

让人们的生活更健康  
Make people's lives healthier

# 二代测序技术在肿瘤精准诊断中的应用

## - 肝癌为例

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2019-7-27

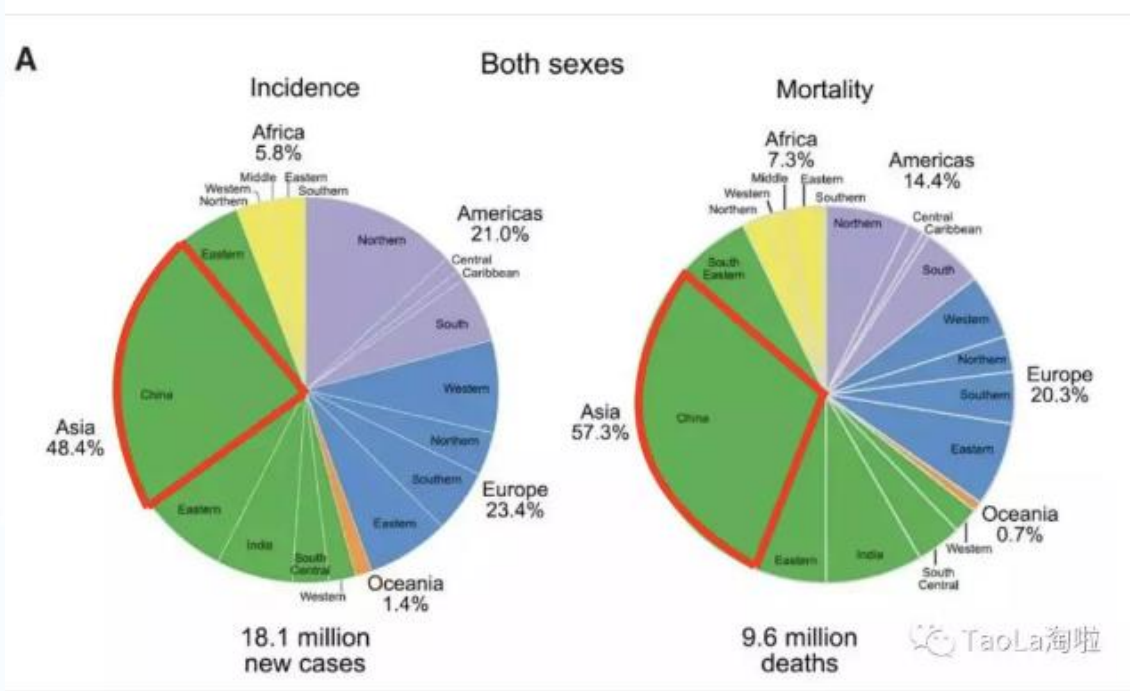


# Outline

- A trend of cancer: global, US and China
- Liver cancer
  - Targeted Therapy
  - Immunotherapy
  - Early detection by NGS
- Next Generation Sequencing (NGS)
  - Whole-exome sequencing of a JMML case
  - Huayin 88-gene myeloid NGS panel

# Cancer



## 2018 全球癌症新发病例和死亡病例





中国的新发病例和死亡病例约占了全球四分之一

## 10 Leading Cancer Types: New Cases and Deaths, US 2019

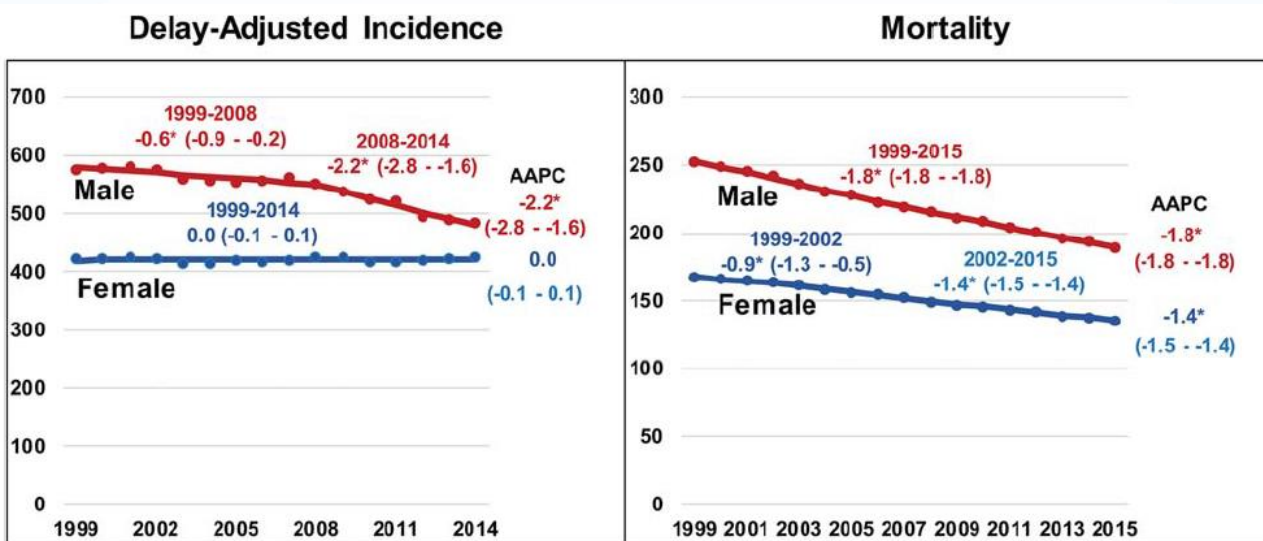
### Estimated New Cases

		Males		Females			
Prostate	174,650	20%			Breast	268,600	30%
Lung & bronchus	116,440	13%			Lung & bronchus	111,710	13%
Colon & rectum	78,500	9%			Colon & rectum	67,100	8%
Urinary bladder	61,700	7%			Uterine corpus	61,880	7%
Melanoma of the skin	57,220	7%			Melanoma of the skin	39,260	4%
Kidney & renal pelvis	44,120	5%			Thyroid	37,810	4%
Non-Hodgkin lymphoma	41,090	5%			Non-Hodgkin lymphoma	33,110	4%
Oral cavity & pharynx	38,140	4%			Kidney & renal pelvis	29,700	3%
Leukemia	35,920	4%			Pancreas	26,830	3%
Pancreas	29,940	3%			Leukemia	25,860	3%
<b>All Sites</b>	<b>870,970</b>	<b>100%</b>	<b>All Sites</b>	<b>891,480</b>	<b>100%</b>		

### Estimated Deaths

		Males		Females			
Lung & bronchus	76,650	24%			Lung & bronchus	66,020	23%
Prostate	31,620	10%			Breast	41,760	15%
Colon & rectum	27,640	9%			Colon & rectum	23,380	8%
Pancreas	23,800	7%			Pancreas	21,950	8%
Liver & intrahepatic bile duct	21,600	7%			Ovary	13,980	5%
Leukemia	13,150	4%			Uterine corpus	12,160	4%
Esophagus	13,020	4%			Liver & intrahepatic bile duct	10,180	4%
Urinary bladder	12,870	4%			Leukemia	9,690	3%
Non-Hodgkin lymphoma	11,510	4%			Non-Hodgkin lymphoma	8,460	3%
Brain & other nervous system	9,910	3%			Brain & other nervous system	7,850	3%
<b>All Sites</b>	<b>321,670</b>	<b>100%</b>	<b>All Sites</b>	<b>285,210</b>	<b>100%</b>		

## All types of cancer overall incidence and mortality rates in US



A 27% decline in cancer death rates since 1991

美国癌症的五年总体生存率已经达到67%

Cronin KA et al. Annual Report to the Nation on the Status of Cancer Cancer. 2018 Jul 1;124(13):2785-2800.

Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019 Jan;69(1):7-34

## 国家癌症中心：2019年中国最新癌症报告

恶性肿瘤是严重威胁我国居民健康的一大类疾病。2019年1月，国家癌症中心发布了最新一期的全国癌症统计数据。



Cancer is the leading cause of death in China

全国分性别主要恶性肿瘤发病前十位

顺位	男性			女性		
	癌症	2015	2014	癌症	2015	2014
1	肺癌	24.17%	24.63%	乳腺癌	17.10%	16.51%
2	胃癌	13.06%	13.62%	肺癌	15.02%	15.43%
3	肝癌	12.74%	12.72%	结直肠癌	9.17%	9.25%
4	结直肠癌	10.46%	10.13%	甲状腺癌	8.49%	7.50%
5	食管癌	8.23%	8.77%	胃癌	6.86%	7.25%
6	前列腺癌	3.35%	3.25%	子宫颈癌	6.25%	6.04%
7	膀胱癌	2.88%	2.87%	肝癌	5.40%	5.68%
8	胰腺癌	2.51%	2.47%	食管癌	3.88%	4.29%
9	淋巴瘤	2.42%	2.24%	子宫体癌	3.88%	3.79%
10	脑癌	2.32%	2.27%	脑癌	3.21%	3.15%
	其他	17.86%	17.04%	其他	20.74%	21.11%
合计		100%	100%		100%	100%



全国分性别主要恶性肿瘤死亡前十位

	男性		女性	
	癌症	比例	癌症	比例
1	肺癌	29.28%	肺癌	22.96%
2	肝癌	16.36%	胃癌	10.49%
3	胃癌	13.59%	肝癌	9.79%
4	食管癌	9.26%	结直肠癌	9.09%
5	结直肠癌	7.44%	乳腺癌	8.16%
6	胰腺癌	3.25%	食管癌	5.94%
7	白血病	2.16%	胰腺癌	4.20%
8	脑癌	2.10%	子宫颈癌	3.96%
9	淋巴瘤	2.10%	脑癌	2.91%
10	前列腺癌	2.10%	卵巢癌	2.911%
	其他	12.36%	其他	19.59%
合计		100%		100%





## Trends in Cancer Incidence and Mortality in China

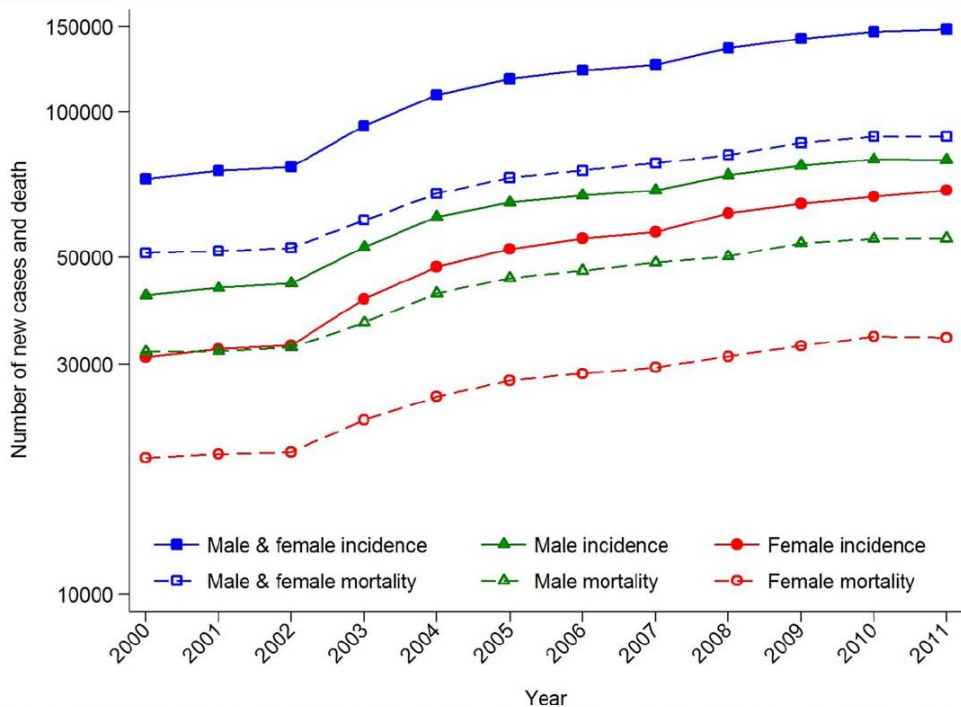


FIGURE 4. Trends in the Number of New Cancer Cases and Deaths for All Cancers Combined by Sex: China, 2000 to 2011.

# Frontier Cancer Therapies

- Targeted Therapy
  - More than 130 new cancer drugs or indications approved by the FDA since 2006
- Immunotherapy
  - Checkpoint inhibitors
    - Combination with other immunotherapies and chemotherapy with better survival rate
  - CAR-T therapy
    - Anti-CD19 CAR-T for refractory B-ALL (FDA 2017)
    - Anti-CD19 CAR-T for refractory DLBCL (FDA 2017)

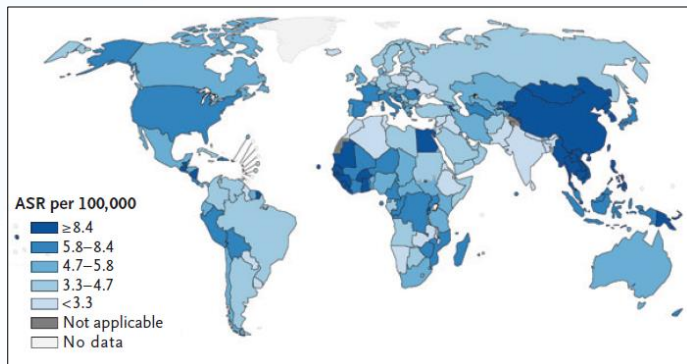
# Liver Cancer

# 肝癌 (Hepatocellular carcinoma)

- Globally liver cancers: 3<sup>rd</sup> most common cause of cancer-related death and 6<sup>th</sup> in incidence
- In China, 2<sup>nd</sup> in cancer death, 3<sup>rd</sup> in incidence
- Mostly as a result of HBV or HCV infection and alcohol abuse
- Increase in nonalcoholic fatty liver disease (NAFLD) in metabolic syndrome and obesity
- 4 new agents with clinical efficacy in the past 2 years with FDA approvals

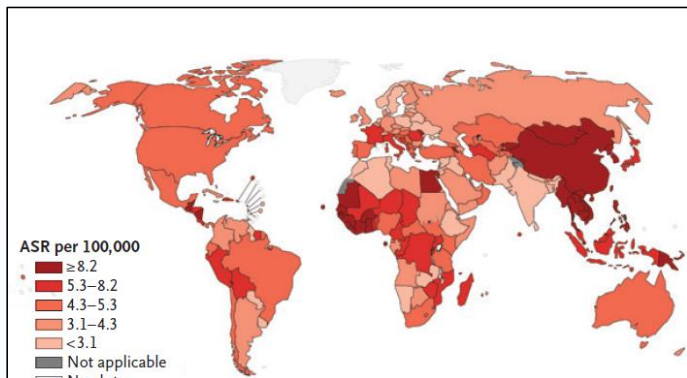
Forner A et al. Hepatocellular carcinoma. Lancet 2018; 391: 1301–14

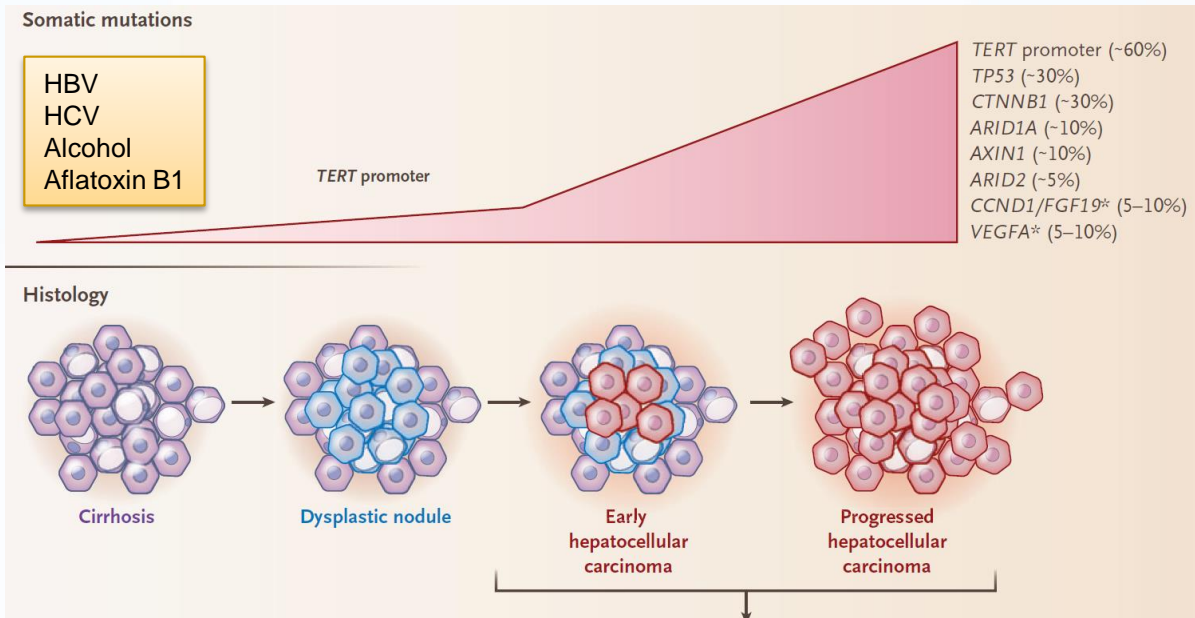
### A. Incidence



### Worldwide Epidemiology of Liver Cancer in 2018

### B. Mortality

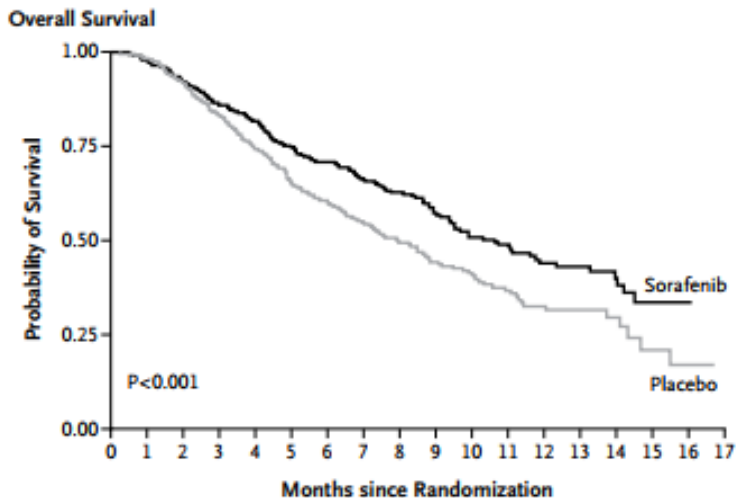




HBV, HCV, alcohol > Chronic hepatic inflammation > Fibrosis > **Cirrhosis** > Aberrant hepatocyte regeneration > dysplastic nodules (preneoplasia) > Hepatocellular carcinoma with proliferative, invasive, and survival advantages

ORIGINAL ARTICLE

## Sorafenib in Advanced Hepatocellular Carcinoma



A phase 3 trial, 602 patients.  
An oral multikinase inhibitor that blocks RAF signalling and vascular endothelial growth factor, platelet-derived growth factor, and KIT.

SHARP trial: survival increased from a median of 7.9 months with placebo to 10.7 months with sorafenib

Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008; 359: 378-90.

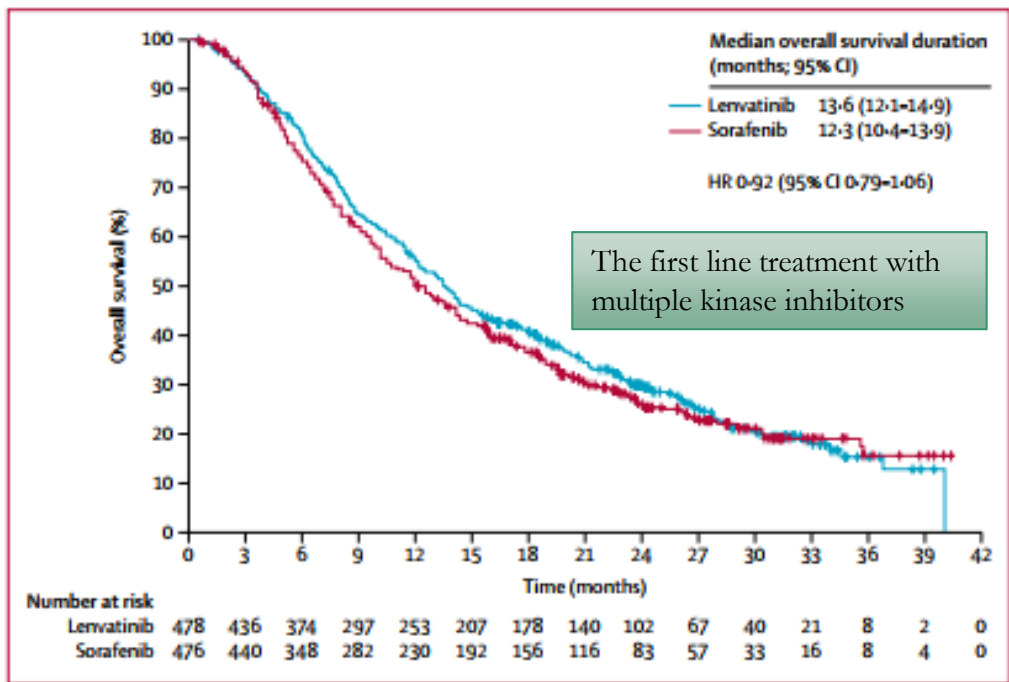


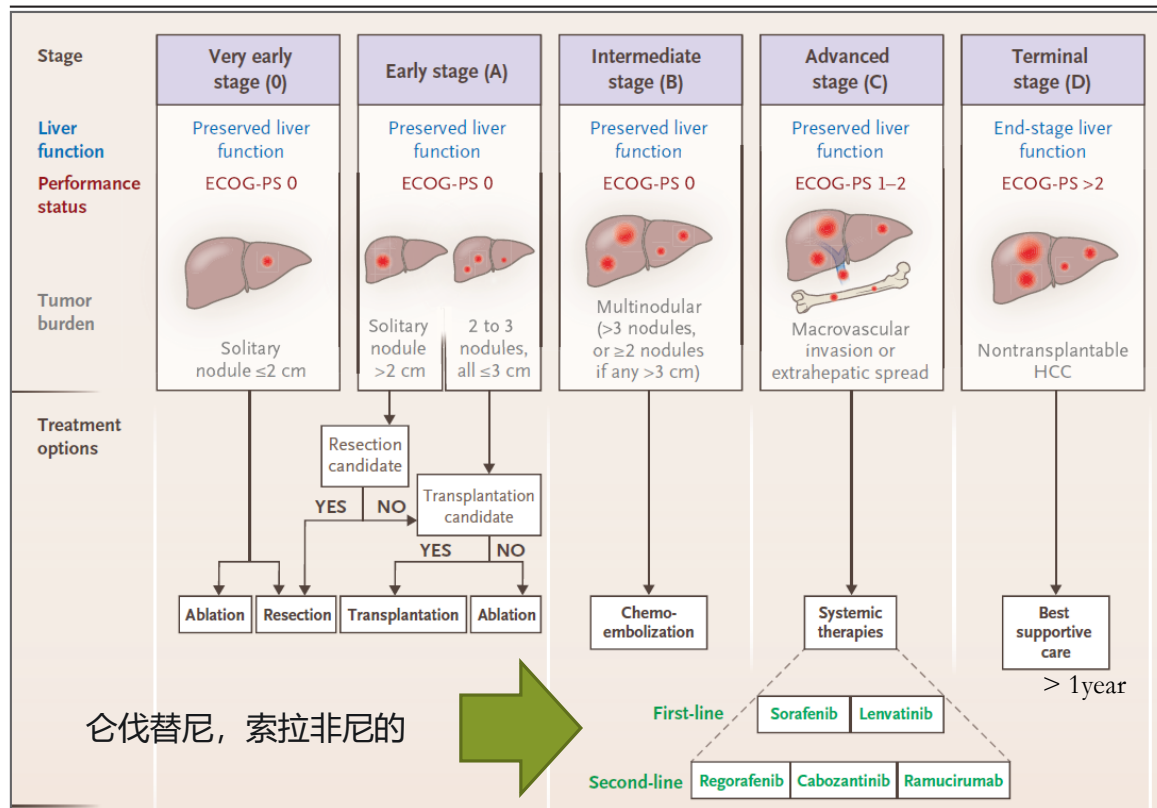
Figure 2: Overall survival outcomes  
Kaplan-Meier estimates of overall survival by treatment group. HR=hazard ratio.

New trial: 1492 patients in 20 countries, median survival was 13.6 months with lenvatinib and 12.3 months with sorafenib

Kudo M et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. Lancet. 2018 Mar 24;391(10126):1163-1173.



## Clinical Algorithm for the Management of Hepatocellular Carcinoma

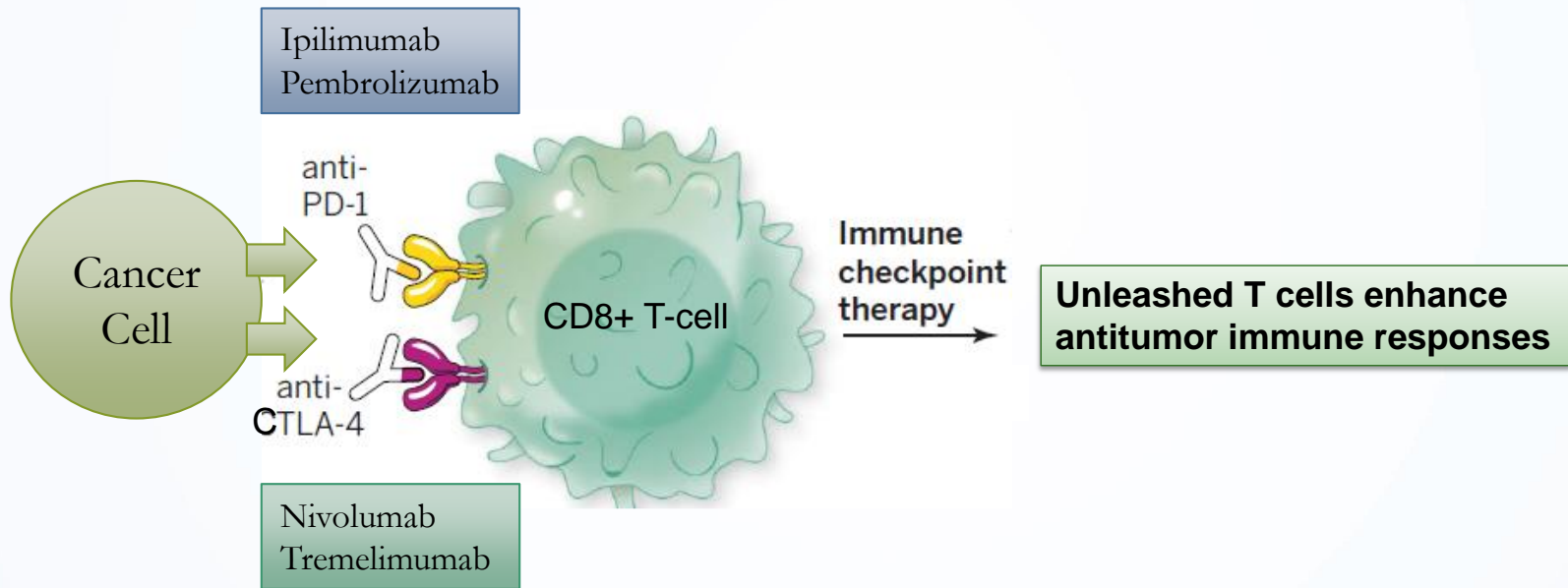




2018 a Nobel Prize was awarded to the researchers found that the immune system could be harnessed to attack cancer

## Principle of Immune Checkpoint Blockade Cancer Therapy

- Inhibiting T-cell adaptive immune resistance



# PD-1 checkpoint inhibitor immunotherapy

- Tremelimumab
- Nivolumab (FDA)
  - 55% response
  - Duration > 12 months
- Pembrolizumab
  - Phase 3
- Combination trials
  - lenvatinib plus pembrolizumab
  - atezolizumab plus bevacizumab

# How to decrease deaths of liver cancer

- Reduce the causes
  - Universal HBV vaccination
  - Improve cure rates of HCV infection
  - Stop alcohol abuse
- Chemoprevention with statin ( ? )
- **Early detection**
- Better clinical management
- New drugs with less toxic and low cost

# Earlier detection of liver cancer by CancerSEEK

- Detecting mutations in cf-DNA by **NGS** and proteins in blood (a 61-amplicon panel, liquid biopsy)
- Sequenced on an Illumina MiSeq instrument.
- Sensitivities ranged from 69 to 98%
- Specificity greater than 99%
- 8 common solid tumor types including **liver**, ovary, stomach, pancreas, esophagus, colorectum, lung, and breast
- Cost less than \$500 per test

Cohen JD, Li L, Wang Y, et al: Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 359:926-930, 2018

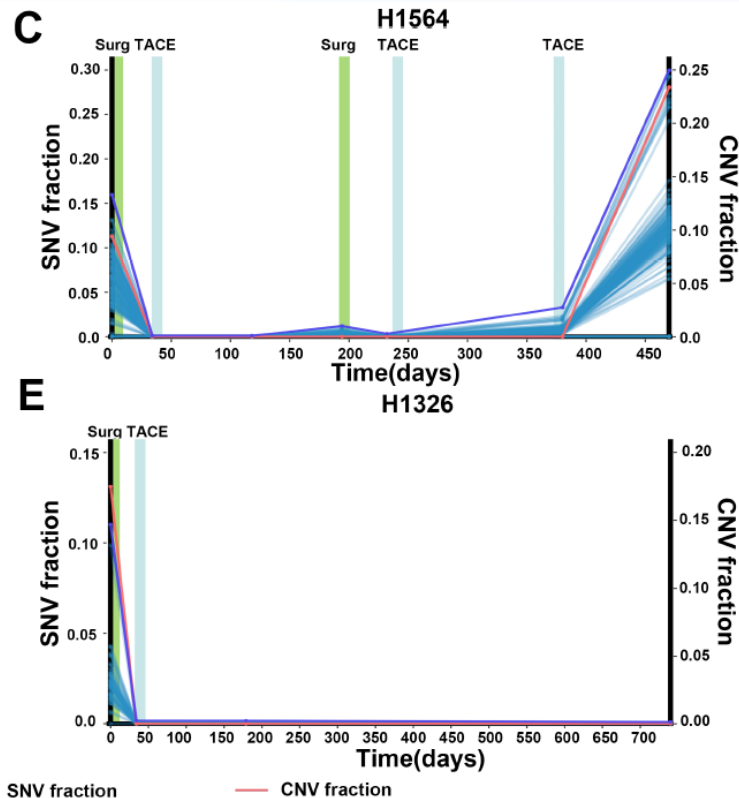
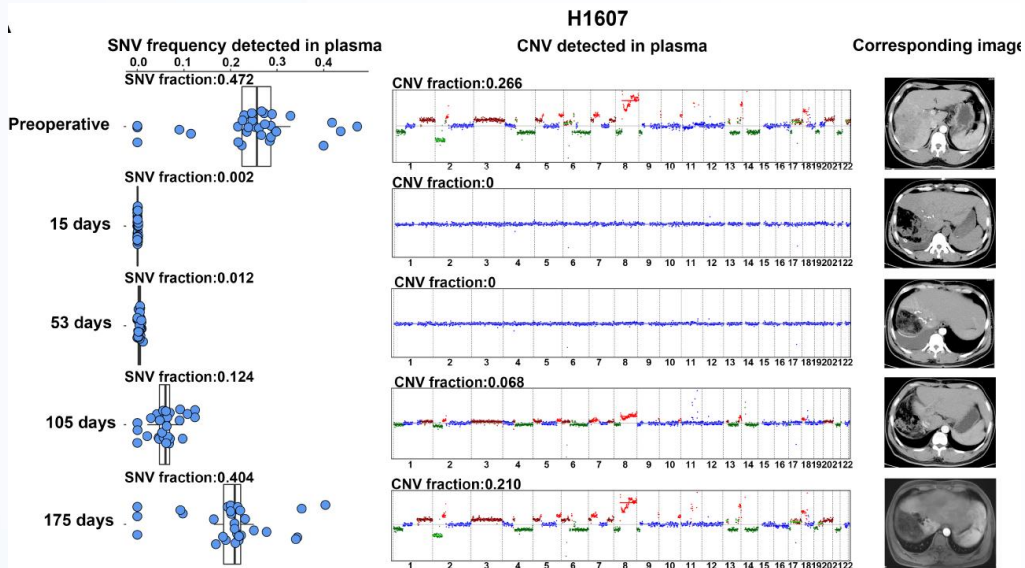
# Clinical Cancer Research

## Comprehensive liquid profiling of circulating tumor DNA and protein biomarkers in long-term follow-up patients with hepatocellular carcinoma

Zhixiong Cai, Geng Chen, Yongyi Zeng, et al.

*Clin Cancer Res* Published OnlineFirst June 19, 2019.

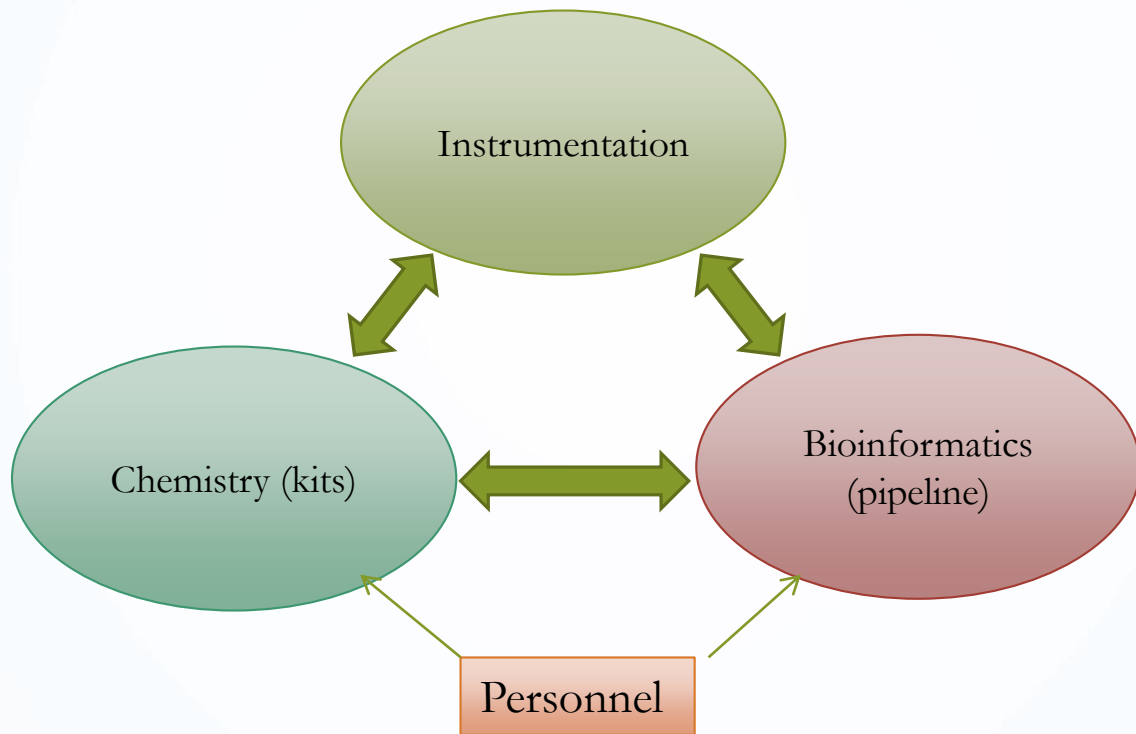
ctDNA SNV and CNV profiling by NGS in HCC patients to detect MRD



# NGS

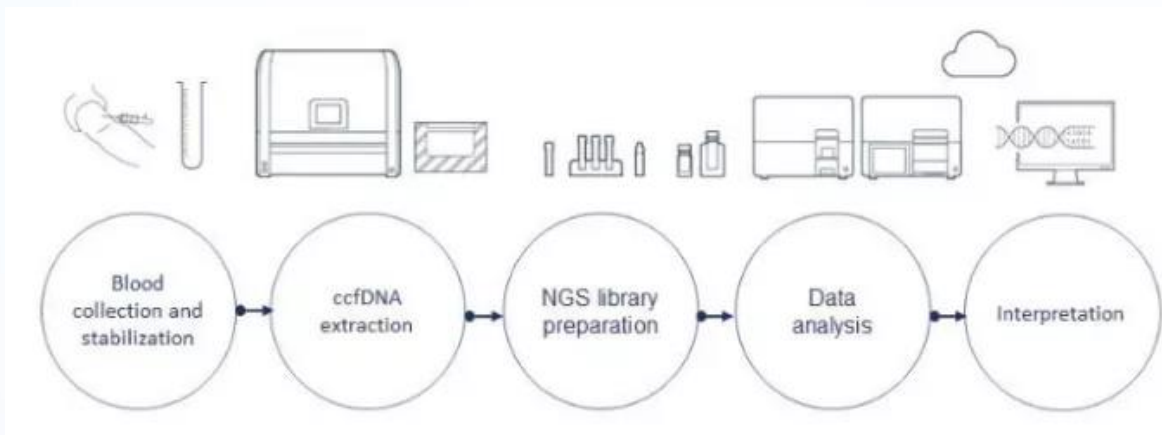


# Components of Diagnostic NGS

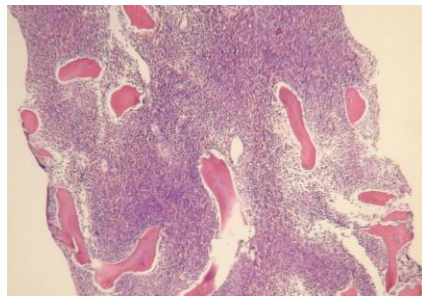
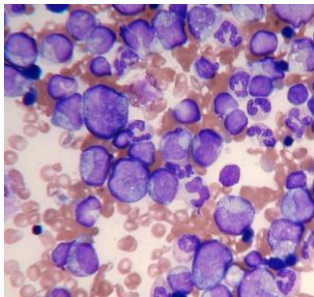
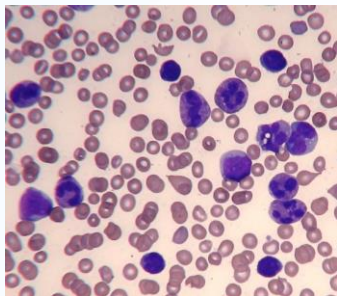


# Whole-exome sequencing reveals PI3K/AKT pathway's involvement in JMML

Saad M Khan, Jason E Denney, Michael X Wang\*, and Dong Xu\*



## Diagnosis of JMML (WHO 2016)



### Clinical and hematological Criteria (need all 4):

- No BCR/ABL fusion gene (FISH-not detected)
- Peripheral blood monocytes  $> 1,000 /\mu\text{L}$  (13,169  $/\mu\text{L}$ )
- Less than 20% blasts in blood and bone marrow (5%)
- Splenomegaly (+)

### Genetic Criteria (need 2 or more):

- Somatic mutations in PTPN11, KRAS or NRAS (PTPN11 mutation, exon 3 181G>T)
- Clinical diagnosis of neurofibromatosis type 1 or NF1 mutation
- Germline CBL mutation and loss of heterozygosity of CBL



### Other Criteria

- Monosomy 7
- Hemoglobin F increased for age (24%)
- Immature granulocytes in peripheral blood smear (5%)
- GM-CSF hypersensitivity in myeloid progenitors in vitro (not performed)
- Hyperphosphorylation of STAT5

## Whole exome sequencing of a JMML sample by NGS

- **Case:** 2-year-old male patient was diagnosed as JMML based on WHO criteria and mutation of *PTPN11* gene. After a consent was obtained, genomic DNA was extracted from a bone marrow sample.
- **NGS:** Whole human exome was captured using Agilent SureSelect All Exon V4 kit and sequenced using Illumina HiSeq 2000 sequencer. The result was verified with a deep sequencing cancer panel.
- **Analysis:** Mercury pipeline for exome was used for analysis.

Reid JG et al. Launching genomics into the cloud: deployment of Mercury, a next generation sequence analysis pipeline. BMC Bioinformatics. 2014 Jan 29;15:30.

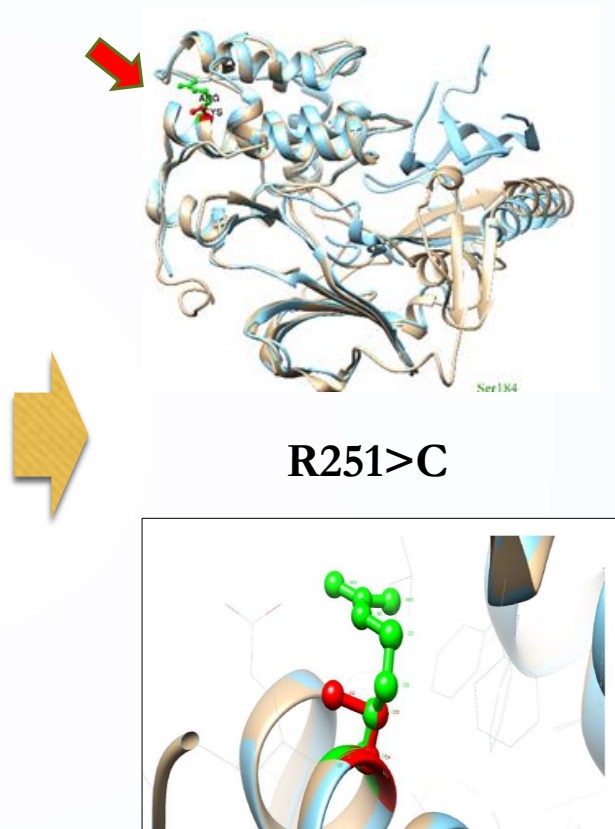
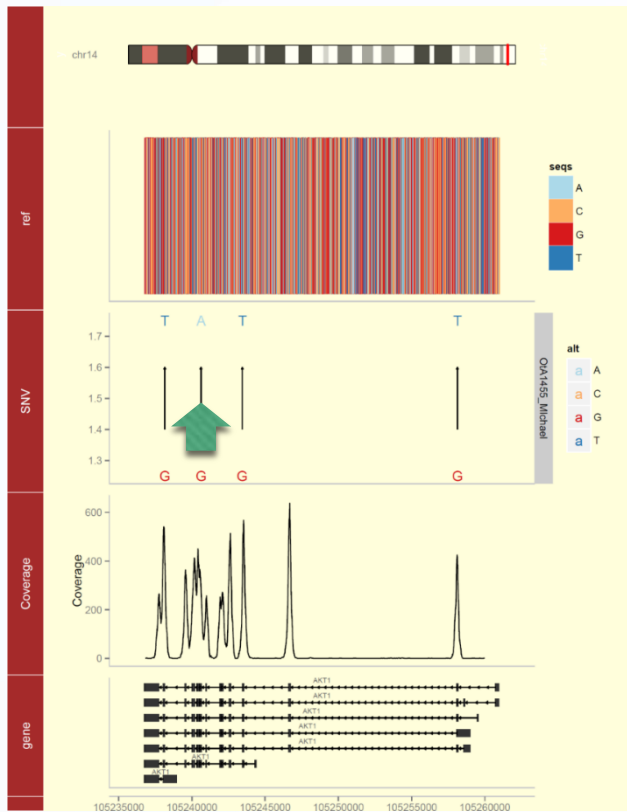
# Alignment Metrics of Whole Exome and Targeted Cancer Panel Sequencing

<b>Exome type</b>	<b>Total no of reads (10<sup>6</sup>)</b>	<b>Mapped Reads (10<sup>6</sup>)</b>	<b>Proper pairs (10<sup>6</sup>)</b>	<b>PCR duplicates (10<sup>6</sup>)</b>	<b>Uniquely mapped (10<sup>6</sup>)</b>
<b>Whole exome</b>	<b>39.41</b>	<b>38.94 (98.82%)</b>	<b>38.74 (98.30%)</b>	<b>1.51 (3.84%)</b>	<b>35.58 (90.3%)</b>
<b>Cancer panel</b>	<b>14.20</b>	<b>13.90 (97.91%)</b>	<b>13.81 (97.28%)</b>	<b>1.23 (8.66%)</b>	<b>12.92 (91.03%)</b>

## Non-synonymous SNVs identified in whole exome sequencing and cancer panel sequencing

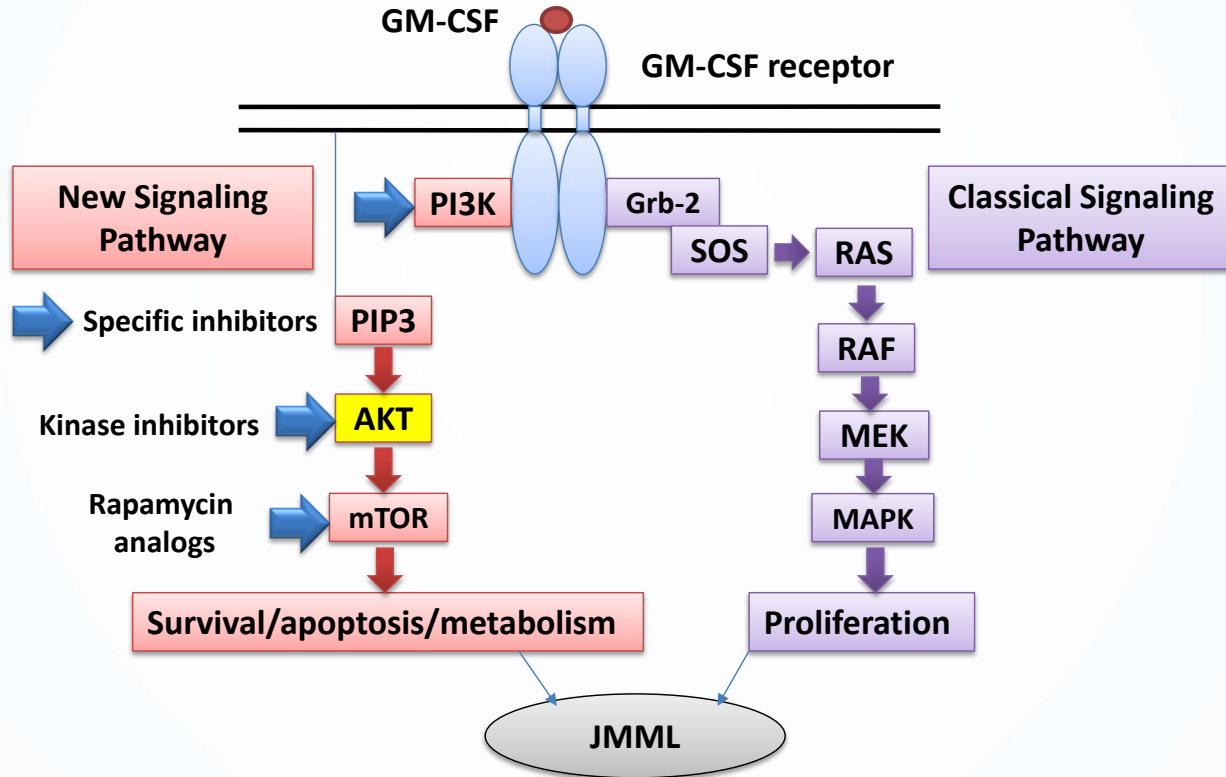
Chromosome	HGNC symbol	SNV position	Reference	Alternative	Mapping quality	Depth	Provean score	Provean Prediction (cutoff = -2.5)	Sift score (cutoff <= -0.5)	Sift prediction	Exon affected	Amino acid change
1	NTRK1	156843653	C	T	60	40	-3.90	Deleterious	0.00	Damaging	Exon8	T360>M
6	IIPR3	33651070	G	A	60/60	40/223	-0.08	Neutral	0.266	Tolerated	Exon35	A1562>T
12	HMG2	66260663	C	G	60	136	-3.00	Deleterious	0.00	Damaging	Exon2	Q7>H
		66260665	G	T	60	134	-2.00	Neutral	0.00	Damaging	Exon2	Q113>K
12	PTPN11	112888165	G	T	60/60	34/139	-7.10	Deleterious	0.00	Damaging	Exon3	D61>Y
14	AKT1	105239869	G	A	60/60	26/259	-6.40	Deleterious	0.013	Damaging	Exon9	R251>C
14	MLH3	75514537	A	T	60/60	48/35	-2.43	Neutral	0.014	Damaging	Exon2	F608>I
22	MYH9	36685257	C	A	60/60	40/187	-3.71	Deleterious	0.030	Damaging	Exon32	K1477>N
X	AR	66765152	T	A	59	26	0.11	Neutral	0.994	Tolerated	Exon1	L55>Q
		66765155	T	A	60	27	0.15	Neutral	0.449	Tolerated	Exon1	L56>Q





Nonsynonymous AKT1 mutation detected

## A Novel Mutation Identified in PI3K/AKT/mTOR Pathway





# 华银NGS Myeloid 88-gene Panel Design

- Literature
  - Genomic Classification and Prognosis in Acute Myeloid Leukemia, NEJM 2015
  - Molecular Minimal Residual Disease in Acute Myeloid Leukemia, NEJM 2018
  - Acute myeloid leukemia ontogeny is defined by distinct somatic mutations, BLOOD 2015
- Professional society recommendation (AMP CMN Working Group DNA Variants in Chronic Myeloid Neoplasms, 2018)
- Commercial product (Illumina TruSight Myeloid Panel)
- WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues – 2016
- Chinese professional guidelines (二代测序技术在血液肿瘤中的应用中国专家共识 (2018年版) 中华血液学杂志, 2018,39(11): 881-886)

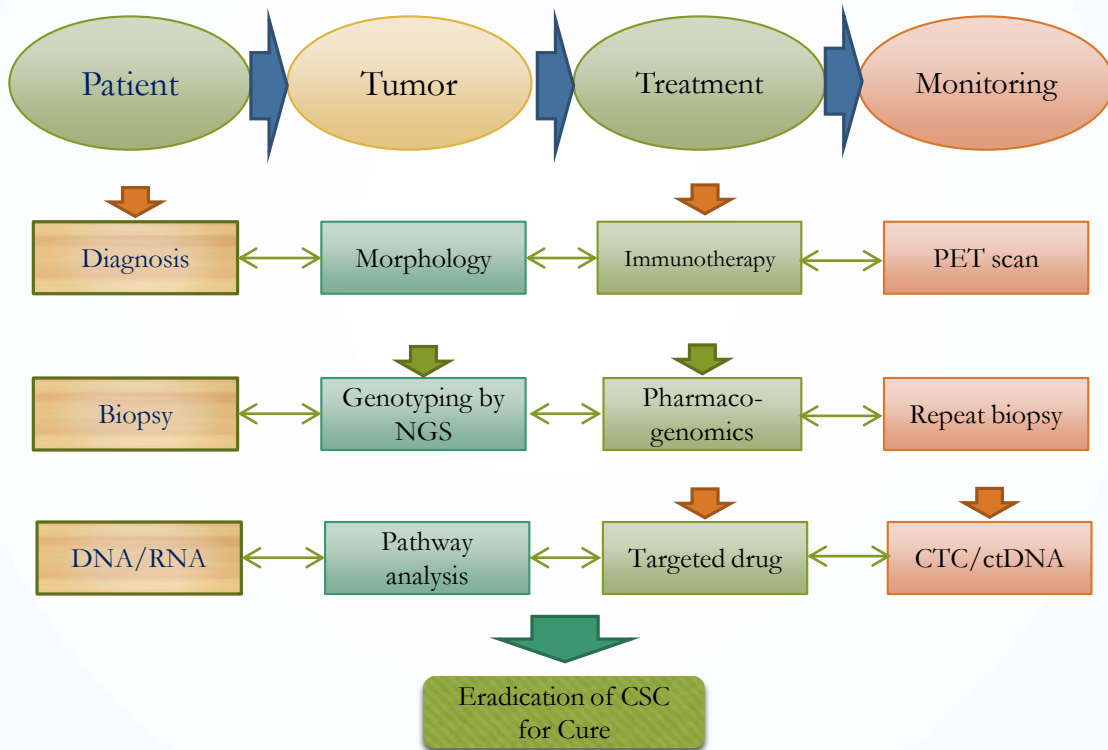
# 华银NGS Myeloid 88-gene Panel

- ABL1
- ANKRD26
- ASXL1
- ATRX
- BCOR
- BCORL1
- BRAF
- CALR
- CBL
- CBLB
- CBLC
- CDKN2A
- CEBPA
- CREBBP
- CSF1R
- CSF3R
- CUX1
- DNMT3A
- EP300
- ETNK1
- ETV6
- EZH2
- FBXW7
- FLT3
- GATA1
- GATA2
- GNAS
- IDH1
- IDH2
- IKZF1
- JAK2
- JAK3
- KDM6A
- KIT
- KMT2A
- KRAS
- MLL2
- MPL
- NF1
- NOTCH1
- NOTCH2
- NPM1
- NRAS
- PDGFRA
- PHF6
- PPM1D
- PRPF8
- PTEN
- PTPN11
- RAD21
- RUNX1
- SETBP1
- SF1
- SF3A1
- SF3B1
- SMC1A
- SMC3
- SRSF2
- STAG2
- TERT
- TET2
- TP53
- U2AF1
- WT1
- ZRSR2
- DDX41
- ANKRD26
- TERC
- TERT

# NGS Validation Process

- **Samples:**
  - Cell lines with known mutations such as DLD1 colon cancer cell line has 8 mutations, 10% dilution to determine sensitivity
  - Clinical samples: >50, different types and tumor content
- **Protocol**
  - Data generation, 9 runs; coverage (>500x)
  - Different types of mutations: point mutation, indels, copy number
  - Verification with the standard methods or another NGS platform
  - Data analysis, summary (PARR+AS/AS) and report
- **Implementation**
  - SOP, personnel training
  - LIS system: ordering, reporting, billing
  - Announcement
  - Communication with clinicians

# Perspectives of Precision Oncology by NGS



# Outline

- A trend of cancer: global, US and China
- Liver cancer
  - Targeted Therapy
  - Immunotherapy
  - Early detection
- Next Generation Sequencing (NGS)
  - Whole-exome sequencing of a JMML case
  - Huayin 88-gene myeloid NGS panel

***THANK YOU***