bio-techne®

AMP 2024 Corporate Workshop

ESR1 & Beyond:

Leveraging Exosomes for Highly-Sensitive Variant Detection on qPCR

Thomas Bittick, Sr. Product Manager, Oncology

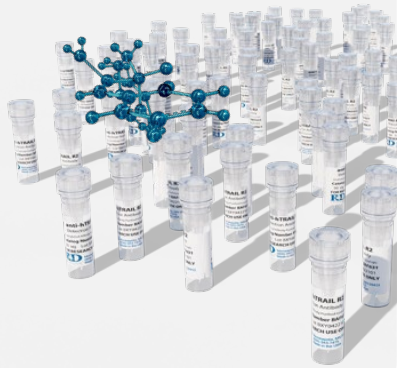
20 November 2024

The Power of Bio-Techne

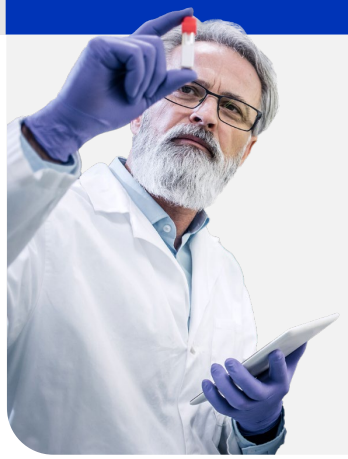
500,000+
Quality Products

3,000+
Committed Employees

45+ Years
of Innovation



500,000+
User Citations



7
Analytical Platforms

bio-techne® // Global Developer, Manufacturer, and Supplier of High-Quality Reagents, Analytical Instruments, and Precision Diagnostics.

INCLUDES R&D Systems™ Novus Biologicals™ Tocris Bioscience™ ProteinSimple™ ACD™ ExosomeDx™ Asuragen® Lunaphore™

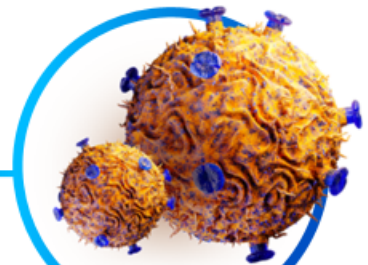
Core Products Enable Advanced Research

DELIVERING COMPREHENSIVE WORKFLOW SOLUTIONS

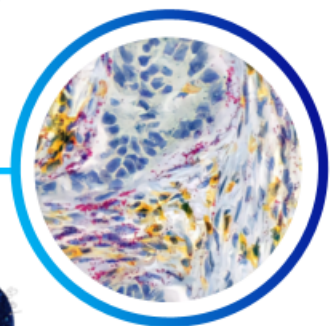
Core Products

- Antibodies
- Proteins
- Immunoassays
- Molecular Test Kits and Lab Services
- Calibrators & Controls
- Small Molecules

Proteomic Analytics



Cell Culture & Gene Therapy



Spatial Biology

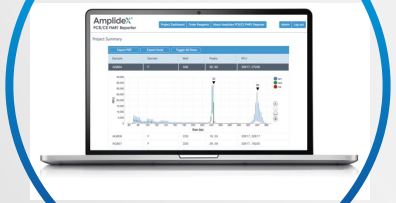
Liquid Biopsy & Molecular Dx

Asuragen Makes Complex Molecular Testing Simple

All-inclusive kits are easy to implement and optimize operational efficiency

Proprietary chemistries and standardized cGMP reagents make testing practical

Provide automated interpretation software



Lab

Run on a variety of widely available platforms

Asuragen Oncology Portfolio

Simplifying detection and workflow with QuantideX®

HemOnc Product Offering

QuantideX qPCR BCR-ABL IS Kit[1,2,3]

- Detects p210 fusion protein
- Direct reporting on the IS

QuantideX qPCR BCR-ABL minor Kit[2,3]

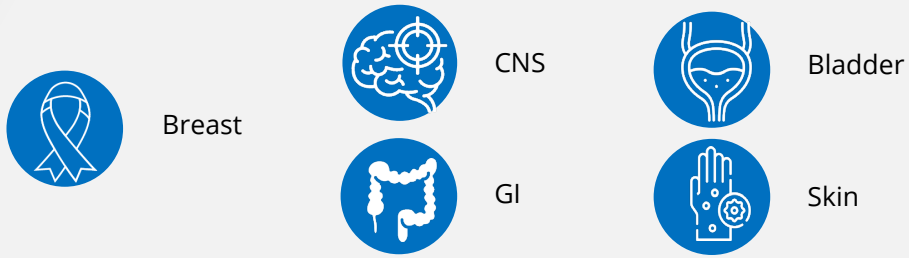
- Detects p190 fusion protein

QuantideX qPCR PML-RARA kit – in development

- Multiplexed design: bcr1, bcr2 & bcr3 and reference genes detected in same well

Solid Tumor Monitoring (Liquid Biopsy)

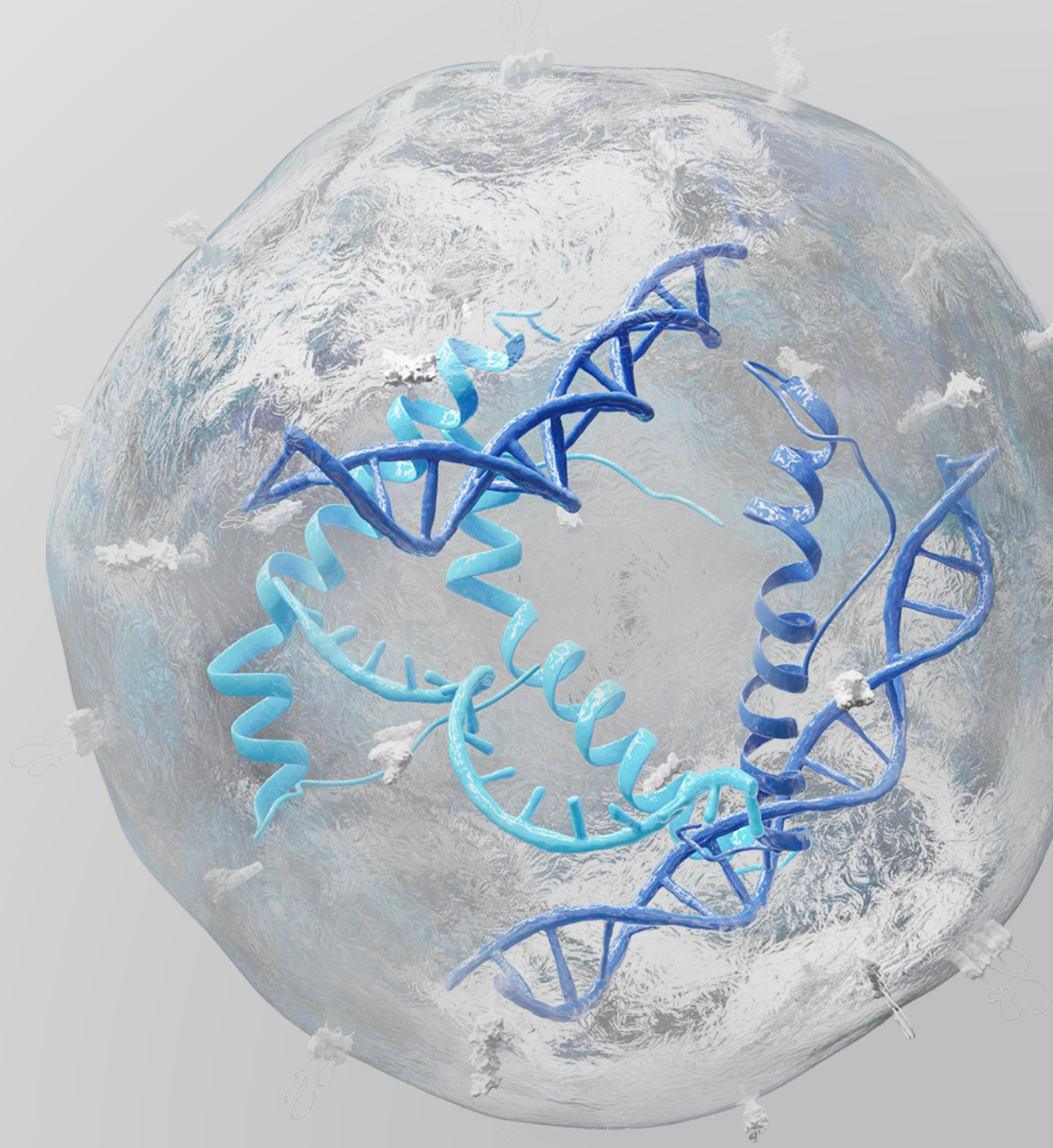
Leverage proprietary exosomal isolation to develop a line of Liquid Biopsy kits for detection of treatment resistance biomarkers associated with breast cancer and other solid tumors.



1. For in vitro diagnostic use.
2. CE-IVD/CE-IVDR. For U.S. export only.
3. For research use only. Not for use in diagnostic procedures.

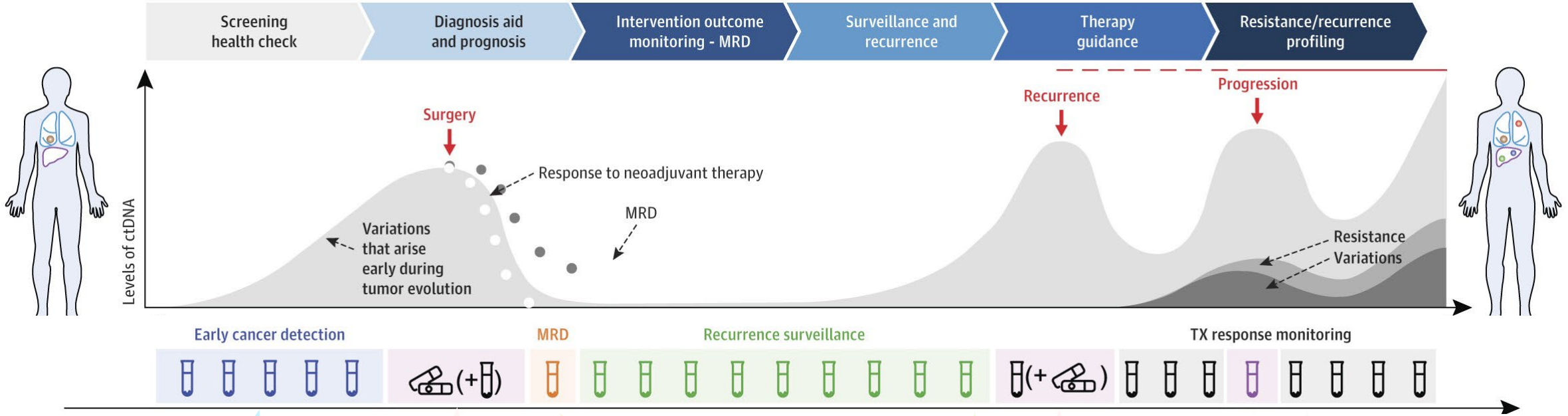
Speaker:
Brian Haynes, PhD
Chief Scientific Officer
Bio-Techne Diagnostics Division

ESR1 & Beyond: Leveraging Exosomes for Highly-Sensitive Variant Detection on qPCR



LBx Applications Span the Entire Cancer Care Continuum

Non-/minimally-invasive testing can support a number of patient management decisions across oncology applications, overcoming challenges with conventional care



Routine, **non-invasive** screen for presence of cancer-derived particles in healthy individuals

Comprehensive genomic profiling (CGP) for diagnosis/ prognosis **without need for surgery**

Sensitive detection of minimal residual disease (MRD) following intent-to-cure treatment (surgery, radiation +/- adjuvant therapy)

Longitudinal monitoring of patients in remission to detect recurrence earlier

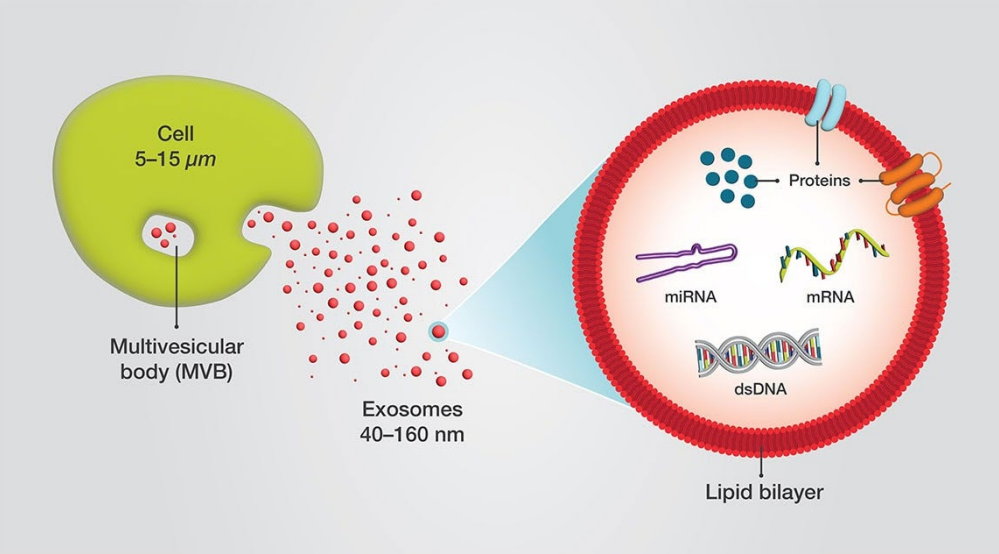
Comprehensive genomic profiling (CGP) for diagnosis/ prognosis **without need for surgery**

Longitudinal testing to monitor for response / resistance to targeted therapy

Source: JAMA Oncol. 2022 Dec 1;8(12):1830-1839.

Leveraging Exosomes for a Differentiated Approach

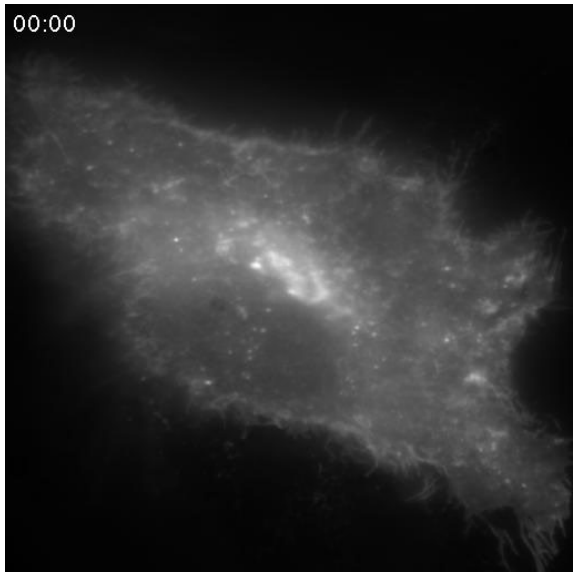
Extracellular vesicles (EVs), such as exosomes, are actively secreted by the cell and carry a snapshot of the body's transcriptome (exosomal RNA); adding exosomal RNA to ctDNA can amplify signal for targets of interest



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.

Exosomes are in great abundance and allow for additional analytes (exoRNA, splice variants) to be examined, which can provide a dynamic disease snapshot and improve downstream assay performance

	ctDNA	Exosomes
Prevalence	Low (0.01-1% total cfDNA)	High
Stability	Short (<2 hours)	Long (days)



Verweij et al., J Cell Biol 2018

ExoLution™

Accessible & reproducible exosome isolation technology

Harnessing the Power of Exosomes to Boost Test Sensitivity

By combining ctDNA with exosomal RNA, test sensitivity can be greatly improved, detecting meaningful events even in conditions where ctDNA shedding may be limited

Improved *EGFR* mutation detection using combined exosomal RNA and circulating tumor DNA in NSCLC patient plasma

Multi-analyte strategy confers improved performance over other highly-sensitive technologies using ctDNA only…

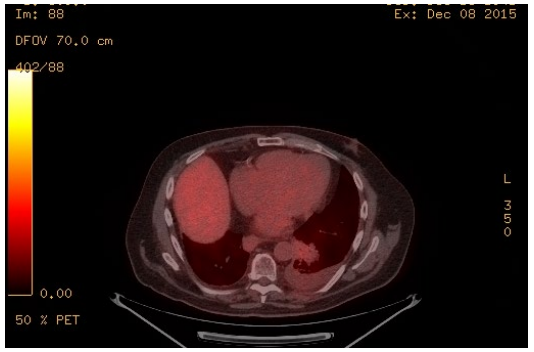
		Tissue Biopsy Result: Activating[a]	Tissue Biopsy Result: T790M		Activating	T790M
TIGER-X Representative Subgroup A ($n = 56$ total, 54 with valid tumor status)						
exoNA (EXO1000)[b]	+	53	44	Sensitivity (exoNA)	98%	90%
	−	1	5			
ctDNA (BEAMing)[b]	+	44	41	Sensitivity (ctDNA)	82%	84%
	−	10	8			
M0/M1a Subgroup C ($n = 21$ total, 19 with valid tumor status)						
exoNA (EXO1000)[c]	+	14	5	Sensitivity (exoNA)	74%	31%
	−	5	11			
ctDNA (BEAMing)[c]	+	5	3	Sensitivity (ctDNA)	26%	19%
	−	14	13			

- In the randomized Tiger-X trial cohort, ExosomeDx achieved **98% sensitivity** for *EGFR* activating mutations and **90% sensitivity** for *EGFR T790M*
- Even in patients with no/limited metastasis (M0/M1a), ExosomeDx showed even greater sensitivity vs BEAMing, highlighting potential applications in early-stage disease

Ann Oncol. 2018 Mar; 29(3): 700–706.

… and our results correlate to improvements in patient outcomes.

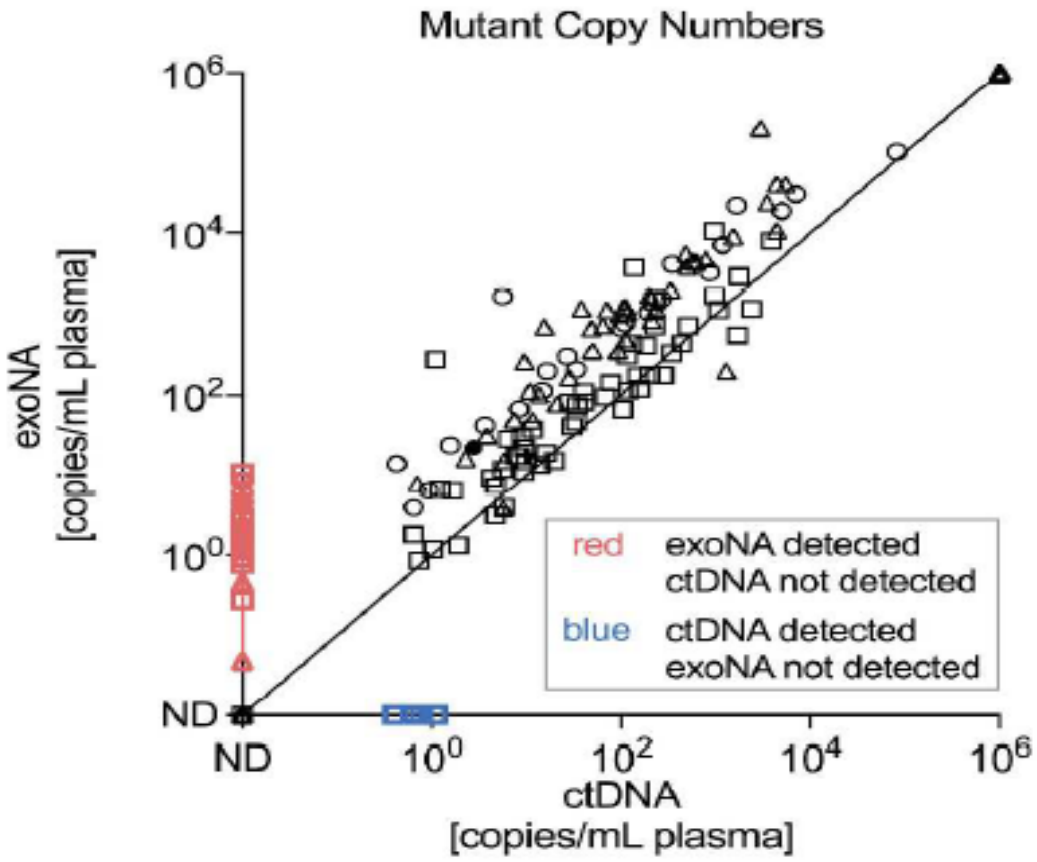
Stage IV NSCLC Adenocarcinoma (EGFR+)



- Patient fails 1L EGFR TKI therapy
- ctDNA test from Guardant ordered → no EGFR T790M detected
- ExoDx ctDNA+exoRNA test → T790M found
- Patient assigned T790M-targeted therapy and immediately responds

The Benefits of the Multi-Analyte Approach

ExoRNA + cfDNA yields a nearly 10-fold increase in mutant copies vs cfDNA alone

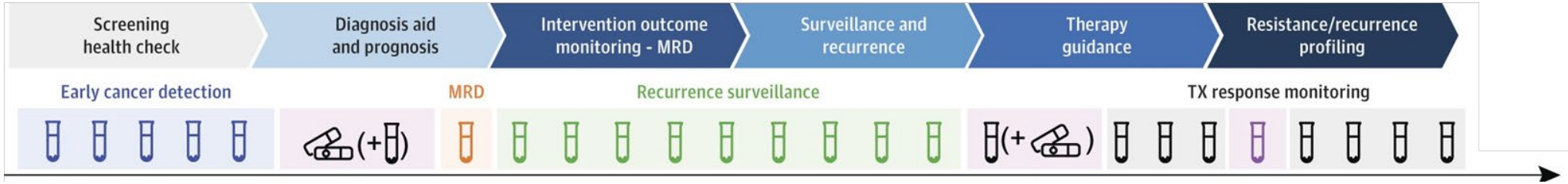


Complete cohort		Mutant cps/mL		
		Median	Range	*p*-value
Activating *EGFR* MUT	exoNA	234	0-202092	<0.0001
	ctDNA	24	0-82406	
T790M *EGFR* MUT	exoNA	12	0-10642	<0.0001
	ctDNA	6	0-3867	

Source: JAMA Oncol. 2022 Dec 1;8(12):1830-1839.

Clinical Consensus Supports Targeted, Decentralized Model for TRM

Treatment response monitoring (TRM) in late-stage disease can be cost-effectively – supported via decentralized technologies like PCR to bring testing closer to the patient



Suitable Methods*

SCED/MCED Panels	CGP	Tumor Informed Panels	CGP	Targeted Panels
NGS				PCR/dPCR

- Patients with actionable mutations in late-stage disease are often treated with targeted therapies
- Focused treatment response monitoring (TRM) and detection of resistance can be achieved with sensitive PCR tests; broad, NGS-based detection is not cost-effective or may not be necessary for routine TRM

Technology and examples		Sensitivity	Application	Fit-for-purpose scale (1 = inappropriate --> 5 = perfect match) Screening	Diagnosis - Prognosis / Prediction	MRD	TRM	Resistance tracking
PCR	qPCR?		• Detection of individual point mutations, including predefined fusions	1	2	4	4	3
	BEAMing		• Screens for known mutations	1	2	2	3	2

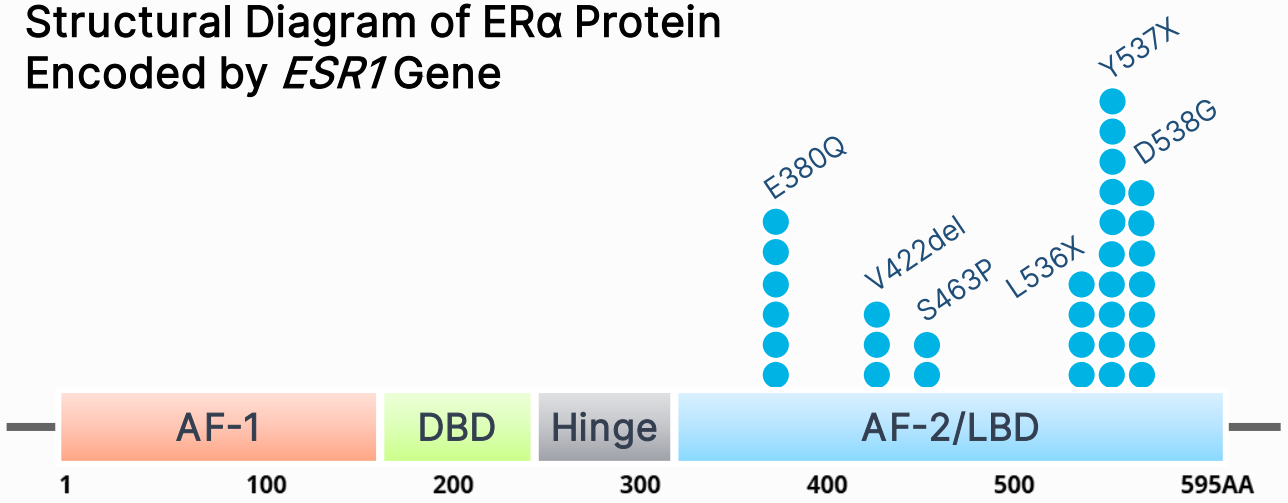
Krebs MG, Malapelle U, André F, et al. Practical considerations for the use of circulating tumor DNA in the treatment of patients with cancer: a narrative review. JAMA Oncol., 2022

* Not limiting

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

Up to 40% of HR+ mBC patients will develop mutations in *ESR1*, causing resistance to endocrine therapy (ET) and disease progression

Structural Diagram of ERα Protein Encoded by *ESR1* Gene



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 ER. *ESR1* mutations in the LBD turn the ER "on" even in the absence of estrogen. Thus, via *ESR1* mutations, estrogen-dependent disease becomes estrogen-*independent*.

- *ESR1* encodes for the ERα protein subunit of the ER
- *ESR1* mutations are extremely rare in treatment-naïve disease; however, up to 40% of HR+ mBC patients on AIs will develop *ESR1* mutations
- Acquired mutations in *ESR1* are concentrated in the ligand-binding domain (LBD), constitutively activating the ER ("always on") even in the absence of estrogen → ET resistance
- Within the LBD, *ESR1* mutations are further concentrated at specific locations (380, 422, 463, 536-538)

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 Clinical 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

ASCO® Feb 6, 2024

The Expert Panel recommends multiple lines of endocrine treatment (ET), frequently paired with targeted agents, with choices informed by prior treatments and by routine testing for activating mutations in *ESR1*, *PIK3CA*, or *AKT1*, or inactivation of *PTEN* (Table 1). Panelists recommend inclusion of CDK4/6 inhibitor therapy with ET in the first line. Second- and third-line therapies reflect targeted options based on tumor genomics.

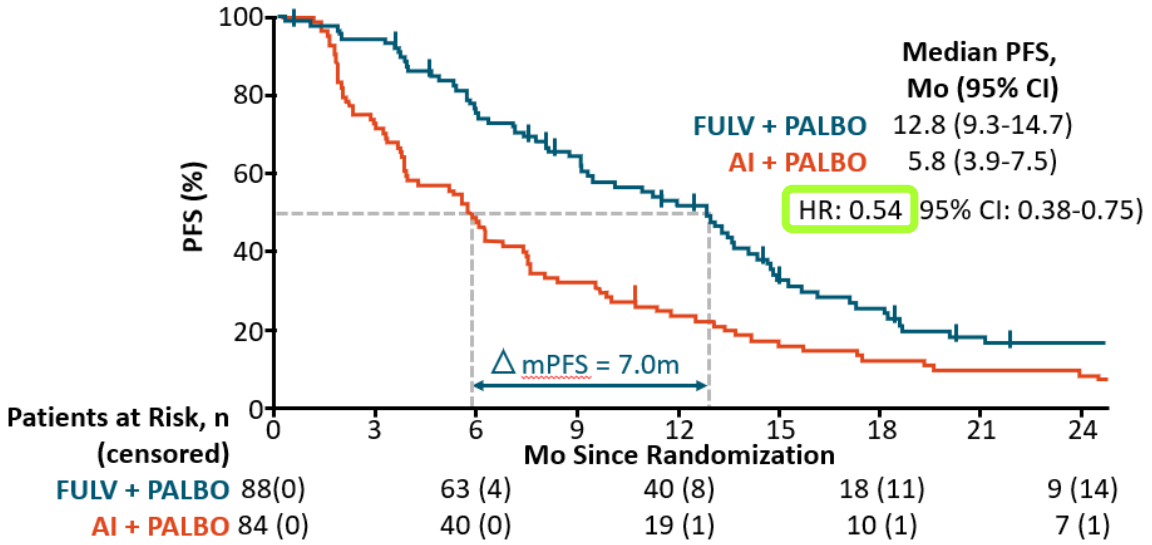
- Following FDA approval of elacestrant, the NCCN guidelines were quickly amended to recommend *ESR1* as a validated biomarker to guide downstream treatment decisions
- Since *ESR1* mutations are extremely rare in primary tumors, which are often molecularly characterized using tissue, the NCCN recommends using a blood test (liquid biopsy, LBx) to detect *ESR1* mutations
- ASCO also recommends routine testing for activating mutations (e.g., *ESR1*) to inform later lines of therapy

National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines: Breast Cancer Version 4.2023; American Society of Clinical Oncology (ASCO) Expert Panel Recommendations: HR+/HER2- Breast Cancer.

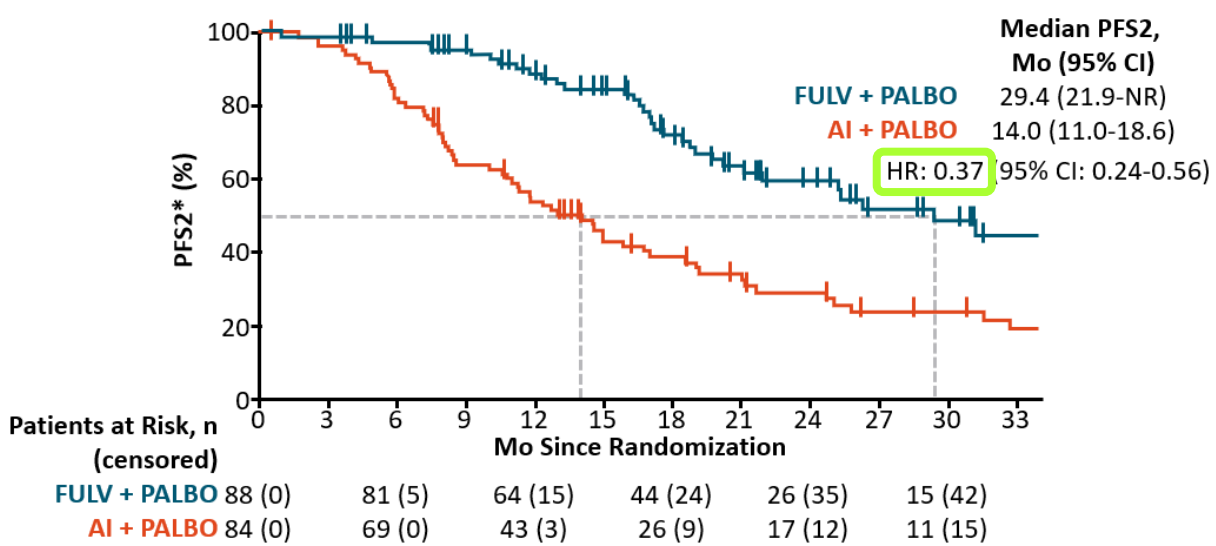
PADA-1: Early Detection = Early Intervention = Better Outcomes

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

mPFS1: Primary Endpoint



mPFS2: Secondary Endpoint



- In PADA-1, serial ctDNA testing was used to identify *ESR1* mutations in mBC patients on standard therapy (AI+CDK4/6i)
- Patients found to have a mutation *prior to disease progression* were randomized 1:1 to continue treatment or switch therapies
- **Switching therapy resulted in over a doubling of median progression-free survival (both mPFS1 and mPFS2)**
- Patients who switched to the new regimen *at radiological progression* had a mPFS of only 3.5 months
- *ESR1* mutation type, %VAF, clonality status were not prognostic/predictive of PFS in either treatment arm

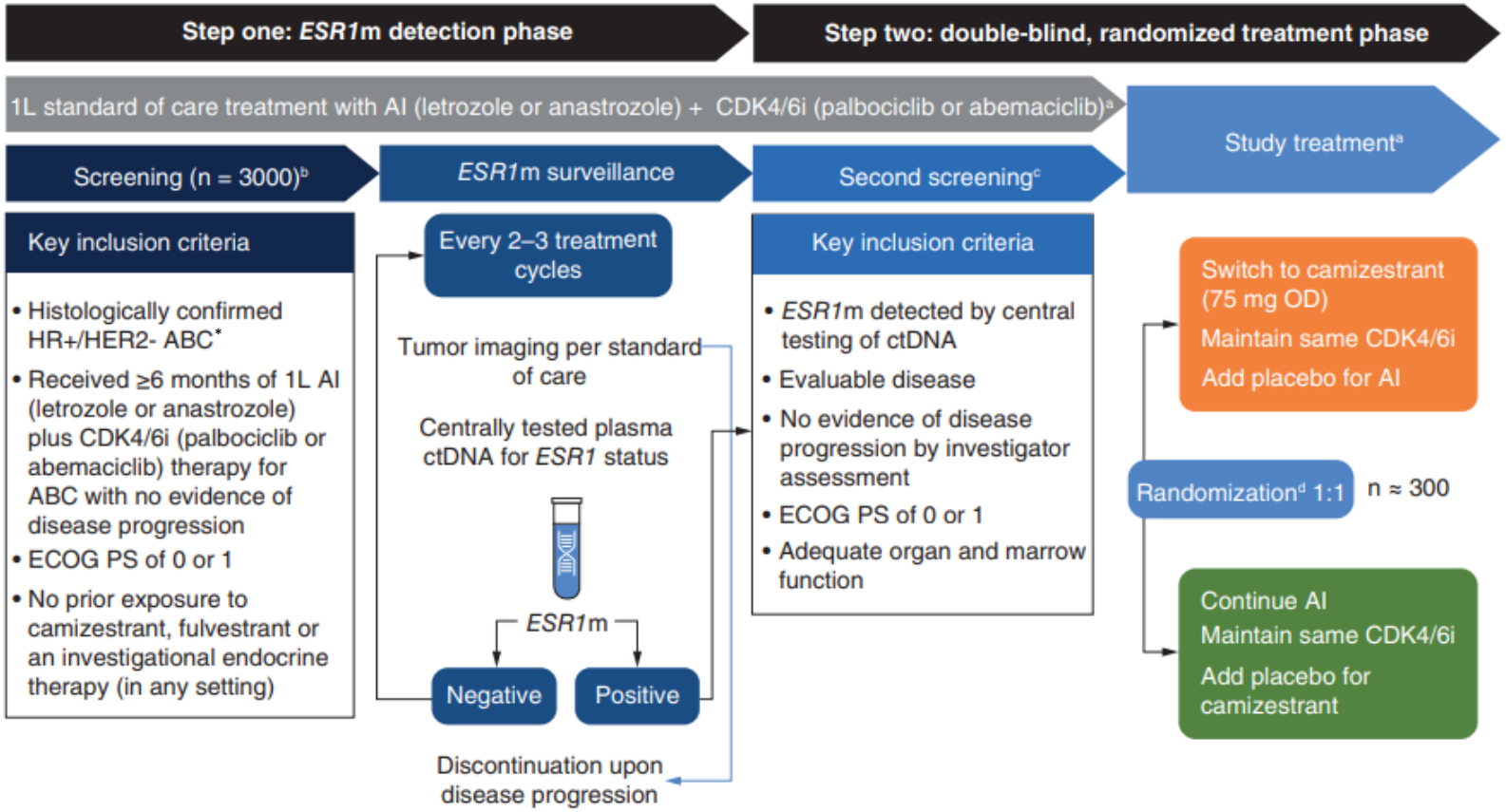
AI = aromatase inhibitor
FULV = fulvestrant (SERD)
PALBO = palbociclib (CDK4/6i)

CCO Independent Conference Highlights of the 2023 ASCO Annual Meeting; Lancet Oncol. 2022;23:1367.

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 (*ESR1m*) vs waiting for clinical progression

SERENA-6 Clinical Trial Design

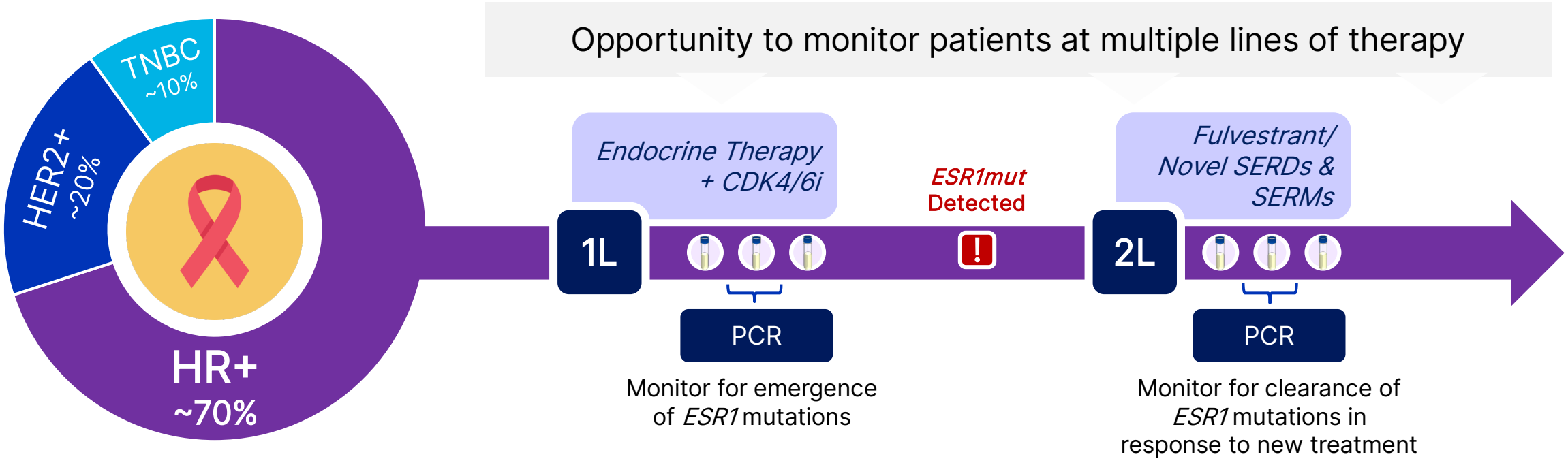


- In **STEP ONE**, patients who had received an AI plus CDK4/6i for ≥6 mos are screened for the first set of inclusion and exclusion criteria
- Those who are eligible are tested for the presence of 11 key *ESR1m* in ctDNA every 2–3 treatment cycles (~8–12 weeks)
- Continuous ctDNA monitoring will occur 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.
* ABC: Advanced Breast Cancer

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., VAF%) is not critical; need to know whether *ESR1* mutations are present or not (qualitative testing is sufficient)
- *ESR1* mutations can 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

Introducing the QuantideX® qPCR *ESR1* exoMutation Kit

11 key *ESR1* LBD mutations. Unprecedented sensitivity on qPCR. Only 2 mL plasma required.

Category	Description & Target Specifications
Coverage	11 *ESR1* ligand-binding domain mutations: E380Q, V422del, S463P, L536H/P/R, Y537S/N/C/D, D538G
Size	50 Reactions
Analyte	cfDNA + exoRNA co-isolated with ExoLution™ Plus Kit
Sample	2 – 4 mL Plasma (double-spun + filtered) K_2EDTA or PAXgene Blood ccfDNA Collection Tubes
Assay Sensitivity	≤0.1% VAF (≤5 copies/mL)
Platforms	ABI 7500 Fast Dx, QuantStudio™ 5 Dx, QuantStudio™ 7 Pro Dx
Regulatory	RUO
Workflow	Sample-to-answer in one day
Data Analysis	Qualitative Mutation Detection via Provided Software

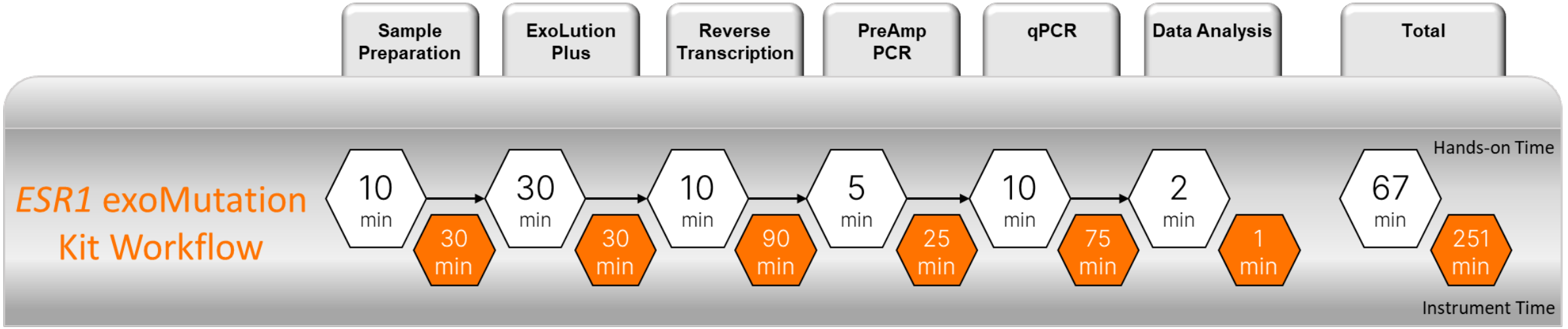
Making Exosome Isolation Accessible with ExoLution™ Plus

Provided with the assay, this new sample prep method enables co-isolation of cfDNA + exoRNA in just an hour using common benchtop centrifuges

- New sample isolation kit to be launched with QuantideX® qPCR *ESR1* exoMutation Kit
- Proprietary, column-based method for **co-isolation of cell free DNA (cfDNA) & exosomal RNA (exoRNA)** in a single step
- Kit will be inclusive of necessary reagents and key consumables to support 50 isolations
- All components stored at room temperature (15 – 25°C)

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*

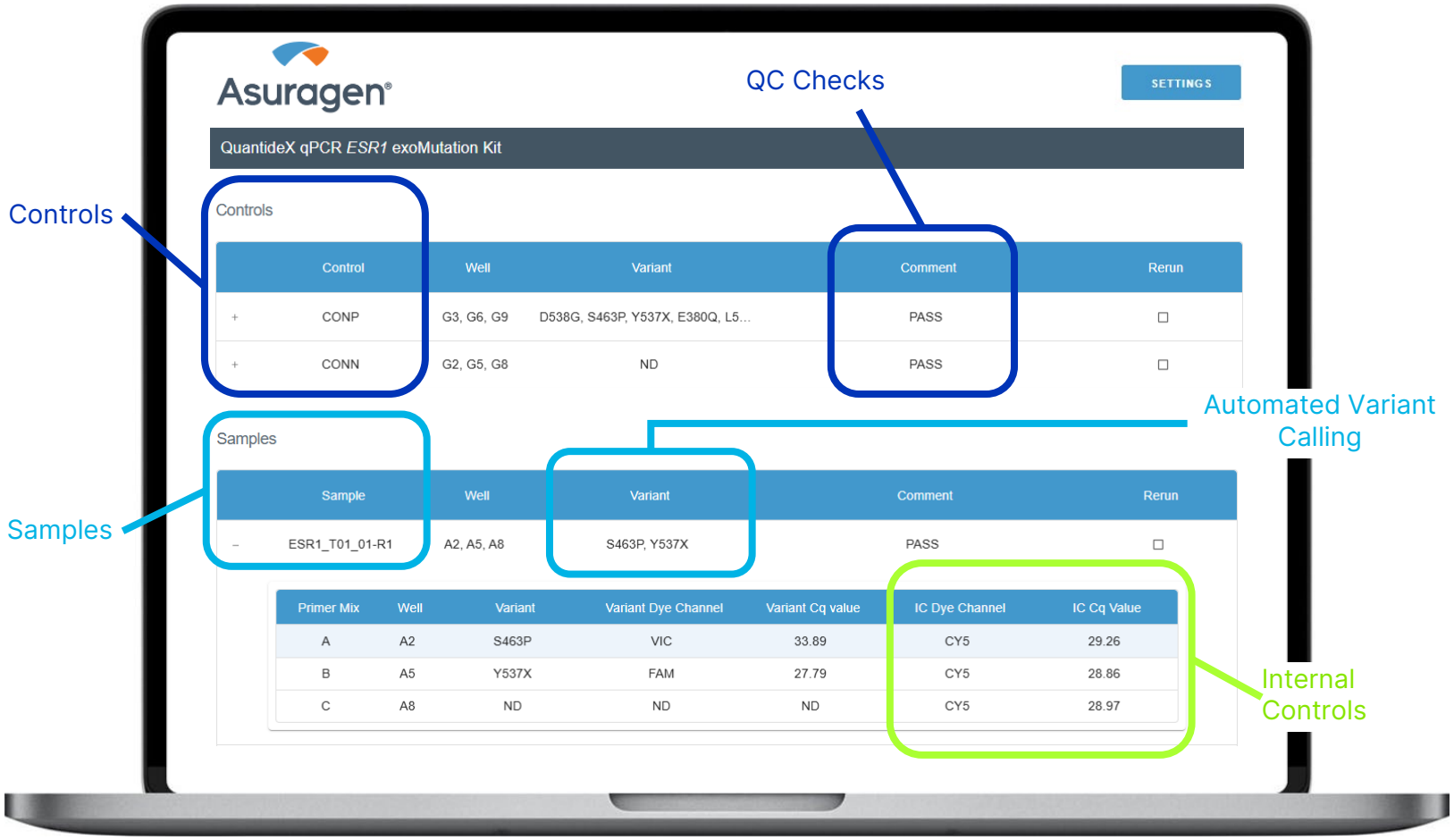
QuantideX qPCR *ESR1* exoMutation Analysis Module*

Sample-to-Results <6 hrs with ~1 hr hands-on time

* For Research Use Only. Not for use in diagnostic procedures.

Automated Results Reporting via Push-Button Software

Included software application streamlines mutation analysis and QC



QuantideX®

qPCR *ESR1* exoMutation Analysis Module

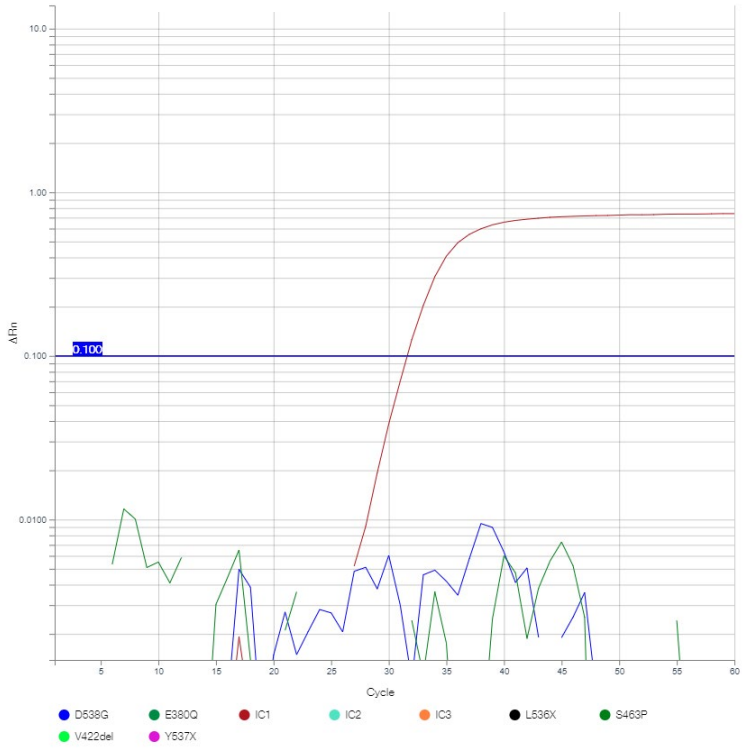
- Automated variant calling
- Batch Control QCs
- Export results in LIMS-compatible format (e.g., .CSV)

QuantideX® qPCR *ESR1* exoMutation Kit: Control Design

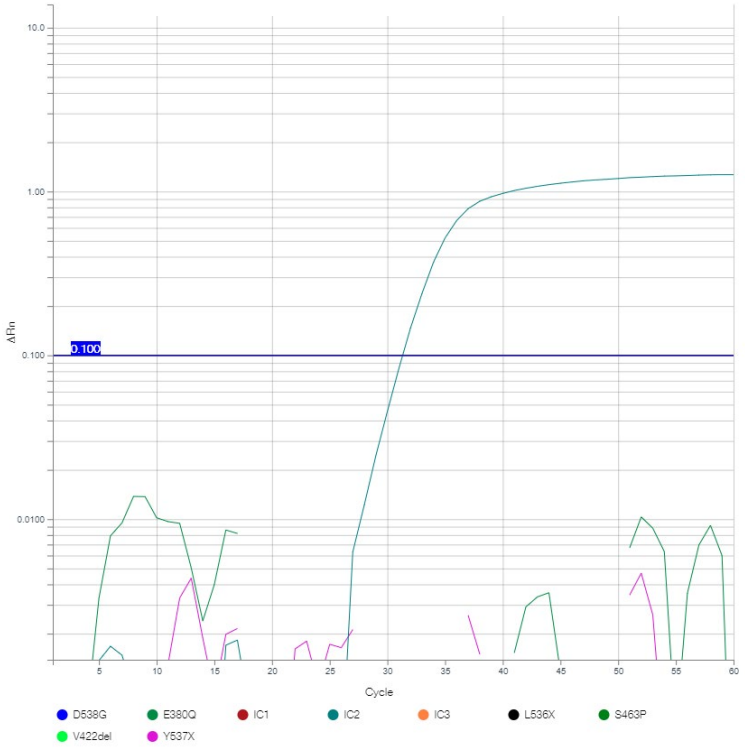
1 Within Sample Control (IC)

- Intent is to control for end user adding adequate sample into testing well across all 3 mixes (IC1, IC2, IC3)
- PAX Blood ccfDNA Tube Clinical Sample Negative for *ESR1* Mutations Shown

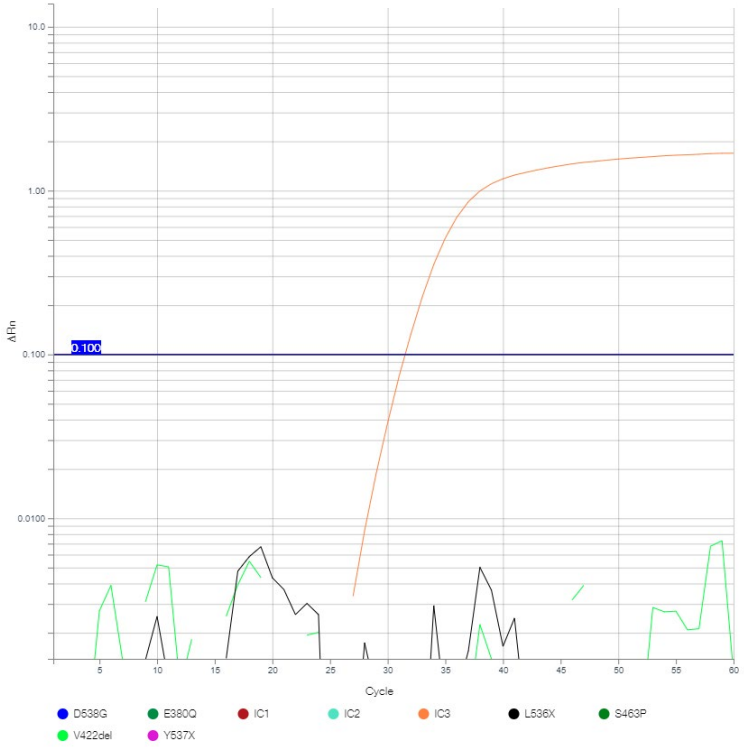
MIX A: D538G + S463P + IC1



MIX B: Y537X + E380Q + IC2



MIX C: L536X + V422del +IC3



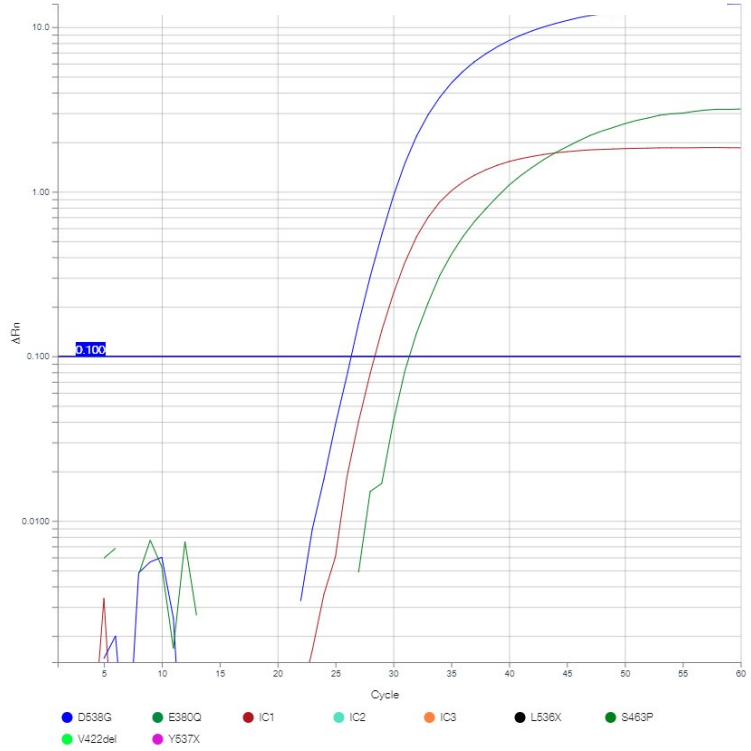
QuantideX® qPCR *ESR1* exoMutation Kit: Control Design

2

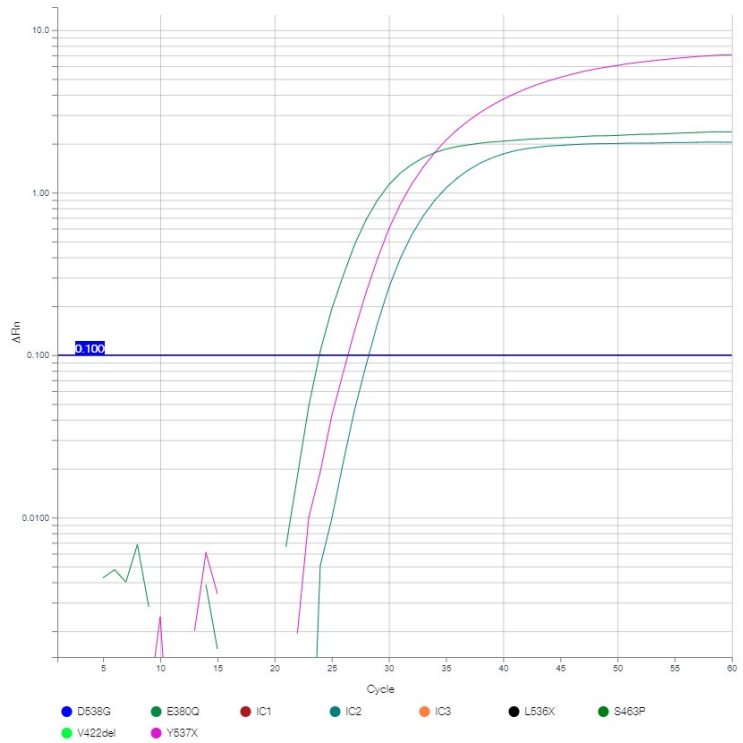
Batch Controls: CONP + CONN

- **CONP: DNA-based Positive Control for 6 of 11 mutations**
  - Intent is to show a successful qPCR via positive signal in all 3 mixes across 2 mutant channels for a total of 6 positive signals
- **CONN: RNA-based Negative Control**
  - Intent is to show successful RT via positive signal in all 3 mixes for the within sample control (IC1, IC2, IC3).
  - Intent is to act as batch contamination control

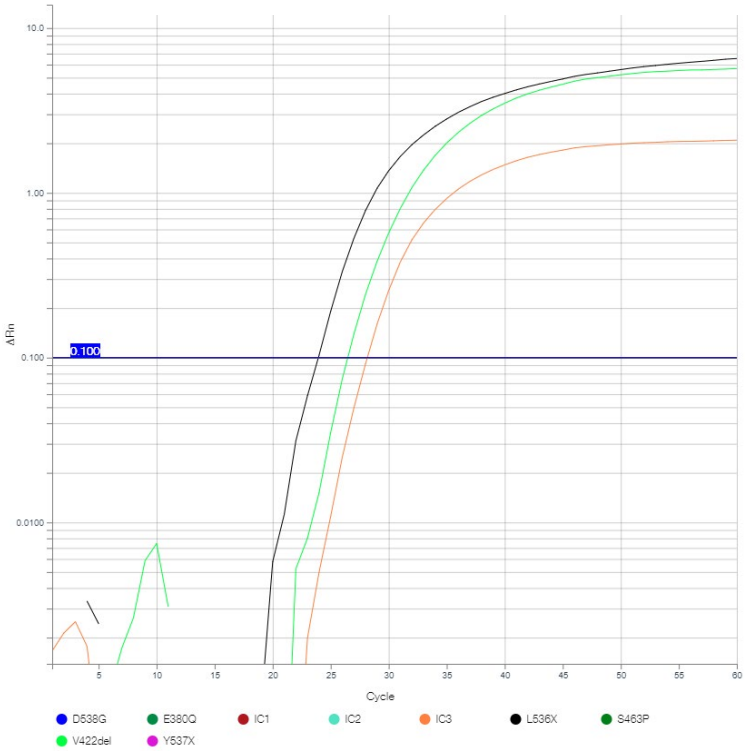
MIX A: D538G + S463P + IC1



MIX B: Y537X + E380Q + IC2



MIX C: L536X + V422del +IC3



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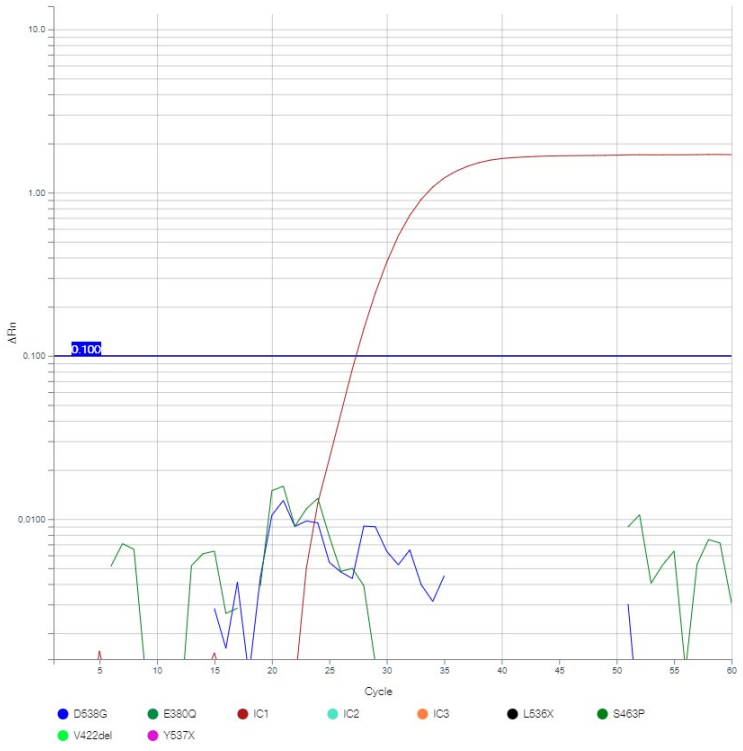
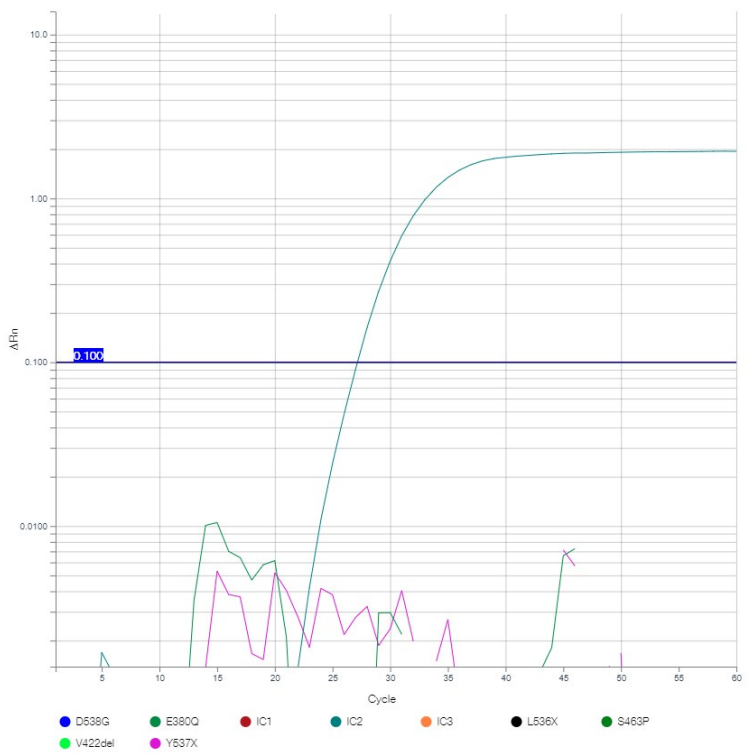
  - Intent is to act as batch contamination control

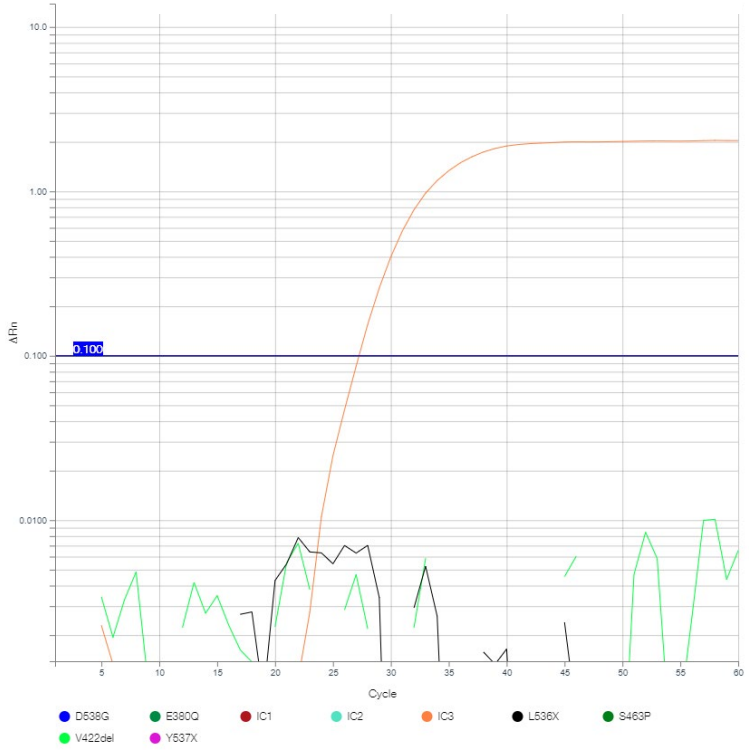
MIX A: D538G + S463P + IC1



MIX B: Y537X + E380Q + IC2



MIX C: L536X + V422del +IC3



High Analytical Specificity Observed Across Both K_2EDTA and PAXgene Tubes

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

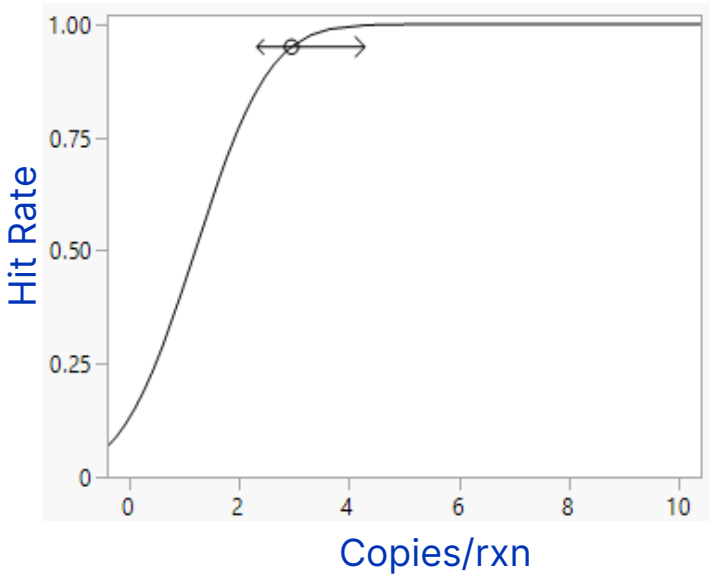
Blood Collection Tube	Negative Percent Agreement (NPA/Specificity)
K_2EDTA	97.1%
PAXgene Blood ccfDNA Tube	97.7%

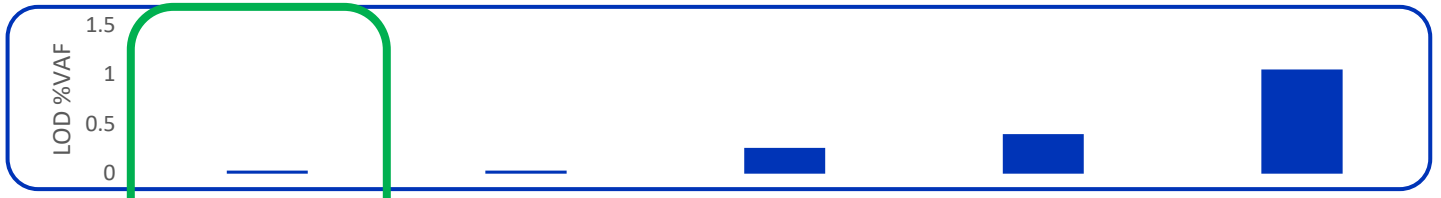
- Utilized ExoLution Plus cfDNA + exoRNA Isolation Kit* workflow for nucleic acid isolation
- Support of multiple collection tubes provides more flexibility to accommodate nuances across laboratory operations

*For Research Use Only. Not for use in diagnostic procedures.

Pushing the Boundaries for Assay Sensitivity on qPCR

Probit analysis of synthetic mutant DNA (0, 1, 3, 5, 10 copies/rxn) titrated in a background of presumed normal fragmented DNA (10,000 total copies) across 20 replicates



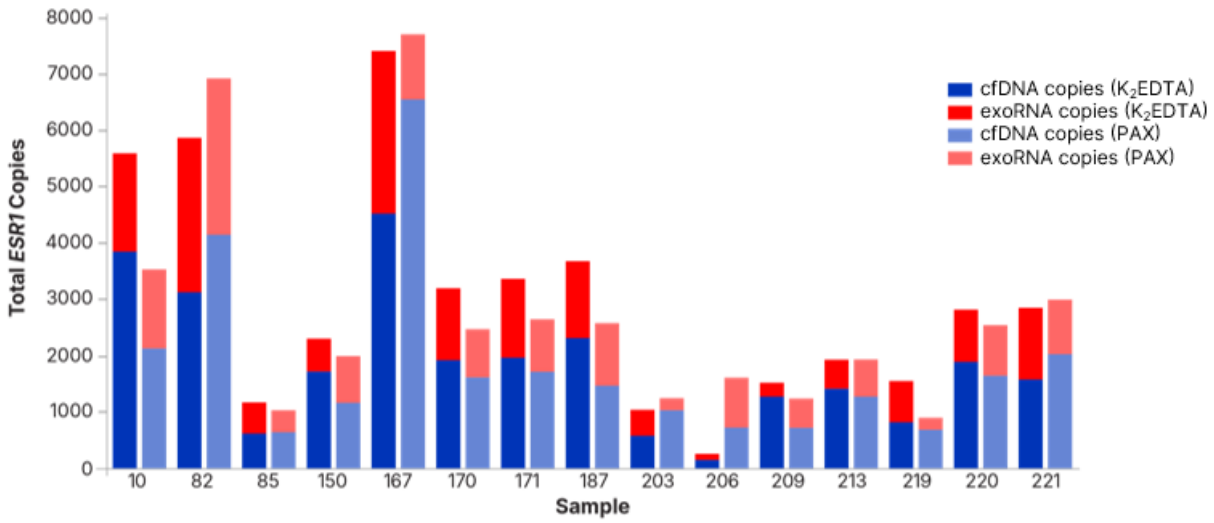


Variant	Asuragen LOD (%VAF)*	Company A (ddPCR)	Company B (qPCR)	Company C (qPCR)	Company D (NGS)
D538G	0.082%	0.01%	0.33%	0.4%	1.1%
S463P	0.066%	0.025%	0.33%	0.08%	2.8%
Y537S	0.025%	0.025%	0.20%	0.1%	1.0%
Y537C	0.028%	0.025%	0.33%	0.4%	Unknown
Y537N	0.025%	0.025%	0.07%	0.2%	Unknown
Y537D	0.030%	-	0.13%	-	Unknown
E380Q	0.028%	0.025%	0.13%	1.0%	1.0%
L536R	0.028%	0.025%	0.26%	0.7%	Unknown
L536H	0.041%	-	0.13%	0.8%	Unknown
L536P	0.028%	-	-	0.9%	Unknown
V422del	0.030%	-	-	-	Unknown

*ABI QuantStudio 5 Dx

Evaluation in presumed normal and mBC cohorts

exoRNA signal increases total evaluable *ESR1* copies



exoRNA increased the total number of measurable *ESR1* copies by >60% as determined by ddPCR in presumed normal female samples

ESR1 resistance mutations detected and confirmed in mBC setting consistent with expected prevalence

Sample ID	Detected Variant(s)	Cqs
202167389	D538G	34.7
17397016	D538G	33.45
17397025	D538G, E380Q, Y537X, L536X	29.31, 33.14, 35.48, 32.43
17397005	D538G, L536X	35.08, 34.29
17397014	E380Q	39.18
17397006	Y537X	36.56
202143632	Y537X	38.7
17397002	Y537X	28.57
17397019	Y537X	26.82

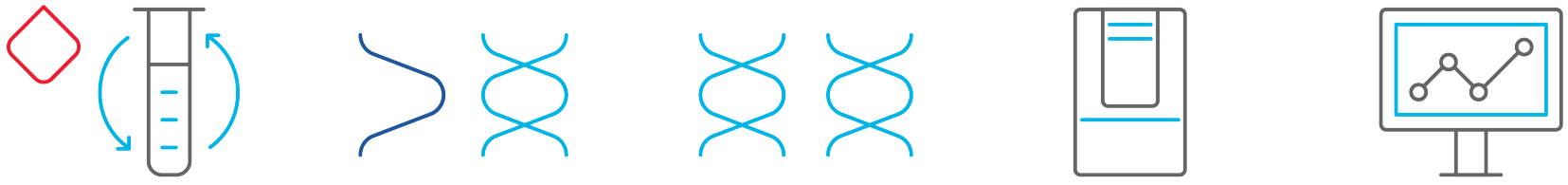
Cohort of 21 mBC samples evaluated, revealing 9/21 ESR1 positives (43%), 7 of 9 were expected by initial screen, the remaining 2 are being confirmed by ddPCR

Simple, Sensitive, and Scalable *ESR1* Mutation Testing Within Your Reach

Providing a complete testing solution including sample prep, RT-qPCR reagents, and analysis software

bio-techne®

Sample Preparation | **ExoLution Plus** | **RT** | **Pre-Amp PCR** | **qPCR**



A minimum of 2 mL of plasma is processed

ExoLution™ Plus Kit isolates both cfDNA and exoRNA in a single step

Test kit contains all PCR reagents to detect target(s) of interest; samples can be batched to maximize throughput

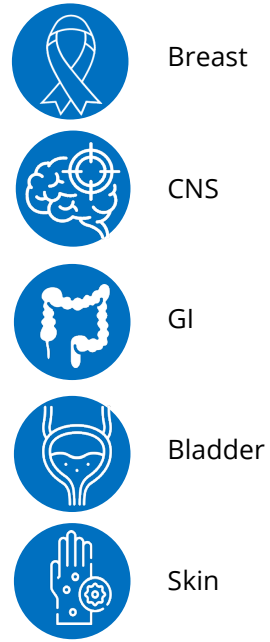
Detects 11 *ESR1* mutations at ≤0.1% VAF

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

Sample-to-Answer in a Single Shift

Total time of <6 hrs with ~1 hr hands-on time*

Oncology Applications



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*For a batch of six samples

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Verification of an RT-qPCR Assay System for Liquid Biopsy Surveillance of Treatment-Resistant *ESR1* Mutations

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