Supplementary Materials

Table S1 Search strategies and results of Science Citation Index

1. Data Base: Science CitaionIndex^{TM} Core Collection(viaThomson Reuters Web of Knowledge platform)
2. The search was performed on 2015-07-03.
3. Timespan: from the year 1986 to 2015
4. Data last updated: 2015-07-01

Set	Results	Search History
#1	7,495	TS=circulating DNA
#2	122,887	TS=((blood OR hemato* OR heamato* OR serum OR plasma) SAME (DNA* OR nucleic acid*))
#3	27,391	TS=((cell free OR cell-free) SAME (DNA* OR nucleic acid*))
#4	145,273	#1 OR #2 OR #3
#5	2,627,976	TS=(blood* OR hemato* OR heamato* OR circulat* OR serum OR plasma)
#6	125,622	#4 AND #5
#7	273,303	TS=((digesti* OR colo* OR rectal*) SAME (tumo* OR cancer* OR carcinom* OR neoplas* OR adenocarcinoma*))
#8	5,551	#6 AND #7
#9	1,204,642	TS=(survival* OR prognos* OR recurren*)
#10	2,680,685	TS=((predict* OR risk* OR clinic*) SAME (factor* OR marker* OR biomarker* OR value* OR role* OR significan*))
#11	3,487,154	#9 OR #10
#12	2,798	#8 AND #11
#13	97,029	TI=((digesti* OR colo* OR rectal*) SAME (tumo* OR cancer* OR carcinom* OR neoplas* OR adenocarcinoma*))
#14	783	#12 AND #13
#15	1,221,669	TI=(mouse OR mice OR rat* OR animal*)
#16	760	#14 NOT #15

Table S2 Search strategies and results of Embase

1. Data base: Embase (via OVIDSP platform)
2. The search was performed on 2015-07-03
3. Timespan: from 1974 to 2015 July 02

Set	Results	Search History
1	797	circulating DNA.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
2	14000	((blood OR hemato* OR heamato*OR serum OR plasma) adj3 (DNA* OR nucleic acid*)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
3	2758	((cell free OR cell-free) adj3 (DNA* OR nucleic acid*)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
4	15955	1 OR 2 OR 3
5	4573321	(blood* OR hemato* OR heamato* OR circulat*OR serum OR plasma).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
6	15045	4 AND 5
7	288242	((digesti* OR colo* OR rectal*) adj3 (tumo* OR cancer* OR carcinom* OR neoplas* OR adenocarcinoma*)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
8	93040	exp colorectal cancer/
9	93040	7 OR 8
10	625	6 AND 9
11	2103686	(survival* OR prognos* OR recurren*).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
12	1457918	((predict* OR risk* OR clinic*) adj3 (factor* OR marker* OR biomarker* OR value* OR role* OR significan*)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
13	498361	exp prognosis/
14	3271583	11 OR 12 OR 13
15	271	10 AND 14
16	122824	((digesti* OR colo* OR rectal*) adj3 (tumo* OR cancer* OR carcinom* OR neoplas* OR adenocarcinoma*)).ti.
17	152	15 AND 16
18	1696705	(mouse OR mice OR rat* OR animal*).ti.
19	150	17 NOT 18

Table S3 Search strategies and results of Pubmed

1. Search strategies and results of Pubmed (viaThomson Reuters Web of Knowledge platform)
2. The search was performed on 2015-07-03.
3. Time span: from the year 1950 to 2015
4. Data last updated: 2015-07-01

Set	Results	Search History
#1	11,621	TS= circulating DNA
#2	197,744	TS=((blood OR hemato* OR heamato* OR serum OR plasma) SAME (DNA* OR nucleic acid*))
#3	38,975	TS=((cell free OR cell-free) SAME (DNA* OR nucleic acid*))
#4	41,271	MH=(DNA, Neoplasm)
#5	265,042	#1 OR #2 OR #3 OR #4
#6	1,967,975	TS=(Blood*OR hemato* OR heamato* OR circulat* OR serum OR plasma)
#7	102,920	#5 AND #6
#8	306,147	TS=((digesti* OR colo* OR rectal*) SAME (tumo* OR cancer* OR carcinom* OR neoplas* OR adenocarcinoma*))
#9	153,632	MH:exp=(Colorectal Neoplasms)
#10	312,323	#8 OR #9
#11	2,579	#7 AND #10
#12	1,643,380	TS=(survival* OR prognos* OR recurren*)
#13	3,057,802	TS=((predict* OR risk* OR clinic*) SAME (factor* OR marker* OR biomarker* OR value* OR role* OR significan*))
#14	135,405	MH=(neoplasm recurrence, local OR blood)
#15	1,155,372	MH:exp=(prognosis)
#16	4,570,655	#12 OR #13 OR #14 OR #15
#17	1,093	#11 AND #16
#18	97,707	TI=((digesti* OR colo* OR rectal*) SAME (tumo* OR cancer* OR carcinom* OR neoplas* OR adenocarcinoma*))
#19	382	#17 AND #18
#20	4,010,145	MH:exp=(animals) NOT MH=(humans)
#21	372	#19 NOT #20

Table S4 PRISMA 2009 checklist of the meta-analysis

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	The prognostic value of circulating cell-free DNA in colorectal cancer: a meta-analysis	1
ABSTRACT			
Structured summary	2	Nine studies including 19 units of analysis were included in the meta-analysis. The pooled HRs with 95% CIs revealed strong associations between cfDNA and RFS (HR [95%CI]=2.78[2.08-3.72], I2=32.23%, n=7) along with OS (HR [95%CI]=3.03[2.51-3.66], I2=29.24%, n=12) in patients with CRC. Entire subgroup analyses indicated strong prognostic value of cfDNA irrespective tumor stage, study size and tumor markers. All the results exhibits that the appearance cfDNA in blood is an indicator for adverse RFS and OS in CRC patients.	2
INTRODUCTION			
Rationale	3	The prognostic studies on cfDNAs in CRC were inconsistent. The prognostic significance of cfDNAs in patients with CRC remains controversial.	3
Objectives	4	To demonstrate the prognostic role of cfDNA in CRC and investigate sources of potential heterogeneity.	3
METHODS			
Protocol and registration	5	The protocols followed the standard methods for prognostic meta-analysis. No registration in advance.	4
Eligibility criteria	6	Please see page###, line### for details.	4
Information sources	7	Citaion IndexTM Core Collection, Embase Classic+Embase, Pubmed (viaThomson Reuters Web of Knowledge platform) OvidSP and Thomson Reuters Web of Knowledge platform.	4
Search	8	Any studies regarding prognosis of CRC using cfDNA were searched. Please See supplemental file 1-3 for details.	4
Study selection	9	Details of the literature search process are outlined in the study selection flow chart (see Figure 1).	15
Data collection process	10	Data extracted from each eligible study were any essential clinical factors, characteristics and survival data, which were relevant to the survival of CRC patients. See Table 1	6
Data items	11	Please see supplemental file 1-3 for details for search strategy including PICOS. The variables were recorded in Table 1.	6
Risk of bias in individual studies	12	We mainly conducted subgroup analyses to evaluate the confounding factors. See Table 2. We used the Newcastle Ottawa Scale (NOS) to assess the quality of each study. See Table S5.	5
Summary measures	13	Hazard ratio and 95% CI intervals were presented for all meta-analyses together with I^2 values.	5
Synthesis of results	14	We pooled the extracted HRs with generic inverse variance method in Comphrensive Meta-analysis program (version2.2, Englewood, NJ, Biostat).	5

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Funnel plots along with Begg's and Mazumdar rank correlation method.	6
Additional analyses	16	Subgroup analysis was performed at first to investigate heterogeneity of included studies. Sensitivity analysis was performed to test reliability of the results. Cumulative meta-analysis	7

		was performed to test the impact of publication year on stability of final results. Figure S1, S2, S3 and S4 in supplemental file 7	
RESULTS			
Study selection	17	A total of 1282 articles were retrieved. And 9 publications were eligible for the analysis. See figure 1	6
Study characteristics	18	As mentioned in manuscript. For details see Table 1.	6
Risk of bias within studies	19	Low bias indicated by NOS assessment.	5
Results of individual studies	20	Individual estimates have been shown in forest plot. (See Table 1 and Figure 2).	6,7
Synthesis of results	21	Combined measures and sub-group analysis were shown in Figure 2 and Table 2.	7
Risk of bias across studies	22	Low risk of bias across studies, please see Figure 3.	8
Additional analysis	23	These were described above and please refer to Table 2 and figure S1-S4 in supplemental file 7.	7
DISCUSSION			
Summary of evidence	24	Entire subgroup analyses indicated strong prognostic value of cfDNA irrespective tumor stage, study size and tumor markers. All the results exhibits that the appearance cfDNA in blood is an indicator for adverse RFS and OS in CRC patients.	8,9
Limitations	25	This was mentioned in the discussion section on page 9, line213-216.	9
Conclusions	26	In conclusion, our meta-analysis has revealed the significant prognostic values of cfDNA for RFS and OS in patients with CRC. Further studies should compare the difference between conventional serum tumor markers and cfDNA as alternatives. More studies are expected to investigate sensitive tumor specific markers and compare multiple time points in different tumor stage group in order to prove the clinical utility of cfDNA.	9
FUNDING			
Funding	27	Funding sources were stated in the manuscript.	10

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Table S5 Quality assessment of eligible studies with the NOS scale

Studies	Score for Selection				Score for Comparability		Score for Outcome			Aggregate score	Quality	References
	Item 1	Item 2	Item 3	Item 4	Item 1	Item 2	Item 1	Item 2	Item 3			
Ryan (2003)	1	1	1	1	0	0	0	1	1	6	High	[54]
Bazan (2006)	1	1	1	1	0	0	0	1	1	6	High	[53]
Trevisiol (2006)	1	1	1	1	0	0	0	1	1	6	High	[57]
Wallner (2006)	1	1	1	1	0	0	0	1	1	6	High	[58]
Herbst (2008)	1	1	1	1	0	0	0	1	1	6	High	[59]
Schwarzenbach (2008)	0	1	1	1	0	0	0	1	1	5	High	[55]
Lin (2014)	1	1	1	1	0	0	0	1	0	5	High	[60]
Philipp (2014)	1	1	1	1	0	0	0	1	1	6	High	[61]
Spindler (2014)	0	1	1	1	0	0	0	1	1	6	High	[56]

Note. Numbered items in each category of the NOS are listed below.

Selection

Item 1) Representativeness of the exposed cohort

Item 2) Selection of the non-exposed cohort

Item 3) Ascertainment of exposure

Item 4) Demonstration that outcome of interest was not present at start of study

Comparability

Comparability of cohorts on the basis of the design or analysis

Item 1) study controls for the most important factor (i.e., age)

Item 2) study controls for any additional factor (treatments for cancer)

Outcome

Item 1) Assessment of outcome

Item 2) Was follow-up long enough for outcomes to occur (maximum follow-up period was over 36 month)

Item 3) Adequacy of follow up of cohorts (over 90%)

Reference

Wells G, Shea B, O'connell D, Peterson J, Welch V, Losos M, Tugwell P: The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. In.; 2000.

Table S6 Details of included subgroups

Study ID-Name(Year)	Country	Male /female ratio	Age (year) Mean/Median(range)	Follow-up (month) Mean/Median(range)	Stage (UICC)	Method	Tumor location	Markers	Marker origin	Sampling time	Positive rate, n/ N(%)	Endpoints	Hazard ratio	Multivariate/univariate analysis	Research quality	Reference No.
Bazan (2006)	Italy	34/32	66/NR/NR	26/NR/(2-48)	I-III	PCR and sequencing	Colorectal	K-RAS	Plasma	Baseline	8/50	RFS	Data explorated	NR	High	[53]
Bazan (2006)	Italy	34/32	66/NR/NR	26/NR/(2-48)	I-III	PCR and sequencing	Colorectal	TP53	Plasma	Baseline	8/50	RFS	Data explorated	NR	High	[53]
Ryan (2003)	Netherland	57/37	66.8/66/NR(Group-B)	NR/NR/(22-36)	I-III	PCR and sequencing	Colorectal	Mut.KRAS2 coden-12,13	Serum	Post-Treatment	15/85	RFS	Reported in text	Multivariate	High	[54]
Schwarzenbach (2008)	Germany	42/13	63/NR/(32-83)	>=24	IV	spectrophotometry	Colon-38/Rectum-17	Total cfDNA	Serum	Baseline	26/55	RFS	Data explorated	NR	High	[55]
Spindler (2014)	Denmark	55/31	NR/66/(37-83)	NR/9.5/NR	IV	q-PCR	Colon-57/Rectum-29	Mut.KRAS	Plasma	Baseline	29/86	RFS	Reported in text	Multivariate	High	[56]
Spindler (2014)	Denmark	55/31	NR/66/(37-83)	NR/9.5NR	IV	q-PCR	Colon-57/Rectum-29	Mut.BRAF	Plasma	Baseline	7/86	RFS	Reported in text	Multivariate	High	[56]
Spindler (2014)	Denmark	55/31	NR/66/(37-83)	NR/9.5/NR	IV	q-PCR	Colon-57/Rectum-29	Total cfDNA	Plasma	Baseline	NR	RFS	Reported in text	Multivariate	High	[56]
Trevisiol (2012)	Italy	46/40	65/66/(41-87)	43.7/41/(25-72)	I-IV	ME-PCR	Colon-69/Rectum-17	Mut.KRAS coden-12	Serum	Baseline	11/86	OS	Data explorated	NR	High	[57]
Wallner (2006)	Germany	58/46	68/NR/(33-92)	>=24	I-IV	Real-Time PCR	Colon-60/Rectum-44	mHLTF	Serum	Baseline	31/104	OS	Reported in text	Univariate	High	[58]
Wallner (2006)	Germany	58/46	68/NR/(33-92)	>=24	I-IV	Real-Time PCR	Colon-60/Rectum-44	mhMLH1	Serum	Baseline	24/104	OS	Reported in text	Univariate	High	[58]
Wallner (2006)	Germany	58/46	68/NR/(33-92)	>=24	I-IV	Real-Time PCR	Colon-60/Rectum-44	mHPP1	Serum	Baseline	13/104	OS	Reported in text	Univariate	High	[58]
Herbst(2008)	Germany	NR	66/(NR)/(33-89)	>=24	I-III	Real-Time PCR	Colorectal	mHLTF	Serum	Baseline	13/106	OS	Reported in text	Multivariate	High	[59]
Herbst (2008)	Germany	NR	66/(NR)/(33-89)	>=24	I-III	Real-Time PCR	Colorectal	mHPP1	Serum	Baseline	6/106	OS	Reported in text	Multivariate	High	[59]
lin (2014)	China	NR	64.9/67/(27-80)	NR/62/(12-84)	I-IV	q-PCR	Colorectal	Total cfDNA	Plasma	Baseline	NR/191	OS	Reported in text	Multivariate	High	[60]
Philipp (2014)	Germany	145/114	64.8/NR/NR	>=24	I-IV	Real-Time PCR	Colon-169/Rectum-90	mHLTF	Serum	Baseline	41/259	OS	Data explorated	NR	High	[61]
Philipp (2014)	Germany	145/114	64.8/NR/NR	>=24	I-IV	Real-Time PCR	Colon-169/Rectum-90	mHPP1	Serum	Baseline	57/259	OS	Data explorated	NR	High	[61]
Spindler (2014)	Denmark	55/31	NR/66/(37-83)	NR/9.5/NR	IV	q-PCR	Colon-57/Rectum-29	Mut.KRAS	Plasma	Baseline	29/86	OS	Reported in text	Multivariate	High	[56]
Spindler (2014)	Denmark	55/31	NR/66/(37-83)	NR/9.5/NR	IV	q-PCR	Colon-57/Rectum-29	Mut.BRAF	Plasma	Baseline	7/86	OS	Reported in text	Multivariate	High	[56]
Spindler (2014)	Denmark	55/31	NR/66/(37-83)	NR/9.5/NR	IV	q-PCR	Colon-57/Rectum-29	Total cfDNA	Plasma	Baseline	NR	OS	Reported in text	Multivariate	High	[56]

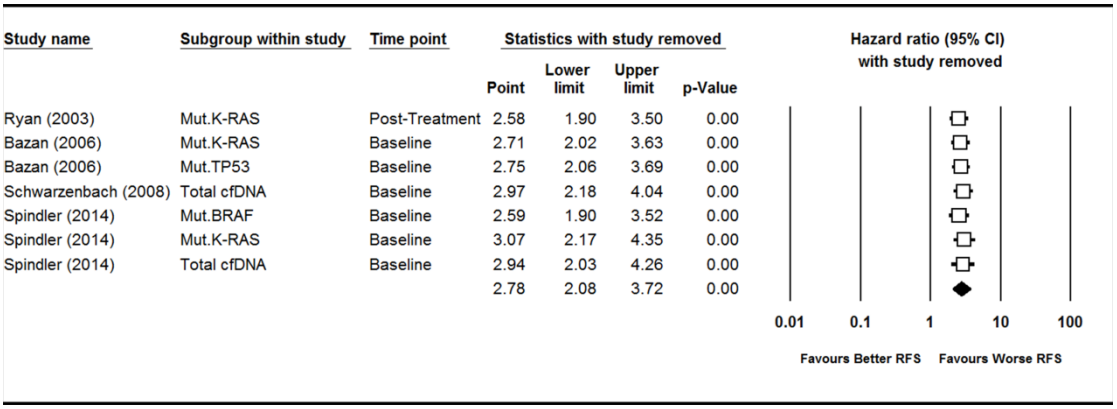


Fig. S1 Sensitivity analysis on RFS by randomly removing one study

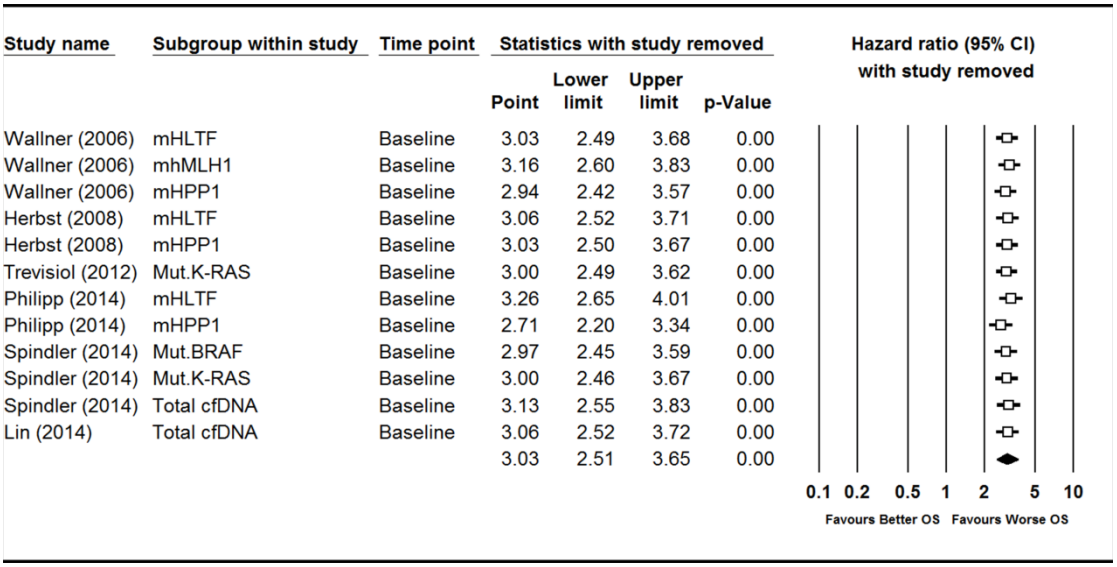


Fig. S2 Sensitivity analysis on OS by randomly removing one study

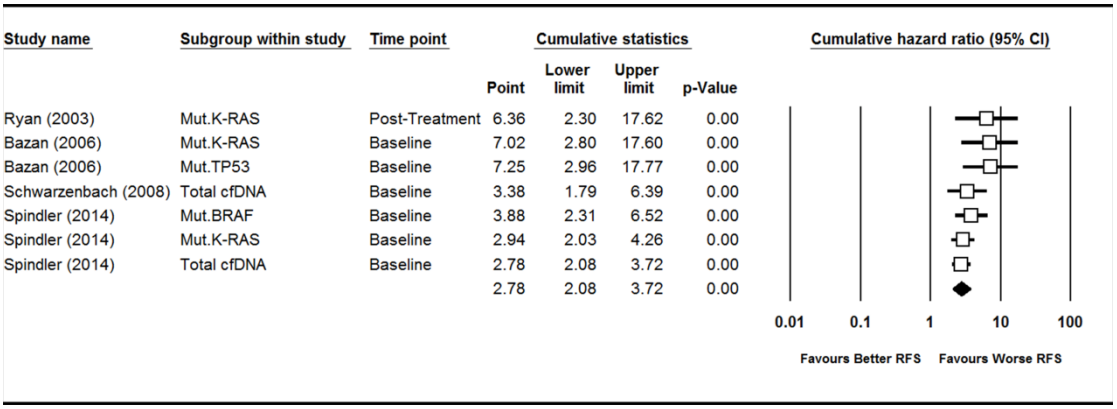
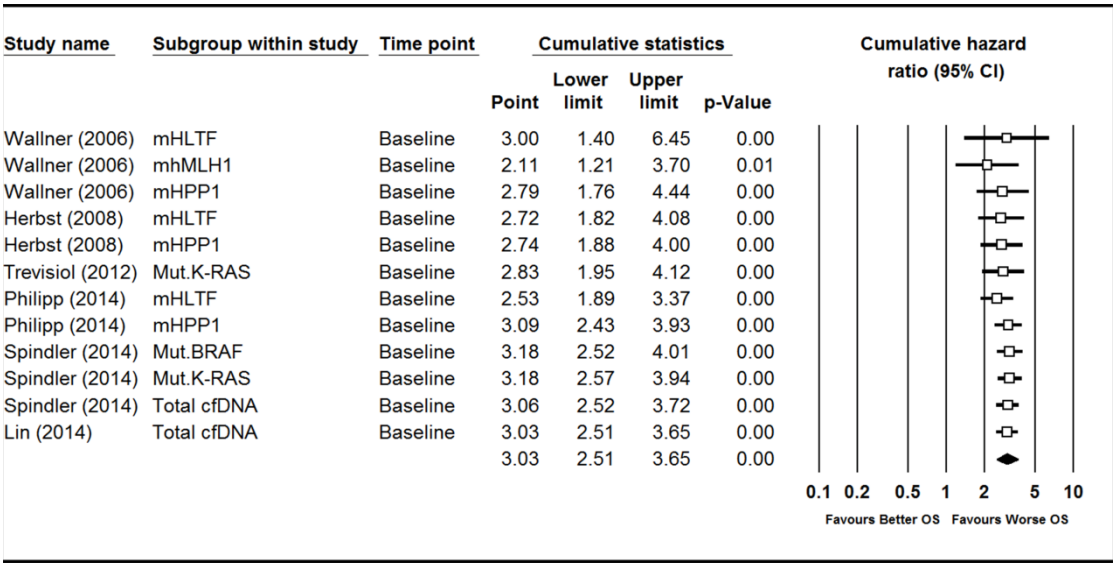


Fig. S3 Cumulative meta-analysis of RFS by publication year



Study name	Subgroup within study	Time point	Cumulative statistics			
			Point	Lower limit	Upper limit	p-Value
Wallner (2006)	mHLTF	Baseline	3.00	1.40	6.45	0.00
Wallner (2006)	mhMLH1	Baseline	2.11	1.21	3.70	0.01
Wallner (2006)	mHPP1	Baseline	2.79	1.76	4.44	0.00
Herbst (2008)	mHLTF	Baseline	2.72	1.82	4.08	0.00
Herbst (2008)	mHPP1	Baseline	2.74	1.88	4.00	0.00
Trevisiol (2012)	Mut.K-RAS	Baseline	2.83	1.95	4.12	0.00
Philipp (2014)	mHLTF	Baseline	2.53	1.89	3.37	0.00
Philipp (2014)	mHPP1	Baseline	3.09	2.43	3.93	0.00
Spindler (2014)	Mut.BRAF	Baseline	3.18	2.52	4.01	0.00
Spindler (2014)	Mut.K-RAS	Baseline	3.18	2.57	3.94	0.00
Spindler (2014)	Total cfDNA	Baseline	3.06	2.52	3.72	0.00
Lin (2014)	Total cfDNA	Baseline	3.03	2.51	3.65	0.00
			3.03	2.51	3.65	0.00

Fig. S4 Cumulative meta-analysis of OS by publication year