FULL PAPER

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A meta-analysis of CXCL12 expression for cancer prognosis

Harsh Samarendra[1], Keaton Jones[2], Tatjana Petrinic[3], Michael A Silva[4], Srikanth Reddy[4], Zahir Soonawalla[4] and Alex Gordon-Weeks*[,5]

[1]Medical Sciences Division, University of Oxford, Oxford, UK; [2]CRUK/MRC Gray Institute for Radiation Oncology and Biology, University of Oxford, Oxford, UK; [3]Cairns Library, John Radcliffe Hospital, Oxford, UK; [4]Department of Hepatobiliary and Pancreatic Surgery, Oxford University Hospitals NHS Trust, Oxford, UK and [5]Nuffield Department of Surgical Sciences, University of Oxford, Room 6607, Level 6, John Radcliffe Hospital, Headley Way, Oxford OX3 9DU, UK

Background: CXCL12 (SDF1) is reported to promote cancer progression in several preclinical models and this is corroborated by the analysis of human tissue specimens. However, the relationship between CXCL12 expression and cancer survival has not been systematically assessed.

Methods: We conducted a systematic review and meta-analysis of studies that evaluated the association between CXCL12 expression and cancer survival.

Results: Thirty-eight studies inclusive of 5807 patients were included in the analysis of overall, recurrence-free or cancer-specific survival, the majority of which were retrospective. The pooled hazard ratios (HRs) for overall and recurrence-free survival in patients with high CXCL12 expression were 1.39 (95% CI: 1.17–1.65, $P=0.0002$) and 1.12 (95% CI: 0.82–1.53, $P=0.48$) respectively, but with significant heterogeneity between studies. On subgroup analysis by cancer type, high CXCL12 expression was associated with reduced overall survival in patients with oesophagogastric (HR 2.08; 95% CI: 1.31–3.33, $P=0.002$), pancreatic (HR 1.54; 95% CI: 1.21–1.97, $P=0.0005$) and lung cancer (HR 1.37; 95% CI: 1.08–1.75, $P=0.01$), whereas in breast cancer patients high CXCL12 expression conferred an overall survival advantage (HR 0.5; 95% CI: 0.38–0.66, $P<0.00001$).

Conclusions: Determination of CXCL12 expression has the potential to be of use as a cancer biomarker and adds prognostic information in various cancer types. Prospective or prospective–retrospective analyses of CXCL12 expression in clearly defined cancer cohorts are now required to advance our understanding of the relationship between CXCL12 expression and cancer outcome.

A feature of most cancers is heterogeneity with regard to treatment response, recurrence and propensity for metastasis. Biomarkers that decipher this heterogeneity, either independently or in addition to current staging systems can help to guide the suitability of radical surgery and chemoradiotherapy, as well as a tailored approach to follow-up. Despite the promise that prognostic biomarkers hold, relatively few have reached clinical practice. This is because of a failure to translate findings from preclinical models to the clinic, a lack of rigorous prospective biomarker validation studies and poor reproducibility between such studies.

In the past two decades, much scientific endeavour has focused on the role that the immune system has in cancer development (de Visser *et al*, 2006; Grivennikov *et al*, 2010). Immune cells contribute to cancer progression, preparation of the premetastatic niche (Psaila and Lyden, 2009) and outgrowth of cancer cells at distant sites. Cytokines are the master regulators of protumourigenic immune cells, orchestrating their recruitment from the bone

*Correspondence: A Gordon-Weeks; E-mail: alex.gordonweeks@gmail.com

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marrow and blood to the tumour and polarising their phenotype once within the tumour microenvironment. These soluble mediators can also promote intravasation of tumour cells or their migration to metastatic sites, drive angiogenesis and inhibit cytotoxic T-cell activity (Balkwill, 2004; Mantovani *et al*, 2008; Chow and Luster, 2014). Certain cytokines may therefore be able to provide prognostic information by identifying tumours that are likely to metastasise or display therapeutic resistance (Ludwig and Weinstein, 2005).

The chemokine CXCL12 (SDF1) binds to the chemokine receptor CXCR4 and is constitutively expressed in tissues that serve as sites for metastasis including the lung, bone marrow and liver. Cancer cells migrate to these organs in a CXCL12-dependent manner (Taichman *et al*, 2002; Ray *et al*, 2015). Preclinical evidence suggests that migration of cancer cells towards CXCL12 in metastatic sites is dependent on simultaneous gain of CXCR4 expression and loss of CXCL12 in the tumour cell, enabling movement away from the primary tumour and towards the metastatic niche (Wendt *et al*, 2006, 2008; Murakami *et al*, 2013). However, this experimentally validated hypothesis is at odds with findings demonstrating that CXCL12 is upregulated in cancer tissues relative to their normal counterparts and that high CXCL12 expression in some human tumours correlates with cancer dedifferentiation and increased tumour grade and stage (Tsuboi *et al*, 2008; Jaafar *et al*, 2009; Machelon *et al*, 2011; Zhong *et al*, 2012).

Given the complex and multifaceted role that CXCL12 has in the progression of primary cancer to metastasis, the prognostic benefit of determining CXCL12 expression in cancer patients is unclear. In an attempt to address this issue, we have performed a meta-analysis of CXCL12 protein expression in the tumour or plasma of cancer patients with the primary independent variable being high *vs* low CXCL12 level. Our primary aims were to determine firstly whether CXCL12 expression predicts survival in cancer patients, and secondly, whether CXCL12 measurement can be considered a valid prognostic biomarker in cancer.

MATERIALS AND METHODS

This meta-analysis was performed in accordance with the Meta-analysis of Observational studies in Epidemiology (MOOSE) group (Stroup *et al*, 2000) and Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidance (Moher *et al*, 2009).

Identification of relevant literature. MEDLINE (PubMed) and EMBASE (Ovid) were search on 10 November 2016 by a health-care librarian (TP) using the strategy shown in Supplementary Figures 1 and 2. All abstracts generated from the search strategy were read and the full text of selected publications was viewed to determine whether the inclusion criteria were met. References from included studies were also hand searched to identify further studies for inclusion.

Inclusion/exclusion criteria. Studies in humans with any solid cancer reporting the effect of CXCL12 expression on absolute, cancer-specific and/or recurrence-free survival were included. We accepted publications reporting any means of CXCL12 protein quantification including ELISA of serum or tumour lysates, or histological analysis of tumour samples. Included studies must have analysed surgical resection histology rather than tumour biopsy. Studies were excluded if they analysed RNA only, or performed a synthesis of publicly available proteomics or RNA data, as were studies of <20 patients. Studies were also excluded if they were not published in English and duplicated data sets from the same institution were also excluded.

Authors were contacted via email if their publication met the inclusion/exclusion criteria, but reported insufficient information for inclusion in the analysis. If no response was obtained they were contacted a second time within 4 weeks.

Assessment of publication quality and risk of bias. The Quality in Prognostic Studies (QUIPS) tool was used to determine risk of bias (Hayden *et al*, 2013). Two authors (HS and AGW) independently assessed each publication meeting the inclusion criteria for the quality domains set out in the QUIPS tool and any discrepancies in their assessment were resolved by joint analysis of the quality domain in question. Risk of bias for each domain was reported using a traffic light system, with red, orange or green indicating a high, moderate or low risk of bias, respectively.

Data extraction and statistical analysis. Data was extracted by one author (AGW) into a spreadsheet and cross-checked by a second author (HS). In all included studies, the independent variable under observation was the level of CXCL12 expression classified as high *vs* low, as defined by each study. The natural logarithm and standard error of the hazard ratio were calculated for outcome measures in each study. Pooled estimates were presented as forest plots and analysed using the random-effects model (DerSimonian and Laird), performed using Review Manager Version 5.3 (Cochrane Collaboration, Oxford, UK). Heterogeneity

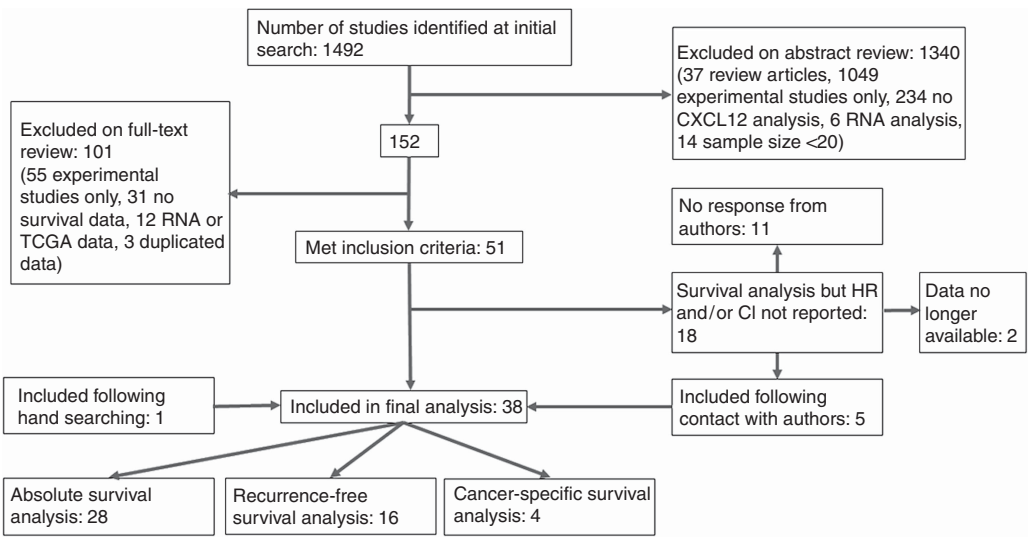

Figure 1. Study flow.

Table 1. Demographics of included studies

First author	Year of publication	Study period	Country	Cancer type	No. of patients	Age *mean (s.d.) $median (range)	% female	% high CXCL12 expression	Median follow-up
Guo *et al* (2016)	2016	?	China	Pancreatic	182	$63 (34–85)	34.7	34.6	12.6
Guo cohort 2 (Guo *et al*, 2016)	2016	?	China	Pancreatic	153	$65 (38–90)	45.1	77.1	10.9
Uchi *et al* (2016)	2016	1997–2007	Japan	Oesophageal	79	$59 (44–79)	89.9	78.45	68
Stanisavljević *et al* (2016)	2016	1993–1996	Norway	Colonic	263	*61.9 (9.3)	52	73.0	?
Stanisavljević cohort 2) (Stanisavljević *et al*, 2016)	2016	2007–2011	Norway	Colonic	239	*73.5 (11.9)	51	89.5	?
Ock *et al* (2017)	2016	2004–2014	Japan	Gastric	70	?	?	48.6	37.8
de Cuba *et al* (2016)	2016	2007–2011	Holland	Colonic (metastatic)	52	*58 (12)	56.6	53.9	22.5
Sterlacci *et al* (2016)	2016	1992–2004	Germany	Lung (NSCLC)	295	?	6.9	53.2	?
Izumi *et al* (2016)	2016	2005–2009	Japan	Gastric	110	?	28.2	58.2	?
Teng *et al* (2016)	2016	2000–2012	China	Endometrial	202	?	100	52.0	?
Rave-Fränk *et al* (2016)	2016	?	Germany	Head and neck	229	?	15.3	42.4	83
Kadota *et al* (2015)	2016	1999–2009	United States of America	Lung adeno.	303	$72 (39–88)	35.0	40.6	49
Tang *et al* (2015)	2015	2009–2014	China	Glioma	42	*44.9 (13)	31.0	21.4	?
Tabernero *et al* (2015)	2015	?	Worldwide	Metastatic colorectal	611	?	37.0	72.2	4.9
Fu *et al* (2015)	2015	2005–2007	China	Cervical	130	?	?	60.8	68
Amara *et al* (2015)	2015	1995–2011	Tunisia	Colorectal	124	?	43.5	71.8	?
Hong *et al* (2014)	2014	?	United States of America	Pancreatic	32	?	15.6	?	38
Wang *et al* (2014)	2014	2002–2006	China	Gastric	84	?	31.3	50.0	59
Martinetti *et al* (2014)	2014	?	Italy	Colonic	50	$60 (51–58)	42	50.0	?
D'Alterio *et al* (2014)	2014	?	Italy	Rectal	68	?	42.6	77.9	64
Walentowicz-Sadlecka *et al* (2014)	2013	2000–2007	Poland	Endometrial	92	*65.1 (9.5)	100	34.8	?
Wang *et al* (2013)	2013	1982–2007	China	Prostatic	148	$65.8 (34–85)	?	18.9	95
Wang *et al* (2012)	2012	2002–2003	China	Renal	97	$55.4 (21–81)	38.1	48.9	?
Lee *et al* (2012)	2012	1998–2009	Korea	Gallbladder carcinoma	72	?	54.2	80.6	?
Schrevel *et al* (2012)	2012	1985–1999	Holland	Cervical	103	$48 (24–87)	100	76.7	137
Popple *et al* (2012)	2012	1984–1997	United Kingdom	Ovarian	172	$61 (24–90)	100	34.3	167
Sakai *et al* (2012)	2012	1999–2007	Japan	Colorectal (metastatic)	92	?	37.0	55.4	38
Machelon *et al* (2011)	2011	2002–2004	France	Ovarian	183	$59 (25–77)	100	47.0	69
Yan *et al* (2011)	2011	?	Australia	Breast	236	$55 (24–87)	100	66.5	131
Kobayashi *et al* (2010)	2010	1995–1999	Japan	Breast	223	$52 (30–82)	100	70.9	74
Liang *et al* (2010)	2010	1990–2005	United States of America	Pancreatic	72	$63 (40–80)	33.3	34.7	?

Table 1. (Continued)

First author	Year of publication	Study period	Country	Cancer type	No. of patients	Age *mean (s.d.) $median (range)	% female	% high CXCL12 expression	Median follow-up
Mirisola *et al* (2009)	2009	2000–2002	Germany	Breast	100	?	100	50.0	?
Akishima-Fukasawa *et al* (2009)	2009	1996–1997	Japan	Colorectal	165	?	38.8	72.1	61
Hassan *et al* (2009)	2009	2000–2003	United States of America	Breast	237	?	100	55.3	40
Gilbert *et al* (2009)	2008	?	United Kingdom	Germ cell	80	$28 (16–65)	0	58.8	75.6
Sasaki *et al* (2009)	2008	1987–1998	Japan	Oesophageal	214	$64 (36–92)	8.4	53.7	42
Ishigami *et al* (2007)	2007	1996–2001	Japan	Gastric	185	$61 (31–82)	29	40.0	?
Pils *et al* (2007)	2007	?	Austria	Ovarian	128	$59 (28–87)	100	68.0	43.7

Abbreviation: NSCLC = non-small-cell lung cancer. Median follow-up is shown in months. *(mean), $(median).

between studies was assessed using the Cochran Q statistic (χ^2 test) and I^2. Heterogeneity was considered high, medium or low if $\geq$75%, 50–75% or $<$50%, respectively (Higgins *et al*, 2003). Funnel plots were constructed for overall, disease-specific and recurrence-free survival analyses and assessed by visual inspection. Subgroup analysis was performed to determine the relationship between CXCL12 expression and outcome in specific cancer types for overall survival. A P-value <0.05 throughout was considered statistically significant. We did not correct the P-value for multiple comparisons within our subgroup analysis as the Cochrane Handbook (V.5.1.0) (Higgins and Green, 2011) currently recommends against this.

RESULTS

Search results. The study flow is shown in Figure 1. A total of 38 studies were included in the meta-analysis of one or more outcome measures totalling 5807 patients (Table 1). Twenty-eight studies reported absolute survival, 16 recurrence-free survival and 4 reported cancer-specific survival. A further 18 studies met the inclusion criteria, but failed to report sufficient data to be included. Of these studies, five authors responded to requests for further data. Of the 11 who failed to respond, 5 reported no association between CXCL12 expression and outcome in the original manuscript.

Study demographics. The demographics of included studies can be seen in Table 1. The total study period ranged from 1982 to 2014, although in 27% of publications, the study period was not identifiable. Forty-seven per cent of studies analysed patients from Australasia, 39% from Europe, 11% from North America and 3% from Africa and 30% of studies analysed data from more than 200 patients. Eighteen studies analysed patients with gastrointestinal cancer (49%), 7 with gynaecological cancer (19%), 4 with breast cancer (11%), 3 with urological cancer (8%) and 2 with lung cancer (5%). The proportion of patients considered to express high levels of CXCL12 varied widely from 18.9% (Wang *et al*, 2013) to 89.5% (Stanisavljević *et al*, 2016). The median follow-up period ranged from 4.9 months in a study of metastatic colorectal cancer (Tabernero *et al*, 2015) to 167 months in a study of ovarian cancer (Popple *et al*, 2012) and 13 studies (35%) did not provide the median follow-up period.

Study methodology and assessment of study quality. The technical detail for included studies, study methodology and technique for CXCL12 protein quantification is shown in Table 2, while an analysis of risk of bias as determined using the QUIPS tool is shown in Supplementary Table 1. There were 3 studies that analysed serum CXCL12 concentration and 34 studies that quantified tumour protein expression using IHC. We did not identify any study that quantified CXCL12 expression in protein from tumour lysate. Most studies simultaneously analysed the expression of other factors with CXCR4 analysed by 23 studies. The antigen retrieval technique and details of the antibody used, sufficient that the methodology could be repeated by readers, were documented by 16 studies (43%). Interestingly, one study reported the use of an antibody with specificity for CXCR4 for the analysis of CXCL12 (Ishigami *et al*, 2007).

The method for defining low and high CXCL12 expression level was reported in 89% of studies, with 65% using an arbitrary method not related to data distribution and only 22% of studies determining CXCL12 value cutoffs based on ROC curve analysis. In 10 studies (26%), the CXCL12 expression data was linked to follow-up data collected in a prospective manner.

Survival analysis. The pooled HR for overall survival in patients with high CXCL12 expression compared with low expression was

Table 2. Technical details of method for CXCL12 quantification

First author	Sample	Quantification method	Other factors analysed	Antigen retrieval method	Sample storage	Antibody/ELISA source (CAT number)	Definition of expression level cutoffs	Follow-up collection	Outcome measures
Guo *et al*, 2016	Tumour tissue	IHC	CXCR7	Autoclave + citrate	FFPE	R&D Systems, Minneapolis, MN, USA	Arbitrary	Retrospective	OS
Guo cohort 26) (Guo *et al*, 2016)	Tumour tissue	IHC	CXCR7	Autoclave + citrate	FFPE	R&D Systems	Arbitrary	Retrospective	OS
Uchi *et al* (2016)	Tumour tissue	IHC	CXCR4	Target retrieval solution (Dako, Santa Clara, CA, USA)	FFPE	R&D Systems (MAB350)	Arbitrary	Retrospective	RFS
Stanisavljević *et al* (2016)	Tumour tissue	IHC	CXCR4	Target retrieval solution (Dako)	FFPE	R&D Systems (MAB350)	Arbitrary	Prospective	RFS
Stanisavljević cohort 2 (Stanisavljević *et al*, 2016)	Tumour tissue	IHC	CXCR4	Target retrieval solution (Dako)	FFPE	R&D Systems (MAB350)	Arbitrary	Prospective	RFS
Ock *et al* (2017)	Serum	Protein array	Multiple (>10)	N/A	Frozen	Bio-Rad Laboratories (Hercules, CA, USA) (Bio-Plex 220 assay)	Data distribution	Retrospective	OS
de Cuba *et al* (2016)	Tumour tissue	IHC	HIF1a, CXCR4, VEGF	?	FFPE	R&D Systems	Arbitrary	Prospective	OS
Sterlacci *et al* (2016)	Tumour tissue	IHC	CXCR4, pCXCR4	?	FFPE	Abcam (Cambridge, UK)	ROC curve analysis	Retrospective	OS
Izumi *et al* (2016)	Tumour tissue	IHC	CXCR4	?	FFPE	R&D Systems AF-310-NA	Arbitrary	Retrospective	OS
Teng *et al* (2016)	Tumour tissue	IHC	CXCR4	?	FFPE	Abcam	Arbitrary	Retrospective	CSS
Rave-Fränk *et al* (2016)	Tumour tissue	IHC	CXCR4	Heat (100 °C, 60 min)	FFPE	R&D Systems	Arbitrary	Retrospective	OS
Kadota *et al* (2015)	Tumour tissue	IHC	Multiple (>10)	?	FFPE	?	Arbitrary	Prospective	OS
Tang *et al* (2015)	Tumour tissue	IHC	CXCR4	Citrate (pH 6.0, 100 °C, 15 min)	FFPE	R&D Systems (MAB350)	Arbitrary	?	OS
Tabernero *et al* (2015)	Serum	ELISA	Multiple (>10)	n/a	Frozen	Assay Gate (Ijamsville, MD, USA)	ROC curve analysis	Prospective	OS, RFS
Fu *et al* (2015)	Tumour tissue	IHC	N/A	?	FFPE	?	Arbitrary	Retrospective	OS
Amara *et al* (2015)	Tumour tissue	IHC	CXCR4	Citrate (pH 9.0, microwave 2–5 min)	FFPE	R&D Systems (?)	Arbitrary	Retrospective	OS
Hong *et al* (2014)	Tumour tissue	IHC	CEA, CA19-9, HGF	?	FFPE	Biovision (Milpitas, CA, USA) (?)	?	Prospective	OS, RFS
Wang *et al* (2014)	Tumour tissue	IHC	N/A	?	FFPE	R&D Systems (MAB350)	ROC curve analysis	Retrospective	OS
Martinetti *et al* (2014)	Serum	ELISA	VEGF, PDGF, osteopontin, CEA	N/A	Frozen	Bio-Rad Laboratories (Bio-Plex 220 assay)	ROC curve analysis	Prospective	OS, RFS
D'Alterio *et al* (2014)	Tumour tissue	IHC	CXCR4, CXCR7	?	FFPE	R&D Systems (MAB350)	Arbitrary	Retrospective	OS
Walentowicz-Sadlecka *et al* (2014)	Tumour tissue	IHC	CXCR4, CXCR7	Epitope Retrieval Solution (Dako)	FFPE	Abcam (AB9797)	Arbitrary	Retrospective	OS

Table 2. (Continued)

First author	Sample	Quantification method	Other factors analysed	Antigen retrieval method	Sample storage	Antibody/ELISA source (CAT number)	Definition of expression level cutoffs	Follow-up collection	Outcome measures
Wang *et al* (2013)	Tumour tissue	IHC	VEGF, MMP9	0.1% zymine (37 °C, 30 min)	FFPE	R&D Systems (?)	Arbitrary	?	RFS
Wang *et al* (2012)	Tumour tissue	IHC	CXCR4, CXCR7	Citrate (pH 6.0, 100 °C, 10 min)	FFPE	R&D Systems (MAB350)	ROC curve analysis	Retrospective	OS, RFS
Lee *et al* (2012)	Tumour tissue	IHC	N/A	Target Retrieval Solution (DAKO)	FFPE	R&D Systems (MAB350)	ROC curve analysis	Retrospective	CSS
Schrevel *et al* (2012)	Tumour tissue	IHC	N/A	Citrate (pH 6.0, microwave, 12 min)	FFPE	R&D Systems (MAB350)	?	Retrospective	RFS
Popple *et al* (2012)	Tumour tissue	IHC	CXCR4	EDTA (pH 9.0, microwave, 10 min)	FFPE	R&D Systems (MAB350)	Arbitrary	Retrospective	OS
Sakai *et al* (2012)	Tumour tissue	IHC	CXCR4, CD133	?	FFPE	R&D Systems (MAB350)	Arbitrary	Retrospective	OS, RFS
Machelon *et al* (2011)	Tumour tissue	IHC	N/A	Citrate (pH 6.0, microwave)	FFPE	Abcam (AB10395)	Arbitrary	Prospective	OS, RFS
Yan *et al* (2011)	Tumour tissue	IHC	FoxP3	Tris/EDTA (pH 9.0, microwave)	FFPE	R&D Systems (MAB350)	Arbitrary	retrospective	CSS
Kobayashi *et al* (2010)	Tumour tissue	IHC	CXCR4	0.5% Tween-20 in PBS	FFPE	R&D Systems (MAB350)	Arbitrary	?	OS, RFS
Liang *et al* (2010)	Tumour tissue	IHC	N/A	EDTA (pH 9.0, 100 °C, 20 min)	FFPE	R&D Systems (?)	ROC curve analysis	prospective	OS, RFS
Mirisola *et al* (2009)	Tumour tissue	IHC	CXCR4	?	FFPE	Dianova (Hamburg, Germany) (?)	ROC curve analysis	?	OS, RFS
Akishima-Fukasawa *et al* (2009)	Tumour tissue	IHC	N/A	Citrate (pH 6.0, 121 °C, 10 min)	FFPE	R&D Systems (?)	Arbitrary	Prospective	OS, RFS
Hassan *et al* (2009)	Tumour tissue	IHC	CXCR4	?	FFPE	R&D Systems (MAB350)	Arbitrary	?	OS
Gilbert *et al* (2009)	Tumour tissue	IHC	CXCR4	?	FFPE	R&D Systems (MAB350)	Arbitrary	Retrospective	RFS
Sasaki *et al* (2009)	Tumour tissue	IHC	CXCR4	Citrate buffer (120 °C, 10 min)	FFPE	R&D Systems (MAB350)	Arbitrary	Retrospective	OS
Ishigami *et al* (2007)	Tumour tissue	IHC	N/A	?	FFPE	R&D Systems (MAB172)	?	?	OS
Pils *et al* (2007)	Tumour tissue	IHC	CXCR4	?	FFPE	R&D Systems (MAB350)	?	?	OS

Abbreviations: CSS = cancer specific survival; ELISA = enzyme-linked immunosorbent assay; FFPE = formalin-fixed paraffin-embedded; IHC = immunohistochemistry; N/A = not applicable; OS = overall survival; RFS = recurrence-free survival.

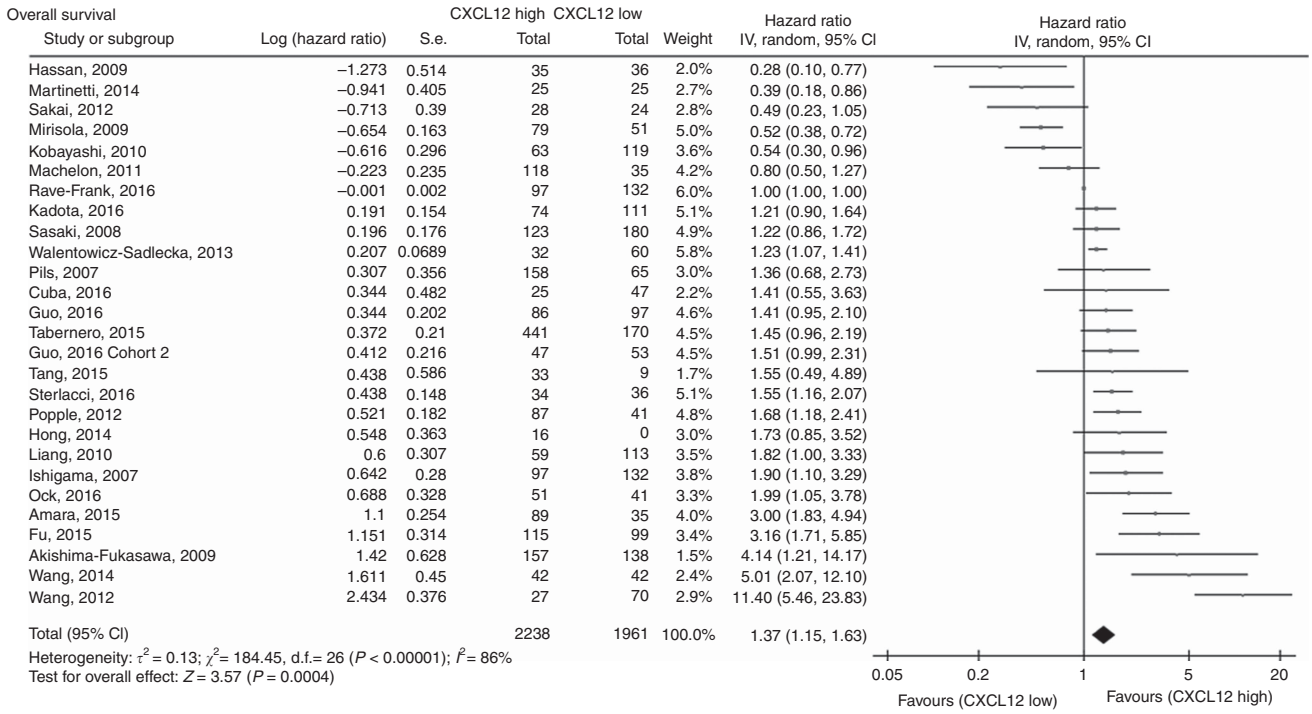

Figure 2. Forrest plot of overall survival for all studies meeting the inclusion criteria listed in order of effect size.

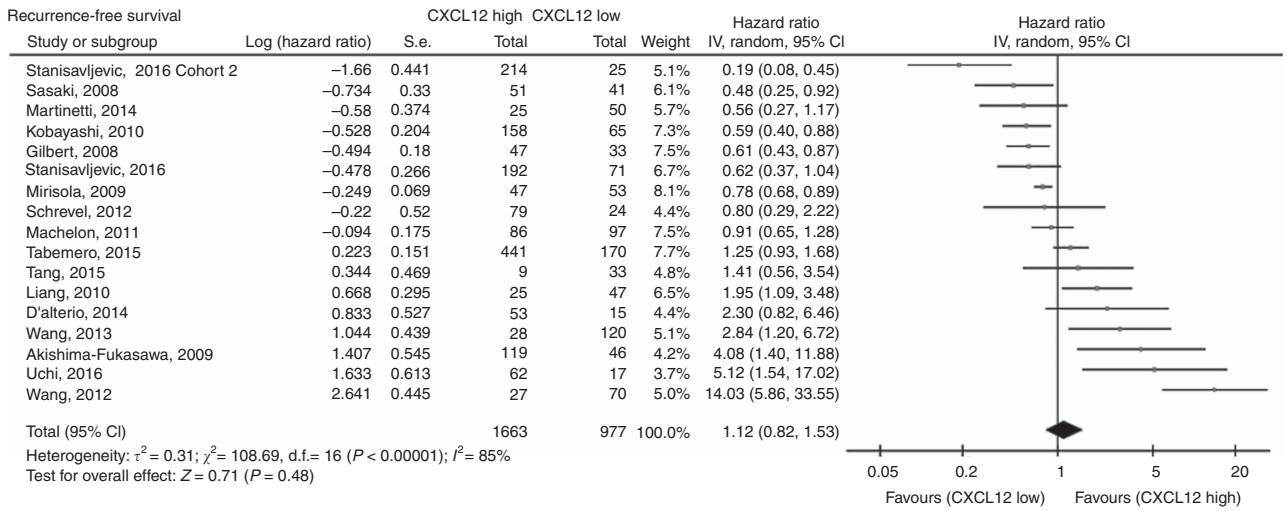

Figure 3. Forrest plot of recurrence-free survival for all studies meeting the inclusion criteria in order of effect size.

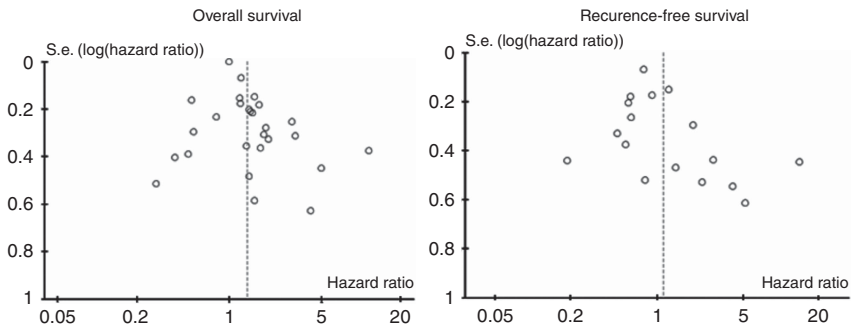

Figure 4. Funnel plots for included studies reporting overall (left) and recurrence-free survival (right).

1.39 (95% CI: 1.17–1.65, $P = 0.0002$), but with a significant degree of heterogeneity ($I^2 = 86\%$) (Figure 2), while the pooled HR for recurrence-free survival was 1.12 (95% CI: 0.82–1.53, $P = 0.48$), again with a high degree of study heterogeneity ($I^2 = 85\%$) (Figure 3). The pool HR for cancer-specific survival, which was only analysed in four studies, was 1.67 (95% CI: 0.43–6.50, $P = 0.46$) again with high study heterogeneity ($I^2 = 87\%$) (results not shown). Funnel plots for overall, recurrence-free and cancer-specific survival demonstrated no evidence of publication bias or small study effects (Figure 4).

Subgroup analysis. Following subgroup analysis, high CXCL12 expression served as a marker of reduced overall survival in oesophagogastric (HR 2.08; 95% CI: 1.31–3.33, $P = 0.002$), pancreatic (HR 1.54; 95% CI: 1.21–1.97, $P = 0.0005$) and lung (HR 1.37; 95% CI: 1.08–1.75, $P = 0.01$) cancers (Figure 5). For colorectal and ovarian cancer, however, there was no relationship between CXCL12 expression and overall survival (HR 1.21; 95% CI: 0.64–2.51, $P = 0.49$) and (HR 1.23; 95% CI: 0.75–2.03, $P = 0.42$), respectively. For breast cancer patients, high CXCL12 predicted better overall survival (HR 0.5; 95% CI: 0.38–0.66,

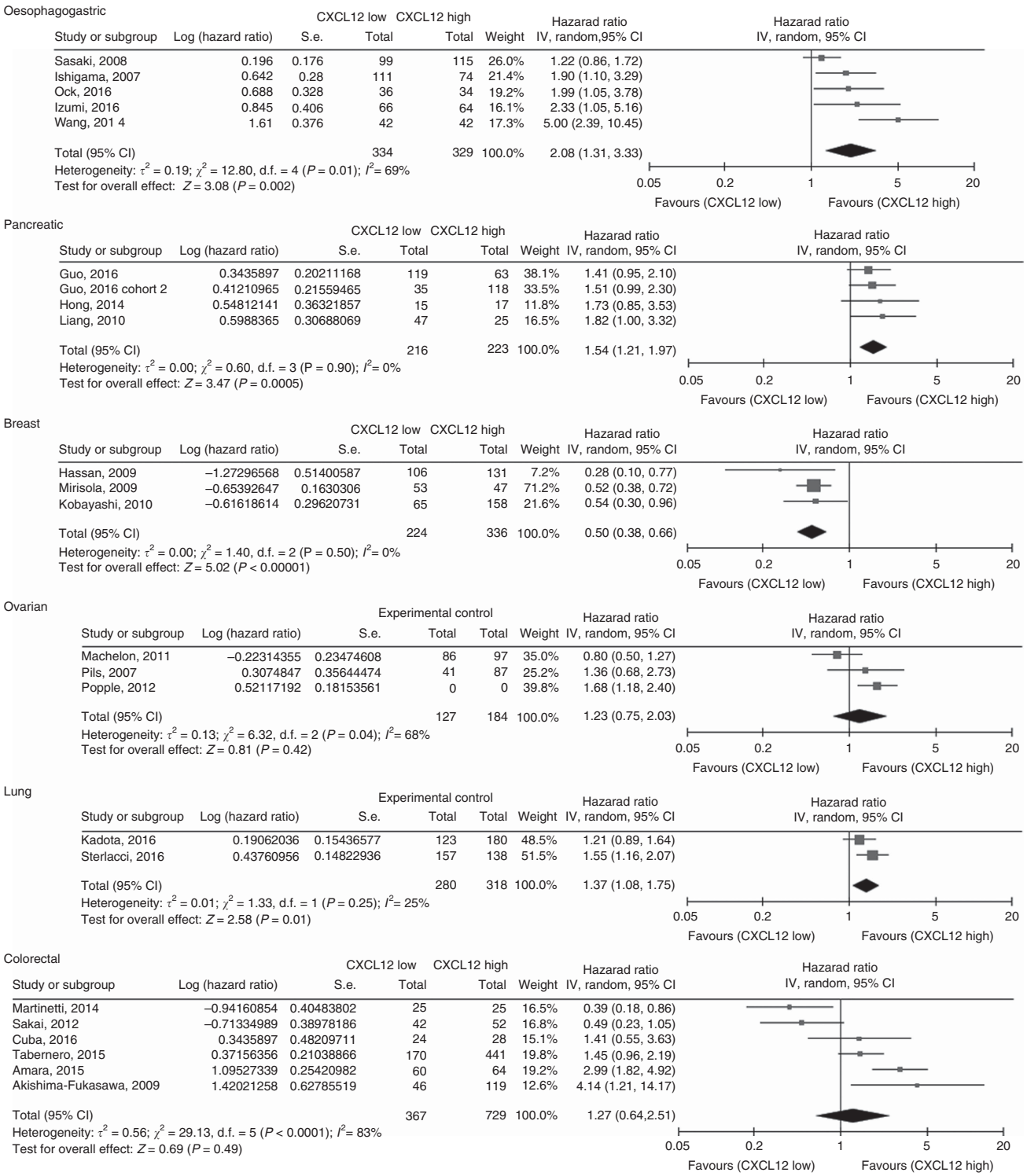

Figure 5. Subgroup analysis by cancer type demonstrating meta-analysis of high *vs* low CXCL12 expression for overall survival.

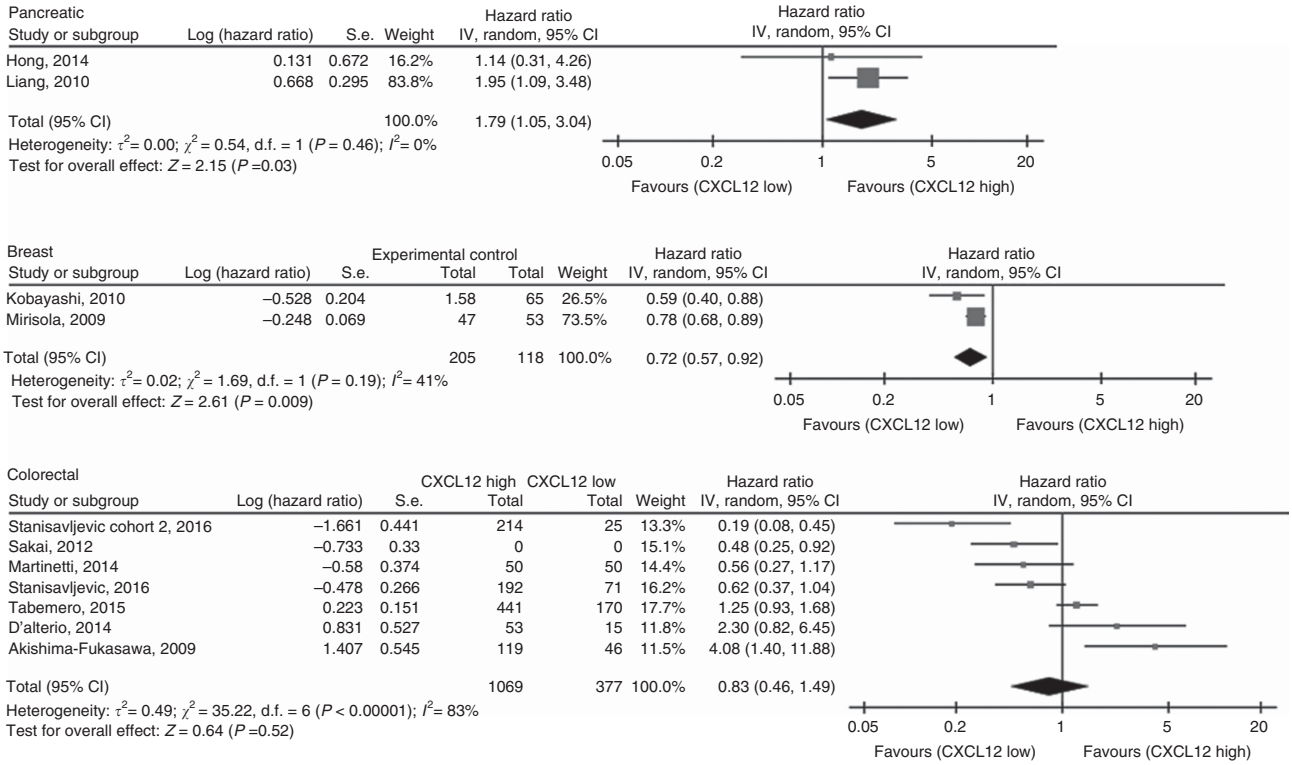

Figure 6. Subgroup analysis by cancer type demonstrating meta-analysis of high *vs* low CXCL12 expression for recurrence-free survival.

$P<0.00001$). Of note, aside from colorectal cancer, statistical heterogeneity across studies was significantly lower in subgroup analyses compared with the analysis of all studies combined.

Relatively fewer studies published data for recurrence-free survival, but where sufficient data was available, it broadly supported the findings for absolute survival. We were able to identify at least two studies reporting recurrence-free survival for pancreatic, breast or colon cancer (Figure 6).

We also performed further subgroup analyses based on the nature of follow-up data collection (prospective *vs* retrospective), study size (>200 patients *vs* <200 patients) or method for defining CXCL12 expression cutoff, reasoning that such analysis might help differentiate studies with a higher level of bias. However, this approach failed to eliminate statistical heterogeneity, which was shared between study types evenly (data not shown).

DISCUSSION

The primary objective of this meta-analysis was to determine whether CXCL12 expression was associated with survival in cancer patients. We found that high CXCL12 expression was associated with reduced absolute survival in patients with oesophagogastric, pancreatic or lung cancer, while the converse was the case for breast cancer patients. Indeed, the major cause of heterogeneity in our meta-analysis resulted from heterogeneity in the relationship between CXCL12 expression and outcome between breast and other cancer types. Our data indicate that determination of CXCL12 expression could be useful for predicting outcome in these cancer types. Although studies of RNA expression were excluded from this meta-analysis, published studies that have assessed CXCL12 mRNA expression in oesophageal (Goto *et al*, 2017) or breast cancer (Razis *et al*, 2012) support our findings, with an association between increased CXCL12 expression and adverse outcome in oesophageal cancer, but the converse in breast cancer.

The cause of the different effect of high CXCL12 expression and outcome in breast compared with other cancers is unclear. The publications of breast, pancreatic, lung and oesophagogastric cancer included in our study all analysed primary rather than metastatic tumours; thus, differences are unlikely to result from sampling differences between cancer types. However, they may result from clinicobiological differences between these cancers.

Thus, breast cancer is rarely fatal unless metastatic, whereas oesophagogastric, lung and pancreatic cancers often cause mortality through local invasion. CXCL12 is able to promote local invasion of cancer cells, while loss of CXCL12 promotes tumour cell migration to organs expressing high levels of CXCL12 such as the liver, bone marrow and lung. Breast cancers may therefore rely on downregulation of CXCL12 to metastasise, whereas in pancreatic, oesophagogastric and lung tumours, high CXCL12 expression may be associated with poor outcome because it promotes local invasion, in turn contributing to mortality.

Alternatively, differences between breast and other cancer types may reflect systemic differences between the demographics of the studies, or the methodologies used. It should also be considered that the source of CXCL12 within the tumour may be important. The majority of included studies did not investigate the cellular source of CXCL12, and while the immunohistochemical images presented in most publications indicate the primary source of CXCL12 is the tumour cell, it is possible that stromal and tumour cell CXCL12 production have different roles in cancer progression. Finally, there is significant redundancy in the chemokine network such that analysis of a single chemokine alone may be insufficient. Thus, the relative ratio of CXCL12 to its receptors CXCR4 and/or CXCR7 may be a better indicator of CXCL12 activity (Luker *et al*, 2012; Wani *et al*, 2014) and there may be differences in these ratios between cancer types.

Our second objective was to determine whether CXCL12 measurement can be used as a prognostic biomarker in cancer patients. The gold standard evidence level for a prognostic biomarker study is a randomised controlled trial (RCT) designed in such a way that participants are randomised to the prognostic

test or a standard prognostic factor and the treatment received dependent on the results of the prognostic test. This type of trial is difficult to perform, requires a very large sample size to be adequately powered and is at significant risk of confounding (Simon *et al*, 2009).

The retrospective analysis of archived tissue specimens collected as part of an RCT may provide as good an indication of the value of a prognostic marker as an RCT of the marker itself, as can the retrospective analysis of tissues linked to prospectively collected follow-up data (a prospective–retrospective design) (Simon *et al*, 2009). In contrast, truly retrospective biomarker studies, where follow-up data is generated retrospectively, are at high risk of bias. We were only able to identify one study that used RCT-generated follow-up data (Tabernero *et al*, 2015) and while a number of other studies were of a prospective–retrospective nature, a significant number were purely retrospective and therefore at risk of bias. This is supported by our analysis of bias using the QUIPS tool, which demonstrated that the majority of studies suffered from at least a moderate risk of bias.

It is however reassuring that the funnel plots generated from studies reporting absolute and recurrence-free survival demonstrate no evidence of publication bias. This is supported by the fact that of the 18 studies that met the inclusion criteria, but reported inadequate outcome data to be included in the meta-analysis, fewer than 50% found that CXCL12 was not associated with outcome; a figure lower than the percentage in the included literature. This suggests that nonsignificant findings with respect to association between CXCL12 expression and outcome are frequently published and were well represented in our analysis.

For a prognostic biomarker to be useful, it must display analytic and clinical validity, as well as clinical utility. Of the studies meeting the inclusion criteria, none robustly determined the validity of the quantification method used and several provided insufficient information such that the method could not be replicated. As a result, the proportion of patients in each study defined as displaying high CXCL12 expression varied considerably. Although such variation may represent true biological differences between tumour types or the patient populations being studied, these factors are unlikely to be the only explanation, as there were significant differences in the proportion of patients with high CXCL12 expression in studies of the same cancer types.

Furthermore, a range of antibodies were used with a sensitivity or specificity for CXCL12 that was not thoroughly determined by the research group, while only two studies repeated their analysis in an independent validation cohort. Based on these findings, although we have identified an association between CXCL12 expression and cancer survival, a standardised, agreed method for CXCL12 quantification has not been reached, and, therefore, CXCL12 can at best be considered an exploratory biomarker at the present time (Goodsaid and Frueh, 2007; Chau *et al*, 2008). Studies are now needed that accurately report the comparison of several methods for CXCL12 measurement in a prospective–retrospective manner in order that a consensus is reached as to the most appropriate test methodology to take forward for further investigation.

The potential clinical validity of CXCL12 is tested in the subgroup analysis presented here. These data indicate that CXCL12 has clinical validity as a biomarker for breast, pancreatic, lung and oesophagogastric cancer. Studies of colon cancer, whether primary or metastatic, demonstrated heterogenous results over a large number of patients, indicating that measuring CXCL12 alone in colon cancer patients is less likely to be useful for prognostication. Despite this, two colorectal cancer studies assessed the effect of the CXCL12:CXCR4 ratio on survival, with both finding that this approach provided prognostic information. Indeed, the publications by Stanisavljević *et al* (2016) and D'Alterio *et al* (2014) found that patients with a combination of low CXCL12 and high CXCR4 expression in the primary tumour experienced reduced recurrence-free or overall survival, respectively. Unfortunately, we were unable to identify other studies that combined the measure of CXCL12 and CXCR4 expression in this way, but the data from these studies indicate that this approach may provide more useful information than measuring either factor alone.

The data identified in our meta-analysis provide only limited information about the precise clinical utility of CXCL12 in specific groups of cancer patients. This is in part because of our broad inclusion criteria that identified a heterogeneous set of studies, and also because of a failure of many included studies to define adequately their cancer population. We found that even simple demographic data such as age, sex and tumour stage was not always reported. Future studies in this area should therefore clearly report the analysis of CXCL12 expression in a subset of cancer patients that are defined on the basis of clinical, histopathological and preferably genomic data such that the clinical utility of CXCL12 in clearly defined cancer patients can be better determined.

In summary, the strengths of this meta-analysis are a wide search strategy identifying multiple studies from differing populations and a pragmatic subgroup analysis highlighting potential differences in the relationship between CXCL12 expression and prognosis between cancer types. Through critical and systematic appraisal, this review has led to guidance points that, if followed, will ensure the generation of higher quality data in the investigation of CXCL12 as a prognostic biomarker. These strengths need to be balanced against the fact that our conclusions are drawn from predominantly retrospectively analyses of survival data, which are by definition prone to bias. The majority of included studies also failed to blind the outcome assessor to participants CXCL12 status, leading to a risk of reporter bias in such studies. Overall, the quality of research in this field needs to improve if progress is to be made in better defining the role of CXCL12 as a prognostic biomarker.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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