Supplementary References

1. Chaudhuri AA, Chabon JJ, Lovejoy AF, Newman AM, Stehr H, Azad TD, Khodadoust MS, Esfahani MS, Liu CL, Zhou L, et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. Cancer Discov 7: 1394-1403, 2017.
2. Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, Le Quesne J, Moore DA, Veeriah S, Rosenthal R, et al. Phylo genetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 545: 446- 451, 2017.
3. Abbosh C, Frankell A, Garnett A, Harrison T, Weichert M, Licon A, Veeriah S, Daber B, Moreau M, Chesh A, et al. Abstract CT023: phylogenetic tracking and minimal residual disease detection using ctDNA in early-stage NSCLC: a lung TRACERx study. Cancer Res 80: CT023, 2020.
4. Moding EJ, Liu Y, Nabet BY, Chabon JJ, Chaudhuri AA, Hui AB, Bonilla RF, Ko RB, Yoo CH, Gojenola L, et al. Circulating tumor DNA dynamics predict benefit from consolidation immunotherapy in locally advanced non-small cell lung cancer. Nat Cancer 1: 176-183, 2020.
5. Gale D, Heider K, Ruiz-Valdepenas A, Hackinger S, Perry M, Marsico G, Rundell V, Wulff J, Sharma G, Knock H, et al. Residual ctDNA after treatment predicts early relapse in patients with early-stage non-small cell lung cancer. Ann Oncol 33: 500-510, 2022.
6. Peng M, Huang Q, Yin W, Tan S, Chen C, Liu W, Tang J, Wang X, Zhang B, Zou M, et al. Circulating tumor DNA as a prognostic biomarker in localized Non-small Cell lung cancer. Front Oncol 10: 561598, 2020.
7. Olsson E, Winter C, George A, Chen Y, Howlin J, Tang MH, Dahlgren M, Schulz R, Grabau D, van Westen D, et al. Serial monitoring of circulating tumor DNA in patients with primary breast cancer for detection of occult metastatic disease. EMBO Mol Med 7: 1034-1047, 2015.
8. Garcia-Murillas I, Schiavon G, Weigelt B, Ng C, Hrebien S, Cutts RJ, Cheang M, Osin P, Nerurkar A, Kozarewa I, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med 7: 302ra1332, 2015.
9. McDonald BR, Contente-Cuomo T, Sammut SJ, Odenheimer-Bergman A, Ernst B, Perdigones N, Chin SF, Farooq M, Mejia R, Cronin PA, et al. Personalized circulating tumor DNA analysis to detect residual disease after neoadjuvant therapy in breast cancer. Sci Transl Med 11(504): eaax7392, 2019.
10. Garcia-Murillas I, Chopra N, Comino-Méndez I, Beaney M, Tovey H, Cutts RJ, Swift C, Kriplani D, Afentakis M, Hrebien S, et al. Assessment of molecular relapse detection in early-stage breast cancer. JAMA Oncol 5: 1473-1478, 2019.
11. Coombes RC, Page K, Salari R, Hastings RK, Armstrong A, Ahmed S, Ali S, Cleator S, Kenny L, Stebbing J, et al. Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence. Clin Cancer Res 25: 4255-4263, 2019.
12. Magbanua MJM, Swigart LB, Wu HT, Hirst GL, Yau C, Wolf DM, Tin A, Salari R, Shchegrova S, Pawar H, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. Ann Oncol 32: 229-239, 2021.
13. Tarazona N, Gimeno-Valiente F, Gambardella V, Zuñiga S, Rentero-Garrido P, Huerta M, Roselló S, Martinez-Ciarpaglini C, Carbonell-Asins JA, Carrasco F, et al. Targeted next-generation sequencing of circulating-tumor DNA for tracking minimal residual disease in localized colon cancer. Ann Oncol 30: 1804-1812, 2019.
14. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, Silliman N, Tacey M, Wong HL, Christie M, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med 8: 346ra92, 2016.
15. Tie J, Cohen JD, Wang Y, Christie M, Simons K, Lee M, Wong R, Kosmider S, Ananda S, McKendrick J, et al. Circulating tumor DNA analyses as markers of recurrence risk and benefit of adjuvant therapy for stage III colon cancer. JAMA Oncol 5: 1710-1717, 2019.
16. Wang Y, Li L, Cohen JD, Kinde I, Ptak J, Popoli M, Schaefer J, Silliman N, Dobbyn L, Tie J, et al. Prognostic potential of circulating tumor DNA measurement in post-operative surveillance of nonmetastatic colorectal cancer. JAMA Oncol 5: 1118-1123, 2019.
17. Reinert T, Henriksen TV, Christensen E, Sharma S, Salari R, Sethi H, Knudsen M, Nordentoft I, Wu HT, Tin AS, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. JAMA Oncol 5: 1124-1131, 2019.
18. Parikh AR, Van Seventer EE, Siravegna G, Hartwig AV, Jaimovich A, He Y, Kanter K, Fish MG, Fosbenner KD, Miao B, et al. Minimal residual disease detection using a plasma-only circulating tumor DNA assay in patients with colorectal cancer. Clin Cancer Res 27: 5586-5594, 2021.
19. Chen G, Peng J, Xiao Q, Wu HX, Wu X, Wang F, Li L, Ding P, Zhao Q, Li Y, et al. Postoperative circulating tumor DNA as markers of recurrence risk in stages II to III colorectal cancer. J Hematol Oncol 14(1): 80, 2021.
20. Schøler LV, Reinert T, Ørntoft MW, Kassentoft CG, Árnadóttir SS, Vang S, Nordentoft I, Knudsen M, Lamy P, Andreasen D, et al. Clinical implications of monitoring circulating tumor DNA in patients with colorectal cancer. Clin Cancer Res 23: 5437-5445, 2017.
21. Tie J, Cohen JD, Wang Y, Li L, Christie M, Simons K, Kosmider S, Wong R, Yip D, Lee M, et al. Serial circulating tumour DNA analysis during multimodality treatment of locally advanced rectal cancer: a prospective biomar ker study. Gut 68: 663-671, 2019.
22. Khakoo S, Carter PD, Brown G, Valeri N, Picchia S, Bali MA, Shaikh R, Jones T, Begum R, Rana I, et al. MRI tumor regression grade and circulating tumor DNA as complementary tools to assess response and guide therapy adaptation in rectal cancer. Clin Cancer Res 26: 183-192, 2020.
23. McDuff SGR, Hardiman KM, Ulintz PJ, Parikh AR, Zheng H, Kim DW, Lennerz JK, Hazar-Rethinam M, Van Seventer EE, Fetter IJ, et al. Circulating tumor DNA predicts pathologic and clinical outcomes following neoadjuvant chemoradiation and surgery for patients with locally advanced rectal cancer. JCO Precis Oncol 5: PO.20.00220., 2021.

24. Azad TD, Chaudhuri AA, Fang P, Qiao Y, Esfahani MS, Chabon JJ, Hamilton EG, Yang YD, Lovejoy A, Newman AM, et al. Circulating tumor DNA analysis for detection of minimal residual disease after chemoradiotherapy for localized esophageal cancer. Gastroenterology 158: 494-505, 2020.
25. Jiang J, Ye S, Xu Y, Chang L, Hu X, Ru G, Guo Y, Yi X, Yang L, Huang D. Circulating tumor DNA as a potential marker to detect minimal residual disease and predict recurrence in pancreatic cancer. Front Oncol 10: 1220, 2020.
26. Sausen M, Phallen J, Adleff V, Jones S, Leary RJ, Barrett MT, Anagnostou V, Parpart-Li S, Murphy D, Kay Li Q, et al. Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. Nat Commun 6: 7686, 2015.
27. Groot VP, Mosier S, Javed AA, Teinor JA, Gemenetzis G, Ding D, Haley LM, Yu J, Burkhart RA, Hasanain A, et al. Circulating tumor DNA as a clinical test in resected pancreatic cancer. Clin Cancer Res 25: 4973-4984, 2019.
28. Christensen E, Birkenkamp-Demtröder K, Sethi H, Shchegrova S, Salari R, Nordentoft I, Wu HT, Knudsen M, Lamy P, Lindskrog SV, et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by Ultra-Deep sequencing of plasma cell-Free DNA in patients with urothelial bladder carcinoma. J Clin Oncol 37(18): 1547-1557, 2019.
29. Hilke FJ, Muyas F, Admard J, Kootz B, Nann D, Welz S, Rieß O, Zips D, Ossowski S, Schroeder C, et al. Dynamics of cell-free tumour DNA correlate with treatment response of head and neck cancer patients receiving radiochemo therapy. Radiother Oncol 151: 182-189, 2020.
30. Yang J, Gong Y, Lam VK, Shi Y, Guan Y, Zhang Y, Ji L, Chen Y, Zhao Y, Qian F, et al. Deep sequencing of circulating tumor DNA detects molecular residual disease and predicts recurrence in gastric cancer. Cell Death Dis 11: 346-354, 2020.
31. Tan L, Sandhu S, Lee RJ, Li J, Callahan J, Ftouni S, Dhomen N, Middlehurst P, Wallace A, Raleigh J, et al. Prediction and monitoring of relapse in stage III melanoma using circulating tumor DNA. Ann Oncol 30(5): 804-814, 2019.

Table SI. Current commercial platforms for ctDNA MRD detection in solid tumors.

Assays/ platforms	Company	Methods		Sample required		Alterations queried	Reported LOD	Turnaround time	Stage
		Baseline	MRD/monitoring	Baseline	MRD/monitoring				
Tumor-informed commercial platforms									
Signatera™	Natera, Inc.	WES	Multiplex PCR-based NGS (ultra-deep sequencing)	FFPE block or slides + 6 mL whole blood (EDTA tube)	20 ml whole blood (Streck tubes)	SNVs, indels	0.01% VAF	2-3 weeks for initial test design; 5-7 days for cfDNA analysis	Clinical use
PCM™	ArcherDX	WES	Anchored Multiplex PCR (AMP™)-based NGS	Tumor tissue	Plasma	SNVs, indels, CNAs	0.008% VAF	\	RUO
RaDaR™	Inivata	WES	Multiplex PCR-based NGS (deep sequencing)	10x10 μm FFPE tissue	20 ml whole blood	SNVs, indels, CNAs	0.0011% VAF	4 weeks for personalized assays design; 7days for cfDNA analysis	Clinical use
MRDetect	C2i Genomics	WGS	WGS	Tumor tissue	2-3 ml whole blood	SNVs, CNAs	0.001% TF	\	RUO
PhasED-Seq	Foresight Diagnostics	WGS	Hybrid capture-based NGS	Tumor tissue or plasma	Plasma	PVs, SNVs	<0.0001 % TF	\	RUO
Tumor-agnostic commercial platforms									
PredicineALERT™	Predicine, Inc.	\	Integrated approch, including a target panel for hotspot, a LP-WGS assay for copy number burden and a whole genome methylation assay	Plasma, urine or tumor tissue	10 ml whole blood; 4 ml plasma (down to 0.4 ml); 40 ml urine	SNVs, indels, CNAs, methylation	0.005% VAF	10 days	RUO
Reveal™	Guardnant Health	\	Hybrid capture-based NGS	\	4 ml plasma	SNVs, indels, CNAs, fusions	0.01% VAF	1 week from sample receipt	Clinical use
AVENIO	Roche	\	Hybrid capture-based NGS	\	4 ml plasma	SNVs, indels	0.1% VAF	5 days	RUO

MRD, minimal residual disease; LOD, limit of detection; NGS, next generation sequencing; WES, whole exome sequencing; SNV, single nucleotide variants; VAF, variant allele frequency; PVs, phased variants; CNA, copy number alteration; indel, insertion or deletion; TF, tumor fraction; FFPE, formalin-fixed paraffin-embedded; RUO, research use only; WGS, whole genome sequencing

Table SII. Summary of clinical evidence of ctDNA MRD in solid tumors.

Authors/(Refs.)	Tumor type(s) /stages	Sample size	Approaches		No. of variants monitored	Sensitivity	Specificity	Lead time (range)
			Detection method	Tumor-informed				
Chaudhuri *et al* (1)	Lung cancer/I-III	40	CAPP-Seq (128 genes)	No	-	94%	100%	5.2 months
Abbosh *et al* (2)	NSCLC/I-III	24	Multiplex-PCR NGS (Signatera™ assay)	Yes	16	93%	90%	70 (10-346) days
Abbosh *et al* (3)	NSCLC/I-III	78	Anchored-multiplex PCR (ArcherDX assay)	Yes	50	82%	100%	151 (0-984) days
Moding *et al* (4)	NSCLC/ IIB-IIIB	12	CAPP-Seq (139 genes)	Yes	6.2 (median)	100%	100%	4.1 months
Gale *et al* (5)	NSCLC/IA-IIIB	88	Multiplex-PCR NGS (RaDaR™ assay)	Yes	Mutiple	95%	99%	212.5 days
Peng *et al* (6)	NSCLC	77	cSMART assay (127 gene panel)	No	-	67%	72.20%	12.6 months
Olsson *et al* (7)	Breast cancer/I-III	20	ddPCR	Yes	~10	93%	100%	11 (0-37) months
Garcia-Murillas *et al* (8)	Breast cancer/early stage	55	dPCR	Yes	Single	80%	96.40%	7.9 (0.03-13.6) months
McDonald *et al* (9)	Breast cancer/ I-III	33	TARDIS	Yes	8-16	91%[a]	96%	\
Garcia-Murillas *et al* (10)	Breast cancer	170	dPCR	Yes	Single	75%	92%	10.7 (8.1-19.1) months
Coombes *et al* (11)	Breast cancer/ I-III	49	Multiplex-PCR NGS (Signatera™ assay)	Yes	16	89%	100%	8.9 (0.5-24) months
Magbanua *et al* (12)	Breast cancer/ II-III	84	Multiplex-PCR NGS (Signatera™ assay)	Yes	12-16	83%		
Tarazona *et al* (13)	Colon cancer/I-III	150	ddPCR	Yes	2	87.50%	\	11.5 (3-18) months
Tie *et al* (14)	Colon cancer/II	230	Safe-SeqS	Yes	Single	48%	100%	167 (81-279) days
Tie *et al* (15)	Colon cancer/III	96	Safe-SeqS	Yes	Single	42%	92.30%	51 (9-470) days
Wang *et al* (16)	CRC/I-III	58	Safe-SeqS	Yes	Single	100%	90.60%	4 (2-31) months
Reinert *et al* (17)	CRC/I-III	130	Multiplex-PCR NGS (Signatera™ assay)	Yes	16	88%	98.30%	8.7 (0.8-16.5) months
Parikh *et al* (18)	CRC /I-IV	84	Guardant Reveal™ test	No	-	91%	100%	-
Chen *et al* (19)	CRC/II-III	154	Geneseeq Prime™ 425-gene panel	No	-	83%	94%	5.01months
Schøler *et al* (20)	CRC/I-IV	45	ddPCR	Yes	Single	100%	100%	9.4 (0.4-14.9) months
Tie *et al* (21)	LARC	159	Safe-SeqS	Yes	Single	48%	94%	-
Khakoo *et al* (22)	LARC	47	ddPCR	Yes	Up to two	75%	100%	78 (SD, 53.0) days
McDuff *et al* (23)	LARC	29	ddPCR	Yes	One or more	100%	87%	-
Azad *et al* (24)	Esophageal cancer/IA-	45	CAPP-Seq (607 genes)	No	-	71.40%	100%	2.8 months

	IIIB							
Jiang *et al* (25)	PDAC/I-IV	27	Custom-designed NGS panel (1,017 genes)	No	-	57%	92.30%	-
Sausen *et al* (26)	PDAC	20	ddPCR	Yes	Single	69%	42.90%	6.5 months
Groot *et al* (27)	PDAC	29	ddPCR	No	-	90%	88.00%	84 (25-146) days
Christensen *et al* (28)	MIBC	68	Multiplex-PCR NGS (Signatera™ assay)	Yes	11-16	100%	98%	96 days
Hilke *et al* (29)	HNSCC	20	Custom-designed NGS panel (127 driver mutations)	Yes	Multiple	25%	100%	\
Yang *et al* (30)	Gastric cancer/I-III	46	Custom-designed NGS panel (1,021genes)	No	-	30%	100%	179 days
Tan *et al* (31)	Melanoma/III	99	ddPCR	Yes	Single	55% at 12 months	94% at 12 months	2 months

[a]Analytical performance. Lead time, the time between ctDNA detection and radiologic/clinical recurrence; Abbreviations: MIBC, muscleinvasive bladder cancer; TARDIS, targeted digital sequencing; Safe-SeqS, Safe-Sequencing System; LARC, locally advanced rectal cancer; HNSCC, head and neck squamous cell carcinoma; PDAC, Pancreatic ductal adenocarcinoma; cSMART, circulating single-molecule amplification and resequencing technology; CAPP-Seq, Cancer Personalized Profiling by deep Sequencing; ddPCR, droplet digital PCR.