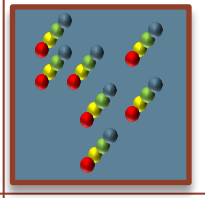
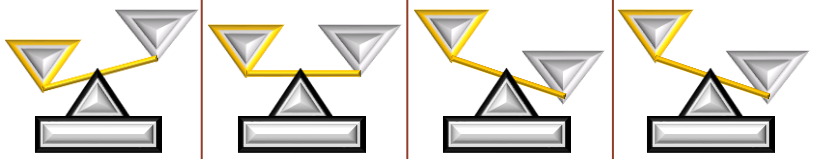
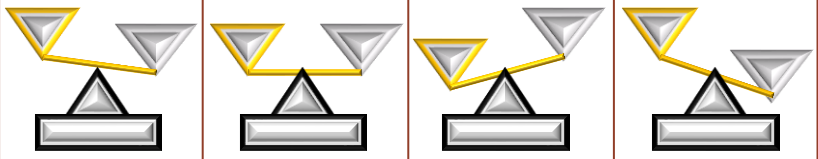
Rolfo C, et al. Crit Rev Oncol Hematol. 2020;151:102978

THE ROLE OF THE PATHOLOGIST IN THE NEXT-GENERATION ERA OF TUMOUR MOLECULAR CHARACTERISATION



Angerilli V, et al. Diagnostics (Basel). 2021;11:339

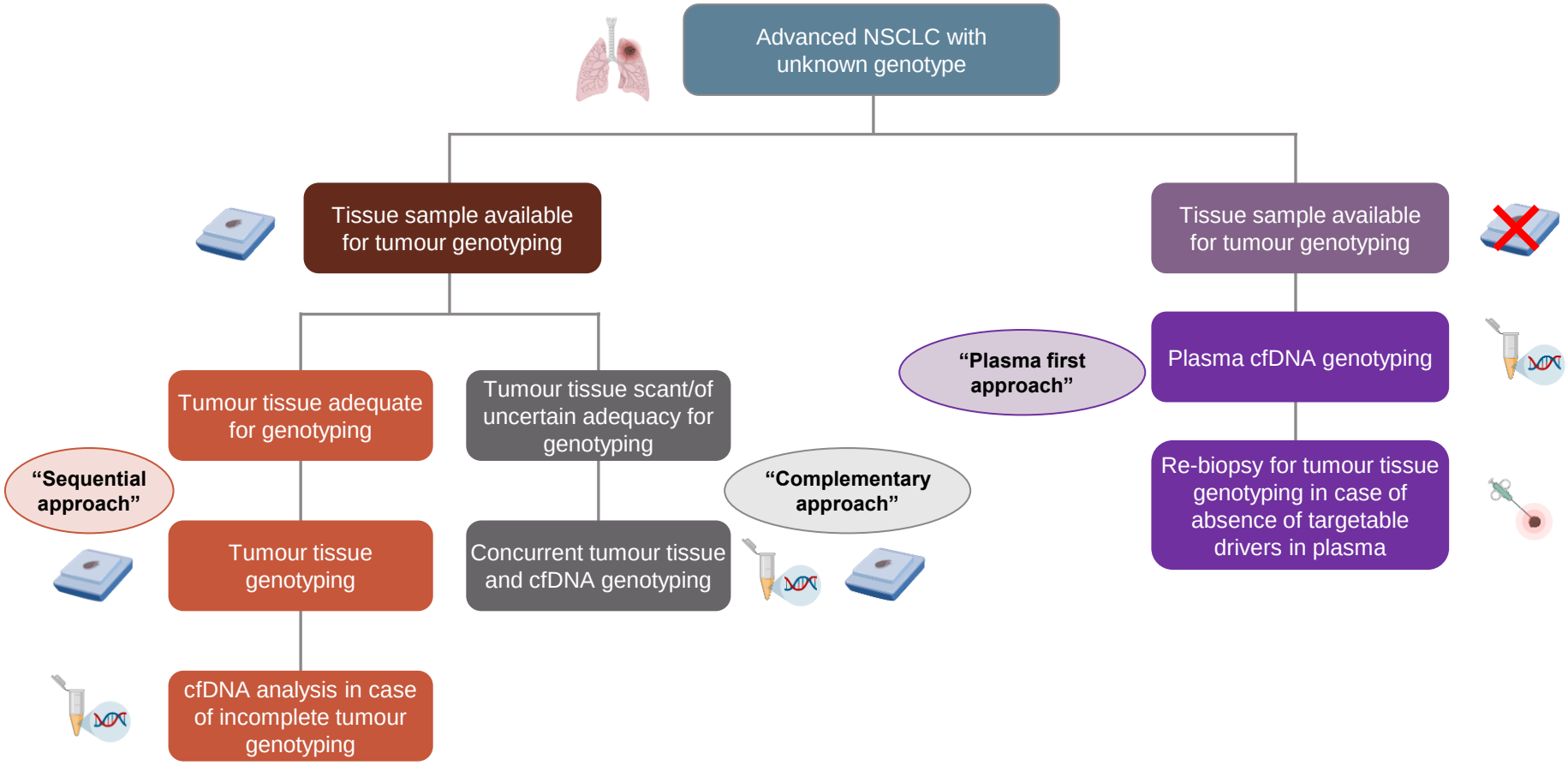
METHODOLOGICAL SCENARIO FOR MOLECULAR TESTING IN NSCLC PATIENTS

	Point mutations and Indels				Protein expression and gene fusions			
	Sanger Sequencing	**Real Time PCR**	**Digital PCR**	**NGS**	**Immuno – histochemistry**	**Fluorescent in situ hybridisation**	**Multiplex digital colour-coded barcode**	**NGS**
								
Limit of detection	10–20%	1–5%	0.1–1%	0.01–5%	Tissue based technique (protein)	Tissue based technique (DNA)	5–10%	0.01–5%
Reference Range	All the mutations present in the analysed gene regions	Only «hot spot» mutations (probe based)	Only «hot spot» mutations (probe based)	All the mutations present in the analysed gene regions	All the fusions - protein (antibody based)	Only specific fusions (probe based)	All the fusions present in the analysed gene regions	All the fusions present in the analysed gene regions
	FP FN	FP FN	FP FN	FP FN	FP FN	FP FN	FP FN	FP FN
								

FN, false negative; FP, false positive; NGS, next generation sequencing; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction

Passiglia F, et al. J Thorac Oncol. 2019;14:2046-2052

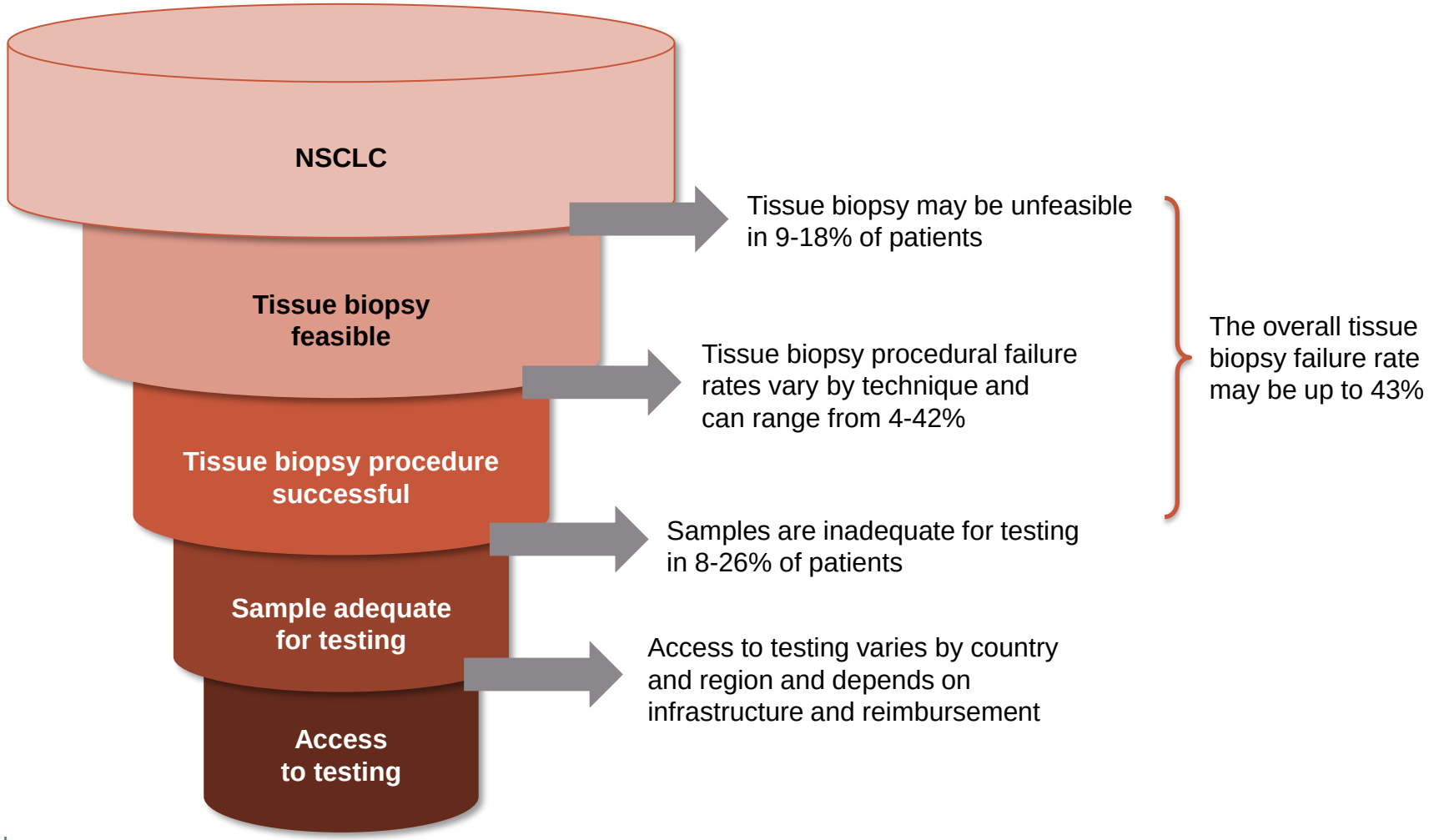
POSITIONING cfDNA GENOTYPING IN TREATMENT NAÏVE ADVANCED NSCLC



cfDNA, cell-free DNA; NSCLC, non-small cell lung cancer

Rolfo C, et al. J Thorac Oncol. 2021;16:1647-1662

WHY PATIENTS MISS OUT ON BIOMARKER TESTING/DIAGNOSIS FROM TISSUE BIOPSY



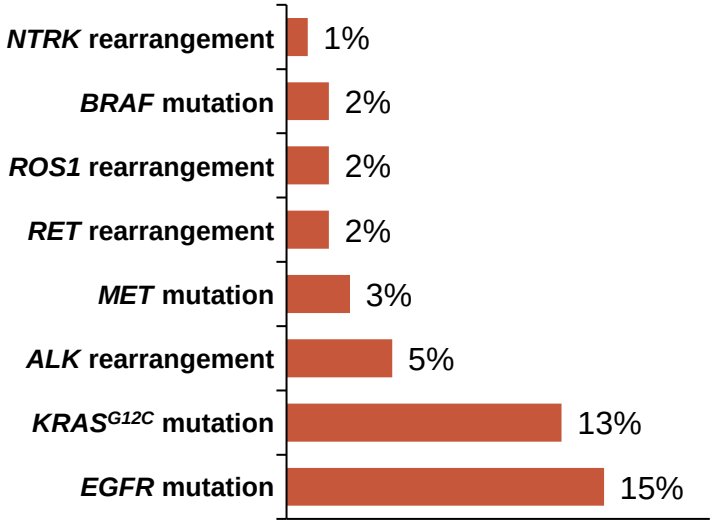
NSCLC, non-small cell lung cancer

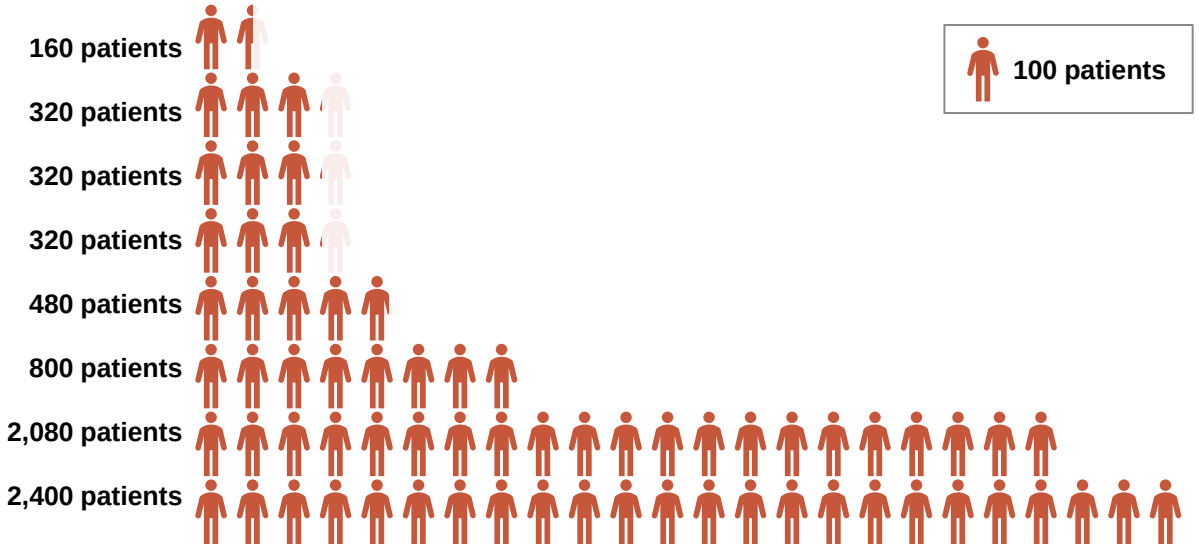
Malapelle U, et al. J Mol Pathol. 2021;2:255-273

ESTIMATE OF PATIENTS IN EUROPE WHO MIGHT BENEFIT FROM LIQUID BIOPSY

In Europe, tissue biopsy may be unfeasible or inadequate, for molecular work-up in an estimated **16,000** patients annually with NSCLC; these patients could potentially benefit from liquid biopsy

Based on published frequencies of NSCLC driver alterations, a molecular diagnosis based on liquid biopsy could allow approximately **6,560** patients annually to benefit from current, emerging, and future targeted treatments

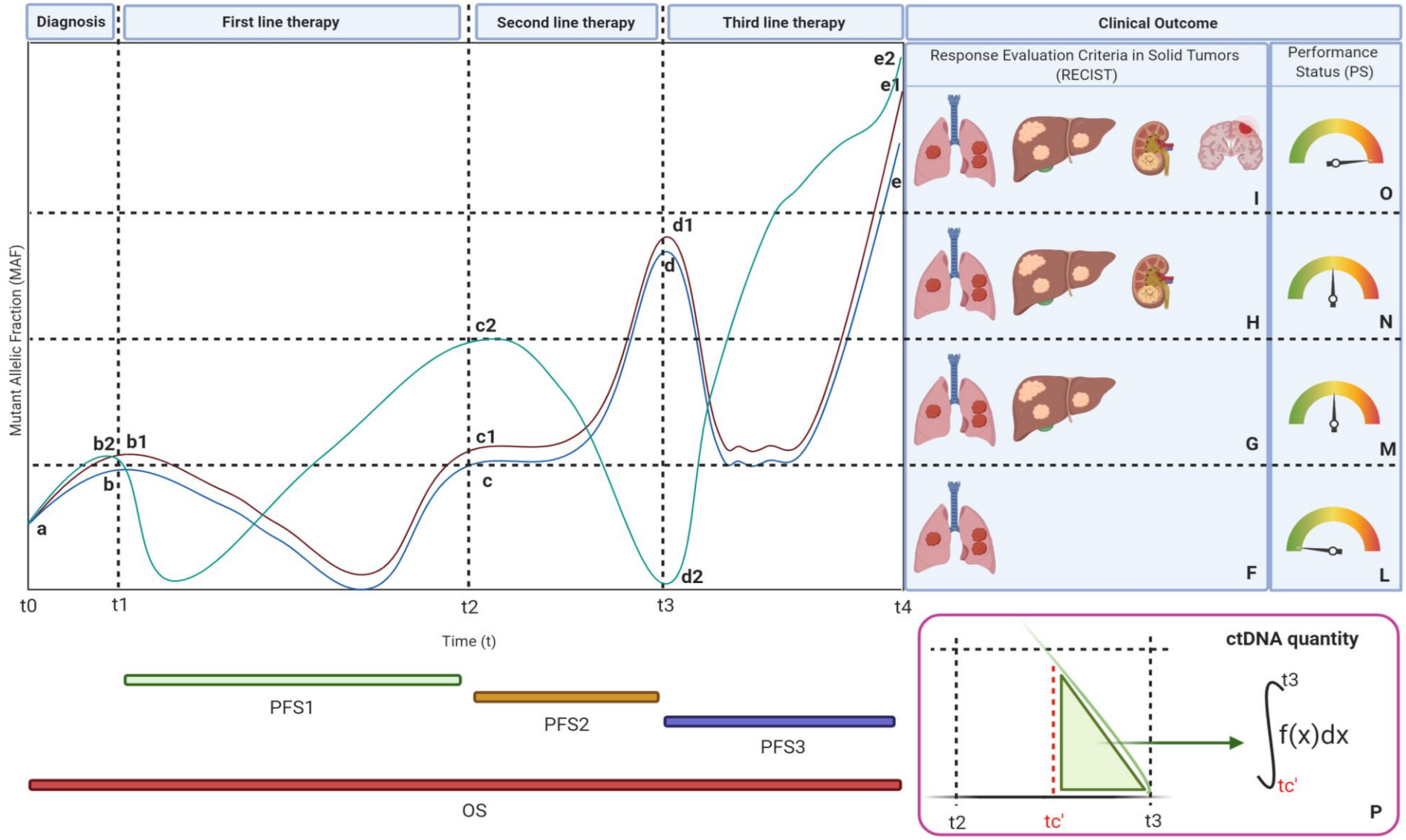




ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homologue; MET, hepatocyte growth factor receptor; NSCLC, non-small cell lung cancer; NTRK, neurotrophic tyrosine receptor kinase; RET, rearranged during transfection; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase

Malapelle U, et al. J Mol Pathol. 2021;2:255-273

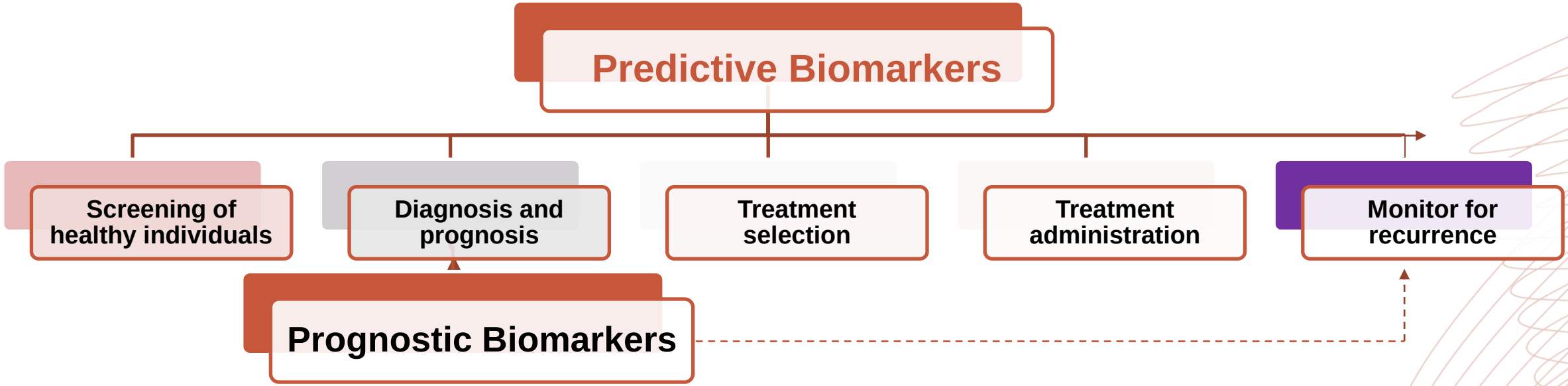
FOLLOWING TUMOUR EVOLUTION THROUGH LIQUID BIOPSY



ctDNA, circulating tumour DNA; OS, overall survival; PFS(1/2/3), progression-free survival (from start of first-, second-, third-line of treatment)

Malapelle U, et al. Manuscript in preparation

EMERGING MOLECULAR BIOMARKERS IN BREAST CANCER



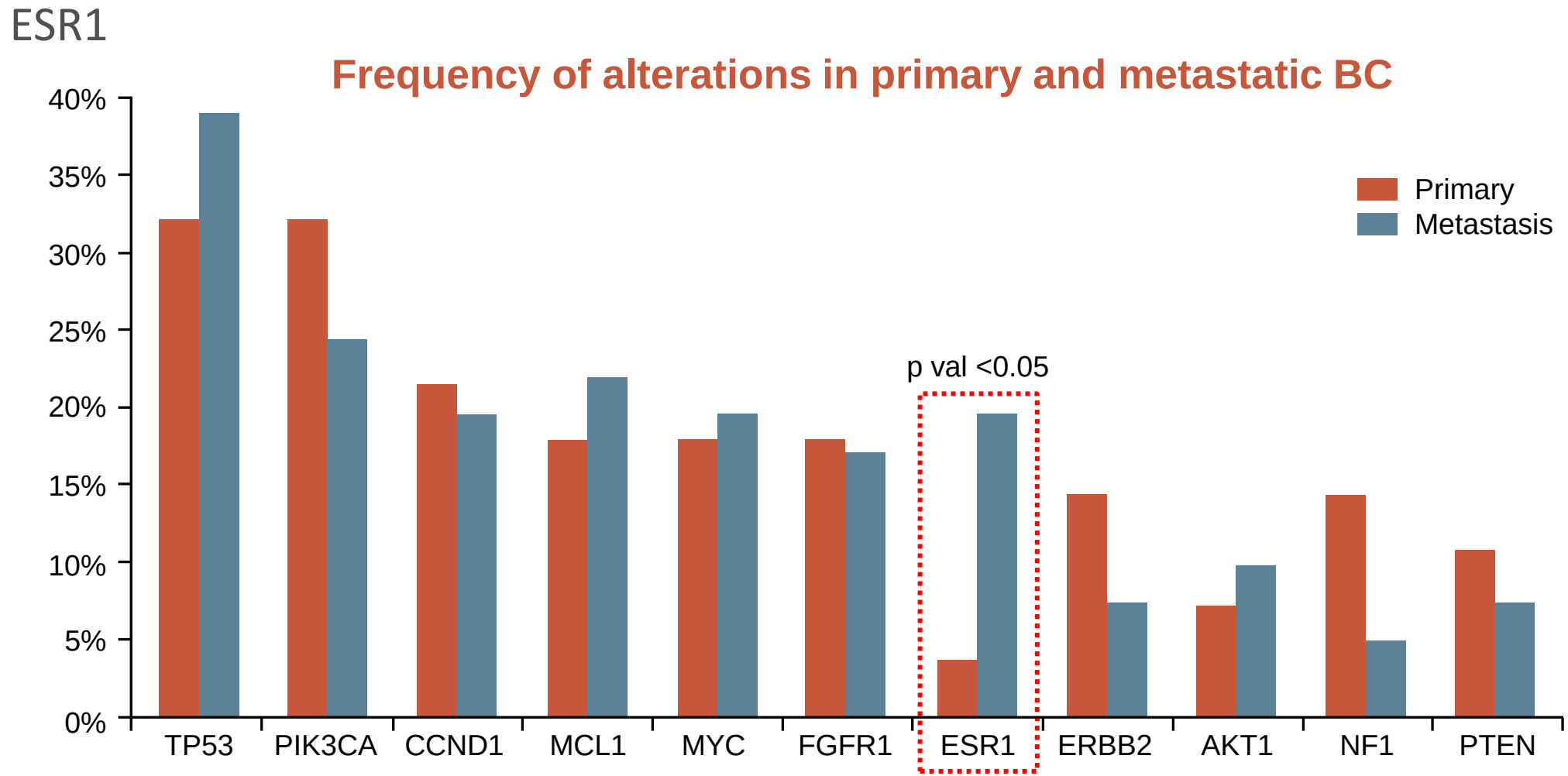
Examples of predictive biomarkers in mBC	ER	BRCA1/2	HER2	21-Gene recurrence score	PD-L1	*ESR1*
Examples of prognostic biomarkers in mBC	HER2	ER/PR	Ki-67	Gene expression panels	DNA methylation	

BRCA1/2, BReast CAncer gene 1/2; ER, estrogen receptor; ESR1, estrogen receptor 1; HER2, human epidermal growth factor receptor 2, mBC, metastatic breast cancer; Ki-67, antigen Kiel 67; PD-L1, programmed death-ligand 1; PR, progesterone receptor

American Cancer Society. Breast Cancer Facts & Figures 2019-2020. Atlanta: American Cancer Society, Inc. 2019; Ross D, et al. Modern Pathology 2018; 32: 81-87; Fu X, et al. Front Cell Dev Biol 2022; 10:813457

ESR1 MUTATION RATE IN mBC PATIENTS

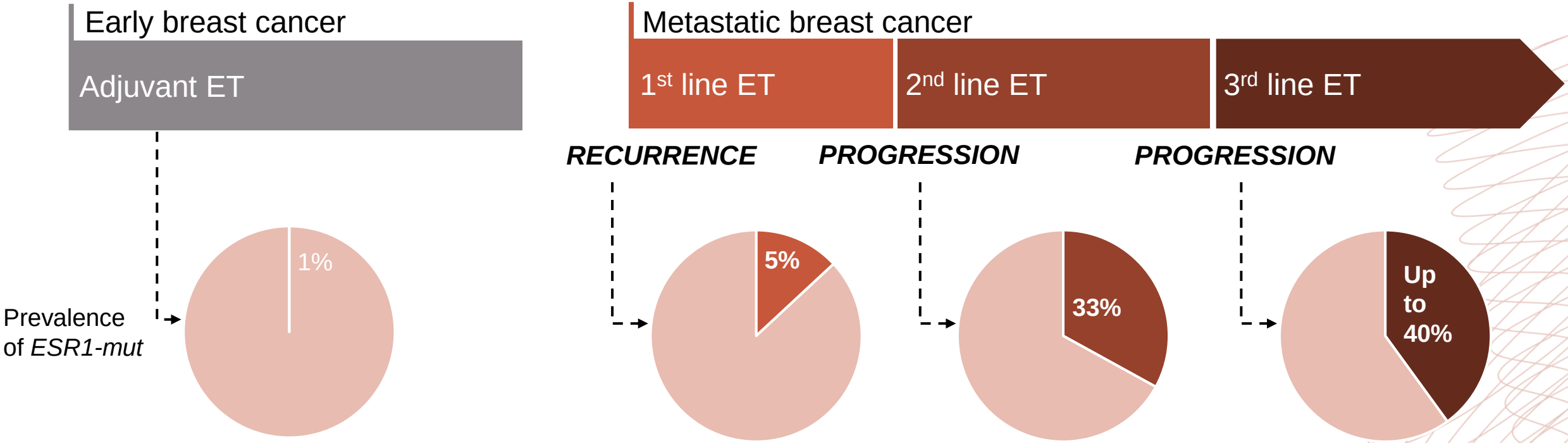
THERE IS A SIGNIFICANT INCREASE IN DETECTION OF ESR1 ALTERATIONS IN METASTATIC SAMPLES



AKT1, AKT serine/threonine kinase 1; CCND1, cyclin D1; ERBB2, Erb-B2 Receptor Tyrosine Kinase 2; ESR1, estrogen receptor 1; FGFR1, fibroblast growth factor receptor 1; (m)BC, (metastatic) breast cancer; MCL1, induced myeloid leukaemia cell differentiation protein; MYC, myelocytomatosis oncogene; NF1, neurofibromin 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; PTEN1, phosphatase and tensin homologue; TP53, tumour protein 53; val, value

Jeselsohn R, et al. Clin Cancer Res. 2014;20:1757-1767

LONGER EXPOSURE TO ET IN 1L INCREASES THE CHANCE OF DEVELOPING *ESR1* MUTATION DURING TREATMENT

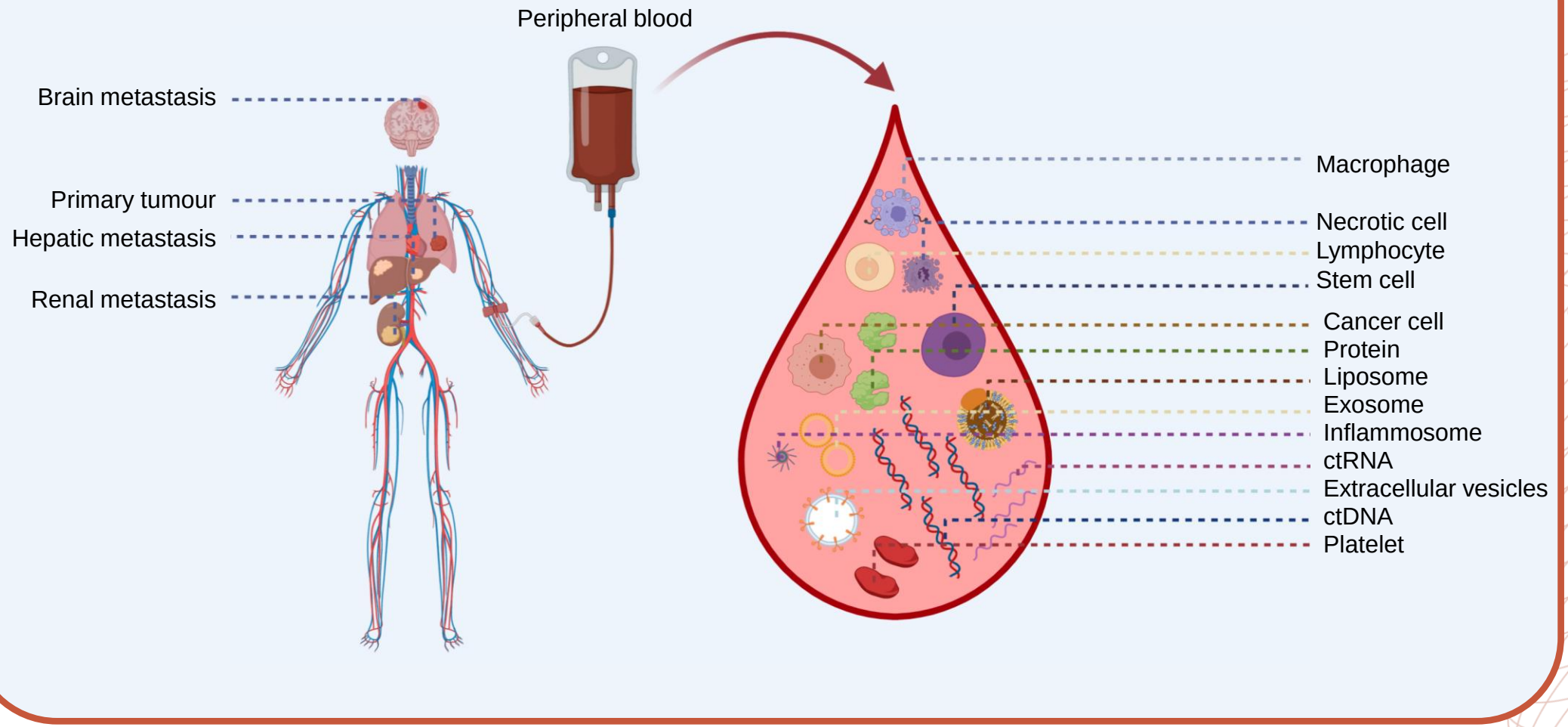


ESR1-mut occur almost exclusively after aromatase inhibitors in the metastatic setting[5]
Testing for _ESR1_-mut should occur at each progression on ET if not detected[11-13]

1L, first line; ET, endocrine therapy; ESR1, estrogen receptor 1

Modified from: 1. Jeselsohn R, et al. Clin. Cancer Res. 2014;20:1757–1767; 2. Jeselsohn R, et al. Cancer Cell. 2018;33:173–186; 3. Allouchery V, et al. Breast Cancer Res. 2018;20:40; 4. Schiavon G, et al. Sci Transl Med. 2015;7(313);313ra182. 5. Brett JO, et al. Breast Cancer Res. 2021;23(1):85; 6. Toy W, et al . Nat Genet. 2013;45(12):1439–1445; 7. Bidard FC et al. J Clin Oncol 2022;40:3246–3256; 8. Jhaveri et al, Annals of Oncology (2023) 34 (suppl_2): S334-S390. 10.1016/annonc/annonc1299; 9. Lin et al, Annals of Oncology (2023) 34 (suppl_2): S334–S390; 10.1016/annonc/annonc1299; 10. Bhave et al, SABCS 2023_PO2-1605; 11. Lee N, et al Int J Mol Sci. 2020;21(22):8807; 12. Gennari A, et al. Ann Oncol. 2021;32(12):1475–1495; 13. Burstein HJ, et al J Clin Oncol. 2023;41(18):3423–3425.

THE “CANCER WORLD” IN A DROP

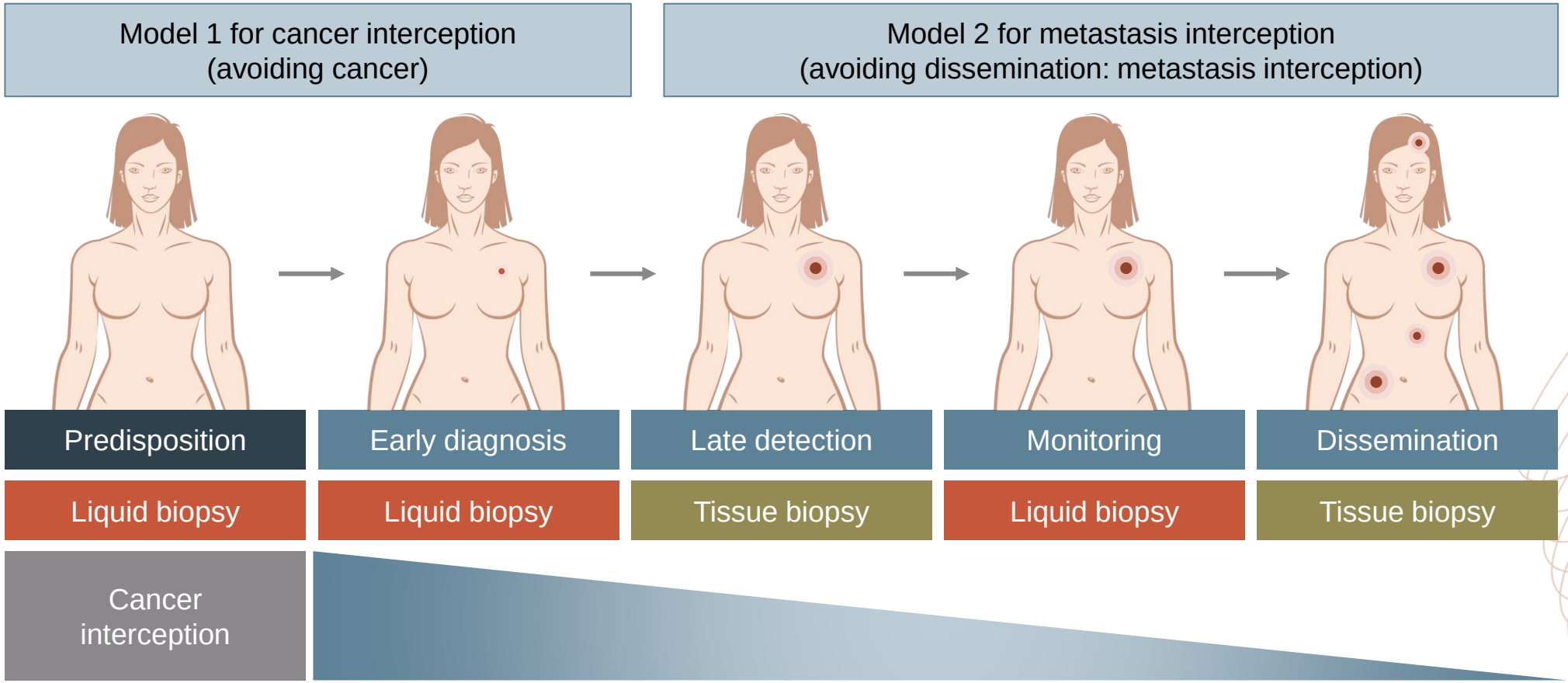


ctDNA, circulating tumour DNA; ctRNA, circulating tumour RNA

Malapelle U, et al. Manuscript in preparation

LIQUID VS SOLID BIOPSIES IN CANCER INTERCEPTION

CHOICE OF SAMPLE DEPENDS ON REQUIREMENT



Adapted from: Serrano MJ, et al. Cancer Discov. 2020;10:1635-1644

SUMMARY

- Liquid biopsy represents a dynamic, less invasive and easy-to-manage diagnostic tool for molecular analysis of actionable genes in clinical practice
- Among the heterogeneous landscape of technical approaches, dPCR and NGS platforms play a pivotal role in the diagnostic testing strategy of liquid biopsies
- *ESR1* molecular analysis is essential in the therapeutic algorithm for ER+ HER2- BC patients
- Tissue and liquid biopsy derived molecular records are integrative for optimising clinical stratification of solid tumour patients

BC, breast cancer; dPCR, digital polymerase chain reaction; ER, oestrogen receptor; ESR1, oestrogen receptor 1; HER2, human epidermal growth factor 2; NGS, next generation sequencing

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