A meta-analysis of CD274 (PD-L1) assessment and prognosis in colorectal cancer and its role in predicting response to anti-PD-1 therapy

Authors: Peter G Alexander[1] , Donald C McMillan, PhD[1], James H Park, PhD[1]
[1] School of Medicine, University of Glasgow, Glasgow, United Kingdom

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Abstract

Background: PD-1 checkpoint inhibitors are novel therapeutic agents in colorectal cancer (CRC). Immunohistochemical staining for CD274 assessment is standardised in upper GI cancer, but not in CRC.
Methods: Methodologies of relevant studies were scrutinized and meta-analysis of survival and CD274/PDCD1 performed. Furthermore, anti-PD-1 therapy clinical trial results in CRC were assessed with particular emphasis on CD274 assessment.
Results: 24 studies were included. CD274 on immune cells was associated with good prognosis. CD274 on tumour cells has heterogenous outcomes and does not meet requirements of a prognostic marker. As a marker of response to anti-PD-1 therapy, CD274 assessment is not standardised in CRC.
Conclusion: CD274 does not appear useful as a prognostic marker. As a marker of response to anti-PD-1 therapy, assessment methodology requires standardisation. As the Combined Positive Score (CPS) is used in upper GI cancer, this seems a logical method to adopt. Thresholds for CRC remain to be determined.

Keywords: Colorectal cancer, Programmed cell death-1, Programmed death ligand-1, Response to adjuvant therapy, DNA Mismatch Repair, Prognosis

Corresponding author: Mr Peter Alexander, University of Glasgow, College of MVLS, New Lister Building, Glasgow Royal Infirmary, 8-16 Alexandra Parade, Glasgow, United Kingdom, G31 2ER. Email: p.alexander.1@research.gla.ac.uk

1. Background

Colorectal cancer (CRC) remains a significant global health issue and was the second leading cause of cancer-related death in 2018 worldwide[1]. Currently, the mainstay of treatment is surgical resection with the addition of chemotherapy in more advanced or inoperable cases, but there is growing interest in the use of immunotherapies to complement both curative and palliative treatment[2].

Programmed cell death protein-1 (PDCD1 or PD-1) is a cell surface protein initially discovered by Honjo and colleagues in the 1990s[3]. Expression of the PDCD1 ligand, programmed death ligand-1 (CD274 or PD-L1), on tumour and antigen presenting cells can cause down-regulation of the adaptive anti-tumour immune response, but monoclonal antibody-mediated inhibition of this interaction facilitates a re-invigorated immune response[4]. More recently, CD274 expression on antigen-presenting immune cells has been shown, by multiplex-fluorescent immunohistochemistry to reduce cytotoxic T-cell and tumour cell interaction[5]. Although it may be assumed that high CD274 expression is a marker of poorer prognosis in patients with CRC, published literature to date has been limited not only by wide variability in reported immunohistochemical techniques and scoring methodologies, but also in the incongruity of which cell populations within the microenvironment were assessed (i.e. tumour or immune).

The tumour percentage score (TPS), a measure of the proportion of strong-staining CD274 tumour cells to total tumours cells, has been proposed as a measure of CD274 activity in patients with lung cancer. However, in gastro-oesophageal cancer, this was not found to accurately identify those who will respond to immune checkpoint inhibitors[6]. Therefore, the combined positive score (CPS) was developed, which is

calculated by dividing the total cells above the threshold for CD274 positivity (both tumour and immune) by the total number of viable tumour cells. This was found to be an effective measure of response to anti-PD-1 therapy, particularly when using a higher threshold (>10)[7].

However, there is currently no standardised method of measuring PDCD1 or CD274 in CRC. Furthermore, microsatellite instability (MSI) may play a pivotal role in CRC response to immune checkpoint inhibition, with several trials reporting therapeutic benefit to immune checkpoint inhibitors in only those with MSI tumours[8]. This therefore represents a significant confounder in any published literature that should be taken account of in multivariate analysis.

Despite this, a number of ongoing clinical trials are investigating the potential of anti-PD-1 therapy in patients with MSS CRC. It is known that high immune MSS cancers also have an improved survival[9]. Those who relapse in this group are likely to be developing similar immune escape pathways to MSI tumours. Therefore, a CD274 score that can correctly identify patients who will respond to anti-PD-1 therapy is required moving forward in CRC.

The aims of this study are two-fold. Firstly, to perform a meta-analysis of the prognostic significance of PDCD1/CD274 in patients with CRC and secondly, to review the current anti-PD-1 therapy trial results, with particular reference to those assessing response in the light of CD274 status.

2. Methods

2.1. Search strategy

A literature search was conducted aiming to identify all primary studies in CRC assessing survival in relation to PDCD1 or CD274 by immunohistochemistry (IHC). In addition, trials assessing response to anti-PD-1 therapy were identified, with a particular interest in those using CD274 assessment in order to assess response. A search was made of PubMed, Ovid MEDLINE and EMBASE databases (last search date: 4th March 2020) utilising these search criteria:

1. "Colon cancer" OR "Rectal Cancer" OR "Colorectal cancer" (in Abstract) AND
2. "Survival" OR Prognos$ (in Abstract) AND
3. PD1 OR PD-1 OR PDCD1 OR CD279 OR PDL1 OR PD-L1 OR B7-H1 OR CD274 OR "programmed cell death" (in Abstract) AND
4. Immunohistochemistry (in any field)

Search limitations were set to English language, human studies, published from 1997 to present. An inspection was made of all titles and abstracts by P.G.A. Relevant studies were identified and full texts obtained. Reference lists were also scrutinised to identify other relevant articles. Studies utilizing only multiplex-fluorescent IHC were excluded.

In addition to the above search, current clinical trials including anti-PD-1 therapy (Nivolumab/Pembrolizumab/Spartalizumab/Durvalumab/Atezolizumab/Amp-224/Avelumab/BAT1306/Tislelizumab/Cetrelimab/Camrelizumab/Toripalimab/Cosibelimab/M7824/Sintilimab/Genolizumab/MGA012/BI754091/Zimberelimab/Dostarlimab/XmAb20717[dual PD-1 and CTLA4 inhibitor]) in colorectal cancer (registered

at Clinicaltrials.gov or clinicaltrialsregister.eu) were reviewed, along with any published results or relevant conference abstracts displaying interim results.

2.2. Methodologic and validity assessment

Study inclusion criteria were derived from published REMARK guidelines[10]. Eligible studies were included if meeting criteria in Table 1.

2.3. Data extraction

Eligible manuscripts were reviewed by P.G.A. Any articles that were felt to be contentious were discussed with the other authors (DCM and JHP). Agreement was reached regarding articles to be included or excluded. Extracted datapoints include: publication year; sample size; stage of disease; time period of sample; specimen used for assessment (TMA including core size, whole section or biopsy); colonic site; antibody used for IHC; method of assessment; MSI status; handling of MSI in statistical analysis; specific survival outcome; adjuvant therapy regimen and response to treatment. Only studies performing multivariate analysis and presenting hazard ratios with 95% confidence intervals were included in meta-analysis. A bias assessment was performed based on REMARK guidelines and the table is presented in supplementary data.

2.4. Statistical analysis

Furthermore, type of survival analysis was noted. Studies with small sample size (less than 80) were excluded from meta-analysis. Where there was only one study of a particular category, the HR and 95% CI for that study are reported. Where there were multiple studies in the same category, a fixed effects summary HR with 95% CI is given. Hazard Ratios of greater than 1.0 indicated worse survival for higher value of a given variable and vice versa. Confidence intervals were considered non-significant if they crossed 1.0. I^2 value is presented to indicate inter-study

heterogeneity. Funnel plot was used to estimate the presence of publication bias. REVMAN systematic review and meta-analysis software, version 5.3, was used to perform meta-analysis.

3. Results

Initial search yielded 230 results (Figure 1), which was reduced to 176 after limiting to English language (*n*=4), human studies (*n*=18) and following deduplication (*n*=32). Abstracts were reviewed and a further 120 were excluded as they did not meet inclusion criteria (Table 1). Full texts were obtained for the remaining 56 relevant texts and, after careful scrutiny, 20 more were excluded for lack of survival analysis (*n*=8), non-standard IHC (*n*=5), replicated results/cohort (*n*=6) or insufficient detail (*n*=1), leaving 36 studies. Finally, for meta-analysis, a further 12 studies had to be excluded for lack of hazard ratios (*n*=6), no multivariate analysis (*n*=2) and cohort size smaller than 80 subjects (*n*=4). The final 24 studies were included in meta-analysis, with methodologies summarised in supplementary table S1. Bias assessment for the included studies is shown in supplementary table S2, all of which were considered low risk for bias, the only study considered moderate risk had been excluded for insufficient detail.

Of the studies included in the meta-analysis, 11 performed assessment on TMAs, 10 on whole sections, 1 on pre-treatment biopsies and 2 on a combination of pre-treatment biopsy and post-resection TMA. TMA core sizes varied between 0.6mm and 3mm, with only 1 study not stating size of core. Eighteen studies documented blinding of assessors, whereas 8 studies did not comment on blinding. Sample size varied between 89 and 1105, with four studies having a sample size >500. Median sample size was 190 with an interquartile range of 117 to 338. Fifty percent of studies assessed MSI, although 2 of these studies did not include MSI in the survival analysis. There were over 25 variables included in the different multivariate analyses, of which the most common were age (*n*=19), sex (*n*=16), tumour grade (*n*=16), T-stage (*n*=14),

N-stage (*n*=13), other inflammatory assessment (*n*=13), venous/lymphatic/peri-neural invasion (*n*=11), tumour site (*n*=10), TNM (*n*=9), M-stage (*n*=7) and MSI (*n*=5). There was also a wide variation in assessment methods. Two studies only assessed CD274 on immune cells, whereas 9 studied CD274 only on tumour tissue. Four studies performed a combined assessment of CD274 on tumour tissue and immune cells, whereas 5 assessed CD274 on tumour tissue and immune cells separately. Others included assessment of PDCD1, with 1 study only assessing PDCD1 on immune cells, another assessing CD274 on tumour tissue and PDCD1 on immune cells, another CD274 on tumour tissue, but both CD274 and PDCD1 on immune cells and another combined tumour and immune cell CD274 assessment and separate PDCD1 assessment on immune cells.

Studies also differed on whether they assessed membranous staining (*n*=9), cytoplasmic staining (*n*=2), combined membrane and cytoplasmic staining (*n*=1) or any staining (*n*=9). Finally the cutoff used by each study differed, with tumour tissue cutoffs of 1% (*n*=3), 5% (*n*=7), 50% (*n*=2), semiquantitative assessment (*n*=4), immunoreactivity score or weighted histoscore (*n*=3), or arbitrary cutoff, such as median (*n*=2). Immune cell cutoffs were 1% (*n*=2), 5% (*n*=5), 10% (*n*=1), 20% (*n*=1), >50% (*n*=1), semiquantitative (*n*=2), immunoreactivity score (*n*=1) or arbitrary (*n*=2).

3.1. PDCD1 (PD-1) assessment in immune cells

Immune cell expression of PDCD1 was assessed in 11 studies[11-21], comprising a total of 2498 patients. In 6 of these studies, comprising 1466 patients, PDCD1 immune cell expression was found to have a statistically significant beneficial survival impact[11-16]. One study of 116 patients found immune PDCD1 expression

to have a significant detrimental survival impact[17], whereas four studies (595 patients) found no impact on survival[18-21].

Six studies assessed for the presence of MSI, of which: two found that MSI was not significant for survival[16, 21]; one study only included those with MSI[19] and they and one other study[16] found PDCD1 not to be significant for survival in MSI patients; another study found that PDCD1 was only significant for survival (beneficial) in MSI patients[15]; two studies found that PDCD1 was only significant for survival (beneficial) in MSS patients[12, 16]; and one study found that survival according to PDCD1 expression was dependent on MSI status in multivariate analysis[11].

Five studies, with 6 cohorts of patients met inclusion criteria for meta-analysis (Table 2, Figure 2): three assessed DFS (HR 0.50; 95% CI: 0.34-0.73), with no significant heterogeneity; four cohorts were studied assessing OS (HR 0.74; 95% CI: 0.60-0.89), with significant heterogeneity; and all-cause survival (HR 0.72; 95% CI: 0.59-0.87) was significantly heterogeneous. Funnel plots did not suggest any significant publication bias, although numbers of studies were small (Figure 2).

3.2. CD274 (PD-L1) assessment in immune cells

Immune cell expression of CD274 was assessed in 19 studies[11, 14, 16, 18, 19, 22-35], comprising a total of 3729 patients. One study[27] must be presumed to have an overlap of 18 MSI patients with another in the same centre[26]. The dates only overlapped for the MSI cohort in this study[27]. In 11 studies, comprising 2718 patients, CD274 immune cell expression was found to have a beneficial survival impact[11, 14, 16, 19, 22-28]. Two small studies, comprising 93 patients, found immune cell CD274 expression to have a detrimental survival impact[18, 29], both of

which assessed only stage IV disease, whereas of the other two studies assessing stage IV disease, one found a beneficial survival impact[14] and one found no survival impact[31]. Six studies of 918 patients found no survival impact[30-35].

Eight studies assessed for the presence of MSI, of which: one found that MSI was not significant for survival[24]; one did not include MSI in survival analysis[27]; two reported immune CD274 to be independent of MSI[11, 28]; four presented results for MSI cohorts of which two were significant for survival (beneficial)[19, 26], whereas two were not significant[16, 30]; two presented MSS cohorts, both of which were significant for survival (beneficial)[16, 26].

Eight studies, with 9 cohorts met inclusion criteria for meta-analysis (Table 2, Figure 3): four assessed DFS (HR 0.43; 95% CI: 0.31-0.60), with moderate heterogeneity; five cohorts were studied assessing OS (HR 0.50; 95% CI: 0.43-0.59), with mild heterogeneity; and all-cause survival (HR 0.49; 95% CI: 0.42-0.57) was moderately heterogeneous. Funnel plots suggested possible publication bias against smaller, non-significant studies (Figure 3).

3.3. CD274 (PD-L1) assessment in tumour tissue

Tumour tissue expression of CD274 was assessed in twenty-eight studies[11, 12, 14-19, 22-28, 30-33, 36-44], comprising 7054 patients. One study[27] must be presumed to have an overlap of 18 MSI patients with another in the same centre[26]. The dates only overlapped for the MSI cohort in this study[27]. In 4 studies, comprising 1636 patients, expression of CD274 on tumour tissue was found to have a significant (beneficial) impact[12, 16, 36, 37]. Whereas nine studies, comprising 1461 patients, found CD274 in tumour tissue to be associated with significant detrimental survival impact[15, 17, 22, 25, 27, 38-41]. Fifteen studies (3636 patients) assessing tumour

tissue CD274 expression did not find any significant survival impact[11, 14, 18, 19, 23, 24, 26, 28, 30-33, 42-44].

Fourteen studies assessed for the presence of MSI, of which tumour tissue CD274 was not significant for survival in nine[11, 19, 24, 26, 28, 30, 42-44], three of these assessing MSI only cohorts[19, 26, 30], although MSI was associated with higher expression of CD274[44]. Of the other 5 studies, only 2 patients had MSI in one[36], two found that CD274 was associated with survival (beneficial) in the MSS subgroup[12, 16], one found CD274 had a significant association with survival (detrimental) in the MSI subgroup[15] and one did not include MSI in survival analysis[27].

Seventeen studies, with eighteen cohorts met inclusion criteria for meta-analysis (Table 2, Figure 4): seven assessed DFS (HR 1.10; 95% CI: 0.87-1.38), with significant heterogeneity; three studies assessed CSS (HR 1.85; 95% CI: 1.19-2.88), with no significant heterogeneity; 15 cohorts were studied assessing OS (HR 0.87; 95% CI: 0.83-0.91), with significant heterogeneity; and all-cause survival (HR 0.88; 95% CI: 0.84-0.92) was significantly heterogeneous. Funnel plot analysis did not suggest any publication bias (Figure 4).

3.4. CD274 (PD-L1) combined assessment in tumour tissue and immune cells

Four studies performed combined assessment of CD274 in tumour tissue and immune cells, comprising 835 patients[21, 23, 45, 46]. Two studies (542 patients) found a significant beneficial survival impact[21, 23], whereas one (175 patients) found a significant detrimental survival impact[45]. One (118 patients) found no impact on survival[46].

Two studies assessed for the presence of MSI, neither of which found MSI to be significant for survival[21, 46].

All four studies met inclusion criteria for meta-analysis (Table 2, Figure 5) of which: two assessed DFS (HR 0.98; 95% CI: 0.71-1.34), with significant heterogeneity; one assessed CSS finding no significant survival association; two assessed OS (HR 1.06; 95% CI: 0.86-1.30), with significant heterogeneity; and all-cause survival (HR 1.00; 95% CI: 0.82-1.21) was significantly heterogeneous. Funnel plot analysis did not suggest any publication bias, although numbers were small (Figure 5).

3.5. CD274 (PD-L1) and response to anti-PD-1 therapy in CRC

There have been results published for 11 trials[8, 47-56] of anti-PD-1 therapy in CRC, as well as published abstracts with interim results for a further 20 trials[57-75] (Table 3). Of these, only 9 trials reported assessment of CD274 expression[8, 48, 50, 52-56, 70]. However, in 5 of these the number of individuals assessed for CD274 were either small[54, 55, 70], or the authors did not account for CD274 in survival analysis[50, 56].

Le et al[8] found CD274 to be expressed only on MSI tumours, which responded well to pembrolizumab. O'Neil et al[52] presented results for 23 patients, who all met inclusion criteria of tumour CD274 expression ≥1%, in a trial of Pembrolizumab in CRC. However, the only patient that responded to immunotherapy was an individual who also had MSI[52]. Overman et al[53], in a cohort of 74 MSI CRC, took account both tumour CD274 status and immune cell CD274 status in response to Nivolumab monotherapy. They did not find any difference in response according to tumour CD274 expression but found that higher immune cell CD274 expression with a semiquantitative cutoff was associated with better response[53]. Eng et al[48], in the

only phase III trial of anti-PD-1 therapy in CRC published to date, randomised patients to receive Atezolizumab monotherapy, Atezolizumab + Cobimetinib or Regorafenib monotherapy. There were 363 patients overall of which 347 were confirmed MSS and 6 were confirmed MSI (3 in Atezolizumab arm and 3 in Atezolizumab + Cobimetinib arm). The Objective Response Rate (ORR) was 2% for the both monotherapy arms and 3% for the combined arm. For MSI CRC in this trial, the response rate was 50%, with 1 of 3 responding in the Atezolizumab arm and 2 of 3 responding in the combined arm. Despite the low response rate in this trial, however, CD274 expression did appear to dictate response to therapy somewhat. In the Regorafenib monotherapy arm, low CD274 appeared to favour Regorafenib over either of the immunotherapy arms. Conversely, there was a non-significant trend towards high CD274 favouring both immunotherapy arms over Regorafenib[48].

4. Discussion

It is clear that immune cell expression of PDCD1 or CD274 is, on the whole, associated with a beneficial impact on survival. However, when compared with various other immune cell assessments that have been validated for their prognostic role[9], it is not clear whether immune cell CD274 holds any additional value as a prognostic marker. When considering the expression of CD274 on tumour tissue the data are heterogenous with just as many studies/participants demonstrating a detrimental impact on survival as a beneficial impact and many studies finding no survival impact. Therefore, as a prognostic marker, tumour CD274 assessment appears to be of little value.

In terms of MSI, there is evidence of higher expression of CD274 in both tumour tissue and immune cells compared with MSS tumours[8, 15, 42]. In those studies assessing purely MSI patients, tumour CD274 expression was found to have either a detrimental survival impact[15] or no survival impact[19, 26, 30] and immune cell CD274 had either a beneficial survival impact[19, 26] or no impact[30].

However, while CD274 may not be particularly useful as a prognostic marker, it may have a role in determining the efficacy of PD-1 checkpoint inhibitor therapy.

The first published trial assessing Nivolumab in solid tumours included 18 CRCs, but results were disappointing with an ORR of 0% in the CRC subgroup[47]. Neither MSI status, nor CD274 expression were considered in this trial. However, there was a turning point for immunotherapy in CRC when Pembrolizumab was given to a variety of MSI cancers including 10 MSI CRC, as well as 18 MSS CRC, with a 40% Immune-Related ORR (IRORR) in the MSI arm, compared with 0% in the MSS arm[8]. This led to the American Food and Drug Administration (FDA) licensing Pembrolizumab for MSI CRC. Trials studying MSI CRC consistently report ORRs

>30% for anti-PD-1 monotherapy[51, 53]. These results in MSI are even more impressive when anti-PD-1 therapy is combined with other checkpoint inhibitors, with ORRs >50%[57].

Le et al[8] also reported in the Pembrolizumab trial that CD274 expression was only present in the MSI patients studied, but postulated that MSS tumours expressing high levels of CD274 may also respond to anti-PD-1 therapy[76]. Following on from this O'Neil et al[52] presented results for a trial of largely MSS CRC in individuals with tumour CD274 expression of >1%. However, the only patient in this trial that responded to immunotherapy was an individual who also had MSI. Further trials of immunotherapy in MSS CRC have yielded poor results, alone or combined with other treatments.

The challenge, therefore, is to find a disease biomarker for MSS CRC that will identify those patients who will respond to anti-PD-1 therapy. The data on CD274 as a marker of disease response in CRC is sparse and the heterogeneity in CD274 assessment methodology among the published trials precludes any meaningful analysis.

In upper GI cancers, CD274 assessment as a biomarker of anti-PD-1 therapy response has been standardised, with a combined percentage score (CPS) cutoff of >1 determining response to Pembrolizumab[77]. However, a CPS of >10 has recently been described as a better biomarker for response to immunotherapy[7]. The CPS method also uses a standardised antibody (22C3 pharmDx IHC assay)[77]. One study in CRC compared the reproducibility of CD274 scoring using three different antibodies and multiple cut-points for survival[27]. There was wide variability in the quality of staining and therefore reproducibility of scoring depending on which

antibody was used: those by Cell Signalling and Dako being the most specific of the three antibodies studied.

The other crucial question under investigation in multiple trials is whether the efficacy of immunotherapy in MSS CRC can be improved in combination with other treatments, such as radiotherapy or standard chemotherapy. Encouragingly, the interim results of a trial of standard chemotherapy vs standard chemotherapy with the addition of anti-PD-1 and CTLA4 inhibitors found response rates to be significantly better in the checkpoint inhibitor arm[62]. Furthermore, a single arm trial of Pembrolizumab + mFOLFOX with 3 MSI and 22 MSS CRC found an ORR of 64% (1 complete responder with MSI)[73]. Finally, a phase II trial of neo-adjuvant Nivolumab + Ipilimumab in early colon cancer found pathological complete response in 60% patients with MSI and 13% patients with MSS[56]. There are a further 9 phase III trials assessing immunotherapy and CRC with no published results yet, in addition to many other phase I and II trials. A catalogue of all 223 registered trials comparing various different combinations of therapies with PD-1 inhibitors was made (supplementary table S3).

Of course, it must be recognised that while the expression of PDCD1 and CD274 have been discussed largely in isolation in this review, there are many other factors that influence the expression of cell surface proteins at a genetic and epigenetic level. These factors include environmental stimuli, such as obesity, exercise, systemic inflammation, diet, smoking and the microbiome. The integration of cancer immunology and epidemiology research has been termed molecular pathology epidemiology (MPE) and has also been utilised alongside precision medicine to investigate the influence of environmental factors on treatment outcomes[78, 79].

Future studies should take account of environmental factors and their influence on cancer immunology and treatment response.

5. Conclusion

While CD274 expression on immune cells is largely associated with better survival, there are many other immune cell assessments that have been validated for their prognostic role and therefore immune cell CD274 adds little value as a prognostic marker. CD274 assessment in tumour tissue would appear to be of little use as a prognostic marker, with significant inter-study heterogeneity and many studies finding no prognostic significance. As a marker for response to anti-PD-1 therapy in CRC, the data is sparse. CD274 expression analysis needs to be standardised moving forward. One strategy would be to adopt the CPS method already in use as a marker of response to immunotherapy in upper GI cancer. Once CD274 assessment is standardised, it may be possible to assess thresholds in clinical trials to determine if CD274 can select those MSS CRC patients who will respond to anti-PD-1 therapy. As in upper GI cancers, a CPS cutoff of >10 is more likely to be selective for immunotherapy responders.

6. Declarations

6.1. Conflicts of interest statement

None of the authors have any conflict of interest to declare.

6.2. Funding

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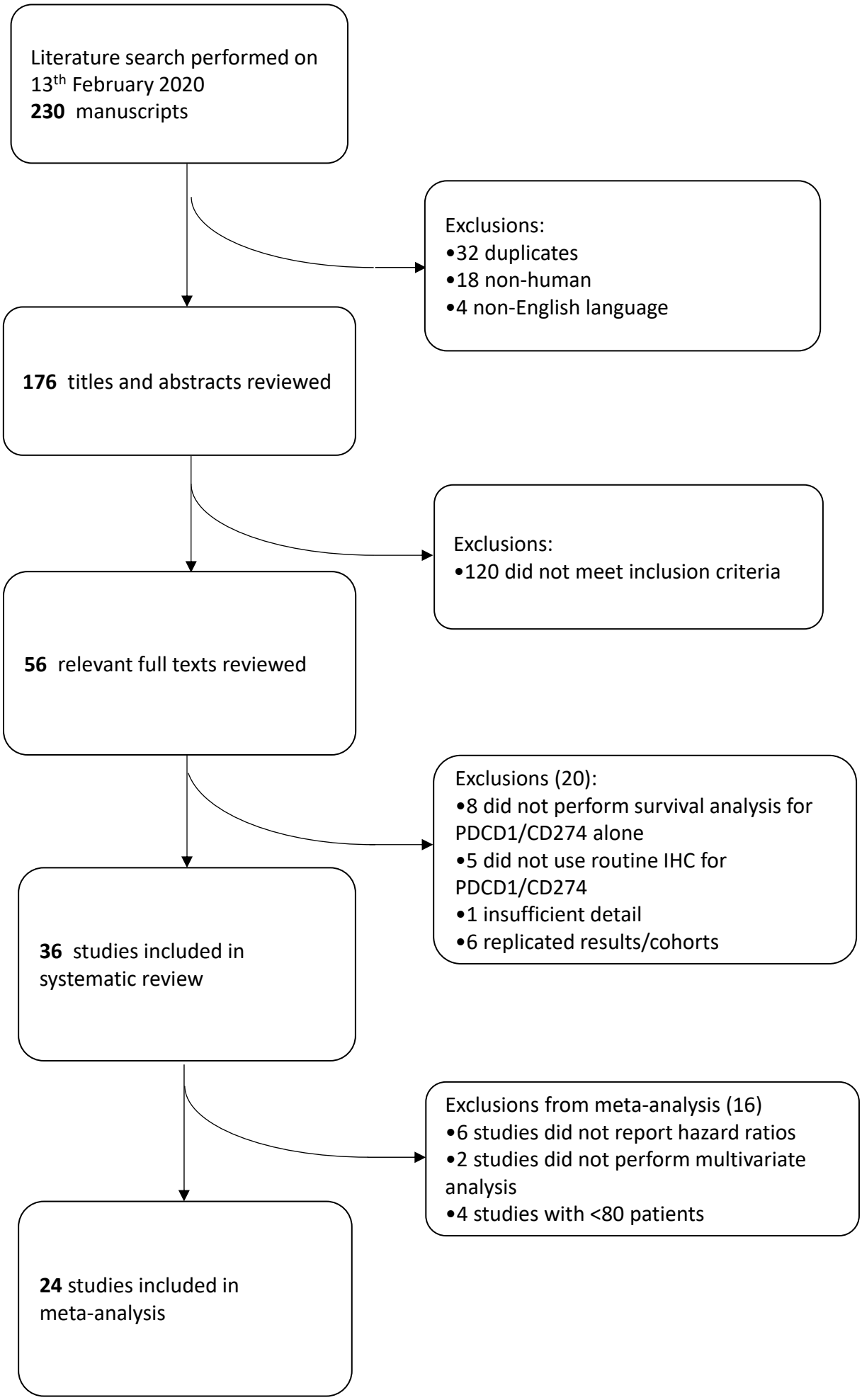

Figure 1. Flow diagram of literature search and included/excluded studies

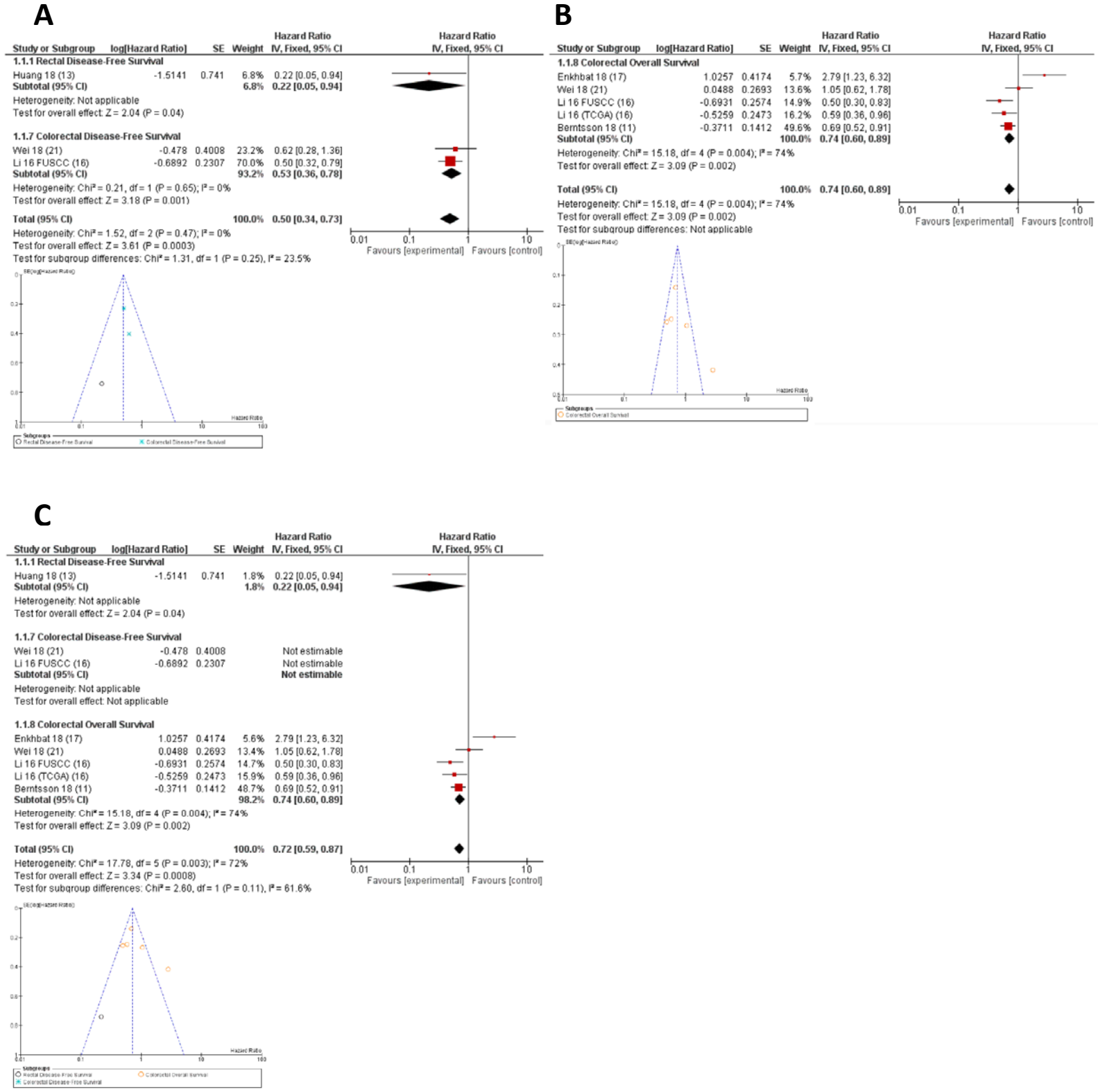

Figure 2. Forest plots and funnel plots for PDCD1 expression on Immune cells for A) DFS, B) OS and C) All survival

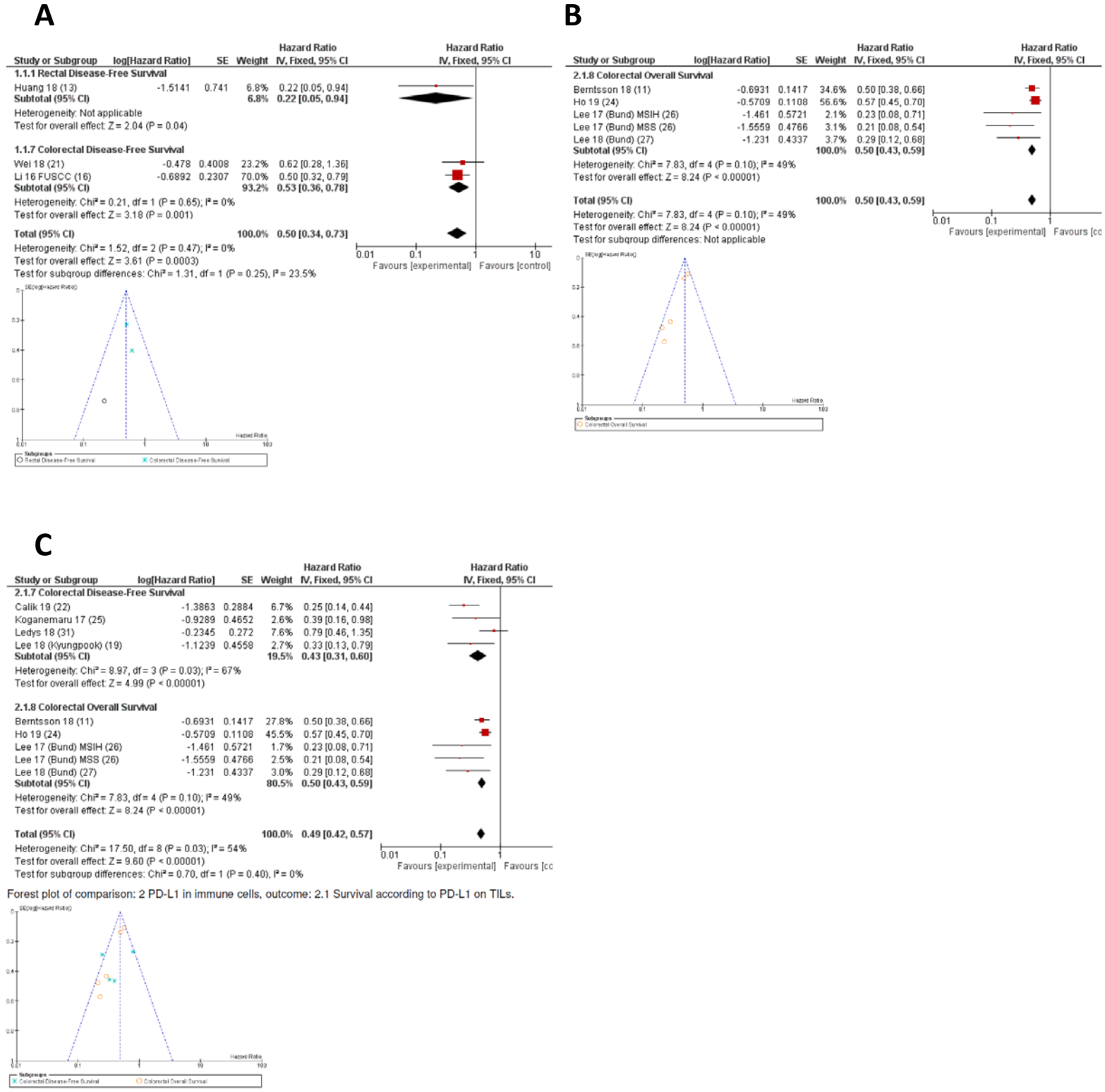

Figure 3. Forest plots and funnel plots for CD274 (PD-L1) expression on Immune cells for A) DFS, B) OS and C) All survival

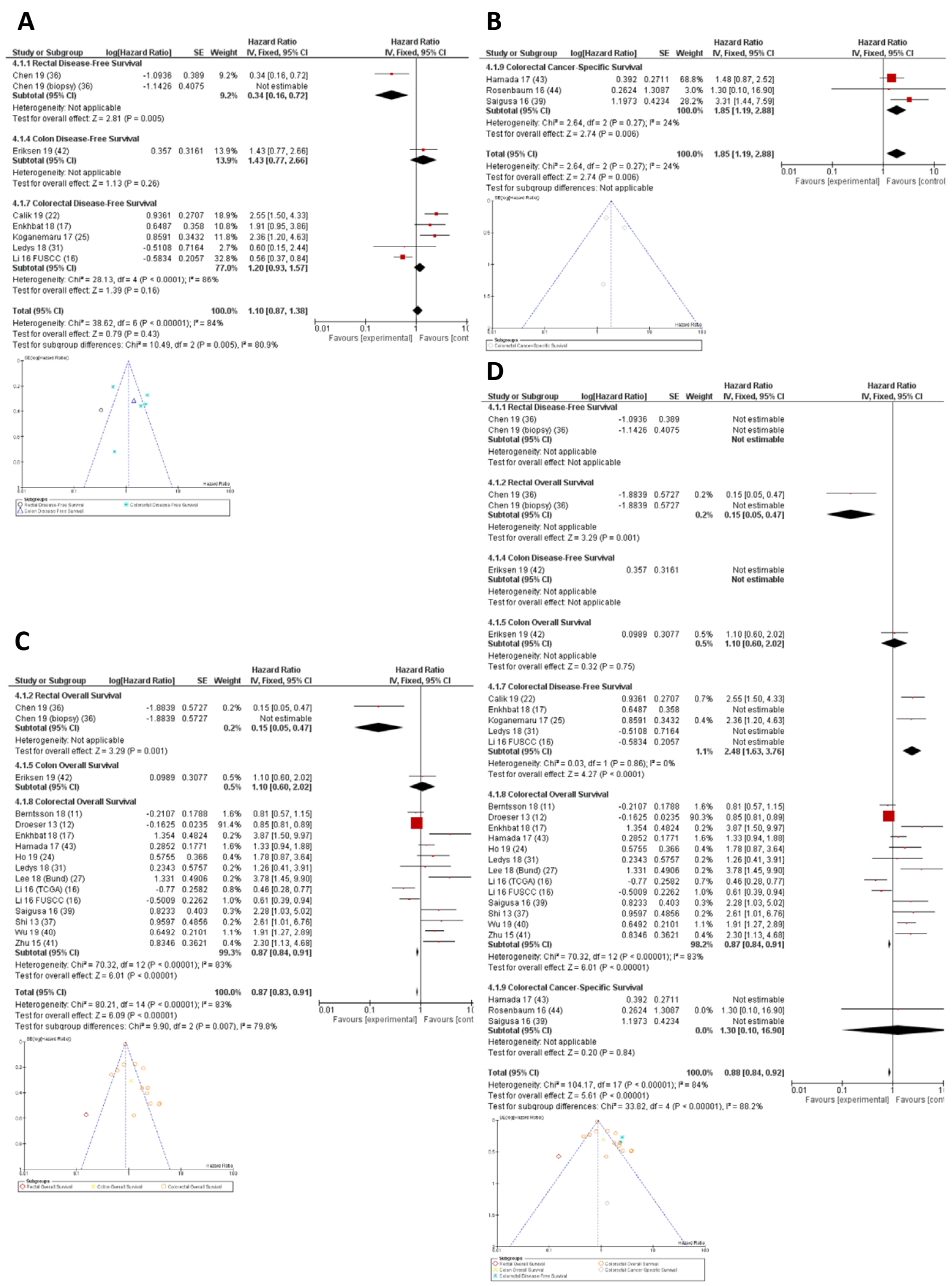

Figure 4. Forest plots and funnel plots for CD274 (PD-L1) expression on Tumour tissue for A) DFS, B) CSS and C) OS and D) All survival

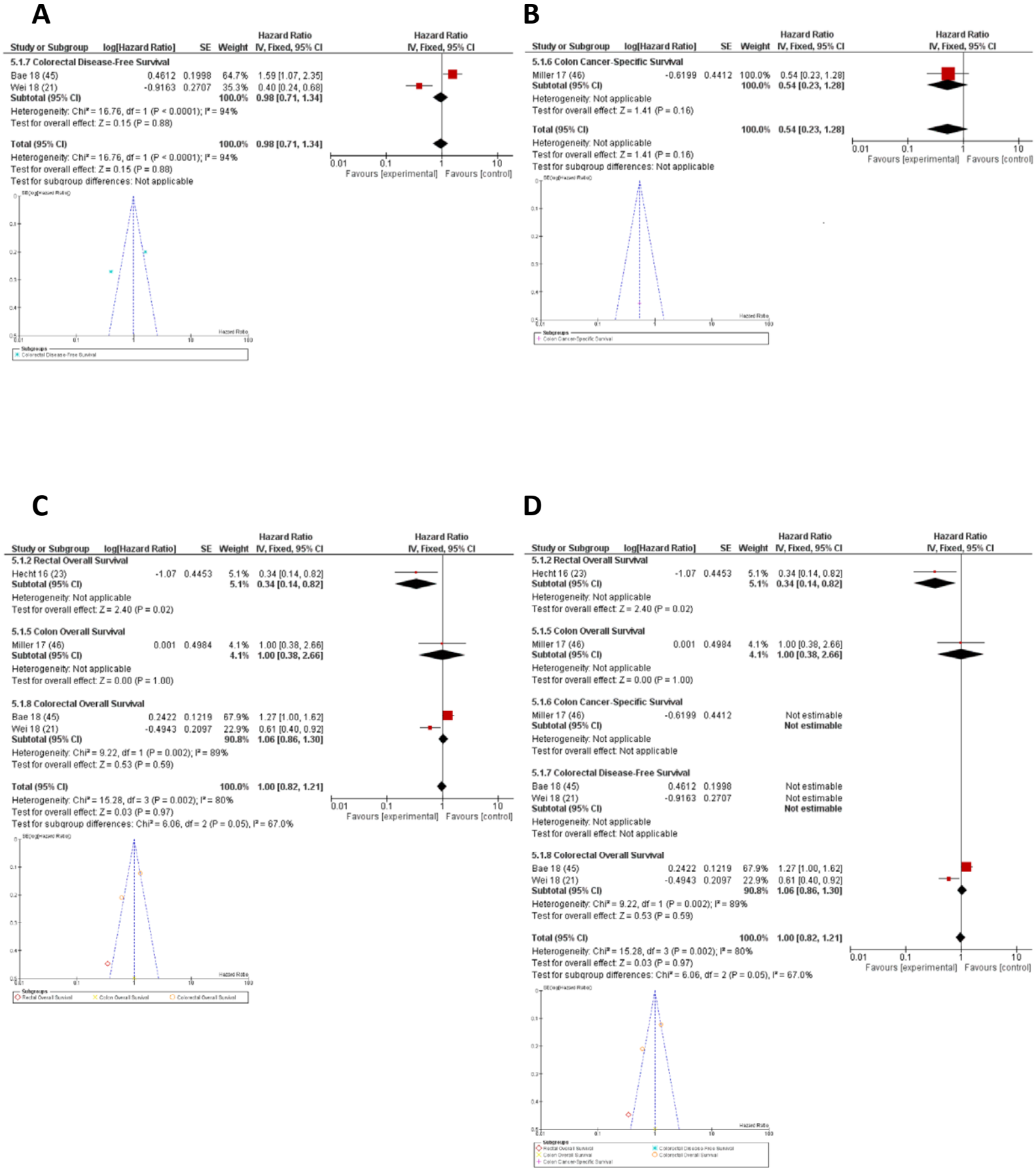

Figure 5. Forest plots and funnel plots for CD274 (PD-L1) expression on combined assessment of Tumour tissue and immune cells for A) DFS, B) CSS and C) OS and D) All survival

Table 1. Inclusion criteria of prognostic studies of PDCD1/CD274 expression in Colorectal cancer

1. Either prospective or retrospective design with a well-defined study population
2. Study of rectal, colon or colorectal cancer primary resections
3. Assessment on FFPE slides using IHC staining for PDCD1 or CD274 on either immune cells or tumour tissue
4. Clear description of the specimen used for assessment, antibodies used and assessment method.
5. Groupings of patients and data cutoffs.
6. Description of statistical analysis methods used.
7. For meta-analysis, only those papers reporting a proportional hazard model used, including details of adjustment variables.

Table 2. Meta-analysis results for survival in colorectal cancer according to PDCD1/CD274 expression on immune and tumour cells

		Overall effect			Heterogeneity		
Colonic site	**Survival type**	**No. of studies**	**HR**	**95% CI**	**I^2 test (%)**	***P*-value**	**First Author Surname/year**
PDCD1 high immune cells							
R	DFS	1	0.22	0.05-0.94	NA		Huang
CR	DFS	2	0.53	0.36-0.78	0	0.65	Wei, Li 16 (FUSCC)
Any	DFS	3	0.50	0.34-0.73	0	0.47	Huang, Wei, Li 16 (FUSCC)
CR	OS	5	0.74	0.60-0.89	74	0.004	Enkhbat, Wei, Li 16 (FUSCC), Li 16 (TCGA), Berntsson
Any	Any	6	0.72	0.59-0.87	72	0.003	Huang, Enkhbat, Wei, Li 16 (FUSCC), Li 16 (TCGA), Berntsson
CD274 high immune cells							
CR	DFS	4	0.43	0.31-0.60	67	0.03	Calik, Koganemaru, *Ledys*, **Lee 18 (Kyungpook)**
CR	OS	5	0.50	0.43-0.59	49	0.10	Berntsson, Ho, **Lee 17 (Bundang; MSIH)**, Lee 17 (Bundang; MSS), Lee 18 (Bundang)
CR	Any	9	0.49	0.42-0.57	54	0.03	Calik, Koganemaru, *Ledys*, **Lee 18 (Kyungpook)**, Berntsson, Ho, **Lee 17 (Bundang; MSIH)**, Lee 17 (Bundang; MSS), Lee 18 (Bundang)
CD274 high tumour cells							
R	DFS	1	0.34	0.16-0.72	NA		Chen
C	DFS	1	1.43	0.77-2.66	NA		Eriksen
CR	DFS	5	1.20	0.93-1.57	84	<0.001	Calik, Enkhbat, Koganemaru, *Ledys*, Li 16 (FUSCC)
Any	DFS	7	1.10	0.87-1.38	84	<0.001	Chen, Eriksen, Calik, Enkhbat, Koganemaru, *Ledys*, Li 16 (FUSCC)
CR	CSS	3	1.85	1.19-2.88	24	0.27	Hamada, Rosenbaum, Saigusa
R	OS	1	0.15	0.05-0.47	NA		Chen
C	OS	1	1.10	0.60-2.02	NA		Eriksen
CR	OS	13	0.87	0.84-0.91	83	<0.001	Berntsson, Droeser, Enkhbat, Hamada, Ho, *Ledys*, Lee 18 (Bundang), Li 16 (FUSCC), Li 16 (TCGA), Saigusa, Shi, Wu, Zhu
Any	OS	15	0.87	0.83-0.91	83	<0.001	Chen, Eriksen, Berntsson, Droeser, Enkhbat, Hamada, Ho, *Ledys*, Lee 18 (Bundang), Li 16 (FUSCC), Li 16 (TCGA), Saigusa, Shi, Wu, Zhu
Any	Any	18	0.88	0.84-0.92	84	<0.001	Chen, Eriksen, Calik, Koganemaru, Berntsson, Droeser, Enkhbat, Hamada, Ho, *Ledys*, Lee 18 (Bundang), Li 16 (FUSCC), Li 16 (TCGA), Saigusa, Shi, Wu, Zhu, Rosenbaum
CD274 high combined tumour and immune cells							
CR	DFS	2	0.98	0.71-1.34	94	<0.001	Bae, Wei
C	CSS	1	0.54	0.23-1.28	NA		Miller
R	OS	1	0.34	0.14-0.82	NA		Hecht
C	OS	1	1.00	0.38-2.66	NA		Miller
CR	OS	2	1.06	0.86-1.30	80	0.002	Bae, Wei
Any	OS/Any	4	1.00	0.82-1.21	80	0.002	Hecht, Miller, Bae, Wei

Bold studies: MSIH only; *Italics* studies: stage IV only.

Abbrevitions: R rectal; C colon; CR colorectal

Table 3. Anti-PD-1 therapy in colorectal cancer trials and the role of immunohistochemistry in predicting response

Trial (NCT)	**Phase**	**CRC *N***	**MSI *N***	**MSS *N***	**Treatment**	**CD274 assessed**	**Method**	**Cut-off**	**Response**
Published results									
Brahmer 2012 (NCT00729664) (47)	I	18	Unknown		Nivolumab monotherapy	N			ORR 0%
Chalabi 2020 (NCT03026140) (56)	II	60 planned	21	20	Neoadjuvant in early colon Ca: Nivolumab + Ipilimumab +- Cox-2	**Y**	IHC (Dako, 1:40) unclear assessment method	Uncl	MPR in 19 patients with MSI (60% pCR); MPR in 3 patients with MSS (13% pCR).
Eng 2019 (NCT02788279) (48) IMblaze 370	III	363	6	347	Randomised controlled trial: Atezolizumab mono (3 MSI) vs Atezolizumab + Cobimetinib (3 MSI) vs Regorafenib mono	**Y**	IHC (Ventana, ?dilution) expressed on immune cells in either primary or metastasis	1%	ORR 2%, 3% and 2%, respectively. Survival was significantly better with Regorafenib in the CD274 low patients. High CD274 patients trended towards better survival in Atezolizumab or combined arms, but not significant. (ORR 50% for all MSI patients and atezolizumab +- cobimetinib)
Floudas 2019 (NCT02298946) (49)	I	15	Unkn	4 known	Amp-224 (anti-PD-1), cyclophosphamide + radiotherapy	N			ORR 0%
Hellman 2019 (NCT01988896) (50)	Ib	84	2	62 (rest unkn)	Cobimetinib + atezolizumab	Y	IHC (Ventana, ?dilution) expressed on tumour tissue and immune cells in primary	5%	ORR 8% (50% for MSI patients and 10% in known MSS); no split per CD274 status given.
Le 2015 (NCT01876511) (8)	II	28	10	18	Pembrolizumab monotherapy	Y	IHC (?antibody) membranous tumour cell expression	Uncl	IRORR 40% for MSI and 0% for MSS, CD274 only found to be expressed in MSI.
Le 2020 (NCT02460198) (51) KEYNOTE 164	II	124	124	0	Pembrolizumab monotherapy	N			ORR 33%
O'Neil 2017 (NCT02054806) (52)	Ib	23	1	22	Pembrolizumab monotherapy	Y	IHC (?antibody) membranous tumour expression or interface between immune cells/tumour	1%	ORR 4% (only patient with MSI responded), all had TPS of >1% for inclusion in trial

Overman 2017 (NCT02060188)* (53) Checkmate-142	II	74 (314 planned)	74	0	Nivolumab monotherapy [vs Nivolumab + (Ipilimumab vs Cobimetinib vs anti-LAG3 vs Daratumumab) vs Nivolumab + Ipilimumab + Cobimetinib] **Results only for monotherapy arm**	Y	IHC (Dako, ?dilution), membranous tumour or immune cell staining (semiquantitative: rare, intermediate, numerous)	1%	ORR 31%; when split by CD274 expression; there were similar ORRs for tumour expression, whereas, in high immune cell expression, there was significantly better ORR (39% vs 24% vs 21% for numerous, intermediate and rare, respsectively).
Yamamoto 2017 (NCT00441337) (54)	I	4 (of 39 solid tumours)	Unknown (3 rectal, 1 colon)		Nivolumab monotherapy	Y	IHC (CD274, Medical&Biological laboratories Co, Nagoya, Japan) staining positive if same as positive control, no further detail.	SQ	ORR 25% (MSI unclear); 8 of 11 patients deemed CD274 high, unclear tumour type, all partial responders in this high group.
Yarchoan 2020 (NCT02981524) (55)	II	17	0	17	GVAX, cyclophosphamide and pembrolizumab, single arm	Y	IHC (Spring Bioscience, ?dilution) tumour expression pre-treatment and subsequent biopsies (only 4 tested)	1%	ORR 0%, of 4 patients tested for CD274, all were initially low, although 1 became high on post-treatment biopsy, all four tumours displayed signs of necrosis on repeat biopsy
Poster results									
Andre 2018 (NCT02060188)* (53) Checkmate-142	II	119	119	0	Nivolumab monotherapy [vs Nivolumab + (Ipilimumab vs Cobimetinib vs anti-LAG3 vs Daratumumab) vs Nivolumab + Ipilimumab + Cobimetinib] **Results only for Nivolumab + Ipilumumab arm**				ORR 55%
Azad 2018 (NCT02437136) (58) ENCORE 601	II	16 (of 202 solid tumours)	0	16	Pembrolizumab + Entinostat (HDAC inhibitor)				IRORR 6%
Boland 2018 (NCT02713373) (59)	Ib/II	9	Unclear		Pembrolizumab + Cetuximab				ORR 0%
Callahan 2017 (NCT01975831) (60)	I	11	Unclear		Durvalumab + Tremelimumab				ORR 9%
Cassier 2019 (NCT02777710) (61)	I	14	Unclear (at least 2MSI)		Durvalumab + Pexidartinib (CSF-1R TKI)				ORR 0%
Chen 2019 (NCT02870920) (62)	II	179	0	179	Standard chemo + Tremelimumab + Durvalumab vs Best supportive care (randomised)				Significantly better OS (median and disease control rate were 6.6months and 22.7% in experimental arm vs 4.1 and 6.6% in standard arm, respectively), although adverse events were higher in experimental arm.

Halama 2019 (NCT03168139) (63)	Ib/II	11	0	11	Pembrolizumab + Olaptesed pegol (CXCL12 inhibitor)				ORR 0%
Hochster 2017 (NCT01633970) (64)	I	10 (of 240 solid tumours)	10	0	Atezolizumab + Bevacizumab arm presented (multiple other combinations)				ORR 30%
Hubbard 2019 (NCT03258398) (65)	II	56	0	56	Avelumab + Tomivosertib (MNK inhibitor)				ORR 2%
Lee 2017 (NCT02260440) (66)	II	31	0	30	Pembrolizumab + Azacitidine				ORR 3%
Monjazeb 2019 (NCT02888743) (67)	II	18	Unclear		Durvalumab + Tremelimumab + radiotherapy				ORR 0%
Patel 2019 (NCT02860546) (68)	II	18	0	18	Nivolumab + Trifluridine/Tipiracil				ORR 0%
Rutkowski 2019 (NCT02908906) (69)	I/II	26	26	0	Cetrelimab monotherapy				ORR 8%
Sanborn 2018 (NCT02335918) (70)	I/II	42 (of 175 solid tumours)	Unclear		Nivolumab + Varlilumab (anti-CD27)	Y	Unclear		ORR 5% (2 of 41; 1 MSI, 1 MSS, both CD274 low)
Segal 2016 (NCT02437071) (71)	II	34	0	26	Pembrolizumab + radiotherapy OR radiofrequency ablation				ORR 9% (1 of 11) in radiotherapy arm, none in RFA arm
Segal 2019 (NCT01693562) (72)	I	36 (of 1022 solid tumours)	36	0	Durvalumab monotherapy				ORR 22%
Segal 2019 (NCT02227667) (72)	II	16	11	0	Durvalumab monotherapy				ORR 27% (unclear whether MSI or high tumour infiltrating lymphocytes)
Shahda 2017 (NCT02375672) (73)	II	30	3	22	Pembrolizumab + mFOLFOX				ORR 64% (1 CR)
Shinozaki 2018 (NCT02851004) (74)	Ib/II	94 MSS	0	12	Pembrolizumab + BBI608 (Napabucasin)				ORR 8% (1 of 12)
Taylor 2019 (NCT02811497) (75) METADUR	II	14	0	14	Durvalumab + Azacitidine				ORR 0%

* same trial, results for different arms (sequential assignment)

Abbreviations: CRC, colorectal cancer; MSI, microsatellite unstable; MSS, microsatellite stable; ORR, objective response rate; MPR, major pathological response; pCR, pathological Complete Regression; IRORR, immune-related objective response rate; IHC, immunohistochemistry