maccura

DIGITAL PCR BASED SOLUTIONS FOR THE DETECTION OF TUMOR SPECIFIC GENE

Maccura digital PCR solutions

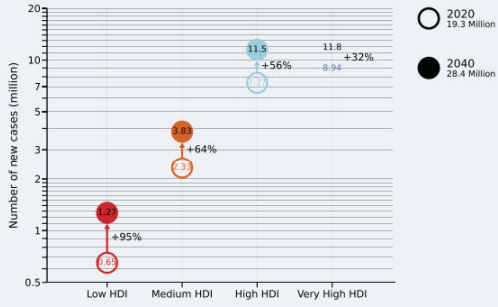
Cancer remains a serious threat to **human health**

According to the data released by the International Agency for Research on Cancer (IARC) of the World Health Organization, there were 19.3 million new cancer cases and 9.9 million deaths worldwide in 2020. The cancer incidence and mortality rates in Asia were as high as 49.3% and 58.3%, respectively. A conservative projection is that there will be 28.4 million new cancers in 2040.

With the continuous development and progress of cancer diagnosis and treatment technology, WHO put forward that: one third of cancers can be completely prevented; One third of cancers can be cured through early detection.

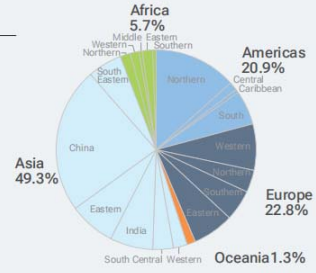
International Agency for Research on Cancer
World Health Organization

Cancer prediction
2020 to **2040**



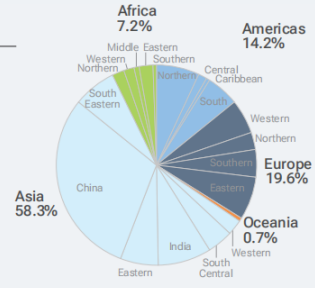
Incidence

19.3 million new cases



Mortality

9.9 million deaths



—Sung.H,et al.CA CANCER J CLIN 2021;71:209–249

Early diagnosis and treatment are the key to improve the survival rate of cancer

The early symptoms of a variety of cancers are not obvious, and most patients are in the middle and late stages when they are diagnosed. However, the survival rate of patients with early diagnosis is significantly improved because of effective treatment, so early diagnosis is crucial to improve the survival rate of cancer patients.

The National Cancer Center of China has issued the guidelines for the screening, early diagnosis and treatment of gastric cancer, colorectal cancer, breast cancer and other cancers to guide the work of cancer diagnosis and treatment and improve the level of national health.

The 5-year relative survival rate comparison

90%	Stage I colorectal cancer	Stage V colorectal cancer	**14%**
55%	Stage I lung cancer	Stage V lung cancer	**5.3%**
90%	Timely treatment of early gastric cancer	Patients undergoing surgery for gastric cancer	**<30%**
95%	Timely treatment of early esophageal cancer	Standardized age of esophageal cancer in 2015	**30.3%**

—Data obtained from the National Cancer Center

Liquid biopsy – a breakthrough in cancer diagnosis and treatment

The diagnostic methods of cancer include tissue biopsy and liquid biopsy, among which liquid biopsy is one of the "top ten breakthrough technologies in 2015". Liquid biopsy can be examined only by obtaining the body fluids of patients, which is not invasive, and can be detected at different time points to dynamically monitor the progress of the disease, which has a wide range of clinical applications and far-reaching significance.

01 Early Diagnosis

02 Risk Assessment

03 Treatment Options

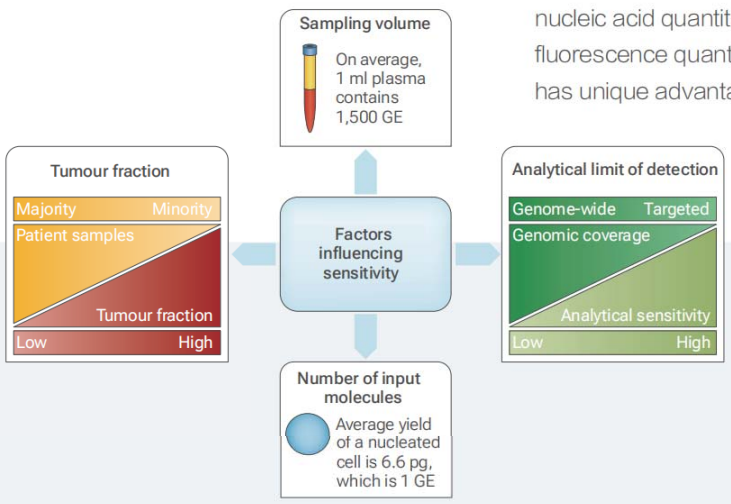
04 Identification of drug targets and resistance mechanisms

05 Surveillance for residual disease

High-sensitivity and high-precision detection method helps the study of tumor course

Tumor-associated DNA can be diagnosed by detecting tumor associ-ated DNA in blood samples, but its content is very low, so it is difficult to screen target DNA from a large number of circulating free DNA (cf DNA) and blood cells. Detection technology and physiological factors limit the widespread implementation of liquid biopsy in clinical practice.

digital PCR(dPCR) technology is an absolute quantitative detection method for nucleic acid. It can accurately quantify nucleic acid without a standard curve, which ensures the accuracy and precision of detection to a great extent, and is resistant to inhibitor interference. It is the third generation of nucleic acid quantitative detection technology after real-time fluorescence quantitative PCR (qPCR) technology, which has unique advantages in tumor liquid biopsy.

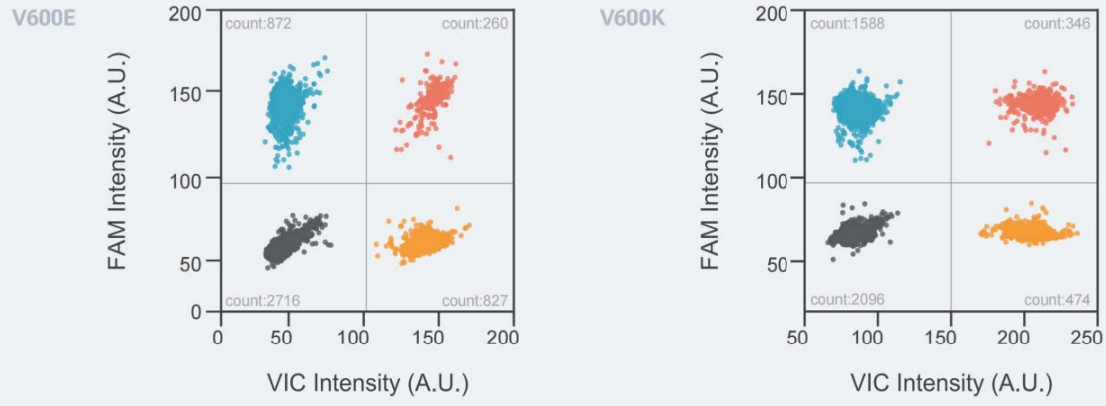


——Heitzer.E,et al.Nat Rev Genet (2019) 20:71–88

BRAF Gene MUTATION Detection

BRAF gene is located on human chromosome 7 and mainly encodes a serine/threonine protein kinase of the RAF family. Mutations in this gene are commonly associated with cancers, such as colorectal cancer, thyroid cancer, and non-small cell lung cancer. The detection of BRAF gene mutation by dPCR can be applied to tumor liquid biopsy and anti-tumor drug development.

The figure below is a representative 2D scatter plot of human BRAF V600E and V600K mutation detection.

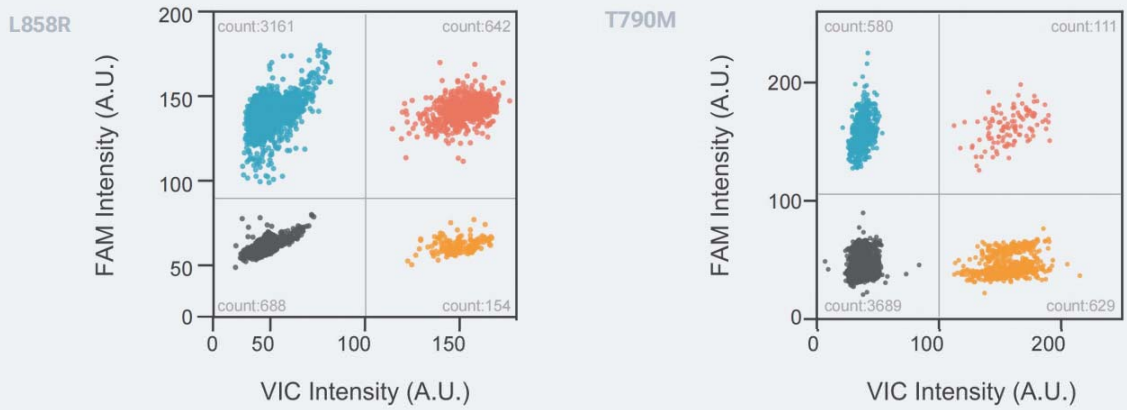


* FAM: mutant type VIC: wild type

EGFR Gene MUTATION Detection

EGFR gene is the receptor for cell proliferation and signal transduction of human epidermal growth factor. This gene has mutations in non-small cell lung cancer. Accurate detection of EGFR gene mutations is of great significance for the selection of clinical treatment drugs.

The following figure is a representative 2D scatter plot of human EGFR gene L858R and T790M mutation detection.



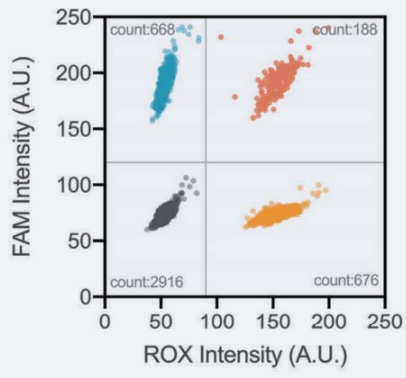
* FAM: mutant type VIC: wild type

BCR-ABL FUSION Gene Detection

Regular monitoring of BCR/ABL expression level in patients with chronic myeloid l eukemia (CML) is the prerequisite to ensure the efficacy. dPCR is more sensitive than qPCR at both the cellular and peripheral-blood levels, increasing the quantit -ative sensitivity by two orders of magnitude. In the monitoring of minimal residual disease, dPCR can provide a deeper molecular biological stratification of CML patients, guide drug use and individualized treatment.

The following figure is a representative 2D scatter plot of BCR/ABLP210 fusion ge ne detection.

BCR/ABL P210



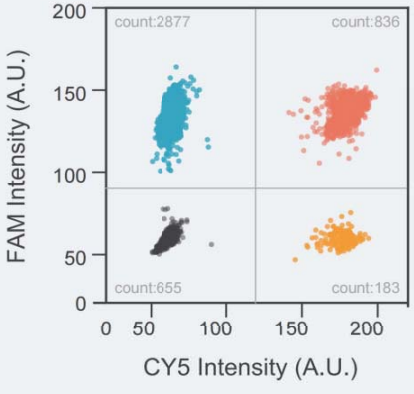
* ROX: mutant type FAM: wild type

PIK3CA Gene MUTATION Detection

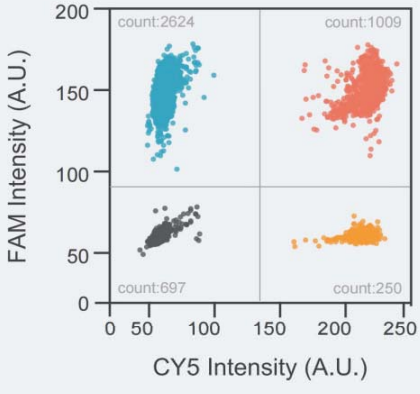
PIK3CA gene is the intracellular homolog of the retroviral v-p3k oncogene, encoding the p110α catalytic subunit of class IA phosphatidylinositol 3-kinase (PI3Ks). PIK3CA mutation is one of the most common types of oncogenic mutations, especially in refractory tumors such as non-small cell lung cancer and triple-negative breast cancer. The establishment of a high sensitivity, rapid and low abundance detection method for PIK3CA mutation is an important way to achieve personalized cancer treatment.

The following figure is a representative 2D scatter plot of human PIK3CA gene Exon9 E542K and Exon9 C420R mutation detection.

Exon9 E542K



Exon9 C420R



* CY5: mutant type FAM: wild type

Collaborative research on MACCURA digital PCR platform

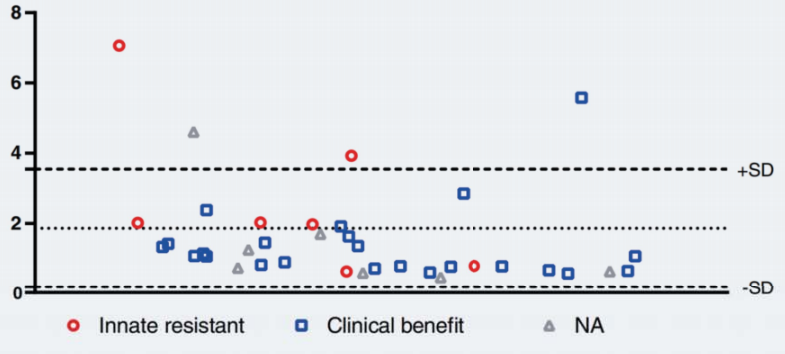
To reveal the molecular mechanisms of primary resistance to palbociclib in patients with $ER^{+}HER2^{-}$ metastatic breast cancer

Professor Fei Ma, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences, and Associate researcher Dan Li, State Key Laboratory of Molecular Oncolog -y, Cancer Hospital, Chinese Academy of Medical Sciences, published a research paper in Molecular Cancer (IF=41.4), entitled "*S6K1 amplification of innate resistance to CDK4/6 inhibitors through activating c-Myc pathway in patients with estrogen receptor-positive breast cancer.*"

CDK4/6 inhibitors —— palbociclib can significantly increase the ER+ HER2 - in patients with metastatic breast cancer (MBC) of progression-free survival and overall survival. However, the presence of drug resistance has hindered the therapeutic efficacy of CDK4/6 inhibitors in patients with ER+ HER2 - MBC. This study included patients who received palbociclib and endocrine therapy as the discovery cohort and found that S6K 1 amplification may be an important cause of primary resistanc -e to palbociclib in ER+HER2-MBC patients.

In this study, S6K1 copy number variation (CNV) detection using the Maccura digital PCR platform showed that the incidence of S6K1 amplification was 28.6% in patients who were naturally resistant to palbociclib, while in patients who had a clinical benefit from palbociclib treatment, the incidence of S6K1 amplification was 28.6%. The incidence of S6K1 amplification was only 4.3%. Digital PCR provides higher detection accuracy and resolution for CNV research.

S6K1:CEP17(ddPCR)



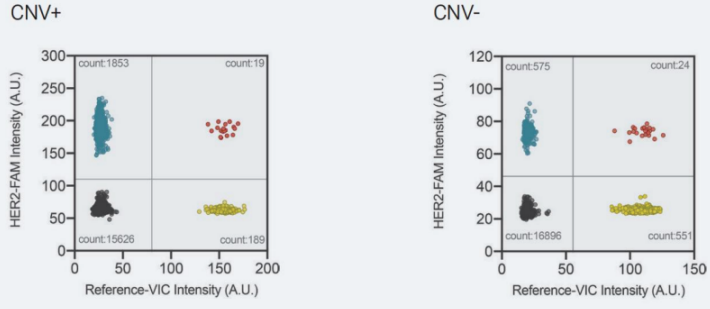
——Mo H,et al. Molecular Cancer (2022) 21:171

Digital PCR has significant advantages in the detection of HER2 copy number variations

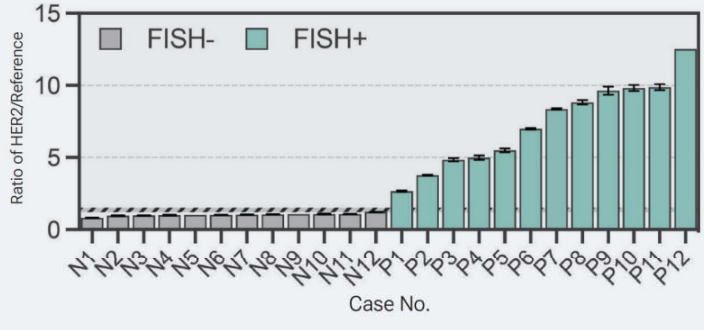
As a proto-oncogene, HER2 gene may be overexpressed in cancers such as breast cancer and lung cancer. Accurate quantification of HER2 gene copy number is of great significance for early screening of breast and lung cancer. The current gold standard method for HER2 gene status detection is FISH assay, but this method has some limitations because of its complexity and reliance on manual counting. Digital PCR can achieve accurate absolute quant-ification of gene copy number, and the detection time is significantly shorter than that of FISH. Quantitative detection of HER2 copy number variations by digital PCR can guide drug use faster and reduce resource waste.

The research group of Dr. Wenbin Du from Institute of Microbiology, Chinese Academy of Sciences and Dr. Cheng Wang from Shanghai Ninth People's Hospital, Shanghai Jiao Tong University Hospital conducted the absolute quan-tification of HER2 copy number variation research project. In this project, D 600 digital PCR platform was used to quantitatively detect HER2 copy number variations, and the results were compared with the actual results of FISH. The results showed that the results of digital PCR were highly consistent with those of FISH, with high accuracy and specificity.

dPCR results



FISH validation results



Product Information

Instrument	D600 Automatic Digital PCR System
Consumables	Universal consumables for digital PCR
Universal Reagents	2 × Universal dPCR MasterMix for DNA 2 × One Step RT-dPCR MasterMix for RNA
Nucleic Acid Extraction Products	N 32 Automatic Nucleic Acid Extraction System N 96 Automatic Nucleic Acid Extraction System Mag-Bind DNA/RNA Extraction Kit
Customized Reagents*	Human PIK3CA E542K Digital PCR Kit Human PIK3CA C420R Digital PCR Kit Human PIK3CA H1047R Digital PCR Kit Human MAP3K3 I441M Digital PCR Kit Human BCR-ABL P210 Digital PCRKit Human EGFR T790M Digital PCR Kit Human EGFR L858R Digital PCR Kit Human EGFR L861Q Digital PCRKit Human BRAF V600E Digital PCR Kit Human BRAF V600D Digital PCR Kit Human BRAF V600K Digital PCR Kit Human MGMT gene methylation Digital PCR Kit Human MLH1 gene methylation Digital PCR Kit

* RUO

Tumor related personalized reagent customization*

Mutation Detection

EGFR	Exon19 del Exon21 L858R Exon18 G719X Exon21 L861Q Exon20 T790M Exon20 ins
KRAS	G12D G12V G12S G12C G12A G12R G13A G13D
NRAS	Exon2 Codon12,13 Exon3 Codon59,61
BRAF	Exon15 V600E Exon15 V600D Exon15 V600K Exon15 V600R
MET	Exon 14 alterations
HER2	Exon20 ins Exon8 alterations Exon17 V659E
PIK3CA	Exon9 E542K Exon9 E545K Exon9 C420R Exon20 H1047R Exon20 G1049G
BRCA1	Exon2 Exon5 Exon11 Exon18 Exon20 Exon21
BRCA2	Exon10 Exon11
C-KIT	Exon11 Exon9 Exon13 Exon17
PDGFRA	Exon18 D842V Exon12 Exon14
IDH1	R132C R132G R132H
JAK2	V617F
MYD88	L265P
TP53	R175H R248L R273C R273H

CNV

HER2 CNV
METCNV

Fusion Gene Detection

ALK
ROS1
RET
BCR-ABL

Gene Methylation Detection

Septin9
SDC2
NDRG 4
BMP6
P16
RASSF1A
SHOX2
CPXM1
HOXA10
DACH6
Cyclin D2
PAX5

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