

**Supplemental Table 1.** Relevant publications on liquid biopsy in the setting of systemic treatment for metastatic melanoma

Treatment	Material	Methods	Results	Reference
DTIC/ temozolomide, <i>n</i> =21 BRAF/MEK inhibitor, <i>n</i> =20 ipilimumab, <i>n</i> =7 others, <i>n</i> =10	CTCs	CellSearch Melanoma kit: HMW-MAA staining and cell size based characterization	pre-treatment CTC detection rate: 40% pre-treatment CTC count (cut-off 2 CTCs per 7.5 ml) is a prognostic marker for OS treatment associated CTC count (cut-off 2 CTCs per 7.5 ml) is a prognostic marker for OS	Khoja et al. 2013 [26]
vemurafenib, <i>n</i> =12 ipilimumab, <i>n</i> =4 DTIC, <i>n</i> = 5 others, <i>n</i> =5	CTCs	multi-markers immunomagnetic enrichment: MCSP, MCAM, ABCB5, CD271	pre-treatment CTC detection rate in vemurafenib patients: 45% pre-treatment CTC count (cut-off 2 CTCs per 4.5 ml) not prognostic for response, PFS and OS decrease in CTC count after treatment associated with longer OS decrease in CTC count after vemurafenib treatment associated with response	Klinac et al. 2014 [27]
biochemotherapy, <i>n</i> = 50 CTC		qRT-PCR for multiple markers: MART-1, MAGE-A3, GalNAc-T	pre-treatment detection rate: 52% pre-treatment numbers (0 versus ≥1) of CTC-markers and of methylated DNA markers were associated with treatment response, PFS and OS pre-treatment CTC marker number correlated with number of detected methylated DNA	Koyanagi et al. 2006 [25]
	serum methylated DNA	methylation-specific RT-PCR for RASSF1A, RAR-beta2		
biochemotherapy, <i>n</i> =41	serum ctDNA	PCR: LOH analysis using nine microsatellite markers	pre-treatment detection rate: 29% absence of pre-treatment LOH ctDNA correlated with response to treatment detection of pre-treatment LOH ctDNA was associated with shorter PFS and OS	Taback et al. 2004 [30]
biochemotherapy, <i>n</i> =49	serum methylated ctDNA	methylation-specific PCR: RASSF1A, RAR-beta2, MGMT	pre-treatment detection rate: 28% (methylated RASSF1A) pre-treatment detection of methylated RASSF1A was more frequent in non-responders and correlated with worse OS pre-treatment detection of methylated RAR-beta2 correlated with worse OS but was not prognostic for treatment response	Mori et al. 2005 [32]
biochemotherapy, <i>n</i> =48	serum ctDNA	qRT-PCR using PNA clamp and FRET LNA probe: BRAF V600E	pre-treatment detection of BRAF V600 ctDNA did not correlate with treatment response (note: no data on mutation status of tumor tissue available) post-treatment detection of BRAF V600 ctDNA associated with non-response and lower OS	Shinozaki et al. 2007 [28]
MEK-inhibitor selumetinib, <i>n</i> =94	serum ctDNA	Taqman-PCR, BRAF V600E/K/D	pre-treatment BRAF V600 mutant ctDNA detection rate (for BRAF-mutant tumor): 56%	Board et al. 2009

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BRAF mutant tumor: n=45			pre-treatment ctDNA detection was not associated with PFS	[33]
targeted therapies, (dabrafenib, trametinib, vemurafenib), n=29 immunotherapies (ipilimumab, pembrolizumab, nivolumab), n=19	plasma ctDNA	droplet digital PCR: BRAF V600E/K PrimePCR: NRAS Q61K/R	pre-treatment ctDNA detection rate: 73% pre-treatment ctDNA <10 copies/ml was associated with response and longer PFS targeted therapy but not immunotherapy: decrease in ctDNA levels in responders detection of ctDNA preceded progression seen by computerized tomography acquired resistance to BRAF inhibitors via NRAS mutations	Gray et al. 2015 [35]
vemurafenib or dabrafenib, n=22	serum and plasma ctDNA	Taqman-PCR, BRAF V600E	pre-treatment detection rate: 50%, correlated with M-stage (M1a 20%, M1b 75%, M1c 61%) pre-treatment ctDNA detection not prognostic for response, PFS and OS BRAF V600E ctDNA levels in plasma generally higher than in serum	Gonzalez-Cao et al. 2015 [37]
dabrafenib, n=376 trametinib, n=200 DTIC, n=52 DTIC/paclitaxel, n=104	plasma ctDNA	BEAMing Technology (emulsion PCR and flow cytometry) for detection of BRAF V600E/K mutation	concordance between ctDNA and tumor: 76% for V600E, 81% for V600K pre-treatment ctDNA detection associated with shorter PFS and OS detection of ctDNA correlated with LDH, performance status, tumor burden (RECIST), and M-stage, ctDNA remained an independent marker for PFS and OS in multivariate analysis	Santiago-Walker et al. 2016 [36]
dabrafenib plus trametinib, n=34 dabrafenib, n=2 (pre-treatment sampling n=16)	plasma ctDNA	automated Idylla™ system, and digital droplet RT-PCR for BRAF V600 E/E2/D/K/R/M mutations	pre-treatment detection rate: 75%, not prognostic ctDNA detected progression in 70% and prior to clinical progression in 44% of cases in case of negative ctDNA at PD: brain or single site progression detectable ctDNA throughout treatment associated with worse PFS	Schreuer et al. 2016 [38]
adoptive T-cell transfer, n=39	serum ctDNA	castPCR for detection of BRAF V600E	pre-treatment detection rate: 54%, pre-treatment values were not prognostic for response or OS early peak of ctDNA correlated with treatment response complete clearance of ctDNA after early peak associated with complete response and prolonged OS	Xi et al. 2016 [39]
chemotherapy, n=76	mRNA	RT-PCR: tyrosinase	pre-treatment detection rate: 73%	Quaglino et al.

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biochemotherapy, <i>n</i> =73			all patients with negative CTCs at first three treatment cycles showed response to treatment pre-treatment CTC status (positive/negative) was a prognostic factor for PFS and OS	2007 [43]

(RT-)PCR – (real-time) polymerase chain reaction, LOH – loss of heterozygosity, CTC – circulating tumor cell, ct-DNA – circulating DNA, PFS – progression-free survival, OS – overall survival, HMW-MAA - high molecular weight melanoma-associated antigen, MART-1 - melanoma antigen recognized by T cells, MAGE-A3 - melanoma antigen gene-A3 family, GalNAc-T - polypeptide N-acetylgalactosaminyltransferase, RASSF1A – Ras association domain family 1, RAR-beta2 – retinoic acid receptor beta2, MGMT –O-methylguanin DNA methyltransferase, MCSP – melanoma chondroitin sulfate proteoglycan, MCAM – melanoma cell adhesion molecule, ABCB5 - ATP-binding cassette sub-family B member.