Biopsia liquida en el manejo del carcinoma hepatocelular

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Disclosures

- **Advisory board / Consulting**: NGM Pharmaceuticals, Natera, Eisai, Astra Zeneca, BMS, Boehringer Ingelheim, Cambridge Healthcare Research, Genentech, Gilead, Espervita, Pioneering Medicine

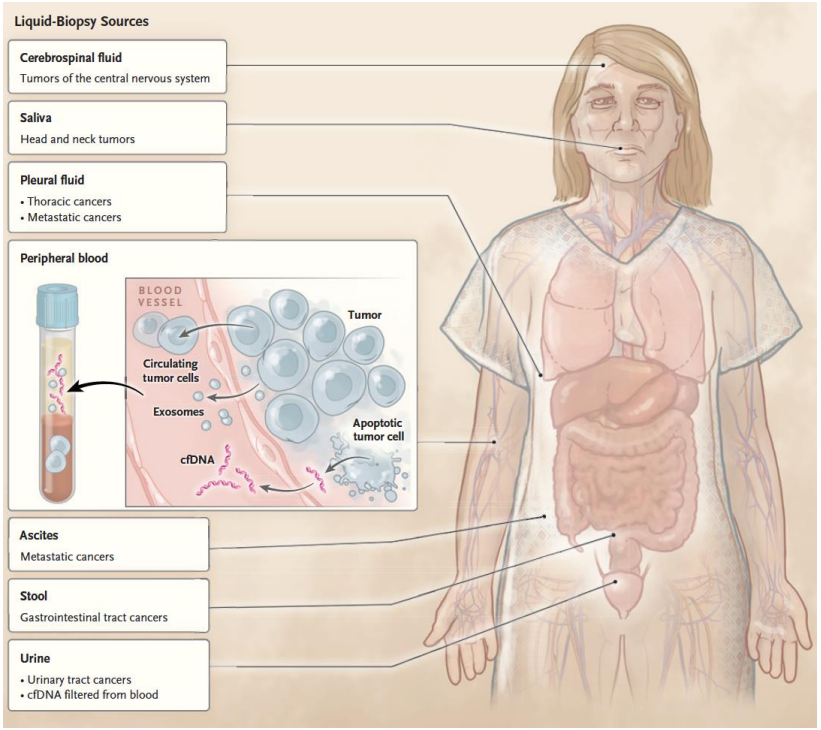
- **Research support**: Eisai Pharmaceuticals

- **Patent**: Small unannotated, non-coding RNAs for the detection of liver cancer (PCT/US20/61441)

Liquid biopsy

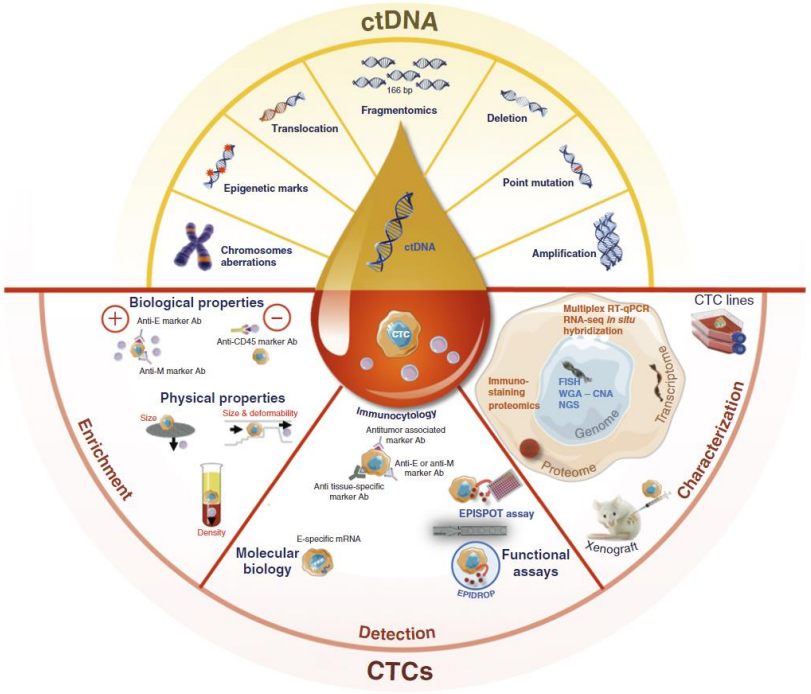
Clinical applications

Tumor components released to fluids



Corcoran RB, NEJM 2018

Molecular analysis in liquid biopsy



Alix-Panabieres, Cancer Disc 2021

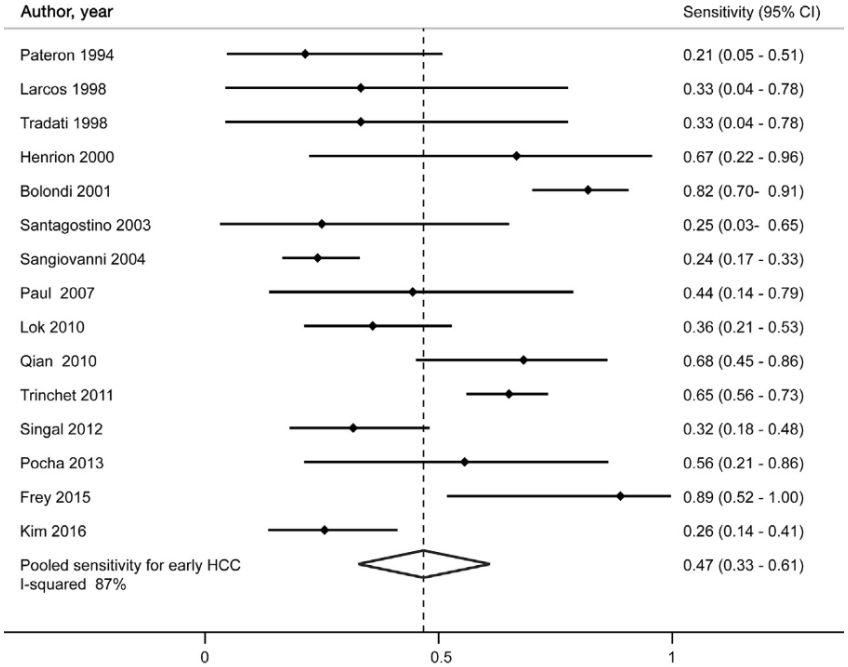
Outline

- Liquid biopsy in the clinical management of HCC
  - Early detection (tumor burden, minimal residual disease)
  - Biomarkers of treatment response

HCC early detection

Recommended tools

Abdominal US



AFP

268 THE LANCET, AUGUST 1, 1970

comparable to that described by Ohno et al.[12] in *Microtus oregoni*, a random loss of one of the Y chromosomes during the spermatogonial divisions[1,2] seems to be an alternative explanation. As pointed out previously,[14,15] the D.N.A.-replication pattern of the two Y chromosomes differs slightly. Although these observations should be confirmed by ^{3}H-TdR pulse labelling, there is evidence that this behaviour reflects a cytological difference between the two Y chromosomes. Under these circumstances, even a preferential anaphase lag of one of the Y chromosomes is conceivable. A proliferative advantage of the 46,XY germ-cells may play an important additional role.[1,2]

We are grateful to Dr. Breit and Dr. Heyd (Munich) and Prof. W. Meyhöfer (Giessen) for performing the testicular biopsies.

Pathologisches Institut, Universität Bonn/Rh.
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W. TINNEFELD
W. FUHRMANN.
E. SCHWINGER.

AGE-DISTRIBUTION OF α-FETOPROTEIN IN HEPATOCELLULAR CARCINOMA

SIR,—Dr. Mawas and his colleagues' report [13] indicating a possible influence of age on the occurrence of α-feto-protein (A.F.P.) in patients with hepatocellular carcinoma and teratocarcinoma is supported by our results in hepato-cellular carcinoma. Using an immunodiffusion method and antiserum supplied by Dr. G. I. Abelev, we have detected A.F.P. in serum from fifteen of twenty-four patients with primary hepatic carcinoma (confirmed by needle biopsy of the liver). Correlation of the presence of A.F.P. with the age of the patients (see accompanying table) indicates that positive tests occurred mainly in the younger patients. The protein was present in 100% of patients under thirty, in 66% of patients between thirty and forty, and in only 22% of patients over forty.

A.F.P. TESTS IN DIFFERENT AGE-GROUPS IN PATIENTS WITH PRIMARY HEPATIC CARCINOMA

Age (yr.)	No. of patients	No. A.F.P.-positive	% A.F.P.-positive
10–20	2	2	100
21–30	7	7	100
31–40	6	4	66
>40	9	2	22
Total	24	15	62·5

An association of A.F.P. production with embryonic cells is suggested by its occurrence in the fetus and in patients with testicular teratoma. In patients with primary hepatic carcinoma the production of A.F.P. may reflect a primitive cell-line. If it is confirmed that A.F.P. occurs predominantly in younger patients, the situation may be comparable to that in other tumours which tend to be poorly differentiated in young patients. Abelev [17] has demonstrated that the well-differentiated strains of mouse hepatoma do not synthesise A.F.P. However, Purves [18] could not demonstrate any correlation between tumour differentiation and A.F.P. in man. Whatever the explanation, it is possible that geographic variations in the occurrence of A.F.P. in hepato-cellular carcinoma reflect the age-incidence of the tumour. Variation in the age at which the tumour occurs may indicate different aetiological factors, but these need not directly influence A.F.P. production by the tumour.

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Departments of Medicine and Physiology, University College, Nairobi, Kenya.
A. BAGSHAWE
A. M. PARKER.

LYMPHOCYTE ABNORMALITY IN CHRONIC MUCOCUTANEOUS CANDIDIASIS

SIR,—Dr. Bryceson's comments (July 11, p. 103) on our paper [1] are constructive and relevant. Although there are no published accounts of graft-versus-host reaction in patients with chronic mucocutaneous candidiasis, we are very much aware of this danger. Therefore, we have not yet, after 6 months, decided whether or not lymphoid-cell transplantation to our patient is justified. We think, however, that the risk here is considerably less than in many other patients with immune deficiencies. The defect in our patient appears to be on the efferent side of cellular immunity, being of migration inhibitory factor (M.I.F.) (molecular weight 60,000–70,000). In fact, further work has shown normal ability to produce mitogenic and cyto-toxic factors. The presence of the latter factor in patient 2 of Chilgren et al.[2] (who probably also had only M.I.F. deficiency) might explain how the unmatched donor lymphocytes given to patient 2 only lasted for 14 days as judged by the transient restoration of cutaneous delayed hypersensitivity to candida. The lymphocytes of patient 2 presumably rejected that graft, possibly using cytotoxic factor. To kill candida, however, may require the activation of macrophages by some substance such as M.I.F. Current evidence [3] suggests that Lawrence's transfer factor has a molecular weight below 10,000 (quite distinct from M.I.F.), and acts on the afferent side. With lymphocytes *not transforming* to candida, injection of such a factor could well activate transformation, going through to the release of M.I.F. However, our patients' lymphocytes can already transform to candida, and can make their own mitogenic factor. The injection of transfer factor is unlikely to enable them to make their own M.I.F.—indeed, our patient had already had 11 units of blood (which presumably contained transfer fac-tors). Treatment with fractionated M.I.F. is impractical and likely to have very localised and transient results. Further-more, Dr. S. Lawler (Royal Masrden Hospital) anticipates a complete HL-A match between the patient and her brother, and this is indeed supported by the mixed lymphocyte re-action, in which there was no stimulation between the patient's and her brother's lymphocytes. We therefore conclude that the risk to our patient is slight—indeed, no graft-versus-host reaction has followed her transfusions. We agree, however, that reported results of giving transfer factor to patients with chronic candidiasis although not very encouraging [3,4] do suggest that this treatment should be tried initially in those patients whose lymphocytes fail to transform to candida antigen.

Dr. Block (July 11, p. 104) writes to question "several

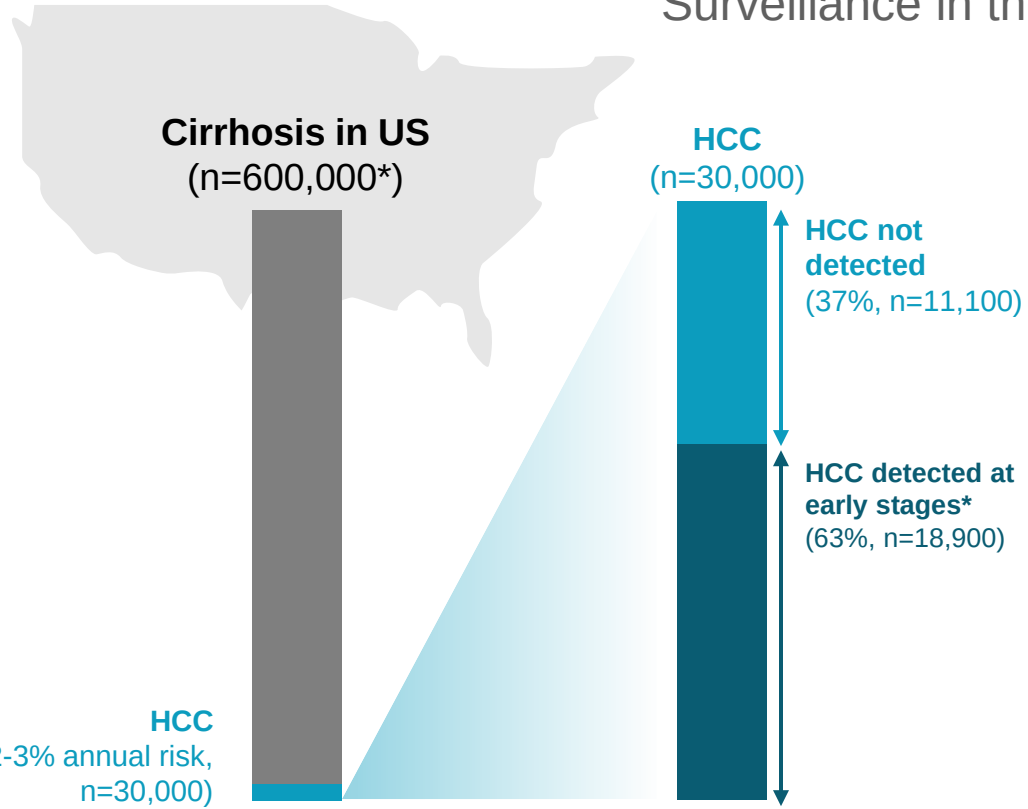
13. Ohno, S., Jainchill, J., Stenius, C. *Cytogenetics*, 1963, **2**, 232.
14. Schwinger, E., Cirotler, P., Gropp, A. *Klin. Wschr.*, 1969, **47**, 548.
15. Murken, J.-D., Bauchinger, M., Kaul, H.-J. *ibid.* 1970, **48**, 62.
16. Mawas, C., Buffe, D., Burtin, P. *Lancet*, 1970, i, 1292.
17. Abelev, G. I., Asseitrova, T. V., Kravevsky, N. A., Perova, S. D., Perevodchikova, N. I. *Int. J. Cancer*, 1967, **2**, 551.
18. Purves, L. R., MacNab, M., Geddes, H. W., Bersohn, I. *S. Afr. med. J.* 1968, **42**, 922.
1. Valdimarsson, H., Holt, L., Riches, H. R. C., Hobbs, J. R. *Lancet*, 1970, i, 1299.
2. Chilgren, R. A., Meuwissen, H. J., Quie, P. G., Good, R. A., Hong, R. *ibid.* 1968, i, 1286.
3. Lawrence, H. S. (editor) Mediators of Cellular Immunity; p. 159. New York, 1969.
4. Kirkpatrick, C. H., Chandler, J. W., Schimke, R. N. *Clin. exp. Immun.* 1970, **6**, 375.

Sensitivity 63%, Specificity 84%

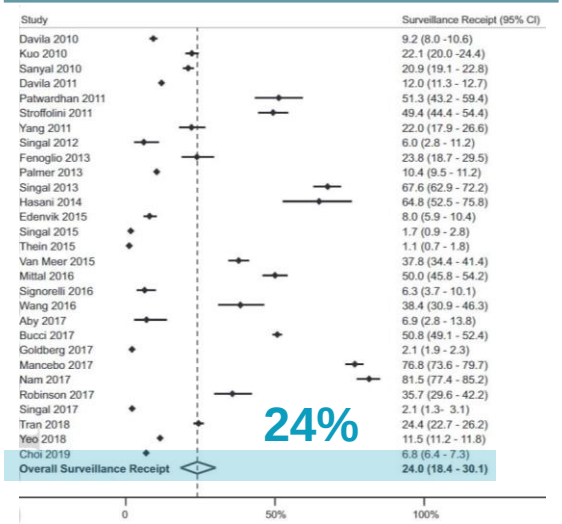
Tzartzeva, Gastroenterology 2018

HCC early detection

Surveillance in the US



Surveillance in cirrhosis (Meta-analysis)

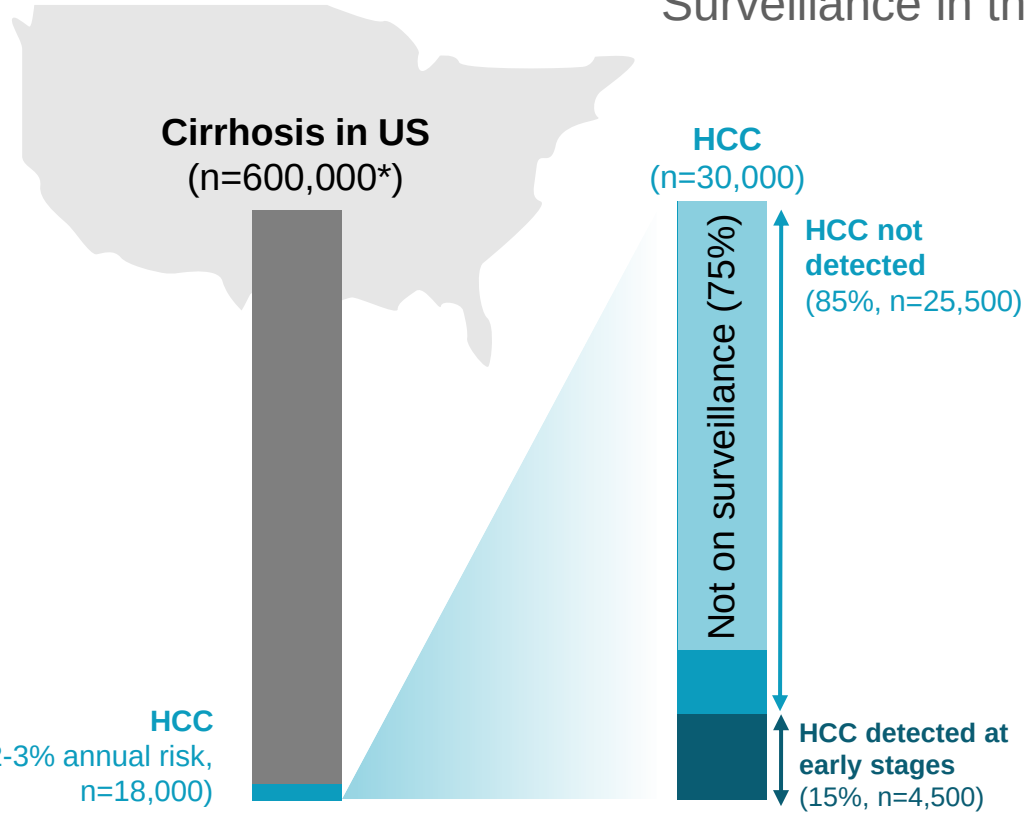


Wolf E et al. Hepatology 2020

*Scaglione, J Clin Gastroenterol 2015
Tzartzeva, Gastroenterology 2018

HCC early detection

Surveillance in the US



Barriers to early HCC detection:

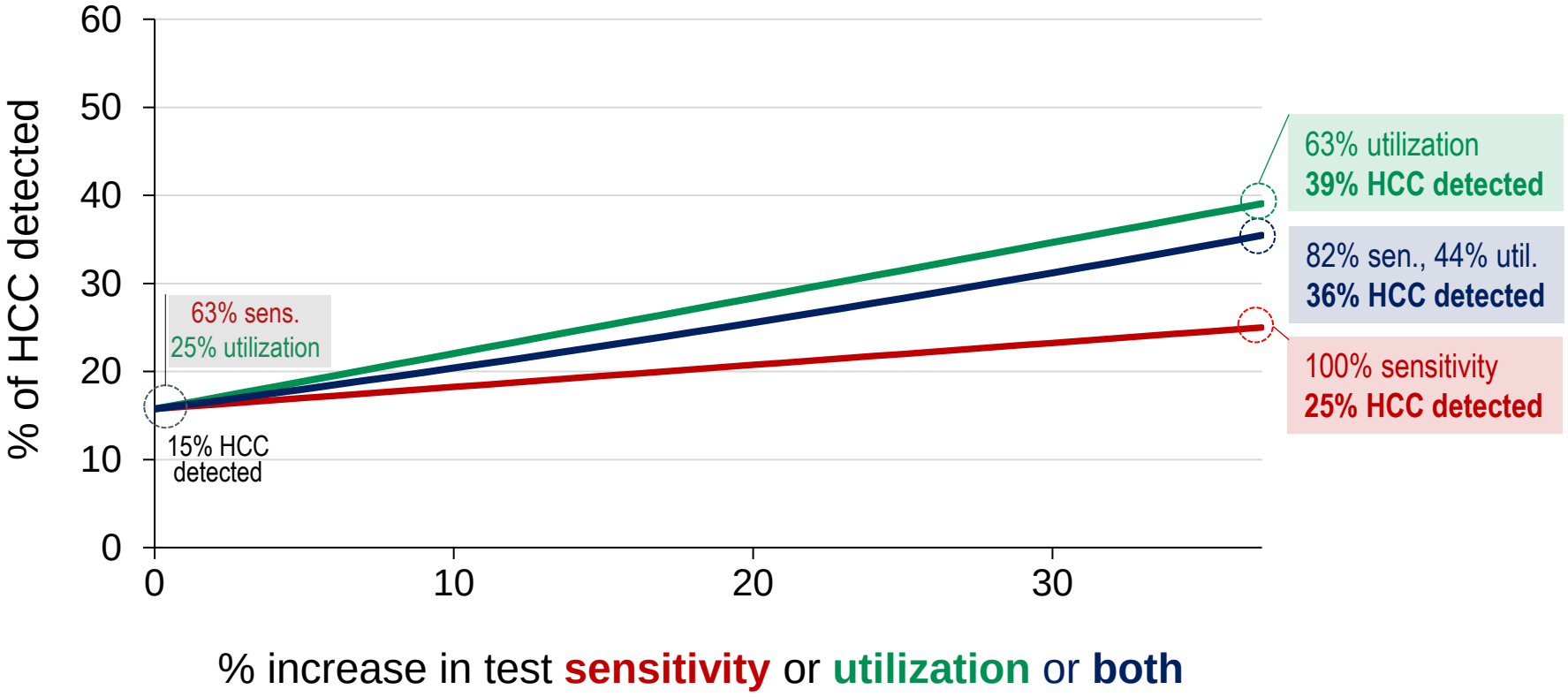
Improve accuracy of the tool

Increase implementation

Scaglione, J Clin Gastroenterol 2015
Tzartzeva, Gastroenterology 2018

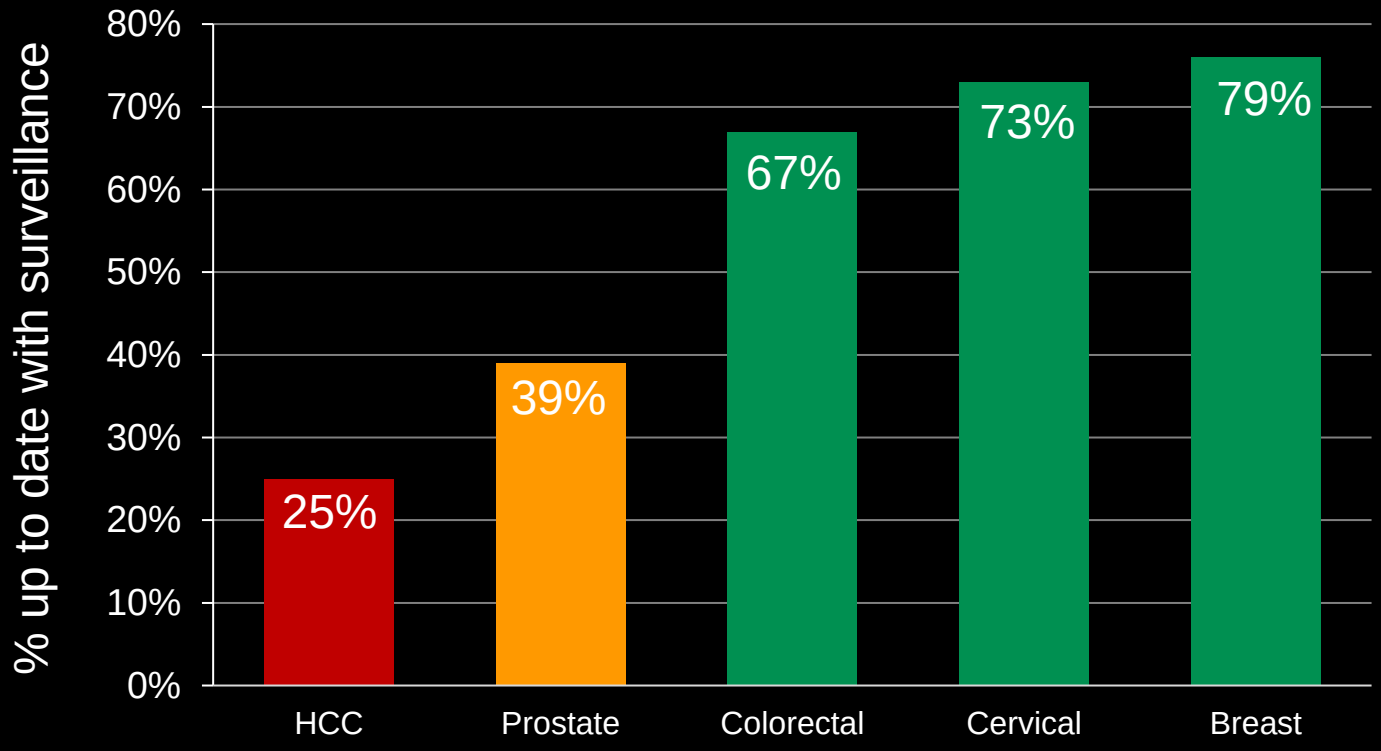
HCC surveillance

Improving sensitivity, utilization or both



HCC surveillance utilization

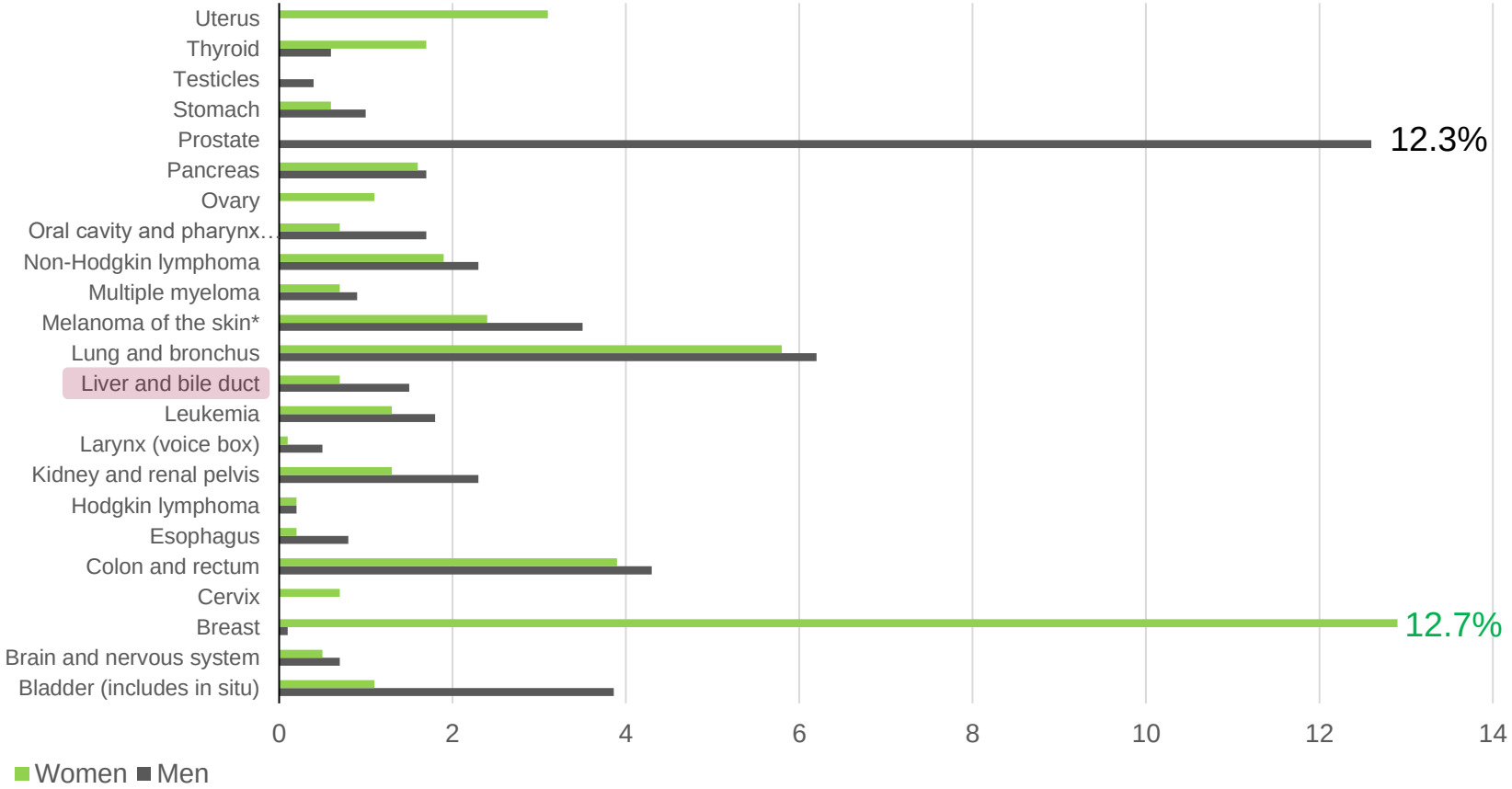
The big picture



NIH – Cancer Trends Progress Report

Lifetime risk of cancer

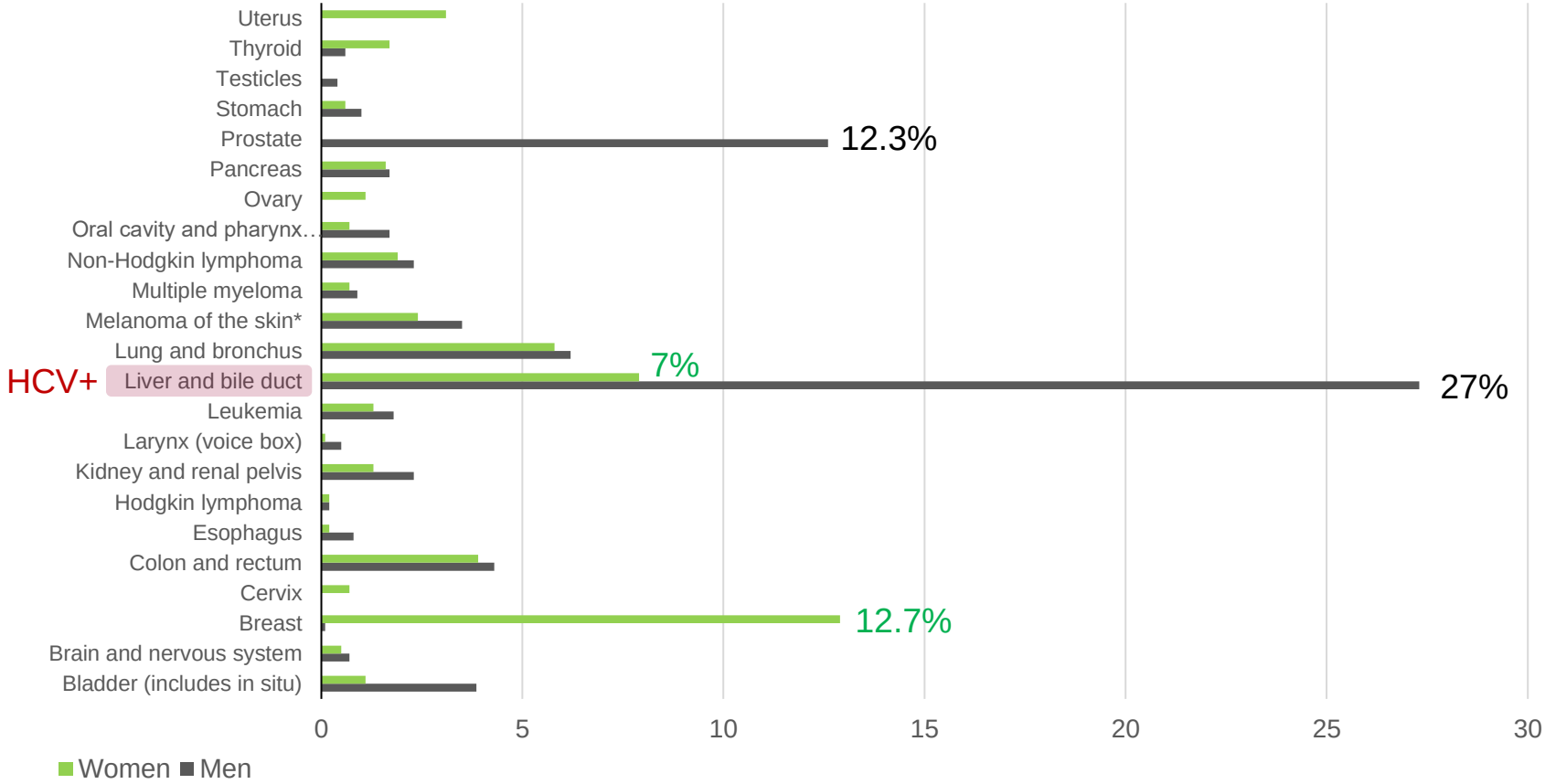
NCI SEER database



https://www.cancer.org/cancer/risk-prevention/

Lifetime risk of cancer

NCI SEER database



Huang, JCO 2011

Liquid biopsy

Methylation of circulating tumor DNA (ctDNA)

Methylation diagnostic signature
(n=1,098 HCC, n=835 control)

Table 1 | Characteristics of ten methylation markers and their coefficients in HCC diagnosis.

Markers	Ref Gene	Coefficients	SE	*z* value	*p* value
		15.595	2.395	6.513	<0.001
cg10428836	BMPR1A	11.543	0.885	−13.040	<0.001
cg26668608	PSD	4.557	0.889	5.129	<0.001
cg25754195	ARHGAP25	2.519	0.722	3.487	<0.001
cg05205842	KLF3	−3.612	0.954	−3.785	<0.001
cg11606215	PLAC8	6.865	1.095	6.271	<0.001
cg24067911	ATXN1	−5.439	0.868	−6.265	<0.001
cg18196829	Chr 6:170	−9.078	1.355	−6.698	<0.001
cg23211949	Chr 6:3	−5.209	1.081	−4.819	<0.001
cg17213048	ATAD2	6.660	1.422	4.683	<0.001
cg25459300	Chr 8:20	1.994	1.029	1.938	0.053

SE: standard errors of coefficients; *z* value: Wald *z*-statistic value.

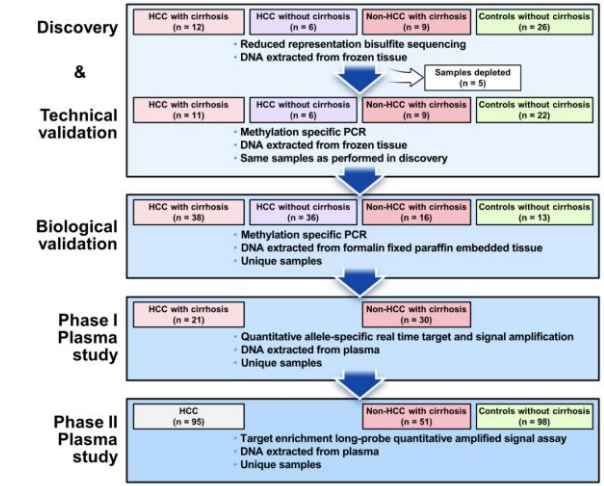
a

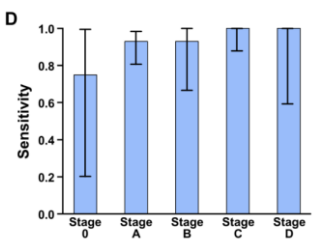
Training dataset	Real HCC	Real normal	
Predict HCC	613	32	
Predict normal	102	528	Totals
Totals	715	560	1,275
Correct	613	528	1,162
Sensitivity (%)	85.7		
Specificity (%)		94.3	

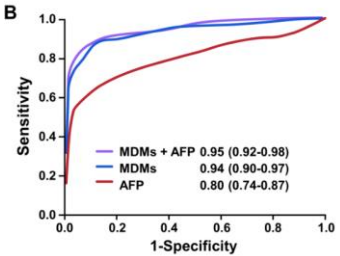
b

Validation dataset	Real HCC	Real normal	
Predict HCC	319	26	
Predict normal	64	249	
Totals	383	275	658
Correct	319	249	568
Sensitivity (%)	83.3		
Specificity (%)		90.5	

Methylation diagnostic signature





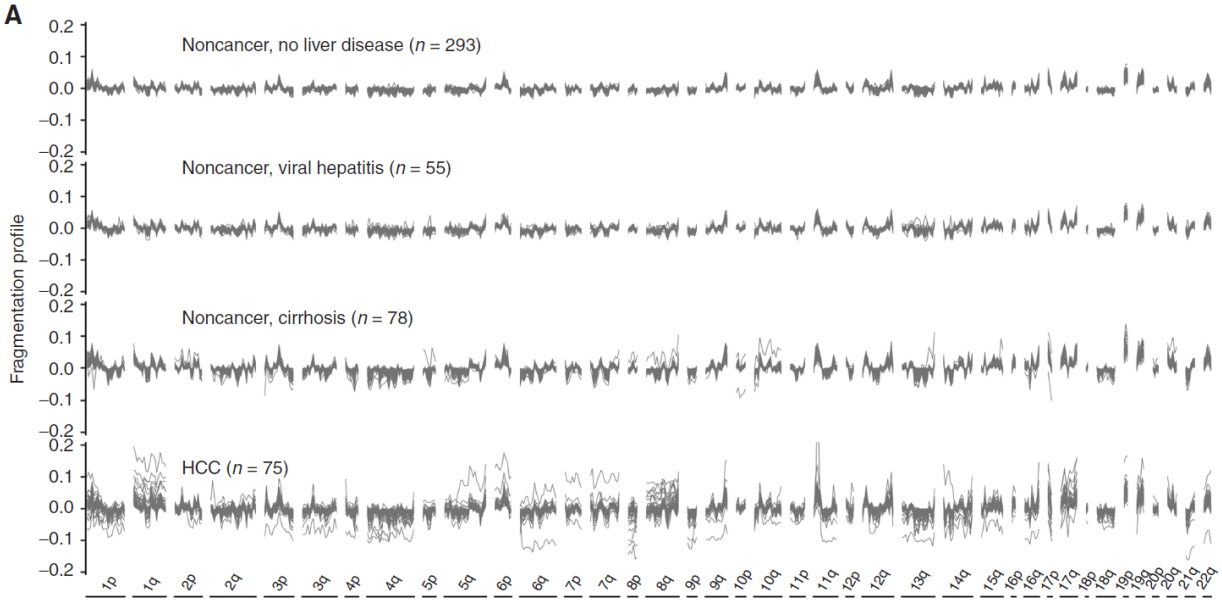


Xu RH, Nat Mater 2018
Kisiel, Hepatology 2018

Liquid biopsy

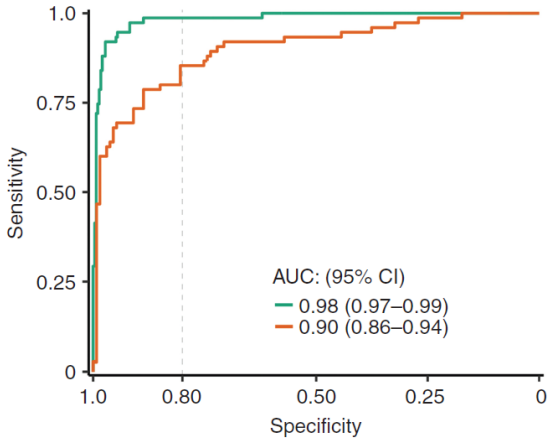
Fragmentomics (ctDNA)

cfDNA fragment profile



HCC detection

(n=75 HCC, 30% BCLC0/A)

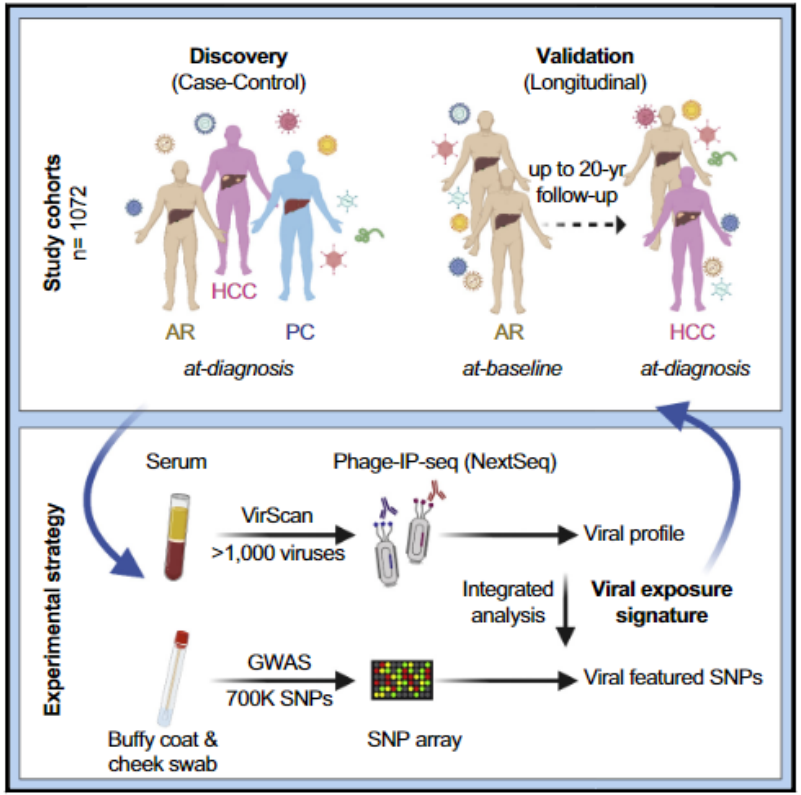


Foda ZH, Cancer Disc 2022

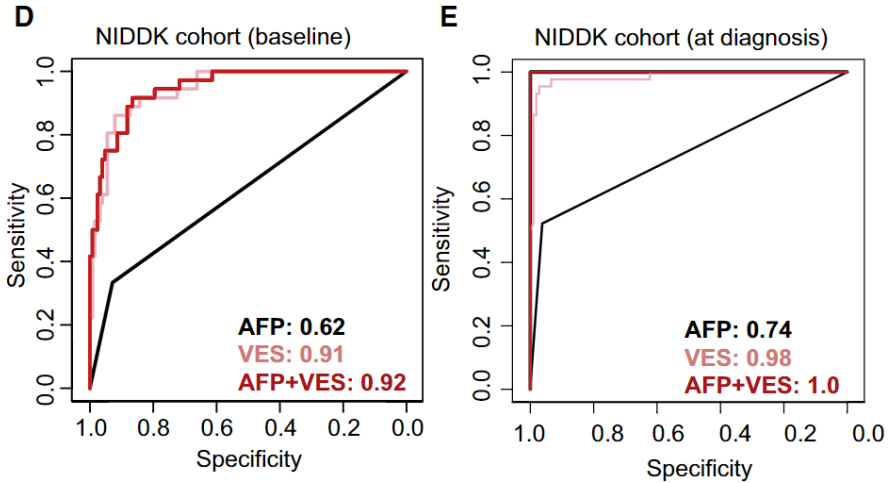
Liquid biopsy

VirScan

Study design (Viral-host interactions)



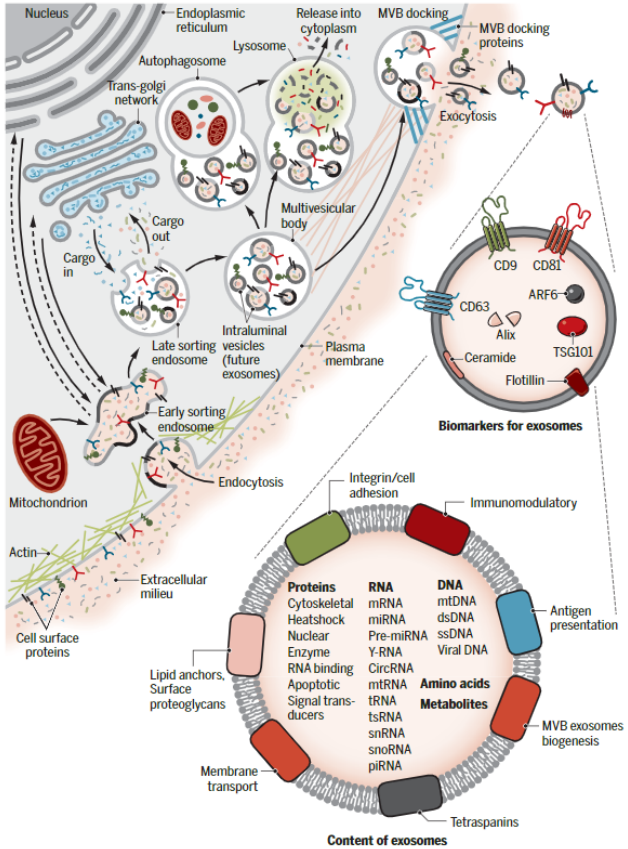
HCC detection performance

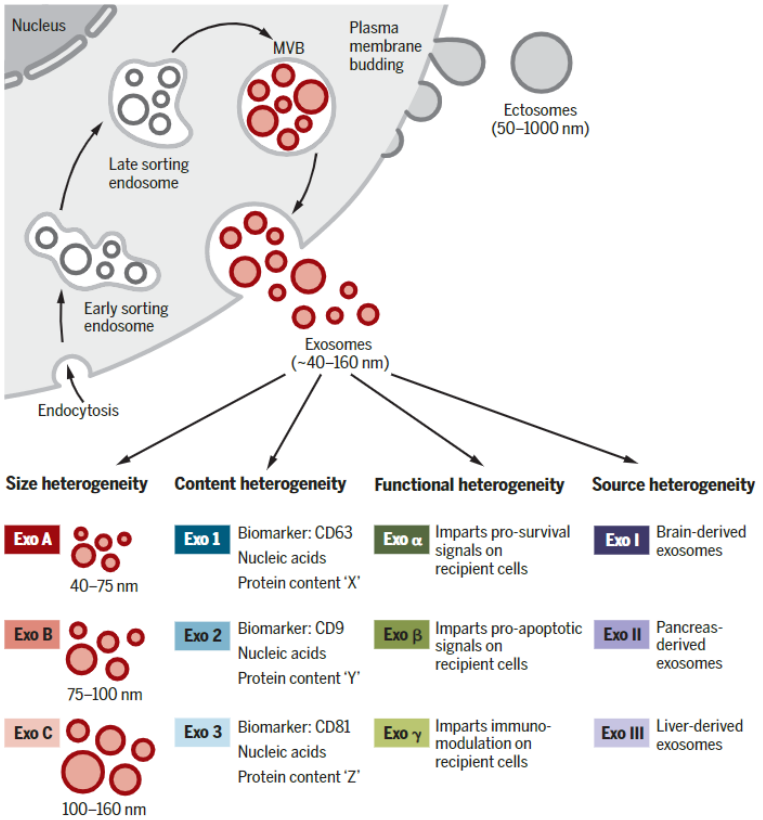


Liu J, Cell 2020

Extracellular vesicles

Definition, biogenesis and function



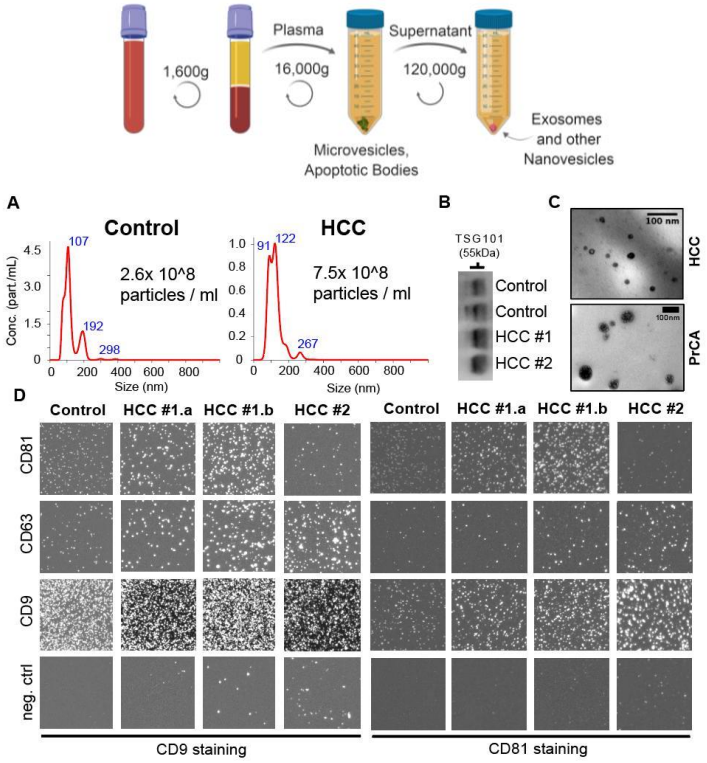


Kalluri, Science 2020

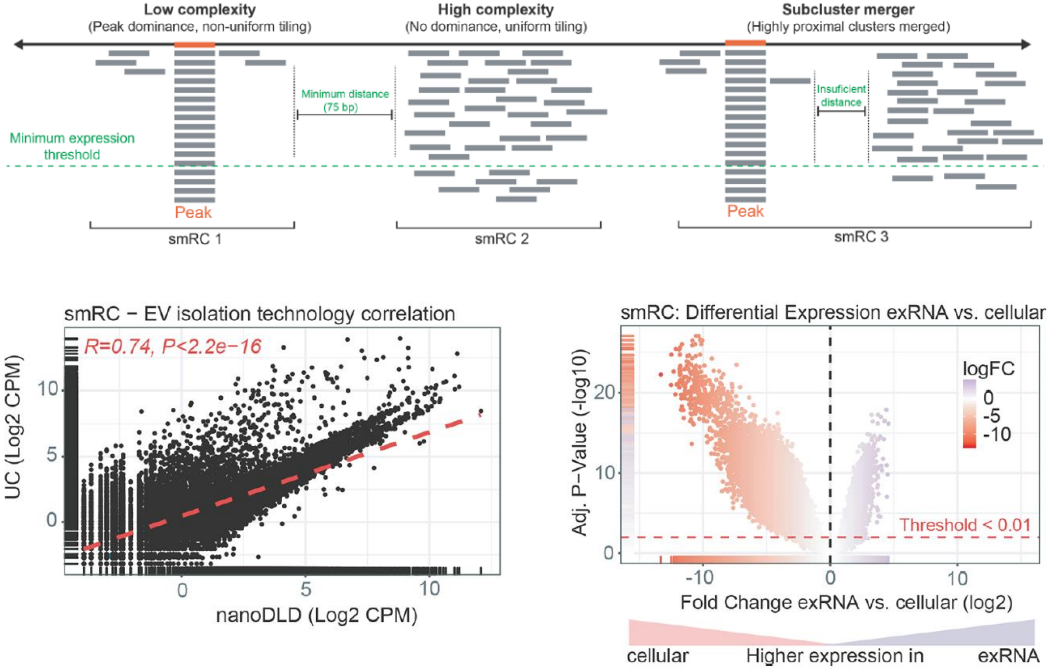
Liquid biopsy

Small RNA clusters – Extracellular vesicles

Exosome isolation (UC)



Small RNA clusters (smRC)

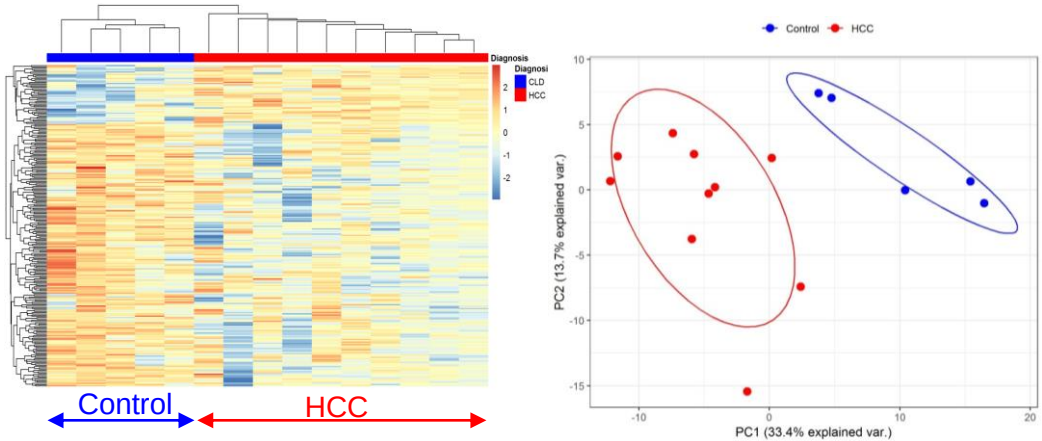


von Felden, Gut 2021

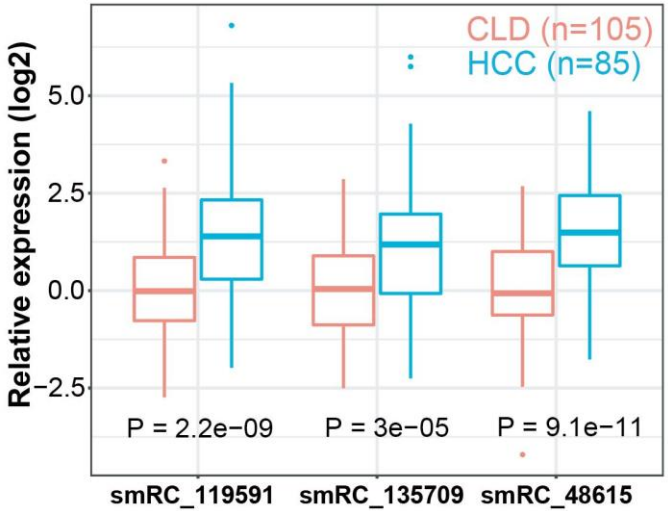
Liquid biopsy

Small RNA clusters – Extracellular vesicles

Exosome-based disease classifier (n=15 patients)



Small RNA in exosomes (n=190)

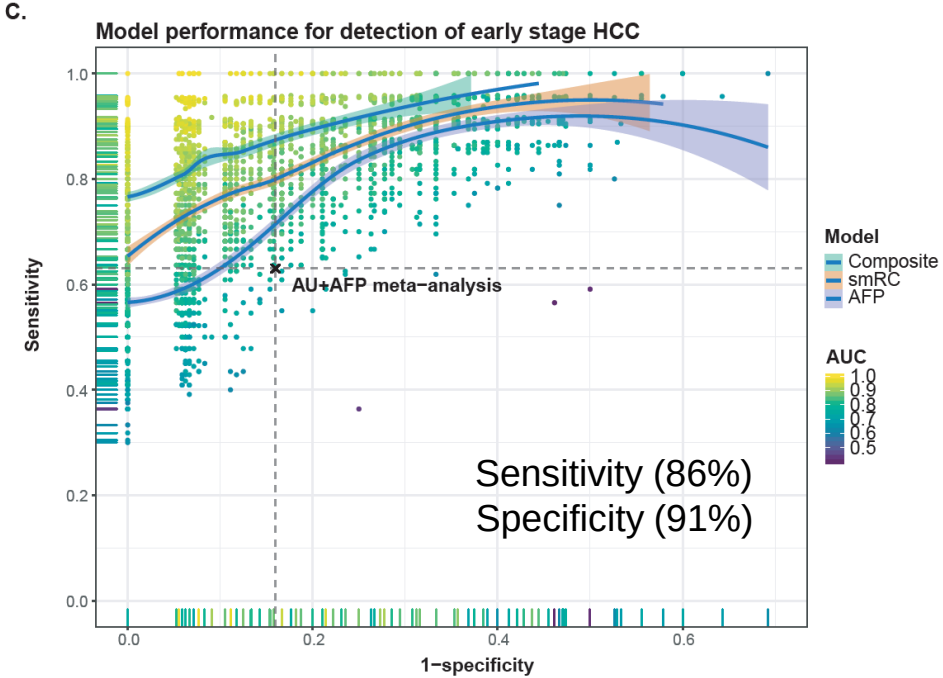


von Felden, Gut 2021

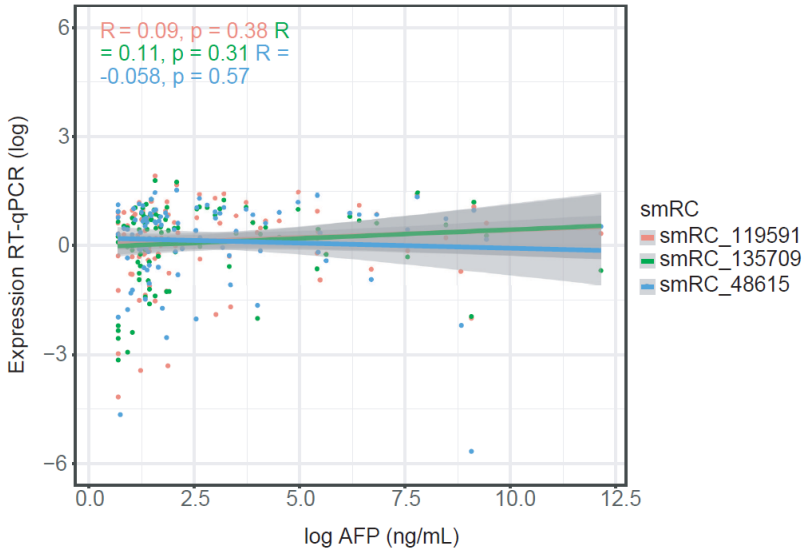
Liquid biopsy

Small RNA clusters – Extracellular vesicles

Exosome-based disease classifier (n=209 patients)



AFP and small RNAs not correlated

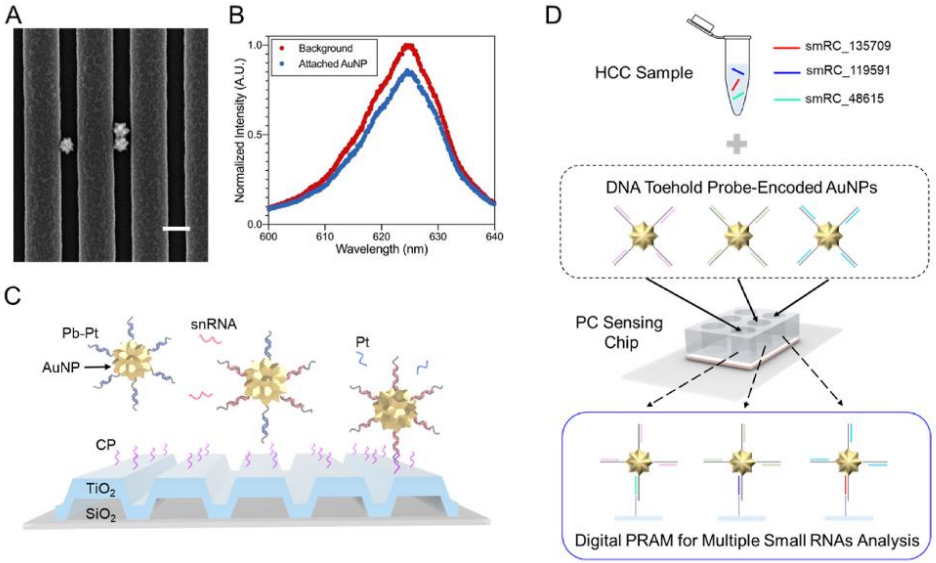


von Felden, Gut 2021

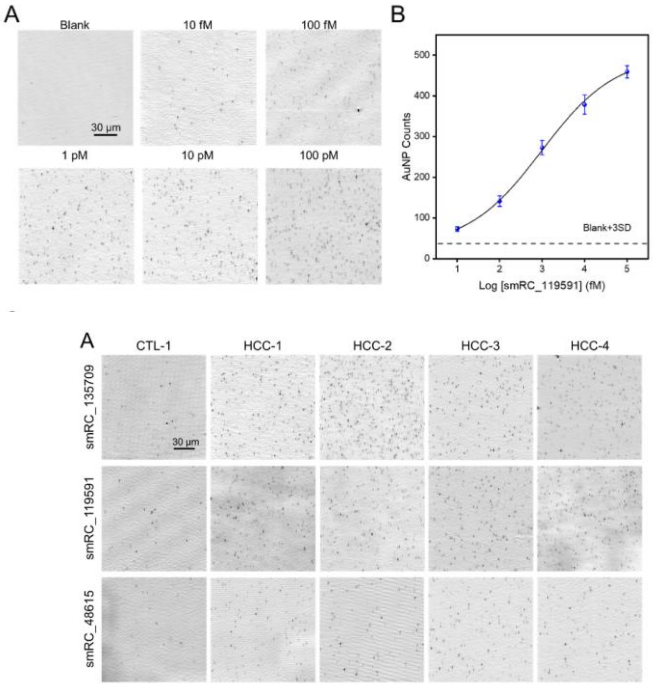
Liquid biopsy

Small RNA clusters – Clinical assay

DNA toehold probe-based photonic resonator absorption microscopy



3-smRCs cluster performance



Zhao B et al. Talanta 2022

Liquid biopsy

HCC surveillance (Biotech landscape)

Company	Product	Sensitivity / Specificity
Exact sciences	ctDNA methylation	81% / 87%
Glycotest	Glycoprotein	95% / 90%
Genetron Health	ctDNA methylation / mutation	85% / 93%
Helio Health	ctDNA methylation	76% / 91%
Glympse bio	Protease biosensors	77% / 77%

NYC Translational Research Center Liver Cancer Surveillance

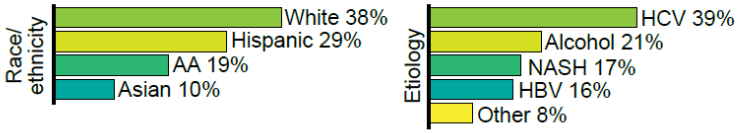
Structure

Mount Sinai — Villanueva, Taouli
Montefiore Einstein — Fortune
Columbia University — Verna
Cornell University — Rosenblatt
DELFI — Dracopoli

Velculescu — Scientific Advisor → Steering Committee

Prospective Biobank n=2,560
(trans-network collaborations)

Clinical Data | Imaging Data | Blood Samples



Project

Aim 1: Phase 2 biomarker study testing liquid biopsy to detect curable HCC

Retrospective Cohort

- Advanced stage HCC n=272
- Liver Disease n=164
- Healthy Liver n=16

Prospective Cohort

- Early stage HCC n=760
- Controls at risk of HCC n=1600

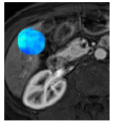
Clinical Data | Liquid Biopsy | Imaging

Aim 2: To integrate clinical data, liquid biopsy and MRI-based radiomics to characterize indeterminate nodules in cirrhosis

Retrospective Cohort

Cirrhosis with indeterminate nodules n=400

Clinical Data | MRI-based Radiomics

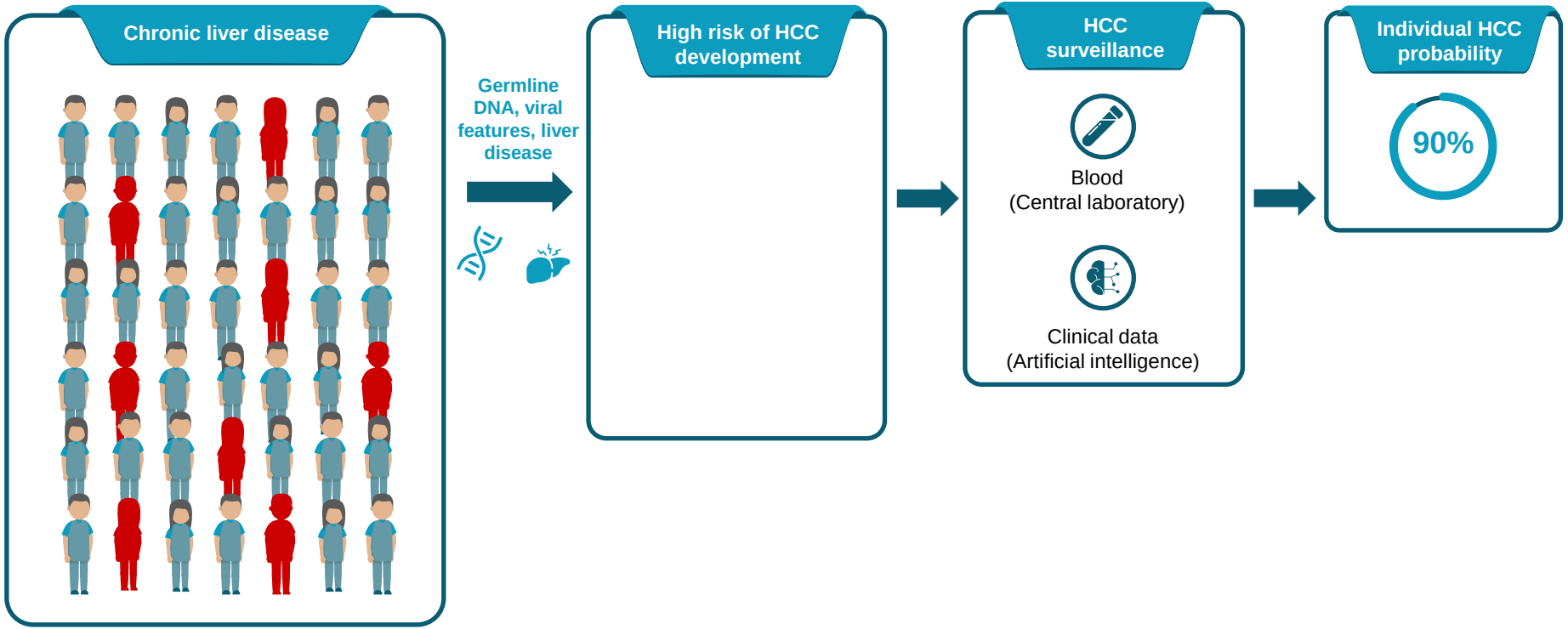


Prospective Cohort

Cirrhosis with indeterminate nodules n=200

Clinical Data | MRI-based Radiomics | Liquid Biopsy

HCC surveillance, 2030

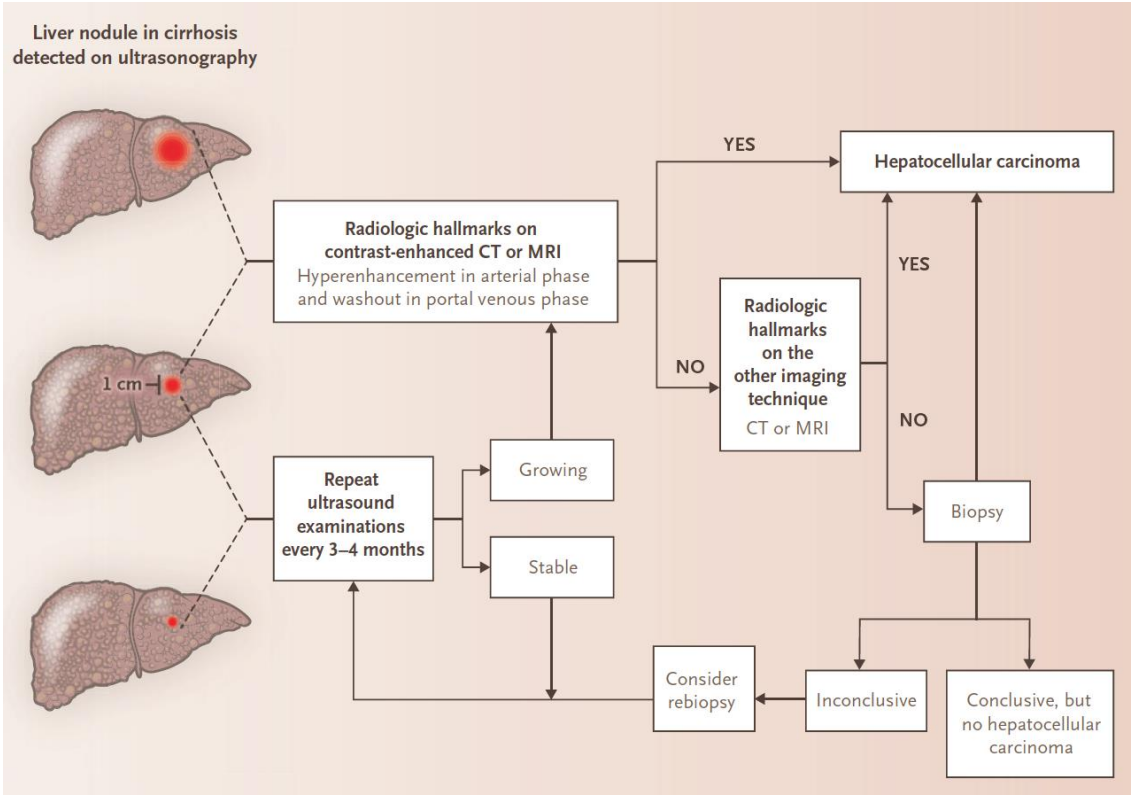


Outline

- Liquid biopsy in the clinical management of HCC
  - Early detection (tumor burden, minimal residual disease)
  - Biomarkers of treatment response

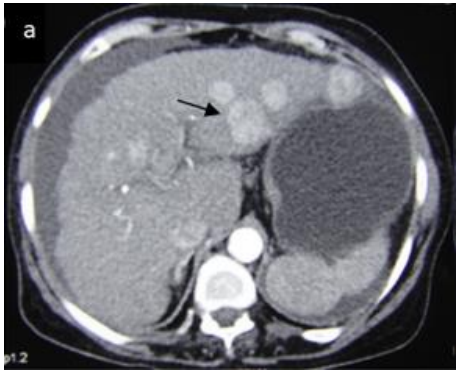
Diagnosis of HCC

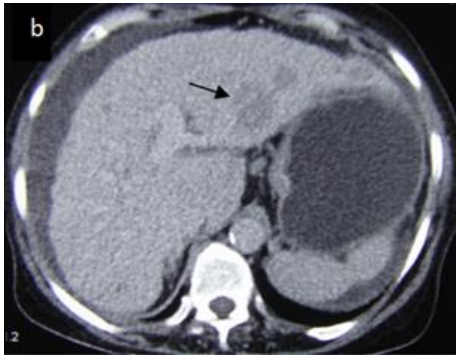
Non-invasive criteria



CT-scan

(Hallmark imaging features)

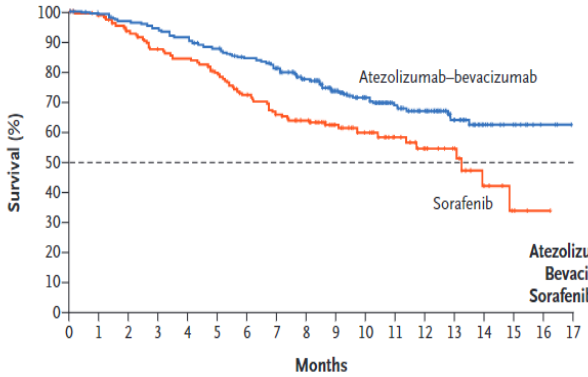




Villanueva A. *N Engl J Med* 2019

Advanced HCC (BCLC-C)

Immune-based therapies (atezolizumab-bevacizumab)



No. at Risk																	
Atezolizumab–bevacizumab	336	329	320	312	302	288	275	255	222	165	118	87	64	40	20	11	3	NE
Sorafenib	165	157	143	132	127	118	105	94	86	60	45	33	24	16	7	3	1	NE

	No. of Events/ No. of Patients (%)	Median Overall Survival (95% CI)	Overall Survival at 6 Mo
		mo	%
Atezolizumab–Bevacizumab	96/336 (28.6)	NE	84.8
Sorafenib	65/165 (39.4)	13.2 (10.4–NE)	72.2

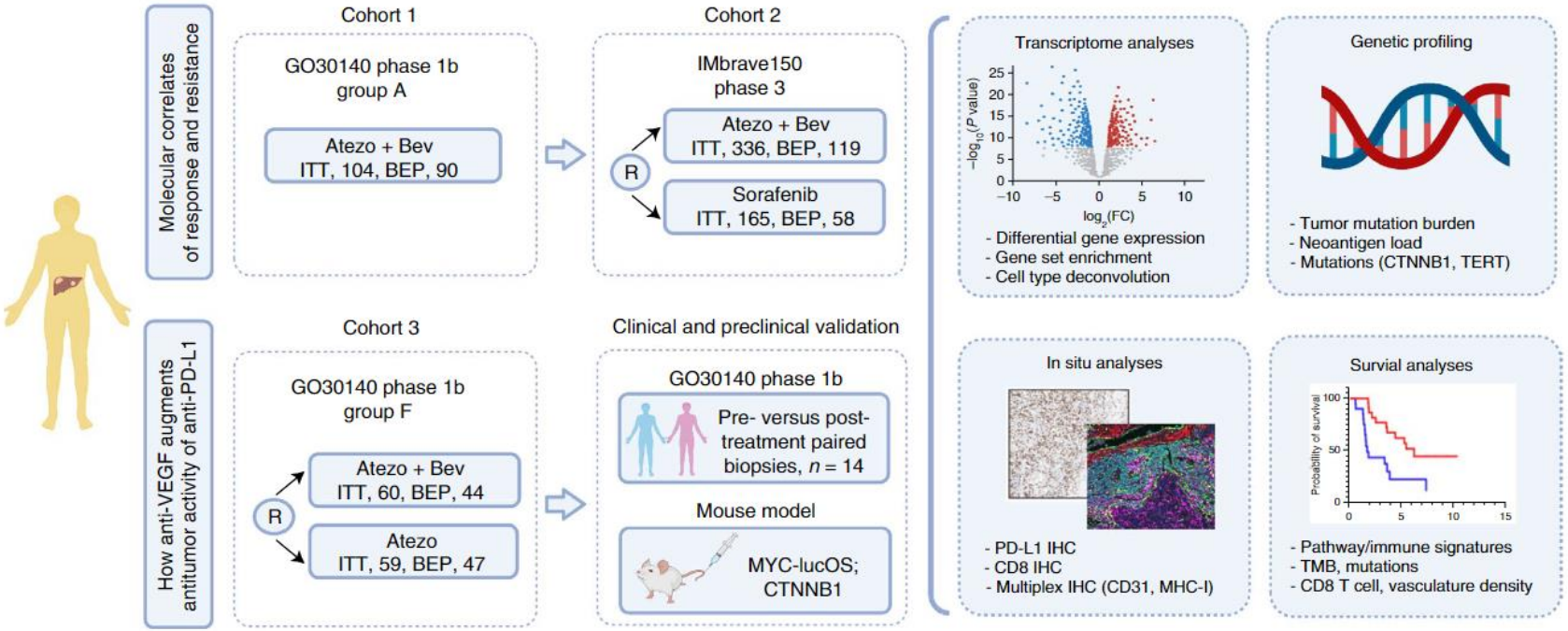
Stratified hazard ratio for death, 0.58 (95% CI, 0.42–0.79)
P<0.001

	IRF RECIST 1.1[a]		IRF HCC mRECIST[b]	
	Atezo + Bev (n = 130)	Sorafenib (n = 60)	Atezo + Bev (n = 128)	Sorafenib (n = 59)
Confirmed ORR, n **(%)** (95% CI)	32 **(25)** (18, 33)	4 **(7)** (2, 16)	38 **(30)** (22, 38)	5 **(8)** (3, 19)
CR, n (%)	5 (4)	0	16 (13)	0
PR, n (%)	27 (21)	4 (7)	22 (17)	5 (8)
Difference in ORR (95% CI), %	18 (7, 29)		21 (9, 33)	
SD, n (%)	59 (45)	25 (42)	52 (41)	24 (41)
PD, n (%)	29 (22)	19 (32)	28 (22)	18 (31)
DCR, n (%)[c]	91 (70)	29 (48)	90 (70)	29 (49)
Ongoing response at data cutoff, n (%)[d]	29 (91)	3 (75)	33 (87)	3 (60)

Finn RS et al. *N Engl J Med* 2020

Advanced HCC (BCLC-C)

Biomarkers of response (atezolizumab-bevacizumab)

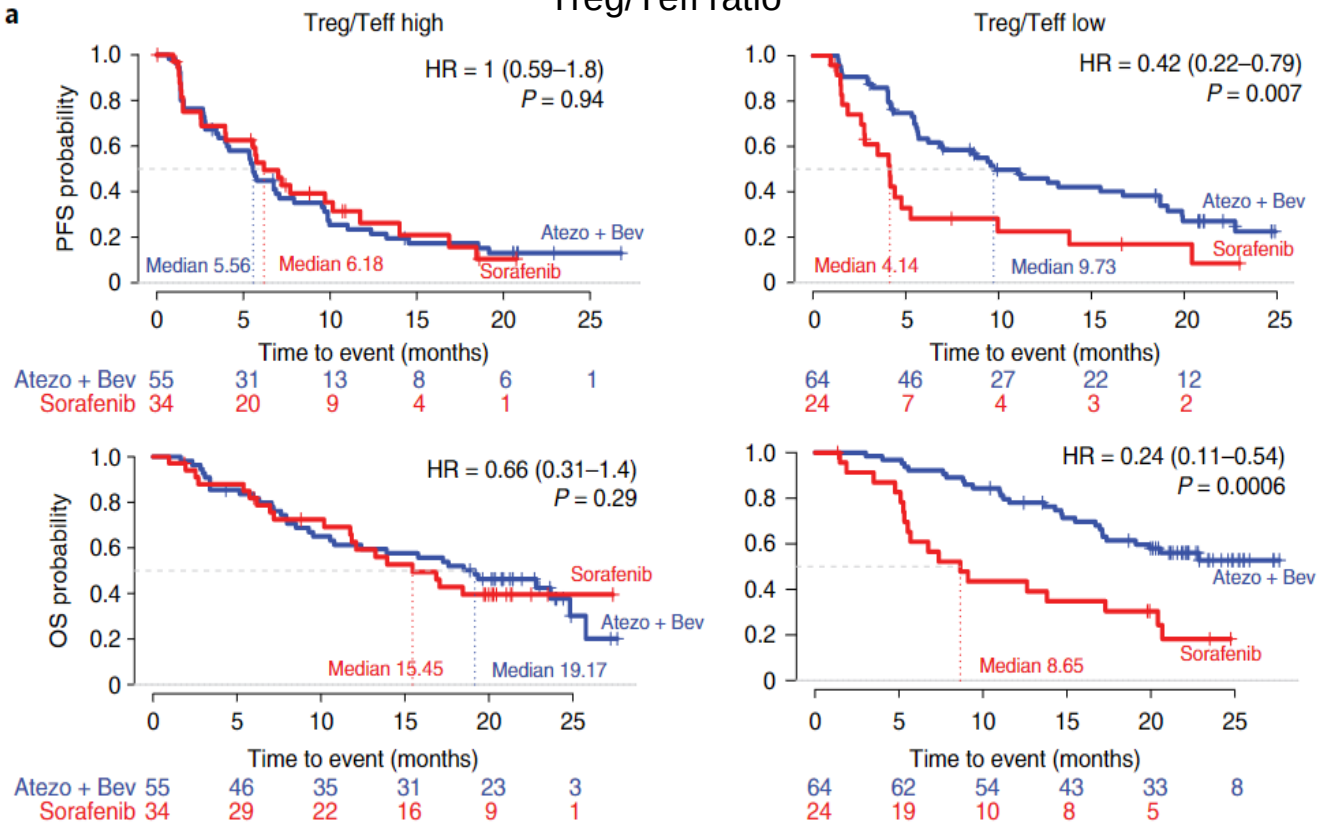


Zhu AX, Nat Med 2022

Advanced HCC (BCLC-C)

Biomarkers of response (atezolizumab-bevacizumab)

Treg/Teff ratio

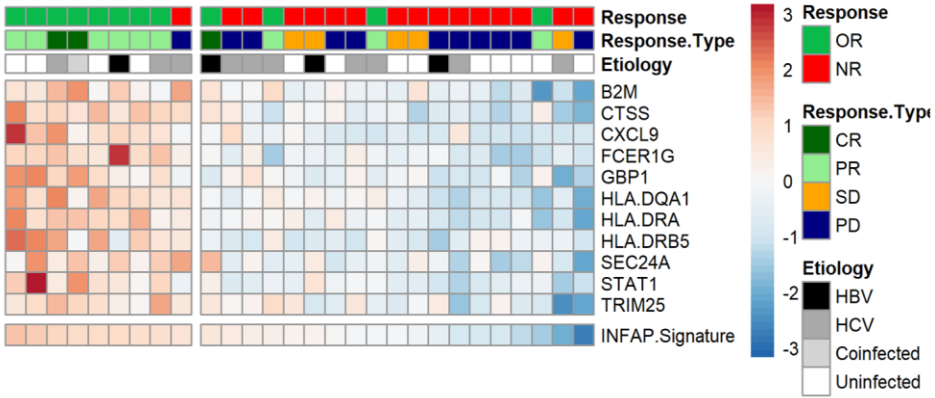


Zhu AX, Nat Med 2022

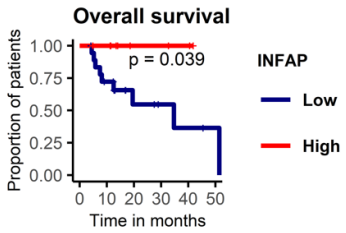
Biomarkers of response to check-point inhibitors

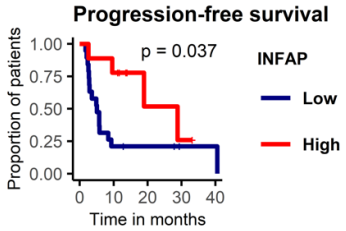
Gene expression

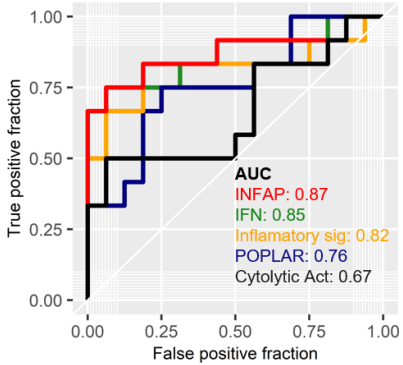
INFAP signature



Predictive performance





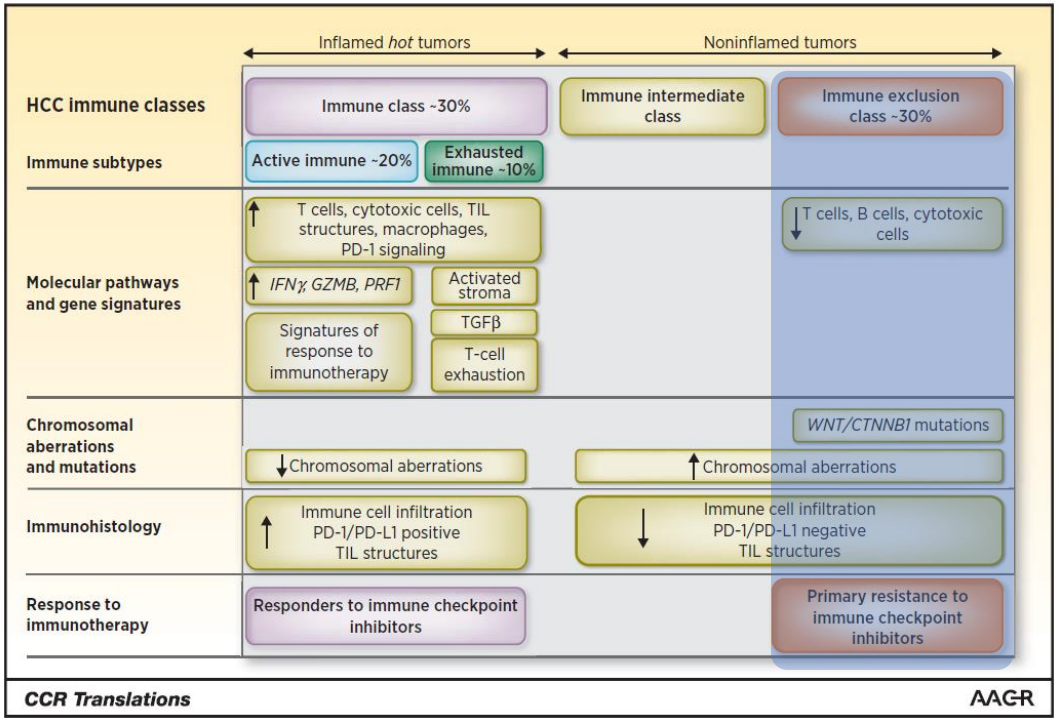


Haber P et al. Gastroenterology 2022

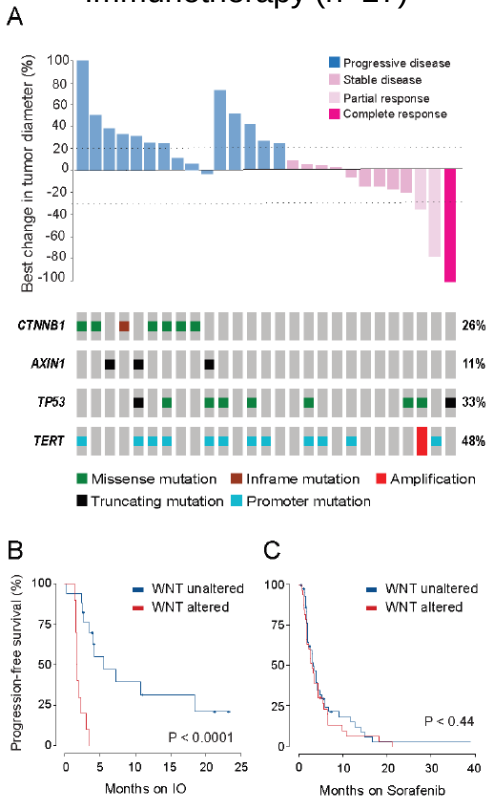
Biomarkers of response to CPI

CTNNB1 mutations (WNT signaling)

Immune landscape in HCC



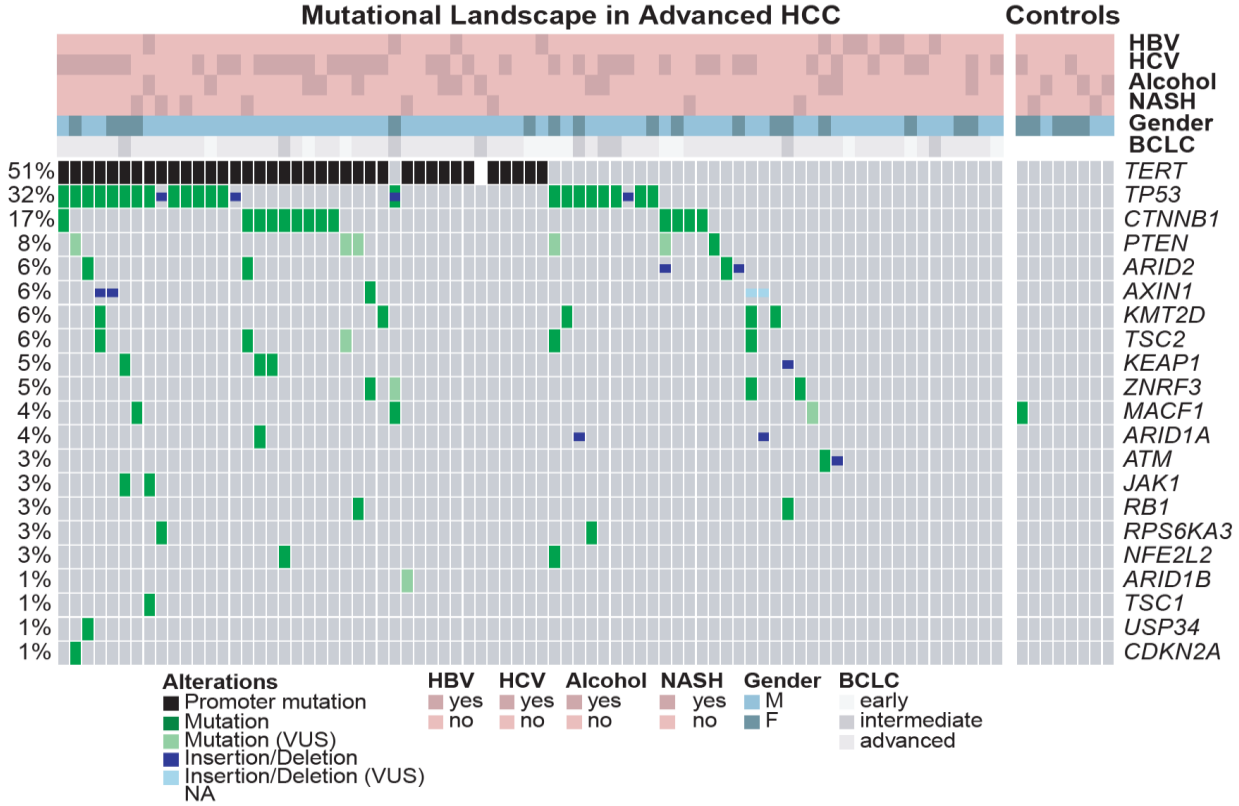
Immunotherapy (n=27)



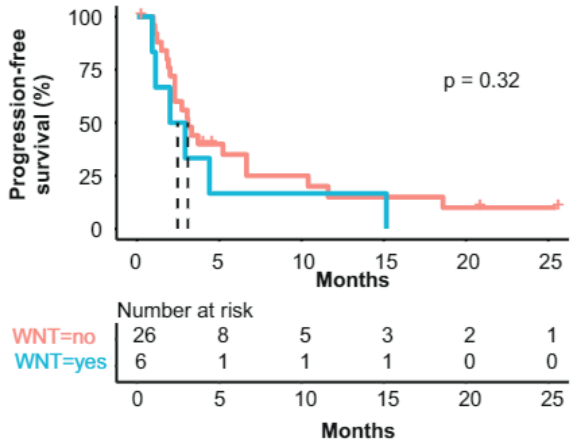
Sia D, Gastroenterology 2017 | Pinyol, *CCR* 2019 | Harding, *CCR* 2018

Mutation profiling of advanced HCC

Ultra-deep sequencing of ctDNA (n=85)



CTNNB1 mutations does not predict lack of response to CPI

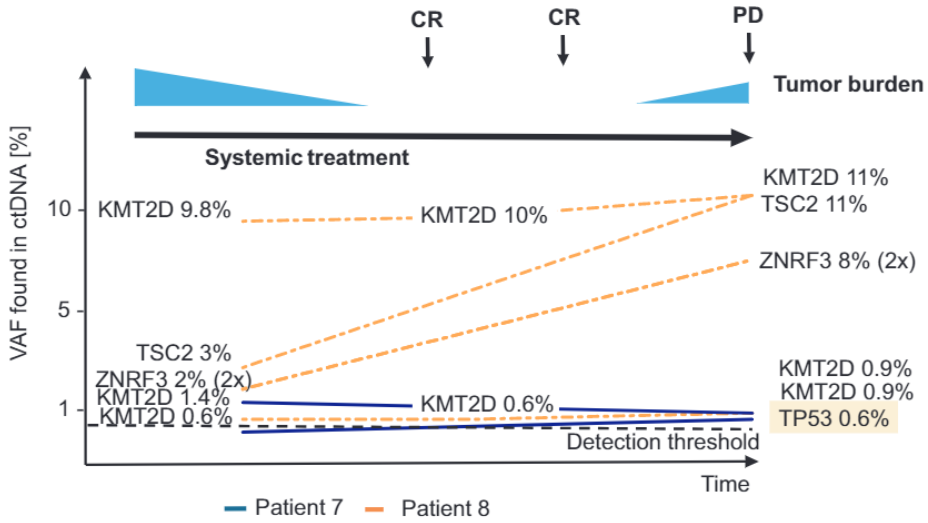


von Felden J, Oncogene 2021

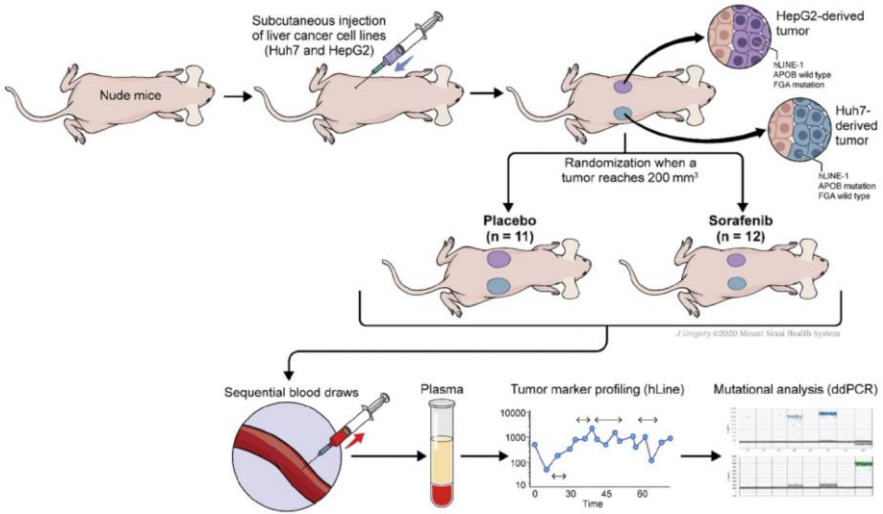
Mutation profiling of advanced HCC

Molecular monitoring

Objective response

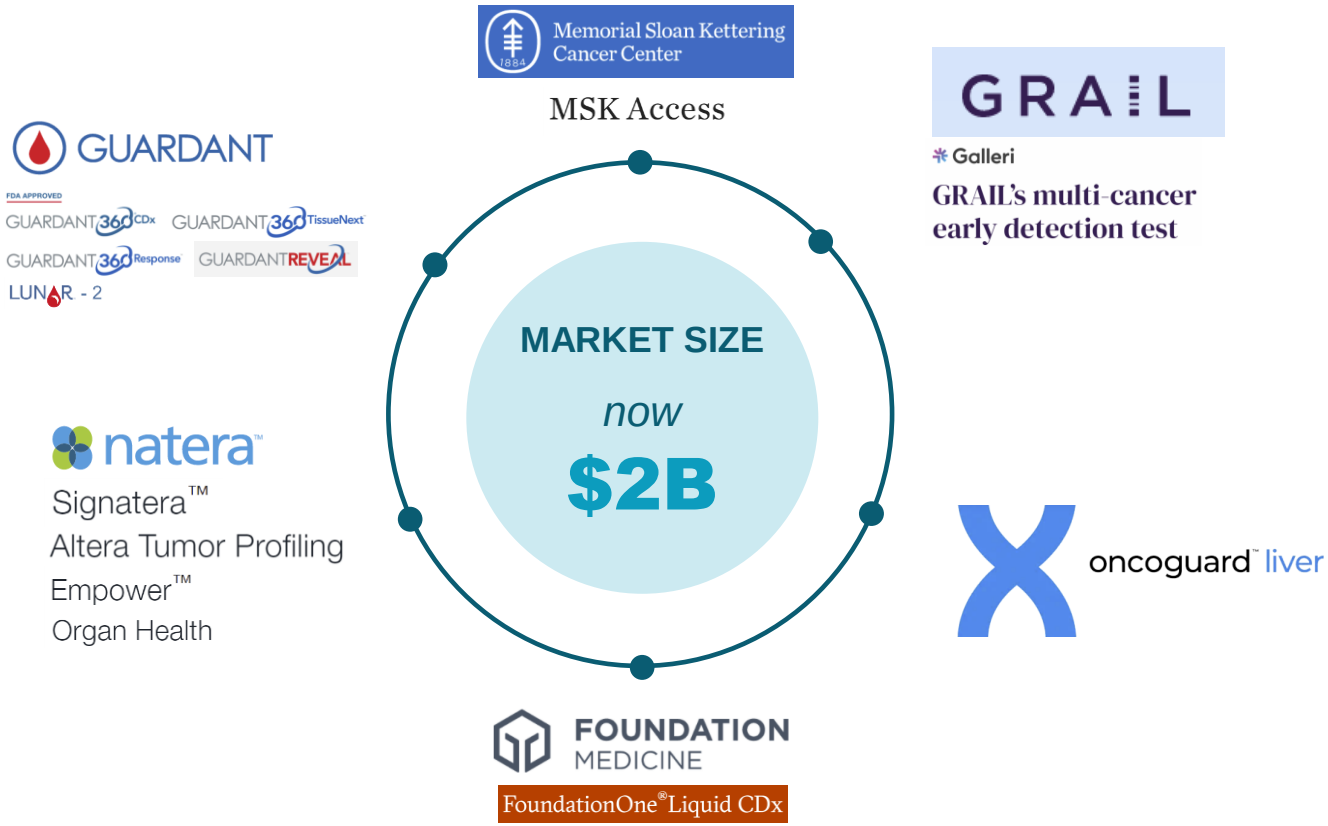


Experimental models of liquid biopsy



von Felden J et al. Oncogene 2021 | Labgaa, Hepatol Commun 2021

Liquid biopsy industry ecosystem



Liquid biopsy industry ecosystem

Use of Circulating Tumor DNA for Early-Stage Solid Tumor Drug Development

Guidance for Industry

FDA, May 2022

Take home messages

- Performance of early detection tools is suboptimal
- Treatment decisions for systemic therapies are not based on prediction of response
- Liquid biopsy has emerged as promising and convenient tool for biomarker development in HCC – Early detection and prediction of treatment response
- Molecular monitoring (‘*real time tracking of molecular alterations in HCC*’) will improve early detection and treatment allocation in HCC

ILTS ILCA

INTERNATIONAL LIVER CANCER ASSOCIATION

Liver Transplantation for Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma

CONSENSUS CONFERENCE 2024

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FEBRUARY 1-2, 2024

Save the Date!