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Turning the Tides

The Importance of Sensitive *ESR1* Liquid Biopsy Surveillance in Overcoming Treatment-Resistant, Hormone Receptor-Positive Metastatic Breast Cancer

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October 2024

Agenda

Bringing High-Sensitivity *ESR1* Mutation Testing to qPCR

- *ESR1* Mutations in HR+ Metastatic Breast Cancer
- Improved Progression-Free Survival through *ESR1* Monitoring
- Heightened *ESR1* Sensitivity via cfDNA and exoRNA

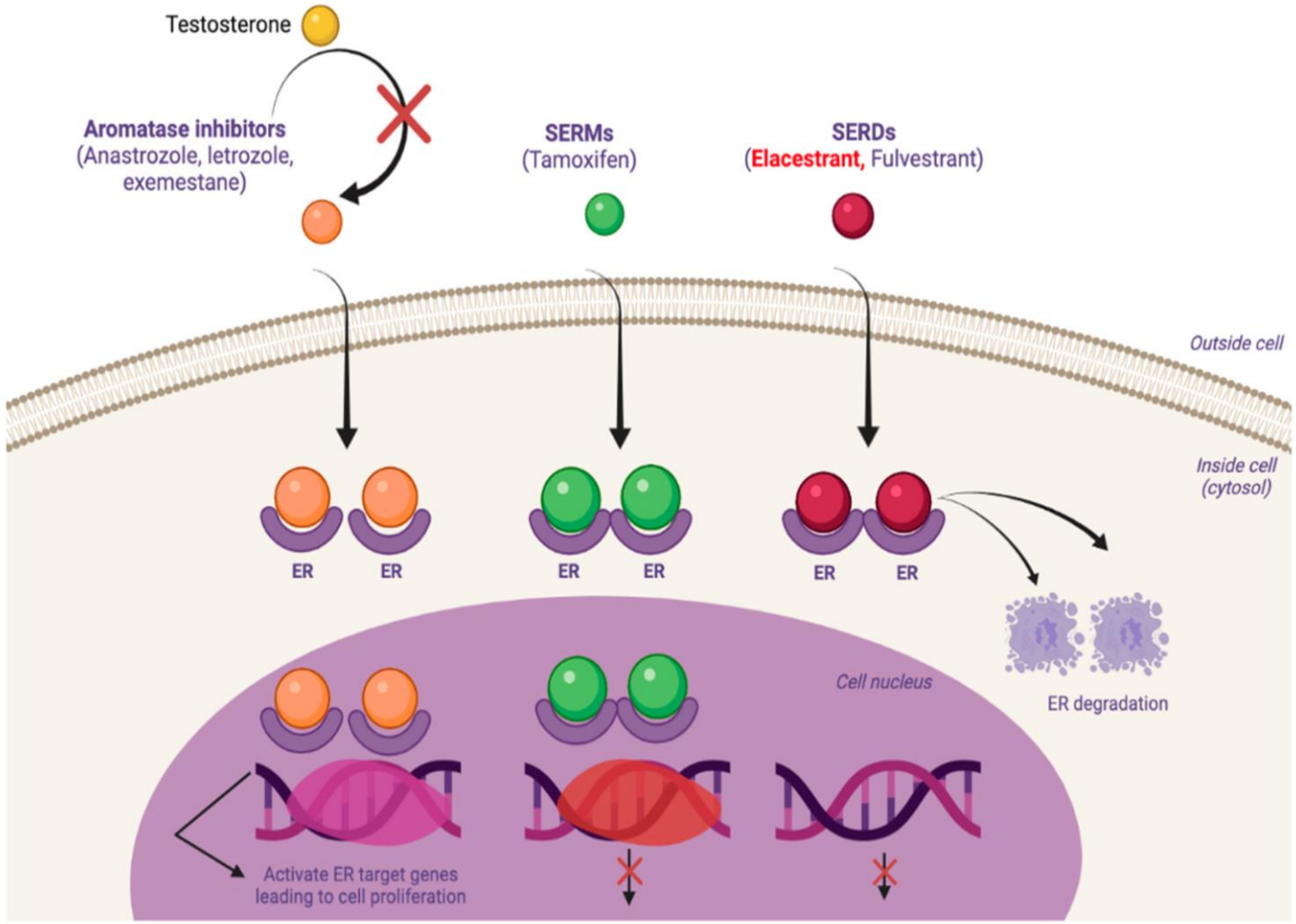
Goals of this session

- Understand how *ESR1* mutations lead to frontline endocrine therapy resistance in hormone receptor-positive metastatic breast cancer (HR-positive mBC)
- Learn how clinical trials showing improved progression-free survival through *ESR1* monitoring to inform treatment decisions and approval of novel second line therapies have influenced NCCN guidelines and the role of *ESR1* liquid biopsy in HR-positive, HER2-negative mBC
- Examine real-time PCR as one example of a cost-effective and efficient approach for *ESR1* liquid biopsy surveillance and how combining cfDNA with exosomal RNA can improve test sensitivity

ESR1 Mutations in HR+ Metastatic Breast Cancer

Primary Treatments for HR+ BC

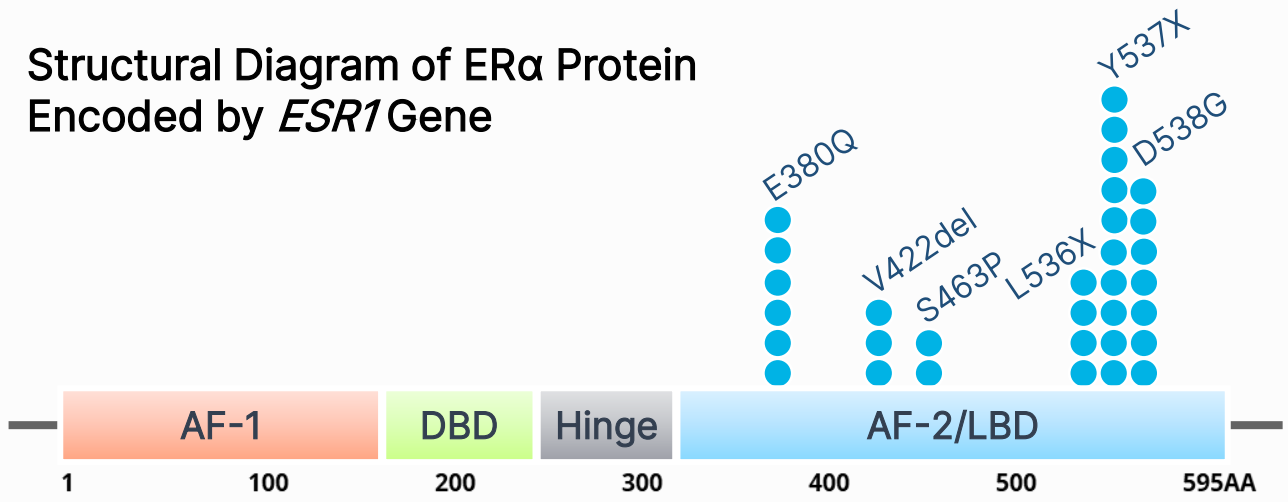
Endocrine therapy is the backbone of treating HR+ BC. There are three distinct mechanisms of action for how these therapies downgrade activity of the ER and arrest progression of disease.



- Endocrine therapies (ET) in breast cancer fall into three categories:
  - Aromatase inhibitors (AIs)
  - Selective estrogen receptor modulators (SERMs)
  - Selective estrogen receptor degraders (SERDs)
- AIs block estrogen production by inhibiting the aromatization of androgens to estrogens.
- SERMs (tamoxifen) competitively inhibit the binding of estrogen to the ER.
- SERDs produce a reduction of SERD-bound ER ability to translocate to the nucleus, inhibiting transcription of ER-regulated genes. The SERD-bound ER subsequently undergoes degradation.

ESR1 Mutations are the Leading Cause of Primary Treatment Resistance in HR+ mBC

~70% of all Breast Cancers are HR+ HER2-



AF-1: activation function-1 | **AF-2**: activation function-2 | **DBD**: DNA-binding domain | **LBD**: ligand-binding domain

Estrogen is the ligand which binds to the Estrogen Receptor (ER). *ESR1* mutations in the LBD turn the ER "on" even in the absence of estrogen. Thus, via $ESR1_{mut}$, estrogen-dependent disease becomes estrogen-*independent*.

- *ESR1* mutations are extremely rare in treatment-naïve disease
- Up to 40% of HR+ mBC patients with prolonged exposure to aromatase inhibitors will develop *ESR1* mutations
- Acquired mutations in *ESR1* are concentrated in the ligand-binding domain (LBD), and results in an "always on" estrogen receptor

HR+ mBC = hormone receptor-positive, metastatic breast cancer | AI = aromatase inhibitor

Nat Rev Clin Oncol. 2015 Oct;12(10):573-83.

ESR1 Mutation Testing Now Included in NCCN Guidelines for HR+ mBC

As *ESR1* mutations are recommended to be tested to guide therapy decisions following front-line treatment failure, the NCCN recommends testing via LBx with PCR and NGS as suitable detection methods

National Comprehensive Cancer Network®

NCCN Guidelines Version 4.2023
Invasive Breast Cancer

ADDITIONAL TARGETED THERAPIES AND ASSOCIATED BIOMARKER TESTING FOR RECURRENT UNRESECTABLE (LOCAL OR REGIONAL) OR STAGE IV (M1) DISEASE

Biomarkers Associated with FDA-Approved Therapies

Breast Cancer Subtype	Biomarker	Detection	FDA-Approved Agents	NCCN Category of Evidence	NCCN Category of Preference
HR-positive/ HER2-negative[v]	*PIK3CA* activating mutation	PCR (blood or tissue block if blood negative)	Alpelisib + fulvestrant[w]	Category 1	Preferred second- or subsequent-line therapy
HR-positive/ HER2-negative[x]	*ESR1* mutation	NGS, PCR (blood)	Elacestrant	Category 2A	Other recommended regimen

- Following FDA-approval of Elacestrant, the NCCN guidelines were quickly amended to recommend *ESR1* as a validated biomarker to guide downstream treatment decisions
- Since *ESR1* mutations are extremely rare in primary tumors, which are often molecularly characterized using tissue, the NCCN recommends using a blood test (liquid biopsy) to detect *ESR1* mutations

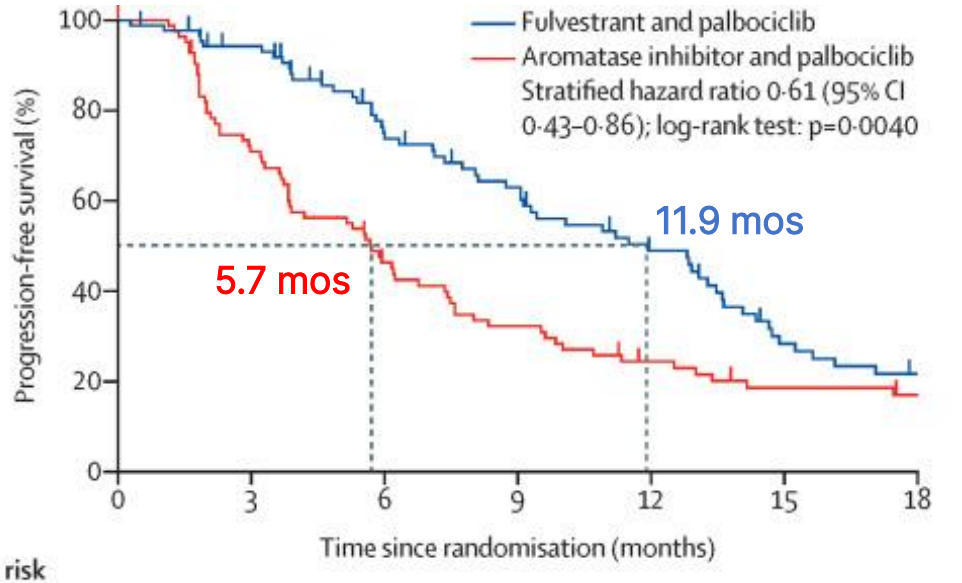
Improved Progression-Free Survival thru *ESR1* Monitoring

PADA-1: Early Detection = Early Intervention = Better Outcomes

Significant benefits to patient outcomes are being realized through monitoring for actionable/ resistance mutations and switching therapy *before* radiological progression

Phase III PADA-1 Trial

Test benefit of therapy change based on detection of *ESR1* mutations in HR+ mBC



Number at risk (number censored)							
Fulvestrant and palbociclib	88 (0)	78 (5)	57 (11)	46 (13)	32 (17)	17 (19)	12 (20)
Aromatase inhibitor and palbociclib	84 (1)	58 (2)	36 (4)	25 (4)	17 (6)	12 (7)	10 (8)

Lancet Oncol. 2022 Nov;23(11):1367-1377.

HR+ BC = hormone receptor-positive breast cancer

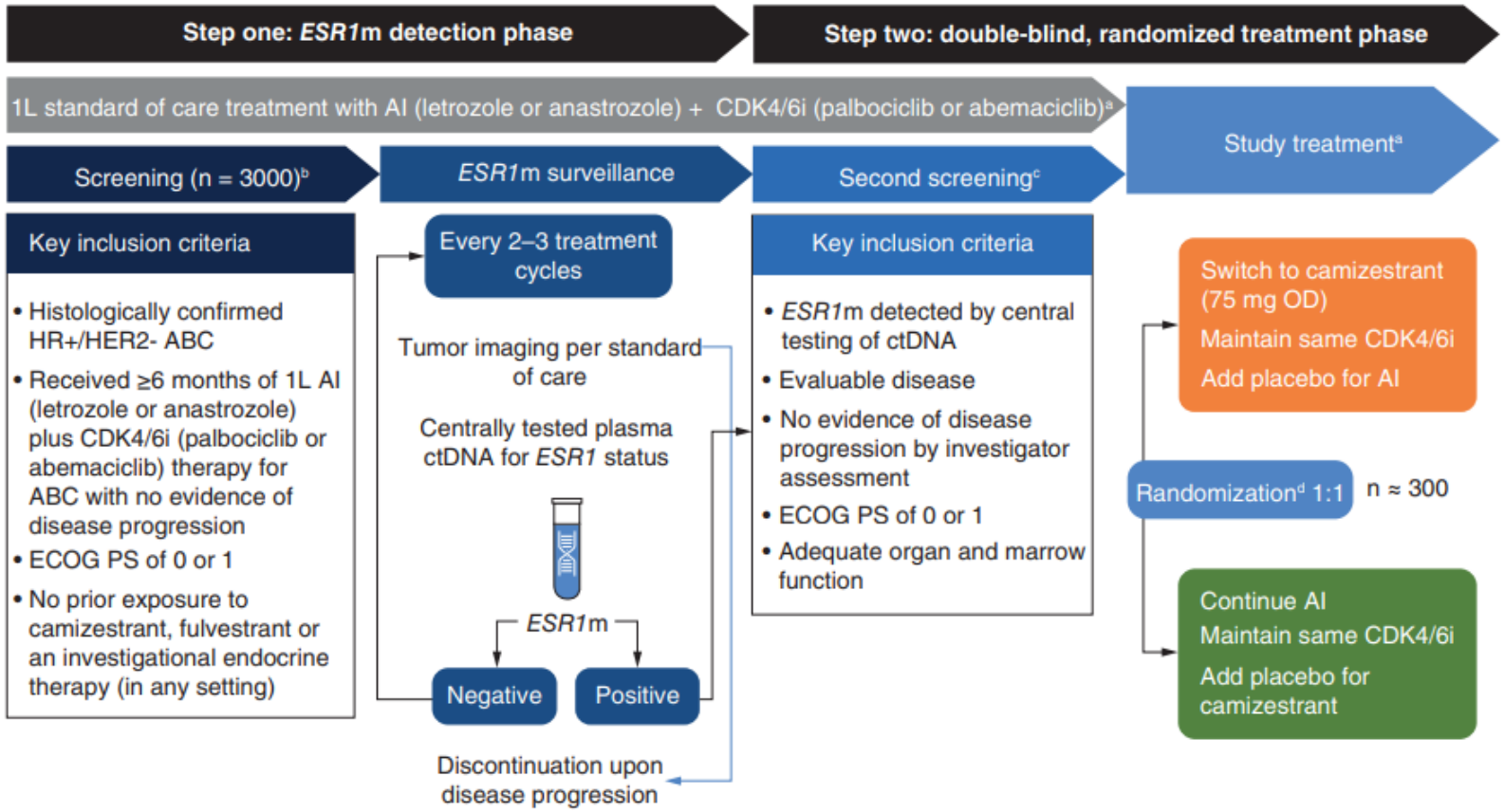
ESR1 mutations can be detected in plasma up to 12 mos before radiological progression, highlighting the clinical benefit of routine testing

- In PADA-1, serial ctDNA testing was used to identify *ESR1* mutations in mBC patients on standard therapy
- Patients found to have a mutation *prior to disease progression* were randomly assigned to continue treatment or switch therapies
- **Switching therapy resulted in a doubling of median progression-free survival**
- Patients who switched to the new regimen *at radiological progression* only had a median progression-free survival of 3.5 months (**smaller benefit if wait for progression vs switch at first detection of *ESR1* mutation)**

SERENA-6: Building Upon PADA-1 with Novel SERD Therapy

Registrational Ph III trial aims to demonstrate improvement to PFS by switching to camizestrant at first detection of *ESR1* mutation vs waiting for clinical progression

SERENA-6 Clinical Trial Design

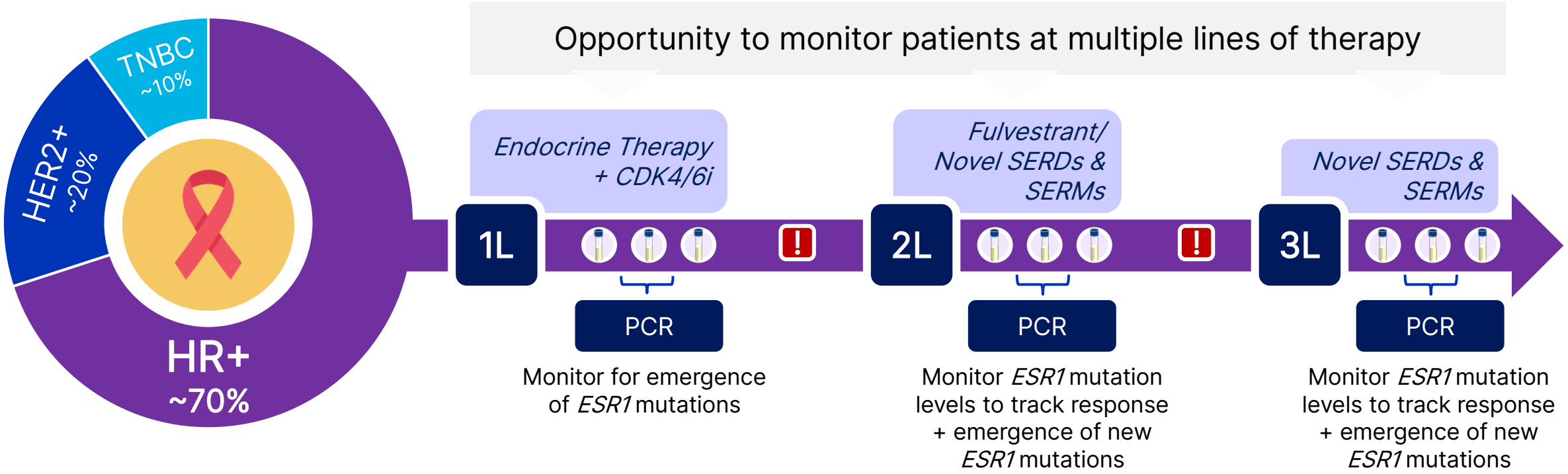


- In **STEP ONE**, patients who had received an AI plus CDK4/6i for ≥6 mos are screened for the first set of inclusion and exclusion criteria
- Those who are eligible are tested for the presence of 11 key *ESR1* mutations in ctDNA every 2–3 treatment cycles (~8–12 weeks)
- Continuous ctDNA monitoring will occur until disease progression is detected → **STEP TWO**
- ESR1m patients are enrolled in **STEP TWO** and randomized 1:1 to SOC or switch to Camizestrant+ CDK4/6i
- Treatment regimen in **STEP TWO** continues until clinical progression, death, or withdrawal

Future Oncol. 2023 Mar;19(8):559-573.

ESR1 Mutation Monitoring in HR+ Breast Cancer

Large patient population presents opportunity for monitoring at multiple lines of treatment



- *ESR1* mutations are acquired from resistance to ET (esp AIs) and are not present in the primary tumor
  - Quantitative measurement (e.g., MAF%) is not as critical; need to know whether *ESR1* mutations are present or not (qualitative testing is sufficient)
- *ESR1* mutations emerge far in advance of radiological progression (~9-12 mos)
  - Early detection of mutations via monitoring before patients show signs of progression can have meaningful clinical benefit → can rescue patients before progression

Heightened *ESR1* Sensitivity via cfDNA and exoRNA

Key Liquid Biopsy (LBx) Terms

CTCs

- **Circulating tumor cells**
- Free-floating tumor cells (not tumor-bound); relatively few in number

cfDNA/ccfDNA

- **Cell-free DNA / circulating cell-free DNA**
- DNA freely circulating in the bloodstream; can come from many sources

ctDNA

- **Circulating tumor DNA**
- Subset of cfDNA from tumor shedding/ necrosis; more prevalent in adv. disease

Exosomes

- Type of **extracellular vesicle (EV)** containing abundance of NAs, proteins, and more
- In relatively great abundance and can reflect disease dynamics in real time

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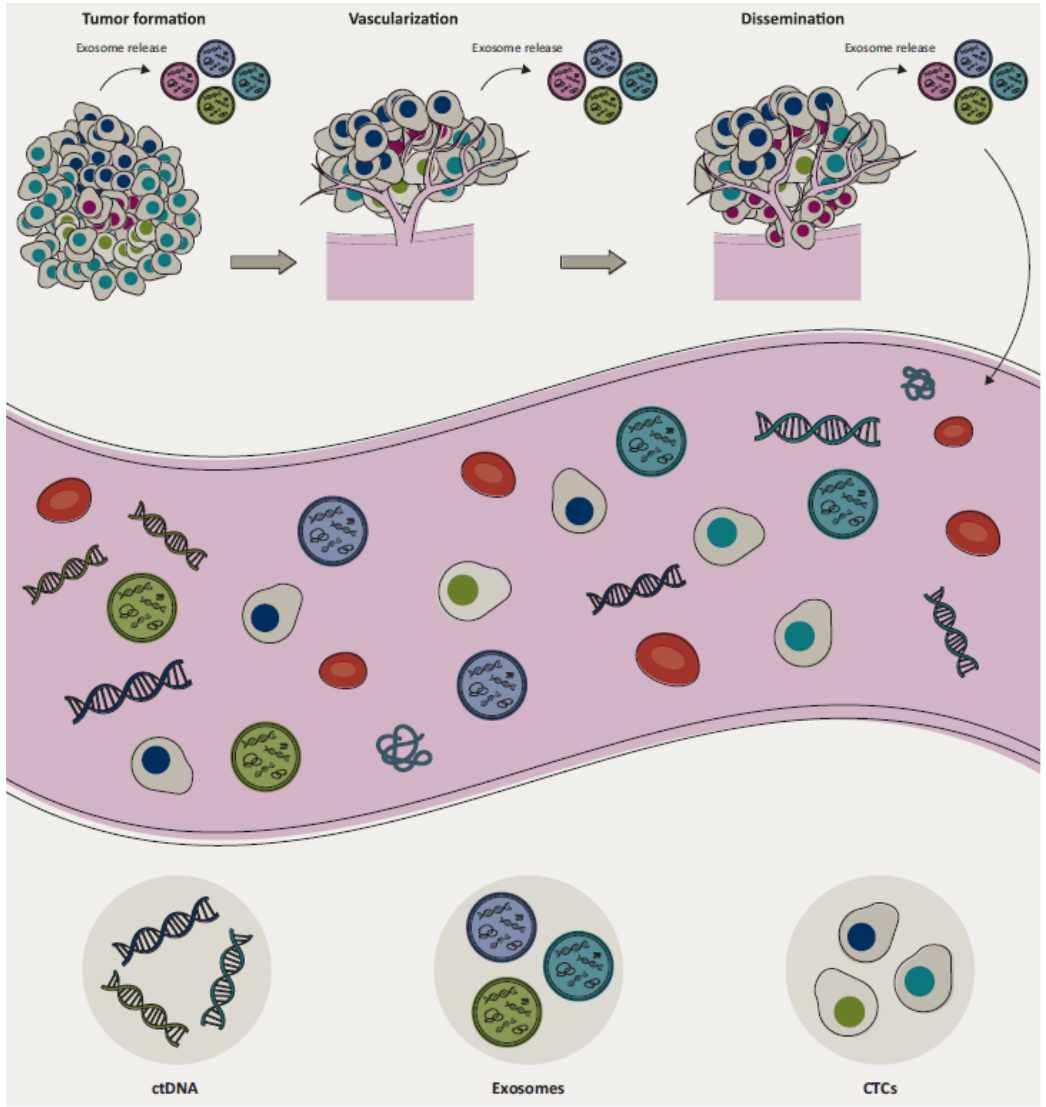
Leveraging Exosomes for a Differentiated Approach

Extracellular vesicles (EVs) are actively secreted by the cell and carry a snapshot of the body's transcriptome (exosomal RNA); combining exosomal NA + ctDNA can enhance variant detection



ctDNA is released from the dying cells through apoptosis and/or necrosis

Allows detection of:

- Mutations/SNPs
- Methylation
- Fragmentomics

Exosomes are actively released from cells

Allows detection of:

- Mutations/SNPs
- RNA transcription pathway analysis
- Protein
- Glycomics/metabolomics
- Enable marker-based enrichment

One cell can secrete tens of thousands of exosomes per day. That same cell secretes no ctDNA until it dies, when it secretes just *two copies* of ctDNA.

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Complete Assay Workflow Optimized for any Laboratory

We aim to provide a complete solution to support targeted *ESR1* mutation detection using widely installed qPCR instruments

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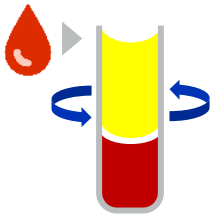
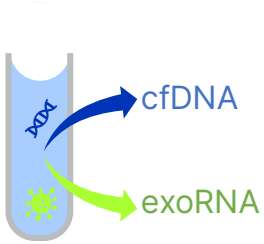
Sample Collection → cfDNA+ exoRNA Isolation → RT-qPCR → Data Analysis



Whole blood collected and spun down to extract plasma



ExoLution Plus™ Kit* extracts both cfDNA + exoRNA in a single step

ESR1 exoMut Kit* contains all reagents to detect target(s) of interest; samples can be batched to maximize throughput

Push-button software to automate and streamline results interpretation for the end user

Complete Solution Includes Sample Prep, RT & PCR Reagents, and Software

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Introducing ExoLution™ Plus cfDNA + exoRNA Isolation Kit*

Proprietary ExoLution Plus Kit* allows for the co-isolation of cfDNA + exoRNA in a single step

New LBx Sample Isolation Kit for Co-isolation of cfDNA + exoRNA

- New sample isolation kit to be launched with QuantideX® qPCR *ESR1* exoMutation Kit*. Will support the growing portfolio of targeted liquid biopsy assays using qPCR
- Proprietary, column-based method for **co-isolation of cell free DNA (cfDNA) & exosomal RNA (exoRNA)** from human plasma
- Provided with purchase of the assay
- Kit will be inclusive of necessary reagents and key consumables to support 50 isolations
- All components stored at room temperature (15 – 25°C)

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Product Overview: QuantideX® qPCR *ESR1* exoMutation Kit*

Exosome-powered RT-qPCR test enabling ultra-sensitive detection of the most common & clinically relevant *ESR1* variants from plasma

Category	Description
Coverage	11 *ESR1* ligand-binding domain mutations: E380Q; V422del; S463P; L536H/P/R (X); Y537S/N/C/D (X); D538G
Kit Size	50 reactions
Analyte	cfDNA + exoRNA
Sample	2 – 4 mL Plasma [K_2EDTA (purple top) or PAXgene® Blood ccfDNA Tube]
Assay Sensitivity	≤0.1% MAF (~5 copies/mL)
Platform	ABI 7500 Fast Dx + QuantStudio 5 Dx + QuantStudio 7 Pro Dx
Workflow	Sample-to-answer in one day (<6 hours)
Regulatory	RUO
Data Analysis	QuantideX qPCR *ESR1* exoMutation Analysis Module

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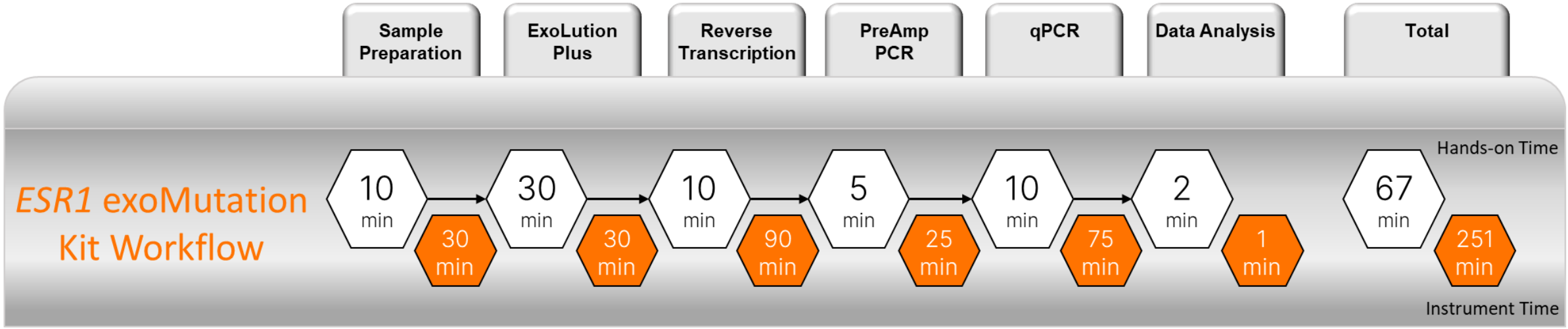
Targeted *ESR1* Testing Enables Rapid, Simple Workflow

Exosome-powered RT-qPCR test enabling ultra-sensitive detection of the most common & clinically relevant *ESR1* variants from plasma



ExoLution Plus cfDNA + exoRNA Isolation Kit*

QuantideX qPCR *ESR1* exoMutation Kit*

QuantideX qPCR *ESR1* exoMutation Analysis Module*

ESR1 results in a single workday → Sample-to-Results <6 hrs with ~1 hr hands-on time

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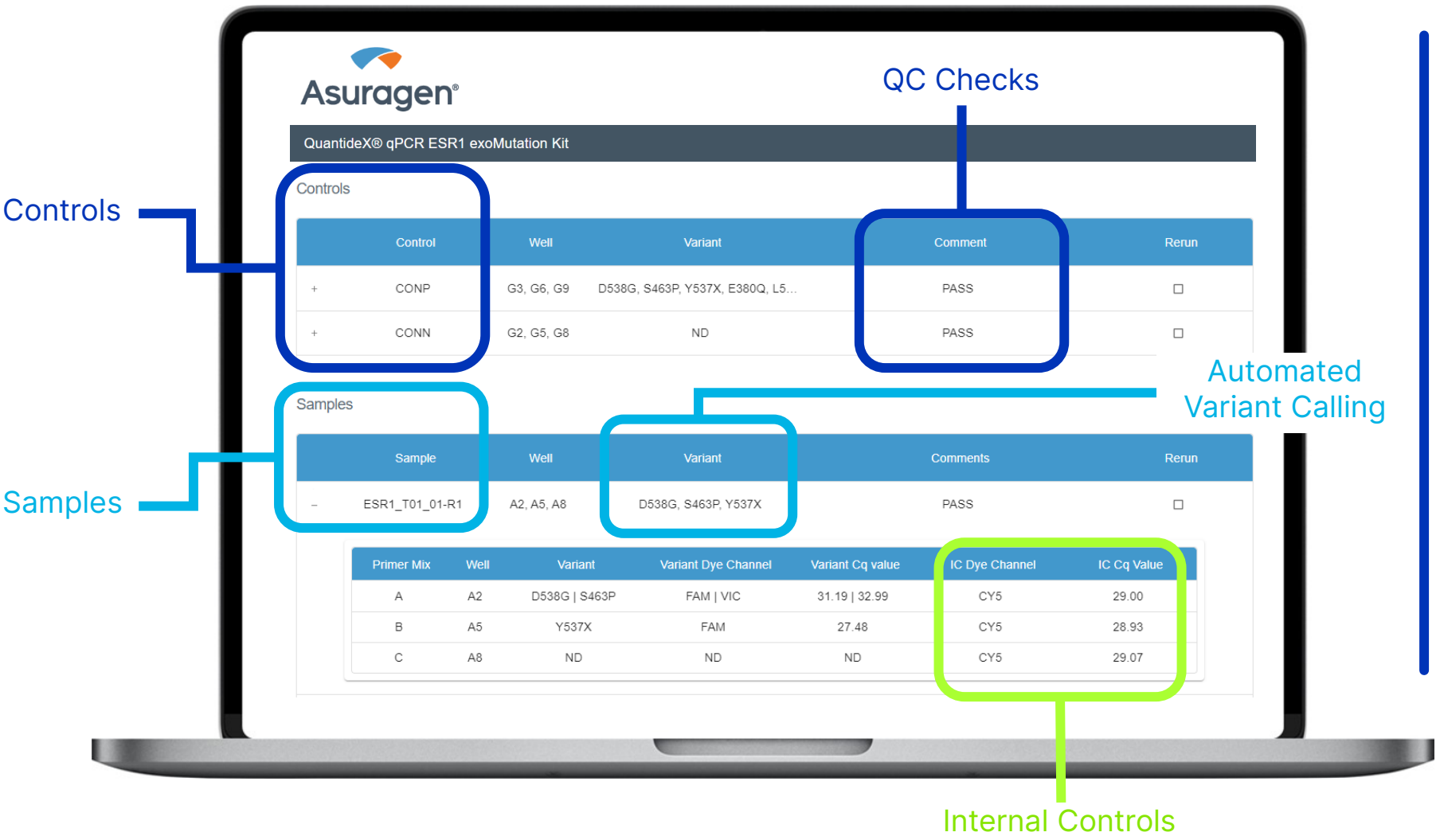
Software Enables Comprehensive, Push-Button Analysis and QC



QuantideX

qPCR *ESR1* exoMutation Analysis Module

- Automated variant calling
- Batch Control QCs
- Export results in LIMS-compatible format (CSV)

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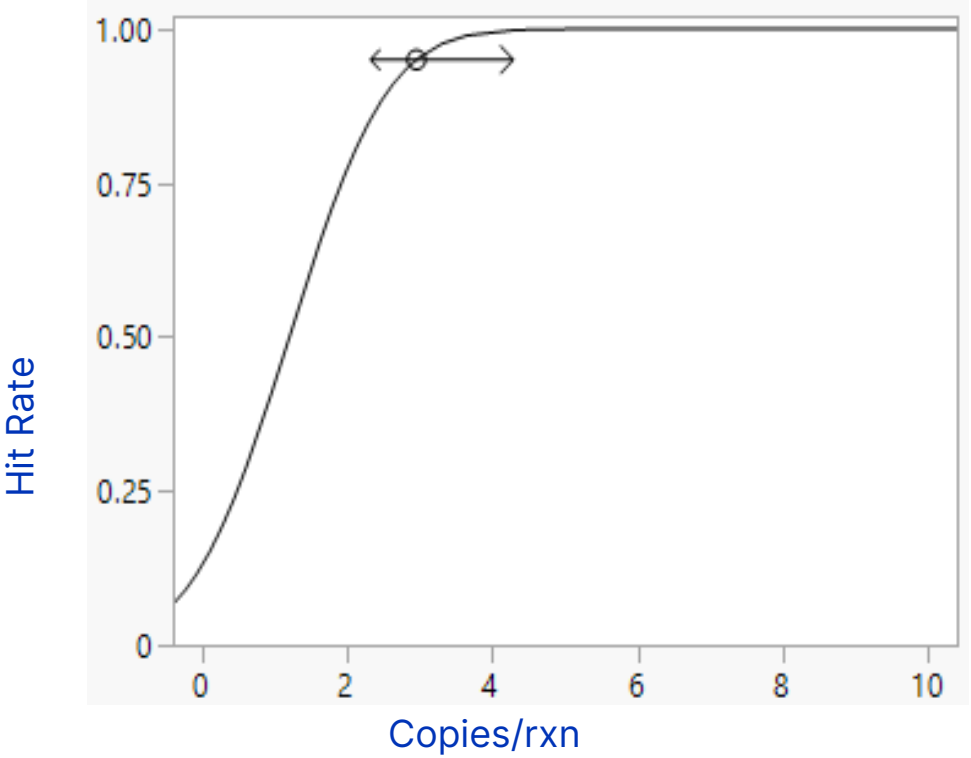
Limit of Detection Demonstrates High Sensitivity

Probit analysis of synthetic DNA (0, 1, 3, 5, 10 copies/rxn) titrated in a background of presumed normal DNA (10,000 total copies)



Variant	LOD (%MAF)**	Company A (qPCR)	Company B (dPCR)
D538G	0.082%	0.4%	0.01%
S463P	0.066%	0.08%	0.025%
Y537S	0.025%	0.1%	0.025%
Y537C	0.028%	0.4%	0.025%
Y537N	0.025%	0.2%	0.025%
Y537D	0.030%	-	-
E380Q	0.028%	1.0%	0.025%
L536R	0.028%	0.7%	0.025%
L536H	0.041%	0.8%	-
L536P	0.028%	0.9%	-
V422del	0.030%	-	-

**ABI QuantStudio 5 Dx

ESR1 exoMutation Kit Exhibits High Analytical Specificity

Evaluation of target specificity (exclusivity) was determined on plasma procured from presumed normal samples

Collection Tube	Negative Percent Agreement
K_2EDTA	98.8%
PAXgene Blood ccfDNA Blood Tube	97.7%

- Utilized ExoLution Plus cfDNA + exoRNA Isolation Kit* workflow for nucleic acid isolation
- High specificity (exclusivity) exhibited for K2EDTA and PAXgene Blood ccfDNA Blood Tube
- Support of multiple collection tubes allows better fit for lab-specific workflows

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ESR1 Liquid Biopsy Surveillance in Treatment-Resistant, HR+/HER2- mBC

ESR1 Mutations in HR+ Metastatic Breast Cancer

- *ESR1* ligand binding domain mutations may be present in up to 40% of AI-treated HR+, HER2- mBC patients
- *ESR1* mutations change cancer cells from estrogen-dependent to estrogen-independent cells

Improved Progression-Free Survival thru *ESR1* Monitoring

- Ongoing clinical studies (e.g., PADA-1, SERENA-6) aim to prove clinical utility for *ESR1* mutation monitoring for patients on frontline endocrine therapies
- NCCN guidelines amended to include *ESR1* mutation testing in LBx (blood)

Heightened *ESR1* Sensitivity via cfDNA and exoRNA

- qPCR provides a cost-effective workflow for *ESR1* mutation monitoring
- Increased sensitivity from LBx (plasma) utilizing cfDNA and exosomal RNA

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Thank You

FOR MORE INFORMATION:

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