

Prognostic significance of isolated tumor cells in patients with colorectal cancer in recent 10-year studies (Review)

YOSHITO AKAGI, TETSUSHI KINUGASA, YOSUKE ADACHI and KAZUO SHIROUZU

Department of Surgery, Kurume University School of Medicine, Kurume, Fukuoka 830-0011, Japan

Received November 1, 2012; Accepted April 18, 2013

DOI: 10.3892/mco.2013.116

Abstract. Circulating tumor cells (CTCs) that detach from the primary tumor and move into the circulation are detected in patients with metastatic cancer. The discovery of such cancer cells has been used as a predictor of recurrence and prognosis, although a consensus regarding such applications has not been reached. Peritoneal cytology may be used for identifying high risk of recurrence or mortality, whereas the intraoperative presence of tumor cells in drainage veins, bone marrow, or the liver is not always useful for evaluating the prognosis. The reported positive rate for tumor cells in the peripheral blood of patients with colorectal cancer, including metastasis, has varied from 10 to 80%; however, numerous studies have demonstrated significant differences in the recurrence and mortality rates between patients with and without isolated tumor cells (ITCs) in the peripheral blood. However, the clinical significance of CTCs as an absolute prognostic factor has not been elucidated, since the measurement methodologies and/or the number of cases differed between the studies. Future prospective studies including larger patient populations may elucidate the utility of routine detection of ITCs in daily practice.

Contents

1. Introduction
2. Tumor cells in lymph nodes
3. Tumor cells in the peritoneal cavity
4. Tumor cells in the peripheral blood
5. Tumor cells in drainage veins
6. Tumor cells in the bone marrow
7. Isolated tumor cells in stage IV patients
8. Conclusion

Correspondence to: Dr Yoshito Akagi, Department of Surgery, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan
E-mail: yoshig@med.kurume-u.ac.jp

Key words: colorectal cancer, isolated tumor cells, circulating tumor cells, disseminated tumor cells, prognosis

1. Introduction

Biomarkers predictive of the prognosis of colorectal cancer have been investigated using various materials and methods. With advances in immunohistochemistry (IHC) and molecular biology, occult tumor cells, including micrometastases in lymph nodes and circulating tumor cells (CTCs) in the peripheral blood, have been detected in patients with gastrointestinal and breast cancers (1-5). By applying highly sensitive and specific diagnostic techniques, several prospective studies suggested that the presence of isolated tumor cells (ITCs) in peripheral areas, drainage veins and the bone marrow is associated with poor outcomes in colorectal cancer patients (6-9). ITCs are single tumor cells or small clusters of cells, ≤ 0.2 mm in greatest dimension, detectable by routine hematoxylin and eosin staining or IHC. Several reviews and meta-analyses evaluated clinical studies on ITCs in lymph nodes, peripheral blood, bone marrow and liver and suggested the prognostic significance of such cells in colorectal cancer patients (10-15).

To elucidate the findings of previous clinical studies assessing the prognostic value of disseminated tumor cells (DTCs) in patients with colorectal cancer, we investigated English literature over the past 10 years, using computer searches of PubMed with the following key words: 'colorectal cancer', 'micrometastasis', 'occult metastasis', 'circulating tumor cell', 'disseminated tumor cell', 'isolated tumor cell', 'lymph node', 'peritoneal cavity', 'peripheral blood', 'drainage vein', 'bone marrow', 'liver', 'prognosis', and 'survival'. After reading all the abstracts and reviewing the available studies, a total of 87 studies were collected and included in this review.

2. Tumor cells in lymph nodes

Between January, 2002 and March, 2012, a total of 31 studies assessed the prognostic value of tumor cells in histologically negative lymph nodes of patients with colorectal cancer (Table I) (16-46). The studies investigated a total of 4,080 patients with colorectal (n=20), colon (n=7) and rectal (n=4) cancer, with a median cohort size of 105 patients. The majority of the studies detected tumor cells using a marker of cytokeratin (CK) and IHC techniques.

Positive rates for tumor cells in histologically negative lymph nodes varied considerably among these studies, ranging from 5 to 88% (median, 30%). With median follow-up periods of 24-128 months (median, 60 months), 16 out of

Table I. Tumor cells in negative lymph nodes.

Author (year)	Cases	Tumor	Stage	Marker	Method	Positive rate (%)	Follow-up (months)	Recurrence/mortality rate	Prognostic value	(Refs.)
Rosenberg, <i>et al</i> (2002)	85	CRC	I/II	CK20	RT-PCR	52	86 ^a	R: 34 vs. 7%; M: 22 vs. 5%	Yes	(16)
Choi, <i>et al</i> (2002)	93	CRC	II	MNF116	IHC	31	66	R: 17 vs. 14%	No	(17)
Noura, <i>et al</i> (2002)	64	CRC	II	CEA	RT-PCR	30	80	R: 39 vs. 12%; M: 22 vs. 5%	Yes	(18)
Merrie, <i>et al</i> (2003)	141	CC	II	CK20	RT-PCR	34	42	M: HR 2.7	Yes	(19)
Shimoyama, <i>et al</i> (2003)	57	RC	I/II	CK7/8/CAM5.2	IHC	19	NA	R: 45 vs. 22%	Yes	(20)
Palma, <i>et al</i> (2003)	38	CRC	II	AE1/AE3	IHC	16	75	R: 71 vs. 76 ^b	No	(21)
Fisher, <i>et al</i> (2003)	399	CRC	I/II	AE1/AE3	IHC	18	NA	R: HR 1.19; M: HR 0.94	No	(22)
Bukholm, <i>et al</i> (2003)	156	CC	II	CAM5.2	IHC	38	NA	M: HR 4.4	Yes	(23)
Kronberg, <i>et al</i> (2004)	90	CRC	I/II	AE1/AE3	IHC	29	91	R: 23 vs. 20%; M: 19 vs. 10%	No	(24)
Laso, <i>et al</i> (2004)	21	CRC	I/II	AE1/AE3	IHC	38	57	M: 55 vs. 77 ^b	No	(25)
Rosenberg, <i>et al</i> (2004)	85	CRC	I/II	CEA/CK20	IHC	27	86	M: 28 vs. 9%	Yes	(26)
Mukai, <i>et al</i> (2005)	124	CRC	II	AE1/AE3	IHC	17	>60	R: 65 vs. 10%; M: 38 vs. 8%	Yes	(27)
Lee, <i>et al</i> (2006)	121	CRC	I/II	MNF116	IHC	50	57	R: 17 vs. 15%	No	(28)
Garcia-Saenz, <i>et al</i> (2006)	105	CRC	II	AE1/AE3	IHC	25	60	R: 23 vs. 20%	No	(29)
Messerini, <i>et al</i> (2006)	395	CRC	IIA	CK20	IHC	38	128	R: 22 vs. 22%	No	(30)
Wang, <i>et al</i> (2007)	55	RC	I/II	CK20	IHC	18	56	R: 50 vs. 20%	Yes	(31)
Hara, <i>et al</i> (2007)	144	RC	I/II	AE1/AE3	IHC	24	81	R: 24 vs. 17%	No	(32)
Fleming, <i>et al</i> (2007)	56	RC	I/II	CAM5.2	IHC	18	98	R: 10 vs. 17%	No	(33)
Steinert, <i>et al</i> (2008)	90	CRC	I/II	CK18	IHC	46	61	R: NS	No	(34)
Davies, <i>et al</i> (2008)	105	CRC	I/II	AE1/AE3	IHC	47	48	R: 16 vs. 18%	No	(35)
Park, <i>et al</i> (2008)	160	CC	I/II	CK20	IHC	5	46	R: 20 vs. 15%; M: 8 vs. 7%	No	(36)
Bosch-Roig, <i>et al</i> (2008)	39	CC	II	AE1/AE3	IHC	10	82 ^a	R: 50 vs. 17%; M: 50 vs. 6%	Yes	(37)
Koyanagi, <i>et al</i> (2008)	67	CRC	I/II	CK20/cMET	RT-PCR	40	34	R: 37 vs. 61; M: 43 vs. 57 ^b	Yes	(38)
Waldman, <i>et al</i> (2009)	257	CRC	I/II	GUCY2C	RT-PCR	88	24	R: 21 vs. 6%	Yes	(39)
van Schaik, <i>et al</i> (2009)	72	CC	I/II	LU5	IHC	24	68	R: 49 vs. 28%; M: 38 vs. 21%	Yes	(40)
Uribarrena-Amezaga, <i>et al</i> (2010)	85	CRC	I/II	AE1/AE3	IHC	36	NA	R: 32 vs. 22%	No	(41)
Haince, <i>et al</i> (2010)	123	CC	I/II	GUCY2C	RT-PCR	20	53	R: 33 vs. 16%	Yes	(42)
Faerden, <i>et al</i> (2011)	126	CC	I/II	CAM5.2	IHC	31	60	R: 23 vs. 7%	Yes	(43)
Oh, <i>et al</i> (2011)	124	CRC	II	AE1/AE3	IHC	27	36	R: 14 vs. 7%; M: 4 vs. 2%	No	(44)
Hyslop, <i>et al</i> (2011)	291	CRC	I/II	GUCY2C	RT-PCR	40	24	R: 41 vs. 2%	Yes	(45)
Mescoli, <i>et al</i> (2012)	312	CRC	I/II	MNF116	IHC	59	63	R: 14 vs. 5%	Yes	(46)

^aMean, ^bsurvival (months). CRC, colorectal cancer; CC, colorectal cancer; RC, rectal cancer; CK, cytokeratin; CEA, carcinoembryonic antigen; GUCY, guanylate cyclase HR, hazard ratio; IHC, immunohistochemistry; RT-PCR, reverse transcriptase-polymerase chain reaction; NA, not available; R, recurrence rate; M, mortality rate; NS, not significant.

the 31 studies (52%) demonstrated a significant difference in recurrence and/or mortality rates between patients with and without ITCs in lymph nodes.

3. Tumor cells in the peritoneal cavity

During the same period, 13 studies assessed the prognostic value of tumor cells in the peritoneal cavity of patients with colorectal cancer (Table II) (34,47-58). The studies investigated a total of 2,434 colorectal cancer patients (median, 125 patients). The majority of these studies used standard cytological methods to detect tumor cells in peritoneal lavage samples.

Positive rates for tumor cells in the peritoneal cavity varied among studies, ranging from 2 to 33% (median, 17%). The median follow-up period was 47 months (range, 25-103 months) and 9 out of the 13 studies (69%) demonstrated significant differences in recurrence and/or mortality rates between patients with and without ITCs in the peritoneal cavity.

4. Tumor cells in the peripheral blood

A total of 22 studies assessed the prognostic value of tumor cells in the peripheral blood of patients with colorectal cancer (Table III) (38,59-79). The studies included a total of 2,857 patients (median, 103 patients), most of whom had colorectal cancer, with the exception of 2 patients with colon and 1 with rectal cancer only. A total of 13 studies included patients with stage I/II/III disease (Dukes' A/B/C), whereas 9 included patients with stage IV disease (Dukes' D). The majority of the studies detected tumor cells using a carcinoembryonic antigen (CEA) or CK marker and reverse transcriptase-polymerase chain reaction (RT-PCR), IHC, immunomagnetic assay (IMA), or membrane assay (MA) techniques.

Positive rates for tumor cells in the peripheral blood ranged from 10 to 62% (median, 38%). Following the exclusion of 9 studies on stage IV patients, the positive rate for tumor cells among the studies was 22-62% (median, 36%). With a median follow-up period of 40 months (range, 24 to >70 months), 15 out of the 22 studies (68%) demonstrated significant differences in recurrence and/or mortality rates between patients with and without ITCs in the peripheral blood. Among the 14 studies including only stage I/II/III patients, 12 (86%) demonstrated a prognostic value of ITCs.

5. Tumor cells in drainage veins

Six studies assessed the prognostic value of tumor cells in drainage veins sampled from the mesenteric or portal vein during surgery (Table IV) (63,65,80-83). The studies investigated patients with colorectal cancer, including a total of 638 patients (median, 94 patients). Tumor cells were detected using a CEA marker and RT-PCR.

The positive rate for tumor cells in the drainage vein varied from 11 to 49% (median, 43%). With a median follow-up period of 46 months (range, 30 to ≥60 months), 4 out of the 6 studies (67%) demonstrated a significant difference in recurrence and/or mortality rates between patients with and without ITCs in the drainage veins.

Table II. Tumor cells in the peritoneal cavity.

Author (year)	Cases	Tumor	Stage or depth of invasion	Marker	Method	Positive rate (%)	Follow-up (months)	Recurrence/mortality rate	Prognostic value	(Refs.)
Guller, <i>et al</i> (2002)	39	CRC	I-III	CEA/CK20	RT-PCR	28	31	R: 82 vs. 7%..	Yes	(47)
Yamamoto, <i>et al</i> (2003)	189	CRC	T3/T4	TC	CYT	6	103	R: 55 vs. 26%	Yes	(48)
Kanellos, <i>et al</i> (2003)	110	CRC	T1-T3	TC	CYT	20	>60	M: 32 vs. 20%	No	(49)
Bosch, <i>et al</i> (2003)	53	CRC	I-III	CK20	CYT/ICC	25	37	R: 62 vs. 28%	Yes	(50)
Baskaranathan, <i>et al</i> (2004)	281	CRC	I-IV	TC	CYT	9	49 ^a	R: 35 vs. 14%	Yes	(51)
Lloyd, <i>et al</i> (2006)	125	CRC	I/II	CEA/CK20	RT-PCR	33	25	R: 29 vs. 4%..	Yes	(52)
Kanellos, <i>et al</i> (2006)	95	CRC	I-III	TC	CYT	26	>60	M: 36 vs. 30%	No	(53)
Gozalan, <i>et al</i> (2007)	67	CRC	I-IV	TC	CYT	9	>24	R: 50 vs. 31%	No	(54)
Steinert, <i>et al</i> (2008)	132	CRC	I-III	CK18	CYT/ICC	22	61	R: NS	No	(34)
Katoh, <i>et al</i> (2009)	91	CRC	III	TC	CYT	11	>24.	R: 30 vs. 9% ^b ; M: 86 vs. 21%.	Yes	(55)
Noura, <i>et al</i> (2009)	697	CRC	0-III	TC	CYT	2	91 ^a	R: 63 vs. 17%; M: 50 vs. 13%	Yes	(56)
Nishikawa, <i>et al</i> (2009)	410	CRC	T3/T4	TC	CYT	8	36	R: 60 vs. 30%; M: 79 vs. 32%	Yes	(57)
Temesi, <i>et al</i> (2012)	145	CRC	T1-T4	TC	CYT	17	47	R: 56 vs. 23%	Yes	(58)

^aMean, ^blocal/peritoneal recurrence. CRC, colorectal cancer; CEA, carcinoembryonic antigen; CK, cytokeratin; TC, tumor cell; RT-PCR, reverse transcriptase-polymerase chain reaction; CYT, cytology; ICC, immunocytochemistry; R, recurrence rate; M, mortality rate; NS, not significant.

Table III. Tumor cells in the peripheral blood.

Author (year)	Cases	Tumor	Stage	Marker	Method	Positive rate (%)	Follow-up (months)	Recurrence/mortality rate	Prognostic value	(Refs.)
Bessa, <i>et al</i> (2003)	66	CRC	I-III	CEA	RT-PCR	55	36	R: 22 vs. 23%	No	(59)
Giacomelli, <i>et al</i> (2003)	41	CRC	I-IV	EGFR	RT-PCR	39	36	R: 94 vs. 4%	Yes	(60)
Chen, <i>et al</i> (2004)	42	CRC	II-IV	GCC	RT-PCR	29	36	R: 50 vs. 7%	Yes	(61)
Zhang, <i>et al</i> (2005)	58	CRC	I-III	CK20	RT-PCR	45	>12	M: 55 vs. 33%	Yes	(62)
Sadahiro, <i>et al</i> (2005)	93	CRC	I-III	CEA	RT-PCR	39	59	R: 6 vs. 19%	No	(63)
Douard, <i>et al</i> (2006)	121	CRC	I-IV	CGM2	RT-PCR	48	NA	R: 28 vs. 29%	No	(64)
Iinuma, <i>et al</i> (2006)	167	CRC	I-IV	CEA/CK20	RT-PCR	10	30	R: NS	No	(65)
Koch, <i>et al</i> (2006)	90	CRC	II	CK20	RT-PCR	25	58	R: 28 vs. 10%	Yes	(66)
Katsumata, <i>et al</i> (2006)	57	CC	I-IV	CK20	RT-PCR	42	>70	R: 25 vs. 12%	No	(67)
Allen-Mersh, <i>et al</i> (2007)	113	CRC	I-III	CEA/VK20	RT-PCR	31	46	R: HR 8.66	Yes	(68)
Sadahiro, <i>et al</i> (2007)	200	CRC	I-III	CEA	RT-PCR	22	52	R: 45 vs. 22%	Yes	(69)
Koch, <i>et al</i> (2007)	45	RC	I-IV	CK20	RT-PCR	38	51	M: 34 vs. 13%	No	(70)
Wang, <i>et al</i> (2007)	157	CRC	I-III	CEA/CK19/20	MA	57	36	M: 50 vs. 12%	Yes	(71)
Friederichs, <i>et al</i> (2007)	37	CRC	I-IV	CK20	RT-PCR	30	40	M: 45 vs. 15%	No	(72)
Uen, <i>et al</i> (2007)	194	CRC	II	CEA/CK19/20	MA	27	40	R: 85 vs. 8%	Yes	(73)
Uen, <i>et al</i> (2008)	438	CRC	I-III	CEA/CK19/20	MA	31	44	R: 68 vs. 16%	Yes	(74)
Yie, <i>et al</i> (2008)	51	CRC	I-IV	Survivin	RT-PCR	41	36	R: 48 vs. 17%	Yes	(75)
Koyanagi, <i>et al</i> (2008)	34	CRC	I-III	CK20/cMET	RT-PCR	47	34	M: 36 vs. 50 ^b	Yes	(38)
Wong, <i>et al</i> (2009)	132	CRC	I-III	CK20	IMA	62	24	M: 52 vs. 17%	Yes	(76)
Vardakis, <i>et al</i> (2011)	265	CRC	II-III	CEA	RT-PCR	37	34	R: 37 vs. 12%; M: 24 vs. 12%	Yes	(77)
Lu, <i>et al</i> (2011)	141	CC	II/III	CEA/CK19/20	MA	36	62	R: 73 vs. 12%	Yes	(78)
Iinuma, <i>et al</i> (2011)	315	CRC	II/III	CEA/CK19/20	RT-PCR	24	37 ^a	R: HR 3.04; M: HR 3.20	Yes	(79)

^aMean, ^bsurvival (months). CRC, colorectal cancer; CC, colon cancer; RC, rectal cancer; CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor; GCC, guanylyl cyclase C; CGM2, carcinoembryonic gene member 2; CK, cytokeratin; RT-PCR, reverse transcriptase-polymerase chain reaction; IMA, immunomagnetic assay; MA, membrane assay; HR, hazard ratio; NA, not available; R, recurrence rate; M, mortality rate; NS, not significant.

Table IV. Tumor cells in drainage veins.

Author (year)	Cases	Tumor	Stage	Marker	Method	Positive rate (%)	Follow-up (months)	Recurrence/mortality rate	Prognostic value (Refs.)
Sunouchi, <i>et al</i> (2003)	37	CRC	I-IV	CEA	RT-PCR	43	33	R: 25 vs. 5% ^b ; M: 31 vs. 5%	Yes (80)
Akashi, <i>et al</i> (2003)	80	CRC	I-III	CEA	RT-PCR	44	52 ^a	R: 20 vs. 5%	No (81)
Sadahiro, <i>et al</i> (2005)	49	CRC	I-III	CEA	RT-PCR	49	59	R: 7 vs. 21%	No (63)
Iinuma, <i>et al</i> (2006)	167	CRC	I-IV	CEA/CK20	RT-PCR	34	30	R: HR 1.744; M: HR 1.517	Yes (65)
Kanellos, <i>et al</i> (2006)	108	CRC	I-III	CEA	RT-PCR	11	>60	R: 50 vs. 15%	Yes (82)
Shimada, <i>et al</i> (2012)	197	CRC	II/III	CEA/CK/CD133	RT-PCR	62	37	R: HR 1.13/1.25 ^c ; M: HR 2.28/1.49 ^e	Yes (83)

^aMean, ^bliver and lung, ^cDukes' B/C. CRC, colorectal cancer; CEA, carcinoembryonic antigen; CK, cytokeratin; CD113, cluster of differentiation 113; RT-PCR, reverse transcriptase-polymerase chain reaction; R, recurrence rate; M, mortality rate; HR, hazard ratio.

Table V. Tumor cells in the bone marrow.

Author (year)	Cases	Tumor	Stage	Marker	Method	Positive rate (%)	Follow-up (months)	Recurrence/mortality rate	Prognostic value (Refs.)
O'Connor, <i>et al</i> (2005)	49	CRC	I-III	CK18	ICC	29	55	R: 29 vs. 31%	No (84)
Koch, <i>et al</i> (2006)	90	CRC	II	CK20	RT-PCR	28	58	R: 5 vs. 17%	No (66)
Steinert, <i>et al</i> (2008)	140	CRC	I-III	CK18	CYT/ICC	64	61	M: 17 vs. 20%	No (34)
Flatmark, <i>et al</i> (2011)	235	CRC	I-III	EpCAM	IMA/ICC	17	112	R: HR 3.0	Yes (85)

CRC, colorectal cancer; CK, cytokeratin; EpCAM, epithelial cell adhesion molecule; ICC, immunocytochemistry; RT-PCR, reverse transcriptase-polymerase chain reaction; IMA, immunomagnetic assay; CYT, cytology; R, recurrence rate; M, mortality rate; HR, hazard ratio.

Table VI. Tumor cells in the peripheral blood of stage IV patients.

Author (year)	Cases	Tumor	Marker	Method	Positive rate (%)	Follow-up (months)	Recurrence/mortality rate	Prognostic value	(Refs.)
Vlems, <i>et al</i> (2003)	22	CRC	CK20	RT-PCR	15	18	R: 75 vs. 64%	No	(86)
Fruhauf, <i>et al</i> (2005)	18	CRC	A45B/B3	IHC	56	31 ^a	R: 76 vs. 10%	Yes	(87)
Koch, <i>et al</i> (2005)	37	CRC	CK20	RT-PCR	30	38	M: 71 vs. 50%	Yes	(88)
Topal, <i>et al</i> (2005)	20	CRC	CEA/CK20	RT-PCR	80	37	NA	No	(89)
Cohen, <i>et al</i> (2008)	413	CRC	CK8/18/19	CSS	26	11	R: 5 vs. 8 ^b ; M: 9 vs. 19 ^c	Yes	(90)
Cohen, <i>et al</i> (2009)	413	CRC	CK8/18/19	CSS	26	26	R: 4 vs. 8 ^b ; M: 9 vs. 21 ^c	Yes	(91)
Tol, <i>et al</i> (2010)	451	CRC	CK8/18/19	CSS	29	17	R: 8 vs. 10 ^b ; M: 13 vs. 22 ^c	Yes	(92)
Rahbari, <i>et al</i> (2011)	63	CRC	CK20	RT-PCR	57	23	R: 58 vs. 44%	Yes	(93)
Pilati, <i>et al</i> (2012)	50	CRC	CD133	RT-PCR	50	36	R: 88 vs. 24%; M: HR 2.611	Yes	(94)

^aMean, ^brelapse-free survival (months), ^coverall survival (months). CRC, colorectal cancer; CK, cytokeratin; CD113, cluster of differentiation 113; CEA, carcinoembryonic antigen; RT-PCR, reverse transcriptase-polymerase chain reaction; IHC, immunohistochemistry; CSS, CellSearch system; R, recurrence rate; M, mortality rate; NA, not available; HR, hazard ratio.

Table VII. Tumor cells in the bone marrow of patients with liver metastasis.

Author (year)	Cases	Tumor	Marker	Method	Positive rate (%)	Follow-up (months)	Recurrence/mortality rate	Prognostic value	(Refs.)
Bjørlund, <i>et al</i> (2003)	29	CRC	MOC31	IMA	7	18	M: 50 vs. 12%	Yes	(95)
Vlems, <i>et al</i> (2003)	22	CRC	CK20	RT-PCR	23	18	R: 80 vs. 65%	No	(86)
Koch, <i>et al</i> (2005)	37	CRC	CK20	RT-PCR	16	38	R: 75 vs. 51%	Yes	(88)
Schoppmeyer, <i>et al</i> (2006)	30	CC	CK	ICC	50	43	M: 47 vs. 53%	No	(96)
Vogelaar, <i>et al</i> (2010)	44	CRC	CK20	RT-PCR	20	24	R: HR 4.11; M: HR 6.40	Yes	(97)
Buxhofer-Ausch, <i>et al</i> (2010)	45	CRC	A45B/B3	ICC	22	35	R: 30 vs. 22%	No	(98)
Hinz, <i>et al</i> (2012)	71	CRC	CK20	RT-PCR	23	41	R: 22 vs. 45 ^a	Yes	(99)

^aRecurrence-free survival (months). CRC, colorectal cancer; CC, colon cancer; CK, cytokeratin; IMA, immunomagnetic assay; RT-PCR, reverse transcriptase-polymerase chain reaction; ICC, immunocytochemistry; M, mortality rate; R, recurrence rate; HR, hazard ratio.

6. Tumor cells in the bone marrow

Four studies assessed the prognostic value of tumor cells in the bone marrow (Table V) (34,66,84,85). The studies included a total of 514 colorectal cancer patients (median, 115 patients). These studies detected tumor cells using a CK marker and immunocytochemistry (ICC) or RT-PCR techniques.

Positive rates of tumor cells in the bone marrow varied from 17 to 64% (median, 29%). With a median follow-up of 60 months (range, 55-112 months), only 1 in 4 studies (25%) demonstrated a significant difference in recurrence and/or mortality rates between patients with and without ITCs in the bone marrow.

7. Isolated tumor cells in stage IV patients

We identified 9 studies assessing the prognostic value of tumor cells in the peripheral blood of patients with metastatic colorectal cancer (Table VI) (86-94). The positive rate of tumor cells in the peripheral blood varied from 15 to 80% (median, 30%). Median follow-up was 25 months (range, 11 to ≥38 months) and 7 studies (78%) demonstrated a significant difference in recurrence and/or mortality rates between patients with and without ITCs in the peripheral blood.

Seven studies assessed the prognostic value of tumor cells in the bone marrow of patients with liver metastasis (Table VII) (86,88,95-99). The median positive rate for tumor cells in peripheral blood was 22% (range, 7-50%). The median follow-up period was 35 months (range, 18 to ≥43 months) and 4 studies (57%) demonstrated a significant difference in recurrence and/or mortality rates between patients with and without ITCs in the bone marrow.

Five studies assessed the prognostic value of tumor cells in the normal liver tissue of patients with liver metastasis, excluding 1 study comprising only stage I/II/III patients (Table VIII) (89,100-103). The positive rate for tumor cells in the peripheral blood varied considerably among the studies (10-70%; median, 37%). With a median follow-up period of 44 months (range, 1 to ≥5 months), 3 studies (60%) demonstrated significant differences in recurrence and/or mortality rates between patients with and without ITCs in the normal liver tissue.

8. Conclusion

Although IHC and molecular techniques are useful for detecting tumor cells in histologically negative lymph nodes, the prognostic significance of such cells is equivocal among recent 10-year studies (104). Peritoneal cytology during curative resection occasionally detects tumor cells and may be useful in identifying a high risk of recurrence or mortality, whereas the presence of tumor cells during surgery in the drainage vein, bone marrow, or liver is not always useful for evaluating the prognosis (105).

Recent studies demonstrated that identifying ITCs in the peripheral blood is useful for estimating the outcome of patients with localized as well as metastatic cancer (106,107). ITCs in the peripheral blood may be measured using the CellSearch system (4,5,108-112) and future prospective studies based on large patient samples and long-term follow-up may elucidate

Table VIII. Tumor cells in the normal liver tissue of patients with liver metastasis.

Author (year)	Cases	Tumor	Marker	Method	Positive rate (%)	Follow-up (months)	Recurrence/mortality rate	Prognostic value	(Refs.)
Yokoyama, <i>et al</i> (2002)	46	CRC	CK20	IHC	70	44	R: 69 vs. 16%; M: 78 vs. 36%	Yes	(100)
Schimanski, <i>et al</i> (2003)	16	CRC	K-ras	RT-PCR	50	NA	M: 165 vs. 240 ^a	Yes	(101)
Linnemann, <i>et al</i> (2004)	54	CRC	K-ras	RT-PCR	26	>1	R: 71 vs. 30%; M: 64 vs. 25%	Yes	(102)
Topal, <i>et al</i> (2005)	19	CRC	CEA/CK20	RT-PCR	37	37	NA	No	(89)
Koch, <i>et al</i> (2007)	100	CRC	CK20	RT-PCR	10	55	R: 11 vs. 23%; M: 20 vs. 23%	No	(103)

^aOverall survival (months). CRC, colorectal cancer; CK, cytokeratin; CEA, carcinoembryonic antigen; IHC, immunohistochemistry; RT-PCR, reverse transcriptase-polymerase chain reaction; NA, not available; R, recurrence rate; M, mortality rate.

the utility of routine examination for ITCs in the daily practice of colorectal cancer surgery.

References

- Hayashi N, Ito I, Yanagisawa A, Kato Y, Nakamori S, Imaoka S, Watanabe H, Ogawa M and Nakamura Y: Genetic diagnosis of lymph-node metastasis in colorectal cancer. *Lancet* 345: 1257-1259, 1995.
- O'Sullivan GC, Collins JK, Kelly J, Morgan J, Madden M and Shanahan F: Micrometastases: marker of metastatic potential or evidence of residual disease? *Gut* 40: 512-515, 1997.
- Mori M, Mimori K, Ueo H, Tsuji K, Shiraishi T, Barnard GF, Sugimachi K and Akiyoshi T: Clinical significance of molecular detection of carcinoma cells in lymph nodes and peripheral blood by reverse transcription-polymerase chain reaction in patients with gastrointestinal and breast carcinomas. *J Clin Oncol* 16: 128-132, 1998.
- Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW and Hayes DF: Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 351: 781-791, 2004.
- Cristofanilli M, Hayes DF, Budd GT, Ellis MJ, Stopeck A, Reuben JM, Doyle GV, Matera J, Allard WJ, Miller MC, Fritsche HA, Hortobagyi GN and Terstappen LW: Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. *J Clin Oncol* 23: 1420-1430, 2005.
- Funaki NO, Tanaka J, Ohshio G, Onodera H, Maetani S and Imamura M: Cytokeratin 20 mRNA in peripheral venous blood of colorectal carcinoma patients. *Br J Cancer* 77: 1327-1332, 1998.
- Taniguchi T, Makino M, Suzuki K and Kaibara N: Prognostic significance of reverse transcription-polymerase chain reaction measurement of carcinoembryonic antigen mRNA in tumor drainage blood and peripheral blood of patients with colorectal carcinoma. *Cancer* 89: 970-976, 2000.
- Guadagni F, Kantor J, Aloe S, Carone MD, Spila A, D'Alessandro R, Abolito MR, Cosimelli M, Graziano F, Carboni F, Carlini S, Perri P, Sciarretta F, Greiner JW, Kashmiri SV, Steinberg SM, Roselli M and Schlom J: Detection of blood-borne cells in colorectal cancer patients by nested reverse transcription-polymerase chain reaction for carcinoembryonic antigen messenger RNA: longitudinal analyses and demonstration of its potential importance as an adjunct to multiple serum markers. *Cancer Res* 61: 2523-2532, 2001.
- Sadahiro S, Suzuki T, Tokunaga N, Yurimoto S, Yasuda S, Tajima T, Makuuchi H, Murayama C and Matsuda K: Detection of tumor cells in the portal and peripheral blood of patients with colorectal carcinoma using competitive reverse transcription-polymerase chain reaction. *Cancer* 92: 1251-1258, 2001.
- Tsavellas G, Patel H and Allen-Mersh TG: Detection and clinical significance of occult tumour cells in colorectal cancer (Review). *Br J Surg* 88: 1307-1320, 2001.
- Conzelmann M, Linnemann U and Berger MR: Detection of disseminated tumour cells in the liver of cancer patients. *Eur J Surg Oncol* 31: 977-985, 2005.
- Iddings D, Ahmad A, Elashoff D and Bilchik A: The prognostic effect of micrometastases in previously staged lymph node negative (N0) colorectal carcinoma: a meta-analysis. *Ann Surg Oncol* 13: 1386-1392, 2006.
- Doekhie FS, Kuppen PJ, Peeters KCMJ, Mesker WE, van Soest RA, Morreau H, van de Velde CJ, Tanke HJ and Tollenaar RA: Prognostic relevance of occult tumor cells in lymph nodes in colorectal cancer. *Eur J Surg Oncol* 32: 253-258, 2006.
- Riethdorf S, Wikman H and Pantel K: Biological relevance of disseminated tumor cells in cancer patients (Review). *Int J Cancer* 123: 1991-2006, 2008.
- Katsuno H, Zacharakis E, Aziz O, Rao C, Deeba S, Paraskeva P, Ziprin P, Athanasiou T and Darzi A: Does the presence of circulating tumor cells in the venous drainage of curative colorectal cancer resections determine prognosis? A meta-analysis. *Ann Surg Oncol* 15: 3083-3091, 2008.
- Rosenberg R, Hoos A, Mueller J, Baier P, Stricker D, Werner M, Nekarda H and Siewert JR: Prognostic significance of cytokeratin-20 reverse transcription-polymerase chain reaction in lymph nodes of node-negative colorectal cancer patients. *J Clin Oncol* 20: 1049-1055, 2002.
- Choi HJ, Choi YY and Hong SH: Incidence and prognostic implications of isolated tumor cells in lymph nodes from patients with Dukes B colorectal carcinoma. *Dis Colon Rectum* 45: 750-755, 2002.
- Noura S, Yamamoto H and Ohnishi T: Comparative detection of lymph node micrometastases of stage II colorectal cancer by reverse transcription-polymerase chain reaction and immunohistochemistry. *J Clin Oncol* 20: 4232-4241, 2002.
- Merrie AE, van Rij AM, Dennett ER, Phillips LV, Yun K and McCall JL: Prognostic significance of occult metastases in colon cancer. *Dis Colon Rectum* 46: 221-231, 2003.
- Shimoyama M, Yamazaki T, Suda T and Hatakeyama K: Prognostic significance of lateral lymph node micrometastases in lower rectal cancer: an immunohistochemical study with CAM5.2. *Dis Colon Rectum* 46: 333-339, 2003.
- Palma RT, Waisberg J, Bromberg SH, Simão AB and Godoy AC: Micrometastasis in regional lymph nodes of extirpated colorectal carcinoma: immunohistochemical study using anti-cytokeratin antibodies AE1/AE3. *Colorectal Dis* 5: 164-168, 2003.
- Fisher ER, Colangelo L, Wieand S, Fisher B and Wolmark N: Lack of influence of cytokeratin-positive mini-micrometastases in 'Negative Node' patients with colorectal cancer: findings from the National Surgical Adjuvant Breast and Bowel Projects protocols R-01 and C-01. *Dis Colon Rectum* 46: 1021-1025, 2003.
- Bukholm IR, Bondi J, Wiik P, Nesland JM, Andersen SN, Bakka A and Bukholm G: Presence of isolated tumour cells in mesenteric lymph nodes predicts poor prognosis in patients with stage II colon cancer. *Eur J Surg Oncol* 29: 862-866, 2003.
- Kronberg U, Lopez-Kostner F, Soto G, Zúñiga A, Wistuba I, Miranda V, Pinto E, Viviani P and Marshall G: Detection of lymphatic micrometastases in patients with stage I and II colorectal cancer: impact on five-year survival. *Dis Colon Rectum* 47: 1151-1157, 2004.
- Laso CA, Gonzalez JJ, Frenco F, Azcano E, Sanz L and Navarrete F: Prognostic value of micrometastases in esophageal and colorectal carcinoma: a clinical experience. *Hepatogastroenterology* 51: 964-967, 2004.
- Rosenberg R, Friederichs J, Gertler R, Hoos A, Mueller J, Nahrig J, Nekarda H and Siewert JR: Prognostic evaluation and review of immunohistochemically detected disseminated tumor cells in peritumoral lymph nodes of patients with pN0 colorectal cancer. *Int J Colorectal Dis* 19: 430-437, 2004.
- Mukai M, Sato S, Ninomiya H, Wakui K, Komatsu N, Tsuchiya K, Nakasaki H and Makuuchi H: Recurrence and 5-FU sensitivity of stage II/Dukes' B colorectal cancer with occult neoplastic cells in lymph node sinuses. *Oncol Rep* 14: 1171-1176, 2005.
- Lee MR, Hong CW, Yoon SN, Lim SB, Park KJ, Lee MJ, Kim WH and Park JG: Isolated tumor cells in lymph nodes are not a prognostic marker for patients with stage I and stage II colorectal cancer. *J Surg Oncol* 93: 13-18, 2006.
- Garcia-Saenz JA, Saenz MC, Gonzalez L, Pérez-Segura P, Puente J, López-Tarruella S, Sastre J, Casado A, López-Asenjo JG and Díaz-Rubio E: Significance of the immunohistochemical detection of lymph node micrometastases in stage II colorectal carcinoma. *Clin Transl Oncol* 8: 676-680, 2006.
- Messerini L, Cianchi F, Cortesini C and Comin CE: Incidence and prognostic significance of occult tumor cells in lymph nodes from patients with stage IIA colorectal carcinoma. *Hum Pathol* 37: 1259-1267, 2006.
- Wang C, Zhou ZG, Yu YY, Li Y, Lei WZ, Cheng Z and Chen ZX: Patterns of lateral pelvic lymph node metastases and micrometastases for patients with lower rectal cancer. *Eur J Surg Oncol* 33: 463-467, 2007.
- Hara M, Hirai T, Nakanishi H, Kanemitsu Y, Komori K, Tatematsu M and Kato T: Isolated tumor cell in lateral lymph node has no influences on the prognosis of rectal cancer patients. *Int J Colorectal Dis* 22: 911-917, 2007.
- Fleming FJ, Hayanga AJ, Glynn F, Thakore H, Kay E and Gillen P: Incidence and prognostic influence of lymph node micrometastases in rectal cancer. *Eur J Surg Oncol* 33: 998-1002, 2007.
- Steinert R, Hantschick M, Vieth M, Gastinger I, Kühnel F, Lippert H and Reymond MA: Influence of subclinical tumor spreading on survival after curative surgery for colorectal cancer. *Arch Surg* 143: 122-128, 2008.
- Davies M, Arumugam PJ, Shah VI, Watkins A, Roger Morgan A, Carr ND and Beynon J: The clinical significance of lymph node micrometastasis in stage I and stage II colorectal cancer. *Clin Transl Oncol* 10: 175-179, 2008.

36. Park SJ, Lee KY and Kim SY: Clinical significance of lymph node micrometastasis in stage I and stage II colon cancer. *Cancer Res Treat* 40: 75-80, 2008.
37. Bosch-Roig CE, Rosello-Sastre E, Alonso-Hernandez S, Almenar Cubells D, Grau Cardona E, Camarasa Lillo N, Bautista D and Molins Palau C: Prognostic value of the detection of lymph node micrometastases in colon cancer. *Clin Transl Oncol* 10: 572-578, 2008.
38. Koyanagi K, Bilchik AJ, Saha S, Turner RR, Wiese D, McCarter M, Shen P, Deacon L, Elashoff D and Hoon DS: Prognostic relevance of occult nodal micrometastases and circulating tumor cells in colorectal cancer on a prospective multicenter trial. *Clin Cancer Res* 14: 7391-7396, 2008.
39. Waldman SA, Hyslop T, Schulz S, Barkun A, Nielsen K, Haaf J, Bonaccorso C, Li Y and Weinberg DS: Association of GUCY2C expression in lymph nodes with time to recurrence and disease-free survival in pN0 colorectal cancer. *JAMA* 301: 745-752, 2009.
40. van Schaik PM, Hermans E, van der Linden JC, Pruijt JR, Ernst MF and Bosscha K: Micro-metastases in stages I and II colon cancer are a predictor of the development of distant metastases and worse disease-free survival. *Eur J Surg Oncol* 35: 492-496, 2009.
41. Uribarrena-Amezaga R, Ortego J, Fuentes J, Raventós N, Parra P and Uribarrena-Echevarría R: Prognostic value of lymph node micrometastasis in patients with colorectal cancer in Dukes stages A and B (T1-T4, N0, M0). *Rev Esp Enferm Dig* 102: 176-186, 2010 (In Spanish).
42. Haince JF, Houde M, Beaudry G, L'espérance S, Garon G, Desaulniers M, Hafer LJ, Heald JI, Lyle S, Grossman SR, Têtu B, Sargent DJ and Fradet Y: Comparison of histopathology and RT-qPCR amplification of guanylyl cyclase C for detection of colon cancer metastases in lymph nodes. *J Clin Pathol* 63: 530-537, 2010.
43. Faerden AE, Sjo OH, Bukholm IR, Andersen SN, Svindland A, Nesbakken A and Bakka A: Lymph node micrometastases and isolated tumor cells influence survival in stage I and II colon cancer. *Dis Colon Rectum* 54: 200-206, 2011.
44. Oh TY, Moon SM, Shin US, Lee HR and Park SH: Impact of prognosis of lymph node micrometastasis and isolated tumor cells in stage II colorectal cancer. *J Korean Sc Coloproctol* 27: 71-77, 2011.
45. Hyslop T, Weinberg DS, Schulz S, Barkun A and Waldman SA: Occult tumor burden predicts disease recurrence in lymph node-negative colorectal cancer. *Clin Cancer Res* 17: 3293-3303, 2011.
46. Mescoli C, Albertoni L, Pucciarelli S, Giacomelli L, Russo VM, Fassan M, Nitti D and Rugge M: Isolated tumor cells in regional lymph nodes as relapse predictors in stage I and II colorectal cancer. *J Clin Oncol* 30: 965-971, 2012.
47. Guller U, Zajac P, Schnider A, Bösch B, Vorbürger S, Zuber M, Spagnoli GC, Oertli D, Maurer R, Metzger U, Harder F, Heberer M and Marti WR: Disseminated single tumor cell as detected by real-time quantitative polymerase chain reaction represent a prognostic factor in patients undergoing surgery for colorectal cancer. *Ann Surg* 236: 768-775, 2002.
48. Yamamoto S, Akasu T, Fujita S and Moriya Y: Long-term prognostic value of conventional peritoneal cytology after curative resection for colorectal carcinoma. *Jpn J Clin Oncol* 33: 33-37, 2003.
49. Kanellos I, Demetriades H, Zintzaras E, Mandrali A, Mantzoros I and Betsis D: Incidence and prognostic value of positive peritoneal cytology in colorectal cancer. *Dis Colon Rectum* 46: 535-539, 2003.
50. Bosch B, Guller U, Schnider A, Maurer R, Harder F, Metzger U and Marti WR: Perioperative detection of disseminated tumour cells is an independent prognostic factor in patients with colorectal cancer. *Br J Surg* 90: 882-888, 2003.
51. Baskaranathan S, Phillips J, McCredden P and Solomon MJ: Free colorectal cancer cells on the peritoneal surface: correlation with pathologic variables and survival. *Dis Colon Rectum* 47: 2076-2079, 2004.
52. Lloyd JM, McIver CM, Stephenson SA, Hewett PJ, Rieger N and Hardingham JE: Identification of early-stage colorectal cancer patients at risk of relapse post-resection by immunobead reverse transcription-PCR analysis of peritoneal lavage fluid for malignant cells. *Clin Cancer Res* 12: 417-423, 2006.
53. Kanellos I, Zacharakis E, Kanellos D, Pramateftakis MG and Betsis D: Prognostic significance of CEA level and positive cytology in peritoneal washings in patients with colorectal cancer. *Colorectal Dis* 8: 436-440, 2006.
54. Gozalan U, Yasti AC, Yuksek YN, Reis E and Kama NA: Peritoneal cytology in colorectal cancer: incidence and prognostic value. *Am J Surg* 193: 672-675, 2007.
55. Katoh H, Yamashita K, Sato T, Ozawa H, Nakamura T and Watanabe M: Prognostic significance of peritoneal tumour cells identified at surgery for colorectal cancer. *Br J Surg* 96: 769-777, 2009.
56. Noura S, Ohue M, Seki Y, Yano M, Ishikawa O and Kameyama M: Long-term prognostic value of conventional peritoneal lavage cytology in patients undergoing curative colorectal cancer resection. *Dis Colon Rectum* 52: 1312-1220, 2009.
57. Nishikawa T, Watanabe T, Sunami E, Tsuno NH, Kitayama J and Nagawa H: Prognostic value of peritoneal cytology and the combination of peritoneal cytology and peritoneal dissemination in colorectal cancer. *Dis Colon Rectum* 52: 2016-2021, 2009.
58. Temesi R, Sikorszki L, Bezsilva J, Botos A, Kovács J and Tihanyi T: Impact of intraabdominal lavage cytology on the long-term prognosis of colorectal cancer patients. *World J Surg* 36: 2714-2721, 2012.
59. Bessa X, Pinol V, Castellvi-Bel S, Piazuolo E, Lacy AM, Elizalde JI, Piqué JM and Castells A: Prognostic value of postoperative detection of blood circulating tumor cells in patients with colorectal cancer operated for cure. *Ann Surg* 237: 368-375, 2003.
60. Giacomelli L, Gianni W, Belfiore C, Gandini O, Repetto L, Filippini A, Frati L, Aglianò AM and Gazzaniga P: Persistence of epidermal growth factor receptor and interleukin 10 in blood of colorectal cancer patients after surgery identifies patients with high risk of relapse. *Clin Cancer Res* 9: 2678-2682, 2003.
61. Chen WS, Chung MY, Liu JH, Liu JM and Lin JK: Impact of circulating free tumor cells in the peripheral blood of colorectal cancer patients during laparoscopic surgery. *World J Surg* 28: 552-557, 2004.
62. Zhang XW, Yang HY, Fan P, Yang L and Chen GY: Detection of micrometastasis in peripheral blood by multi-sampling in patients with colorectal cancer. *World J Gastroenterol* 11: 436-438, 2005.
63. Sadahiro S, Suzuki T, Ishikawa K, Saguchi T, Maeda Y, Yasuda S, Makuuchi H, Yurimoto S and Murayama C: Detection of carcinoembryonic antigen messenger RNA-expressing cells in portal and peripheral blood during surgery does not influence relapse in colorectal cancer. *Ann Surg Oncol* 12: 988-994, 2005.
64. Douard R, Wind P, Sales JP, Landi B, Berger A, Benichou J, Gayral F, Loric S and Cugnenc PH: Long-term prognostic value of detection of circulating colorectal cancer cells using CGM2 reverse transcription-polymerase chain reaction assay. *Surgery* 139: 556-562, 2006.
65. Iinuma H, Okinaga K, Egami H, Mimori K, Hayashi N, Nishida K, Adachi M, Mori M and Sasako M: Usefulness and clinical significance of quantitative real-time RT-PCR to detect isolated tumor cells in the peripheral blood and tumor drainage blood of patients with colorectal cancer. *Int J Oncol* 28: 297-306, 2006.
66. Koch M, Kienle P, Kastrati D, Antolovic D, Schmidt J, Herfarth C, von Knebel Doeberitz M and Weitz J: Prognostic impact of hematogenous tumor cell dissemination in patients with stage II colorectal cancer. *Int J Cancer* 118: 3072-3077, 2006.
67. Katsumata K, Sumi T, Mori Y, Hisada M, Tsuchida A and Aoki T: Detection and evaluation of epithelial cells in the blood of colon cancer patients using RT-PCR. *Int J Clin Oncol* 11: 385-389, 2006.
68. Allen-Mersh TG, McCullough TK, Patel H, Wharton RQ, Glover C and Jonas SK: Role of circulating tumour cells in predicting recurrence after excision of primary colorectal carcinoma. *Br J Surg* 94: 96-105, 2007.
69. Sadahiro S, Suzuki T, Maeda Y, Yurimoto S, Yasuda S, Makuuchi H, Kamijo A and Murayama C: Detection of carcinoembryonic antigen messenger RNA-expressing cells in peripheral blood 7 days after curative surgery is a novel prognostic factor in colorectal cancer. *Ann Surg Oncol* 14: 1092-1098, 2007.
70. Koch M, Antolovic D, Kienle P, Horstmann J, Herfarth C, von Knebel Doeberitz M and Weitz J: Increased detection rate and potential prognostic impact of disseminated tumor cells in patients undergoing endorectal ultrasound for rectal cancer. *Int J Colorectal Dis* 22: 359-365, 2007.
71. Wang JY, Lin SR, Wu DC, Lu CY, Yu FJ, Hsieh JS, Cheng TL, Koay LB and Uen YH: Multiple molecular markers as predictors of colorectal cancer in patients with normal perioperative serum carcinoembryonic antigen levels. *Clin Cancer Res* 13: 2406-2413, 2007.

72. Friederichs J, Gertler R, Rosenberg R, Dahm M, Nekarda H, Holzmann B and Siewert JR: Correlation of CK20-positive cells in peripheral venous blood with serum CEA levels in patients with colorectal carcinoma. *World J Surg* 31: 2329-2334, 2007.
73. Uen YH, Lin SR, Wu DC, Su YC, Wu JY, Cheng TL, Chi CW and Wang JY: Prognostic significance of multiple molecular markers for patients with stage II colorectal cancer undergoing curative resection. *Ann Surg* 246: 1040-1046, 2007.
74. Uen YH, Lu CY, Tsai HL, Yu FJ, Huang MY, Cheng TL, Lin SR and Wang JY: Persistent presence of postoperative circulating tumor cells is a poor prognostic factor for patients with stage I-III colorectal cancer after curative resection. *Ann Surg Oncol* 15: 2120-2128, 2008.
75. Yie SM, Lou B, Ye SR, Cao M, He X, Li P, Hu K, Rao L, Wu SM, Xiao HB and Gao E: Detection of surviving-expressing circulating cancer cells (CCCs) in peripheral blood of patients with gastric and colorectal cancer reveals high risks of relapse. *Ann Surg Oncol* 15: 3073-3082, 2008.
76. Wong SC, Chan CM, Ma BB, Hui EP, Ng SS, Lai PB, Cheung MT, Lo ES, Chan AK, Lam MY, Au TC and Chan AT: Clinical significance of cytokeratin 20-positive circulating tumor cells detected by a refined immunomagnetic enrichment assay in colorectal cancer patients. *Clin Cancer Res* 15: 1005-1012, 2009.
77. Vardakis N, Messaritakis I, Papadaki C, Agoglossakis G, Sfakianaki M, Saridaki Z, Apostolaki S, Koutroubaki I, Perraki M, Hatzidaki D, Mavroudis D, Georgoulas V and Souglakos J: Prognostic significance of the detection of peripheral blood CEACAM5 mRNA-positive cells by real-time polymerase chain reaction in operable colorectal cancer. *Clin Cancer Res* 17: 165-173, 2011.
78. Lu CY, Uen YH, Tsai HL, Chuang SC, Hou MF, Wu DC, Juo SH, Lin SR and Wang JY: Molecular detection of persistent postoperative circulating tumour cells in stages II and III colon cancer patients via multiple blood sampling: prognostic significance of detection for early relapse. *Br J Cancer* 104: 1178-1184, 2011.
79. Iinuma H, Watanabe T, Mimori K, Adachi M, Hayashi N, Tamura J, Matsuda K, Fukushima R, Okinaga K, Sasako M and Mori M: Clinical significance of circulating tumor cells, including cancer stem-like cells, in peripheral blood for recurrence and prognosis in patients with Dukes' stage B and C colorectal cancer. *J Clin Oncol* 29: 1547-1555, 2011.
80. Sunouchi K, Machinami R, Mori M, Namiki K, Hattori S, Murata Y, Tsuchiya T, Mizuno H and Tadokoro M: Clinical impact of carcinoembryonic antigen messenger ribonucleic acid expression in tumor-draining vein blood on postoperative liver metastasis in patients with colorectal carcinoma: a prospective, cohort study. *Dis Colon Rectum* 46: 467-473, 2003.
81. Akashi A, Komuta K, Haraguchi M, Ueda T, Okudaira S, Furui J and Kanematsu T: Carcinoembryonic antigen mRNA in the mesenteric vein is not a predictor of hepatic metastasis in patients with resectable colorectal cancer: a long-term study. *Dis Colon Rectum* 46: 1653-1658, 2003.
82. Kanellos I, Zacharakis E, Kanellos D, Pramateftakis MG, Tsalhalis T, Altsitsiadis E and Betsis D: Prognostic significance of CEA levels and detection of CEA mRNA in draining venous blood in patients with colorectal cancer. *J Surg Oncol* 94: 3-8, 2006.
83. Shimada R, Iinuma H, Akahane T, Horiuchi A and Watanabe T: Prognostic significance of CTCs and CSCs of tumor drainage vein blood in Dukes' stage B and C colorectal cancer patients. *Oncol Rep* 27: 947-953, 2012.
84. O'Connor OJ, Cahill RA, Kirwan WO and Redmond HP: The impact of bone marrow micrometastases on metastatic disease-free survival in patients with colorectal carcinoma. *Colorectal Dis* 7: 406-409, 2005.
85. Flatmark K, Borgen E, Nesland JM, Rasmussen H, Johannessen HO, Bukholm I, Rosales R, Härklau L, Jacobsen HJ, Sandstad B, Boye K and Fodstad Ø: Disseminated tumour cells as a prognostic biomarker in colorectal cancer. *Br J Cancer* 104: 1434-1439, 2011.
86. Vlems FA, Diepstra JH, Punt CJ, Ligtenberg MJ, Cornelissen IM, van Krieken JH, Wobbes T, van Muijen GN and Ruers TJ: Detection of disseminated tumour cells in blood and bone marrow samples of patients undergoing hepatic resection for metastasis of colorectal cancer. *Br J Surg* 90: 989-995, 2003.
87. Fruhauf NR, Kasimir-Bauer S, Goringler K, Lang H, Kaudel CP, Kaiser GM, Oldhafer KJ and Broelsch CE: Peri-operative filtration of disseminated cytokeratin positive cells in patients with colorectal liver metastasis. *Langenbecks Arch Surg* 390: 15-20, 2005.
88. Koch M, Kienle P, Hinz U, Antolovic D, Schmidt J, Herfarth C, von Knebel Doeberitz M and Weitz J: Detection of hemato-genous tumor cell dissemination predicts tumor relapse in patients undergoing surgical resection of colorectal liver metastases. *Ann Surg* 241: 199-205, 2005.
89. Topal B, Aerts JL, Roskams T, Fieuwus S, Van Pelt J, Vandekerckhove P and Penninckx F: Cancer cell dissemination during curative surgery for colorectal liver metastases. *Eur J Surg Oncol* 31: 506-511, 2005.
90. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse M, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LW and Meropol NJ: Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol* 26: 3213-3221, 2008.
91. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse MA, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LW and Meropol NJ: Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. *Ann Oncol* 20: 1223-1229, 2009.
92. Tol J, Koopman M, Miller MC, Tibbe A, Cats A, Creemers GJ, Vos AH, Nagtegaal ID, Terstappen LW and Punt CJ: Circulating tumor cells early predict progression-free and overall survival in advanced colorectal cancer patients treated with chemotherapy and targeted agents. *Ann Oncol* 21: 1006-1012, 2010.
93. Rahbari NN, Reissfelder C, Muhlhuber M, Weidmann K, Kahlert C, Büchler MW, Weitz J and Koch M: Correlation of angiogenic factors with circulating tumor cells and disease recurrence in patients undergoing curative resection for colorectal liver metastases. *Ann Surg Oncol* 18: 2182-2191, 2011.
94. Pilati P, Mocellin S, Bertazza L, Galdi F, Briarava M, Mammano E, Tessari E, Zavagno G and Nitti D: Prognostic value of putative circulating cancer stem cells in patients undergoing hepatic resection for colorectal liver metastasis. *Ann Surg Oncol* 19: 402-408, 2012.
95. Bjørnland K, Flatmark K, Mala T, Mathisen O, Bakka A, Aasen AO, Bergan A, Søreide O and Fodstad O: Detection of disseminated tumour cells in bone marrow of patients with isolated liver metastases from colorectal cancer. *J Surg Oncol* 82: 224-227, 2003.
96. Schoppmeyer K, Fruhauf N, Oldhafer K, Seeber S and Kasimir-Bauer S: Tumor cell dissemination in colon cancer does not predict extrahepatic recurrence in patients undergoing surgery for hepatic metastases. *Oncol Rep* 15: 449-454, 2006.
97. Vogelaar FJ, Mesker WE, Rijken AM, van Pelt GW, van Leeuwen AM, Tanke HJ, Tollenaar RA and Liefers GJ: Clinical impact of different detection methods for disseminated tumor cells in bone marrow of patients undergoing surgical resection of colorectal liver metastases: a prospective follow-up study. *BMC Cancer* 10: 153, 2010.
98. Buxhofer-Ausch V, Ausch C, Kitzweger E, Mollik M, Reiner-Concin A, Ogris E, Stampfl M, Hamilton G, Schiessel R and Hinterberger W: Spontaneous changes in tumour cell dissemination to bone marrow in colorectal cancer. *Colorectal Dis* 12: 776-782, 2010.
99. Hinz S, Bockholst J, Roder C, Egberts JH, Schafmayer C, Kuchler T, Becker T and Kalthoff H: Disseminated tumor cells in the bone marrow negatively influence survival after resection of colorectal liver metastases. *Ann Surg Oncol* 19: 2539-2546, 2012.
100. Yokoyama N, Shirai Y, Ajioka Y, Nagakura S, Suda T and Hatakeyama K: Immunohistochemically detected hepatic micrometastases predict a high risk of intrahepatic recurrence after resection of colorectal carcinoma liver metastases. *Cancer* 94: 1642-1647, 2002.
101. Schimanski CC, Linnemann U, Galle PR, Arbogast R and Berger MR: Hepatic disseminated tumor cells in colorectal cancer UICC stage IV patients: Prognostic implications. *Int J Oncol* 23: 791-796, 2003.
102. Linnemann U, Schimanski CC, Gebhardt C and Berger MR: Prognostic value of disseminated colorectal tumor cells in the liver: results from follow-up examinations. *Int J Colorectal Dis* 19: 380-386, 2004.
103. Koch M, Kienle P, Logan E, Antolovic D, Galindo L, Schmitz-Winnenthal FH, Schmidt J, Herfarth C and Weitz J: Detection of disseminated tumor cells in liver biopsies of colorectal cancer patients is not associated with a worse prognosis. *Ann Surg Oncol* 14: 810-817, 2007.

104. Rahbari NN, Bork U, Motschall E, Thorlund K, Büchler MW, Koch M and Weitz J: Molecular detection of tumor cells in regional lymph nodes is associated with disease recurrence and poor survival in node-negative colorectal cancer: a systematic review and meta-analysis. *J Clin Oncol* 30: 60-70, 2012.
105. Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombes RC, Schlimok G, Diel IJ, Gerber B, Gebauer G, Pierga JY, Marth C, Oruzio D, Wiedswang G, Solomayer EF, Kundt G, Strobl B, Fehm T, Wong GY, Bliss J, Vincent-Salomon A and Pantel K: A pooled analysis of bone marrow micrometastasis in breast cancer. *N Engl J Med* 353: 793-802, 2005.
106. Rahbari NN, Aigner M, Thorlund K, Mollberg N, Motschall E, Jensen K, Diener MK, Büchler MW, Koch M and Weitz J: Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. *Gastroenterology* 138: 1714-1726, 2010.
107. Peach G, Kim C, Zacharakis E, Purkayastha S and Ziprin P: Prognostic significance of circulating tumor cells following surgical resection of colorectal cancers: a systematic review. *Br J Cancer* 102: 1327-1334, 2010.
108. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, Tibbe AG, Uhr JW and Terstappen LW: Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res* 10: 6897-6904, 2004.
109. Budd GT, Cristofanilli M, Ellis MJ, Stopeck A, Borden E, Miller MC, Matera J, Repollet M, Doyle GV, Terstappen LW and Hayes DF: Circulating tumor cells versus imaging - predicting overall survival in metastatic breast cancer. *Clin Cancer Res* 12: 6403-6409, 2006.
110. Hayes DF, Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Miller MC, Matera J, Allard WJ, Doyle GV and Terstappen LW: Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin Cancer Res* 12: 4218-4224, 2006.
111. Riethdorf S, Fritsche H, Müller V, Rau T, Schindlbeck C, Rack B, Janni W, Coith C, Beck K, Jänicke F, Jackson S, Gornet T, Cristofanilli M and Pantel K: Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch System. *Clin Cancer Res* 13: 920-928, 2007.
112. Naoe M, Ogawa Y, Morita J, Omori K, Takeshita K, Shichijyo T, Okumura T, Igarashi A, Yanaihara A, Iwamoto S, Fukagai T, Miyazaki A and Yoshida H: Detection of circulating urothelial cancer cells in the blood using the CellSearch System. *Cancer* 109: 1439-1445, 2007.