PIK3CA Gene Mutation Detection With Digital PCR Technoloy

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01

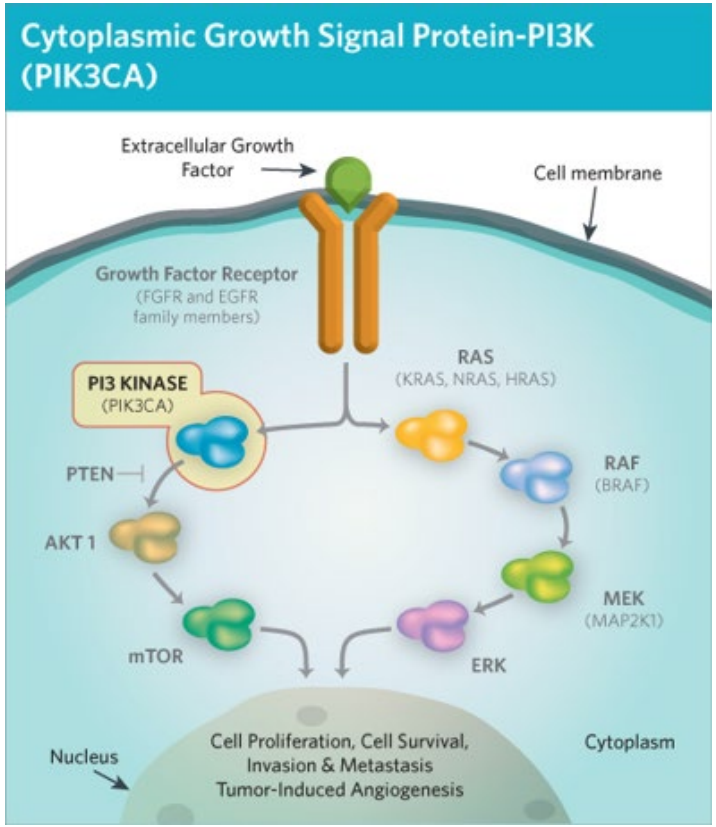
Introduction to PIK3CA gene

Introduction to PIK3CA gene

- PIK3CA is an oncogene. In tumor cells, its kinase activity is enhanced and it can continuously stimulate the downstream AKT signaling pathway, causing cells to proliferate independently of growth factors and increasing cell invasion and metastasis capabilities.
- PIK3CA gene mutations are present in approximately 30% of breast cancers, 25% of endometrial cancers, 15% of colon cancers, 10% of ovarian cancers, and 5% of lung cancers. It is a pan-cancer cancer-causing mutation.
- The PIK3CA gene is located on chromosome 3 and has 20 exons. The PIK3CA gene belongs to the PI3K-Akt signaling pathway, and its main responsibility is to encode p110α protein. Mutations in the PIK3CA gene will cause the PI3K enzyme to be continuously activated, enhance intracellular signal conduction, lead to disorder of the entire pathway, and cause a series of diseases, including cancer, neuropathy, autoimmune diseases, hematopoietic system diseases, etc.

Introduction to PIK3CA gene

- PICK3CA is located in a "hub" position.
- Its upstream is EGFR (also called HER1) and HER2, so patients with PIK3CA mutations may be resistant to some EGFR inhibitors and HER2 inhibitors.
- Downstream is the entire PI3K-AKT-mTOR pathway. Abnormalities in the PI3K-AKT-mTOR pathway are more common than mutations in the PIK3CA gene alone, causing a wider range of cancer types.



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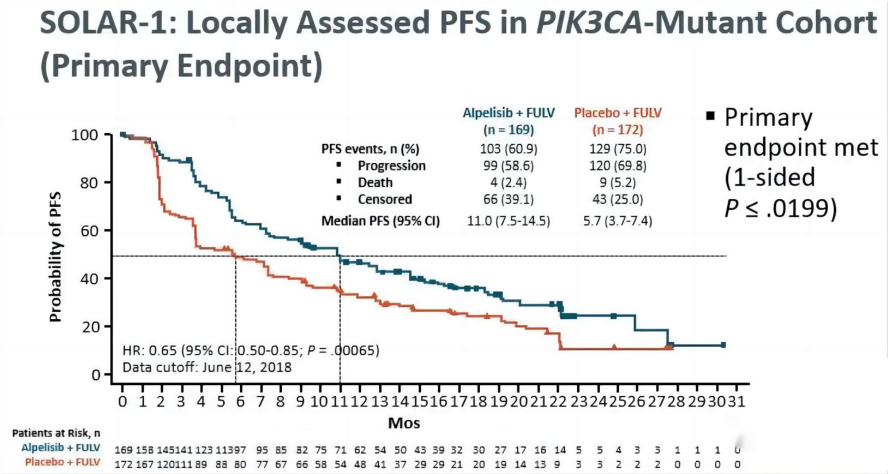
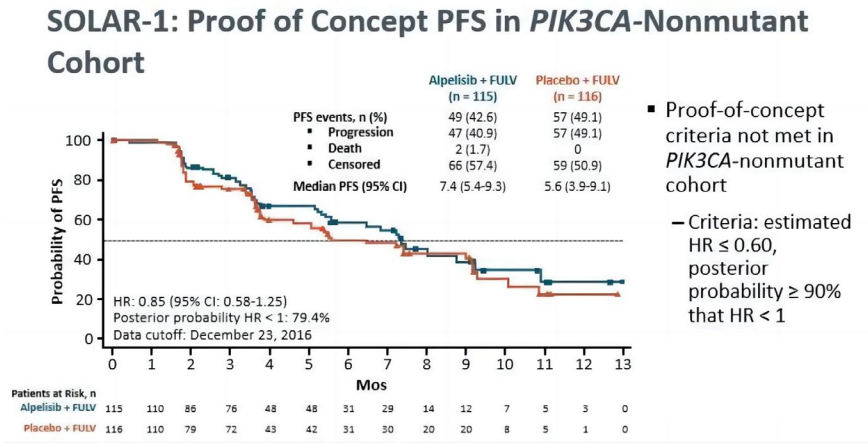
Clinical significance and medication of PIK3CA mutation detection

Clinical significance and medication of PIK3CA mutation detection

- Breast cancer is the most common tumor in women and the leading cause of cancer death
- Approximately 20-40% of BC patients develop metastatic or advanced disease (locally unresectable, stage 3 or 4 disease)
- HR+/HER2-BC subtype accounts for approximately 70% or 2/3 of BC cases
- Endocrine therapy (ET) is the first treatment option for HR+/HER2-MBC
- Approximately 40% of patients eventually develop endothelin resistance, due to dysregulation of the phosphoinositide 3-kinase (PI3K) pathway
- Among patients with HR+HER2- breast cancer, the PIK3CA gene mutation frequency is 50%

Evaluation of an a-selective PI3K inhibitorin HR+/HER2-MBC(SOLAR-1 Phase III trial)

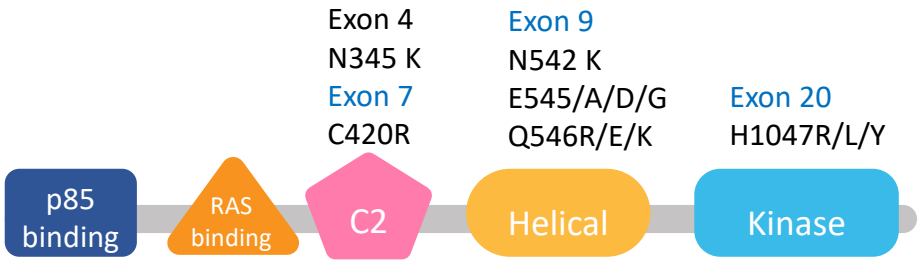




PIK3CA mutation group: mPFS 11.0 months vs 5.7 months

PIK3CA non-mutation group: mPFS 7.4 months vs 5.6 months, no significant difference

Functional domains and key mutations in PIK3CA



- Patients enrolledin the PIK3CA-mutant cohort were those with ⩾1 mutation(s) in exons 7, 9, and 20
- PIK3CA mutations occurred most commonly in exons 9 and 20, 80% in 3 regions: E542K and E545K and H1047(LRY)
- PIK3CA testing was performed on FFPE specimens using qualitative real-time PCR assays
- N345K and N542K were detected by post hoc NGS analyses, efficacy of alpelisib plus fulvestrant inpatients withthese mutations has not been established

Exon	Mutation	Proportion of cohort mutations(N=370)，n （%）
7	C420R	6(1.6)
	E542K	**60 (16.2)**
	E545X(AD/G/K)	**47 (12.7)**
	E545K	**50(13.5)**
9	E545G	4(1.1)
	E545D	5(1.4)
	Q548X(EKR)	2(0.5)
	Q548E	1(0.3)
	Q548R	2(0.5)
	H1047X(LRY)	**106 (28.6)**
20	**H1047R**	**77 (20.8)**
	H1047L	7(1.9)
	H1047Y	3(0.8)

F Andre et al. N Engl J Med 2019;380:1929-40.

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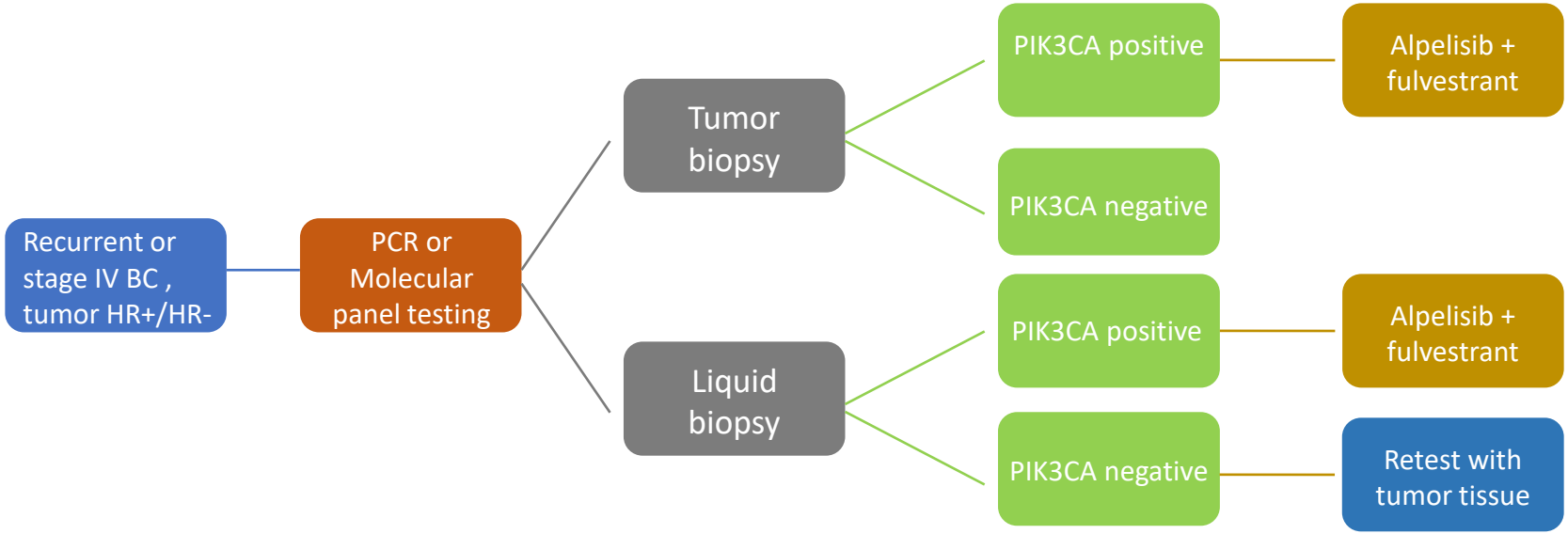
NCCN guidelines for assessment of PIK3CA mutations



PIK3CA mutation medication

PI3K inhibitors correspond to cancers caused by PIK3CA gene mutations. There are not many types of drugs for this target, but patients have great demand, and it is difficult to cure such gene mutations. There are currently three PI3K inhibitors on the market.

pik3CA inhibitor	Indications	
Alpelisib	hematological tumors	FDA approves Piqray (alpelisib, codename BYL719) in combination with fulvestrant for the treatment of postmenopausal women with hormone receptor-positive, HER-2-negative (HR+/HER2-).Piqray is the only targeted drug approved for this subset of breast cancer patients
Idelalisib （Zydelig）		Indications include chronic lymphocytic leukemia (CLL), follicular B-cell non-Hodgkin lymphoma (FL) and small lymphocytic lymphoma (SLL).
Umbralisib （UKONIQ）	Breast cancer among solid tumors	Umbralisib is a dual-target inhibitor of PI3Kδ and CK1-ε. It has obtained orphan drug designation for marginal zone lymphoma and follicular lymphoma indications, and has obtained breakthrough therapy for marginal zone lymphoma indications. specified.

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PIK3CA gene mutation detection method

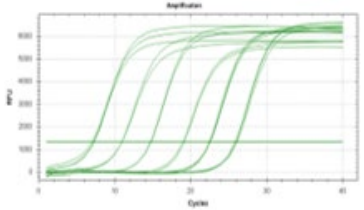
Detection method

PIK3CA mutation detection samples can be tumor tissue or peripheral blood free tumor DNA

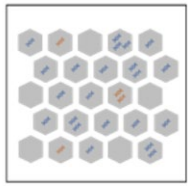
PIK3CA gene mutation detection

Single genes: specific mutation detection

real-time fluorescence PCR



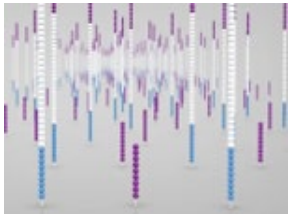
Digital PCR



- Detection of a single known mutation site
- Through the design of primers and probes, multiple mutation sites can be detected simultaneously.

Polygene: hotspot or full gene sequence

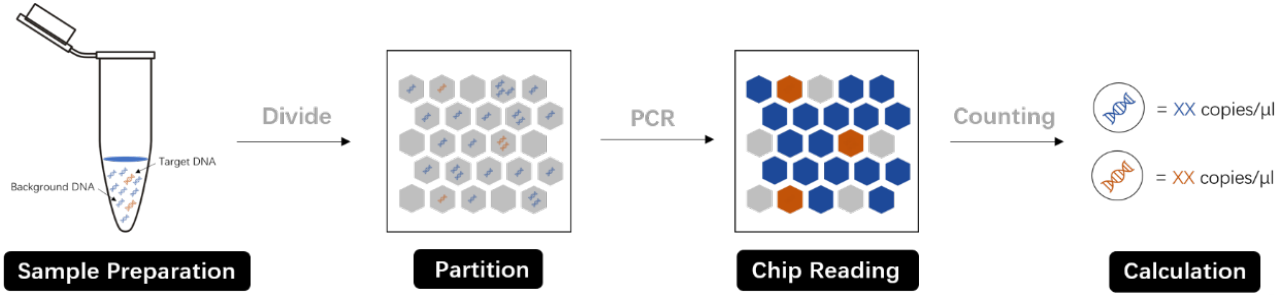
NGS or massively parallel sequencing



You can choose a small panel covering coding exons 9 and 20, or a large panel for clinical liquid biopsy.

Digital PCR detection of PIK3CA mutations

- Detection of circulating free DNA (cfdna) in plasma, also known as "liquid biopsy", avoids the need for biopsy of tumor tissue.
- However, the cfdna content in the blood varies from person to person, and in most cases it is very low. The quality of tumor-derived free nucleic acid (circulating tumor dna, referred to as "ctdna") is even more uneven, and the content varies.
- Currently, there are some methods based on cfdna to detect pik3ca gene mutations, but the sensitivity and specificity of these methods are still unsatisfactory.
- Digital PCR (dPCR) is a powerful technology for targeted mutation detection. Its working principle is to distribute a standard PCR reaction into a large number of tiny reactors, and each reactor may or may not contain one or more copies of the target molecule (DNA template) to achieve "single-molecule template PCR amplification" "After the amplification is completed, the number of positive wells is "counted" by the number of positive reactors, and then the number of positive copies is calculated according to Poisson's formula.



This study designed two multiplex detection methods:

FAM-labeled probes (blue) were used to detect the N345K, C420R, H1047L and H1047R mutations, and a HEX-labeled dropout probe (green) combined with a Cy542-labeled reference probe (red) was used to detect codon 546-5. Two pairs of primers were used to amplify two amplicons simultaneously, a FAM-labeled probe was used to detect the wild-type (WT) sequence, a HEX-labeled probe was used to detect the E542K and E545K mutations, a FAM and Cy1047-labeled probe were used to detect the H5L mutation, and a Cy1047-labeled probe was used to detect the H5L mutation. Labeled probes detect H5R mutations

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Development of multiplex digital PCR assays for the detection of PIK3CA mutations in the plasma of metastatic breast cancer patients

Julien Corné, Fanny Le Du, Véronique Quillien, Florence Godey, Lucie Robert, Héloïse Bourien, Angélique Brunot, Laurence Crouzet, Christophe Perrin, Claudia Lefeuvre-Plesse, Véronique Diéras & Thibault De la Motte Rouge

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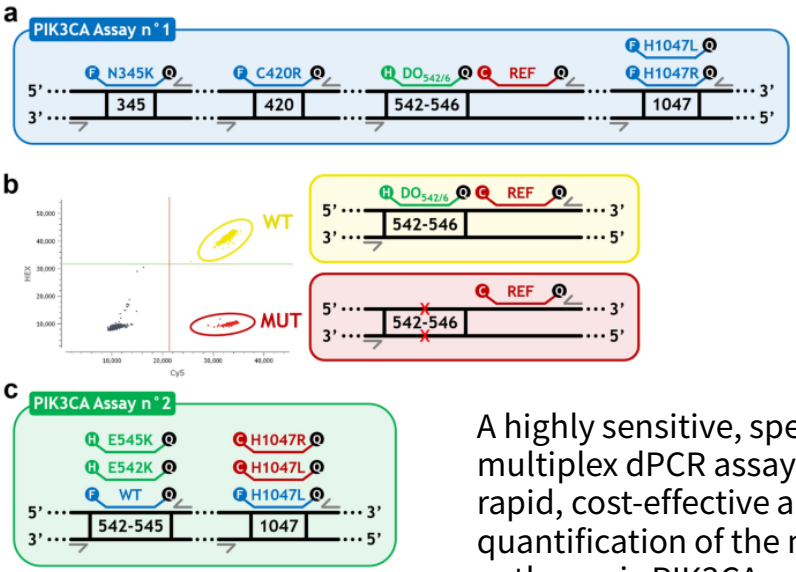

A highly sensitive, specific, and robust multiplex dPCR assay optimized for rapid, cost-effective absolute quantification of the most common pathogenic PIK3CA mutations in breast cancer with 90% coverage.

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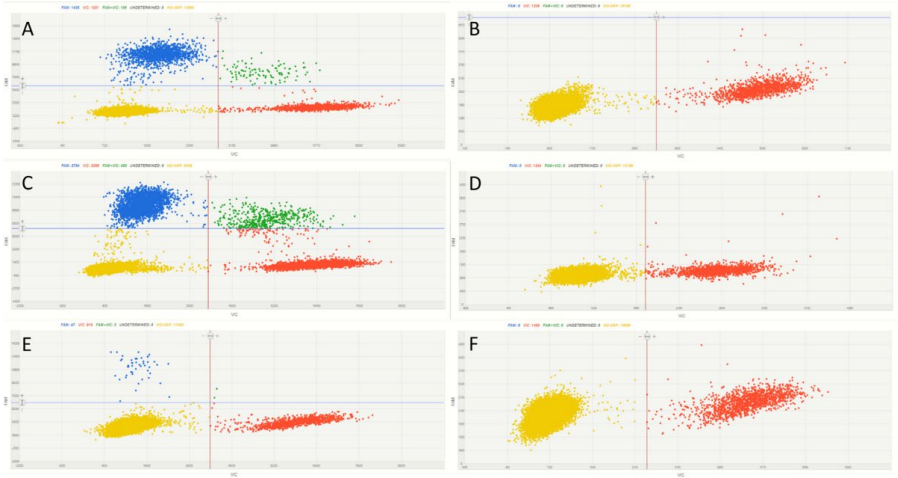
Research article

Chip-based digital Polymerase Chain Reaction as quantitative technique for the detection of *PIK3CA* mutations in breast cancer patients

Stefano Giannoni-Luza [a], Oscar Acosta [a,b], Alexis Germán Murillo Carrasco [a], Pierina Danos [a], José Manuel Cotrina Concha [c], Henry Guerra Miller [c], Joseph A. Pinto [d], Alfredo Aguilar [d], Jhajaira M. Araujo [c], Ricardo Fujita [a], Jose Buleje [a,*]



Tumor samples from 57 breast cancer patients were collected and analyzed by Sanger sequencing and dPCR to detect the three most relevant PIK3CA mutations (p.E545K, p.H1047R, and p.H1047L).
Sanger sequencing detected PIK3CA mutations in 6 patients (10.5%);
Digital PCR detected 7 cases of PIK3CA mutations;
Comparison of dPCR and Sanger sequencing showed a sensitivity of 100% and a specificity of 84.2%.

dPCR showed good performance in detecting PIK3CA mutations with high sensitivity and specificity. In addition, because it can detect low-frequency mutations and mutation coexistences that cannot be detected by Sanger sequencing, dPCR has the potential to become an important tool for genetic testing and personalized treatment.

04

Solutions

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Solutions

Human PIK3Ca gene E542K E545K mutation detection kit

For the qualitative and quantitative detection of PIK3Ca gene E542K E545K mutation in plasma cell-free DNA (cfDNA) of breast cancer, lung cancer, colorectal cancer patients

Human PIK3Ca gene 1047R mutation nucleic acid detection kit

For the qualitative and quantitative detection of PIK3Ca gene 1047R mutation in plasma cell-free DNA (cfDNA) of breast cancer, lung cancer, colorectal cancer patients

- 2~6 color fluorescence detection
- Microcavity chip, uniform cavity
- Accurate quantification, no need to mark curves
- The effective utilization rate of samples reaches 100%

THE END

THANKS

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