The correlations between IL-17 vs. Th17 cells and cancer patient survival: a systematic review

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Keywords: cancer, IL-17, survival, tumor microenvironment, Th17
Abbreviations: FFPE, formalin-fixed, paraffin-embedded; PBMC, peripheral blood mononuclear cells; RORγ, receptor-related orphan receptor gamma; TNM, tumor node metastasis stage.

Both IL-17 and Th17 cells have been ascribed tumor promoting as well as tumor suppressing functions. We reviewed the literature on correlations between IL-17 versus Th17 cells and survival in human cancer, following the PRISMA guidelines. Serum, formalin-fixed, paraffin-embedded (FFPE) tissue and peripheral blood samples were most frequently studied. High IL-17 quantities were correlated with poor prognosis, whereas high Th17 cell frequencies were correlated with improved prognosis. Since Th17 cells are a subpopulation of IL-17⁺ cells and had a different correlation with prognosis than total IL-17, we substantiate that a distinction should be made between Th17 and other IL-17⁺ cells.

Introduction

Interleukin-17 (IL-17) was characterized in 1993 and originally named cytotoxic T lymphocyte-associated-8 (CTLA-8).¹ IL-17 was more recently renamed IL-17A and has five family members: IL-17B–F.² Only IL-17F shows some homology and overlapping functions with IL-17A. The main functions of IL-17 are the attraction of neutrophils and stimulation of inflammation.³ The T-helper 17 (Th17) cell, one of the predominant producers of IL-17 that was characterized in 2005,⁴ is essential to protect the host against pathogens that are not handled well by Th1 and Th2 cells.⁵ This pro-inflammatory cell type plays a dominant role in a variety of autoimmune diseases.⁶ Antibodies targeting IL-17 and its receptor are now used in clinical trials to treat autoimmune diseases like psoriasis, rheumatoid arthritis, and Crohn’s disease.⁶ Since IL-17 can also be produced by innate immune cell types including both lymphoid-derived (e.g., γδ T cells, invariant natural killer T cells, and innate lymphoid cells)⁷,⁸ and myeloid-derived cells (e.g., neutrophils, macrophages, and mast cells),⁹ it may bridge the activities of the innate and adaptive immune system.¹⁰

Much less studied is the role of IL-17 in cancer. Both tumor-suppressing and tumor-promoting functions have been ascribed to the IL-17 protein and Th17 cells.¹¹ This ambiguity about the function of IL-17 and Th17 cells in cancer has limited the potential for targeting the molecule or using cell-based immunotherapy. Part of the ambiguity may have arisen because different aspects of the IL-17 response are studied. Total protein amount or cells expressing IL-17 protein have been measured in serum and tumor-associated fluids by ELISA and in FFPE tissue by immunohistochemistry, respectively. The effect of Th17 cells has been analyzed mainly in peripheral blood, but also in tumor-associated fluids, FFPE and fresh frozen tissue by flow cytometry, immunohistochemistry or RT-PCR. A review on Th17 cells in cancer by Wilke et al. in 2011 already noted that correlations between the IL-17 protein and survival may be different from correlations with the Th17 cell population.¹²

To systematically study the correlations between the IL-17 protein and Th17 cells and survival in human cancer, we investigated all publications in NCBI PubMed, Ovid Embase, and Web of Science addressing this subject. The aim of our study was to identify the correlations between both IL-17 protein and Th17 cells and prognosis in cancer. The studies were classified by the sample type used to study IL-17 or Th17 cells: serum, FFPE tissue, peripheral blood, tumor-associated fluids, and fresh frozen tissue. Subsequently, the effect on survival was analyzed for each of the sample types studied. The implications for further research of IL-17 and Th17 cells are discussed.

Results

Study design and selection criteria

Of the 2,643 publications identified through database searching on IL-17 or Th17 and cancer, 56 studies met the inclusion criteria (Fig. 1). The main reasons for a publication to be excluded were: being a conference abstract (23%), an animal study (24%), no study on cancer (27%), or not reporting on survival data (19%). Two articles were excluded due to lack of other references on the same method and survival analysis. One article reported on an IL-17 polymorphism analysis,¹³ while the other studied RNA levels of Th17 cell expressed retinoic acid receptor-related orphan receptor gamma (RORγt).¹⁴ Neither of these...
studies found a correlation with survival. An overview of the included studies sorted by sample type and clinical outcome is shown in Table 1.

Studies reporting on survival analysis or risk of recurrence were included regardless of the outcome of the study. A potential publication bias was caused by excluding articles that reported on correlations with other clinico-pathological parameters but not survival. This bias was minimized by screening all articles that reported on correlations with clinico-pathological parameters for having performed a survival analysis. The survival criterion enabled us to focus on studies that are relevant for the potential targeting of IL-17 or Th17 cells in a clinical setting.

Generally, a random or consecutive group of patients was analyzed for relatively objective measures (see Tables S1–4). Although most studies did not provide details on the sample selection method, the majority of the studies did specify that they used a continuous variable or categorized IL-17 or Th17 cell numbers in groups based on the presence, mean or median to analyze the effect by Kaplan–Meier and Cox regression analyses. Potential risks of bias identified in categorizing IL-17 or Th17 expression were optimal cut-off values chosen arbitrarily or using a minimum p value, receiver operating characteristic (ROC) curve, or regression tree analysis. Furthermore, one study compared the six long (>3 y) vs. short (<1.5 y) surviving patients. Another study reported that post-chemotherapy samples were used when no pretreatment samples were available for immunohistochemistry. A final potential risk factor was observed in a study of leukemia patients treated with allogeneic stem cell transplantation after myeloablative conditioning, which included donors that varied from related to unrelated and different prophylaxis regimens to prevent graft-vs.-host disease. Additional study details and concerns are listed per sample type in Tables S1–4. Clinico-pathological characteristics of the different studies per measurement method are provided in Tables S5–8.

High IL-17 serum levels are correlated with poor survival

Serum, paraffin tissue, peripheral blood mononuclear cells (PBMCs) and occasionally tumor-associated fluids or fresh frozen tissue were used to measure IL-17 protein or RNA and Th17 cells. Since the cell source and related activity...
measured may differ in different sample types, we sorted and analyzed the studies by sample type. The amount of IL-17 protein in serum was measured by ELISA (Table 1.1). Since total protein quantity was measured, the IL-17 could have been derived from Th17 cells but also from innate immune cell types. Five studies out of ten reported that a high amount of serum IL-17 protein was correlated with poor survival.\textsuperscript{17,24-26,31} One study showed a correlation between high IL-17 and improved survival in leukemia.\textsuperscript{32} Four studies did not observe a significant correlation between high serum IL-17 levels and survival,\textsuperscript{33-36} although one group did find a trend toward poor prognosis ($p = 0.05$).\textsuperscript{36} Overall, a high amount of IL-17 protein in serum has predominantly been correlated with poor survival (Table 2).

\begin{table}[h]
\centering
\caption{Correlation between IL-17 quantification in serum. All studies describing a correlation between a measurement of IL-17 in serum by ELISA and overall or disease-free survival are shown. The analyses were sorted by clinical outcome, cancer type and study size. Sample size indicates the number of patients on which the correlation between the IL-17 measurement and survival was reported. The column ‘Correlation’ indicates whether a high measurement was correlated with poor or improved survival. A multivariate analysis including both the IL-17 measurement as well as another variable also containing this IL-17 measurement (e.g., a ratio) was not included in our analysis since the potential effect might be lost by correcting for it. An asterisk behind a reference number indicates that the study was not included in the quantitative analyses in Table 2 and Fig. 2. Whether or not the correlation found was independently correlated with survival when corrected for clinico-pathological parameters in a multivariate Cox regression analysis is also indicated. A multivariate analysis including both the IL-17 measurement as well as another variable also containing this IL-17 measurement (e.g., a ratio) was not included in our analysis since the potential effect might be lost by correcting for it. Measurement deviations are indicated under ‘Notes’. CLL = chronic lymphocytic leukemia; AC = adenocarcinoma; CRC = colorectal carcinoma; HCC = hepatocellular carcinoma; NA = not applicable or not mentioned in the article; NSCLC = non-small cell lung carcinoma.} 
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Cancer type & Sample size (n) & Outcome & Correlation & Multivariate Cox $p < 0.05$ & Notes & Ref # \\
\hline
NSCLC & 128 & OS & Poor & Yes & & 25 \\
HBV-related HCC & 105 & OS, DFS & Poor & DFS: Yes OS: NA & & 26 \\
Leukemia treated with myelo-ablative conditioning and SCT & 95 & DFS & Poor & Yes & & 31 \\
Gastric carcinoma & 85 & OS & Poor & Yes & & 17 \\
CRC & 80 & DFS & Poor & NA & & 24 \\
Acute leukemia & 93 & OS & Improved & No & & 32 \\
CLL & 294 & OS & No correlation & & sample type: plasma & 33 \\
CLL & 84 & OS & No correlation & & & 34 \\
Pancreatic AC & 62 & OS & Trend toward poor & & & 36 \\
Multiple myeloma & 50 & OS & No correlation & & peripheral blood, bone marrow & 35 \\
\hline
\end{tabular}
\end{table}

A high number of IL-17$^+$ cells in tissue is correlated with poor survival
The total number of IL-17$^+$ cells was quantified on cancer tissue FFPE whole slides or tissue microarrays using immunohistochemistry. This type of analysis offers quantification of the total number of IL-17$^+$ cells within the tumor microenvironment. IL-17 is expressed by different types of tumor infiltrating immune cells in cancer, predominantly neutrophils and mast cells.\textsuperscript{37-39} The total number of IL-17$^+$ cells was correlated with poor prognosis in 18

\begin{table}[h]
\centering
\caption{Correlations per measurement type. The number of analyses per sample and measurement type of IL-17 protein or Th17 cells showing a correlation with improved or poor prognosis or no effect is indicated. The final column denotes the ratio of the number of analyses showing a correlation with improved prognosis over the number of analyses showing a correlation with poor prognosis, as an indication of the factor difference. A white box indicates a correlation with improved survival, a dark gray box a correlation with poor survival and a light gray box no clear correlation} 
\begin{tabular}{|l|c|c|c|c|c|c|}
\hline
Target & Sample type & Measurement method & #analyses & #analyses poor prognosis & #analyses no effect & total # analyses & Factor difference \\
\hline
IL-17 & Serum & ELISA & 1 & 5 & 4 & 10 & 0.2 \\
& FFPE tissue & IHC & 5 & 18 & 4 & 27 & 0.3 \\
& Tumor-associated fluid & ELISA & 1 & 1 & 0 & 2 & 1.0 \\
\hline
Total & & & 7 & 24 & 8 & 39 & 0.3 \\
\hline
Th17 & FFPE tissue & IHC,Th17 & 1 & 0 & 1 & 2 & NA \\
& Peripheral blood & Flow cytometry & 4 & 2 & 3 & 9 & 2.0 \\
& Tumor-associated fluid & Flow cytometry & 2 & 0 & 0 & 2 & NA \\
& Fresh frozen tissue & RT-PCR & 1 & 0 & 0 & 1 & NA \\
\hline
Total & & & 8 & 2 & 6 & 16 & 4.0 \\
\hline
\end{tabular}
\end{table}
out of 27 studies (Table 1.2).\textsuperscript{15,16,18-20,30,37,40-50} Five studies reported on a correlation between a high number of IL-17\textsuperscript{+} cells and improved survival.\textsuperscript{21,28,29,51,52} It is important to note that in two of these five studies, the IL-17\textsuperscript{+} cells were scored in areas with the densest lymphocytic infiltrate, one of which was on pancreatic ductal adenocarcinoma patients who had received immunotherapy (the correlation between IL-17 and survival was based on 12 patients).\textsuperscript{21,29} Four studies did not observe a significant correlation between total IL-17\textsuperscript{+} cells in the tumor and survival.\textsuperscript{22,23,53,54} Again the scoring in 2 of these 4 studies had been performed in hot-spot or dense lymphocytic infiltrate areas, while only 3 of the 18 studies reporting on a negative correlation had focused on hot-spots. Three more studies did not focus on IL-17\textsuperscript{+} tumor-infiltrating immune cells and are included with their reported correlations in Table 1 for completeness, but not in the quantitative analyses.\textsuperscript{39,55,56}

Collectively, 18 studies reported on a significant correlation between high IL-17 and poor prognosis, over 3.5 times more than the studies showing a correlation with improved prognosis ($n = 5$, Table 2). To visualize the overall correlation, forest plots are shown for the hazard ratio of a high number of IL-17\textsuperscript{+} cells on overall (OS) (Fig. 2A) and disease-free survival (DFS) (Fig. 2B). Of the 22 studies reporting on overall survival, 7 were excluded from the meta-analysis due to insufficient Cox regression data. Of the 16 studies reporting on DFS, 4 were excluded due to insufficient Cox regression data.

### Correlation between IL-17 RNA expression in fresh frozen tissue and survival inconclusive

Two studies have analyzed IL-17 RNA expression in fresh frozen samples using RT-PCR (Table 1.3). Both studies, on small study populations, did not find associations with survival. One

| Cancer type | Sample size (n) | Outcome | Correlation | Multivariate Cox $p < 0.05$ | Notes | Ref # |
|-------------|----------------|---------|-------------|-----------------------------|-------|-------|
| HCC         | 323            | OS, DFS | Poor        | No                          |       | 40    |
| HCC         | 300            | OS, DFS | Poor (intratumoral) | NA             |       | 18    |
| HCC         | 150            | OS, DFS | Poor (intratumoral) | No peritumoral IL-17\textsuperscript{+} cells correlated with improved survival |       | 41    |
| HCC         | 108            | OS, DFS | Poor (intratumoral) | Yes hot-spot areas scored |       | 19    |
| HCC         | 56             | OS, DFS | Poor DFS    | NA both intra- and peritumoral cells scored |       | 42    |
| Intrahepatic cholangiocarcinoma | 43     | OS, DFS | Poor     | Yes |       | 43    |
| CRC         | 123            | OS      | Poor (intratumoral) | Yes |       | 44    |
| CRC         | 104            | DFS     | Poor       | NA both tumor center and invasive margin scored |       | 20    |
| CRC         | 102            | OS      | Poor       | NA |       | 45    |
| CRC         | 52             | OS      | Poor       | Yes |       | 16    |
| NSCLC       | 102            | OS      | Poor       | NA |       | 46    |
| NSCLC       | 52             | OS, DFS | Poor        | Yes same research group as ref\textsuperscript{16} |       | 15    |
| Breast carcinoma | 207 | OS, DFS | Poor DFS      | Yes scores in tumor center and front hot-spots averaged |       | 30    |
| Gastric carcinoma | 112 | OS      | Poor       | Yes mainly mast cells were IL-17\textsuperscript{+} |       | 50    |
| Cervical SCC | 109          | OS      | Poor (TNM stage I) | Yes NS in all TNM stages |       | 37    |
| Gallbladder carcinoma | 104 | OS, DFS | Poor OS | No |       | 47    |
| Laryngeal SCC | 71           | DFS    | Poor       | No |       | 49    |
| Pancreatic AC | 46          | OS      | Poor       | NA condensed expression areas scored |       | 48    |
| Gastric AC   | 192            | OS      | Improved   | Yes |       | 51    |
| Esophageal SCC | 181         | OS      | Improved   | No |       | 52    |
| Cervical carcinoma | 153 | DFS     | Improved  | Yes densest lymphocytic infiltrates scored |       | 21    |
| Recurrent ovarian carcinoma | 47 | OS, DFS | Improved DFS | No |       | 28    |
| Pancreatic ductal AC treated with vaccine, CT, RT | 12 | OS | Improved | NA lymphoid aggregates scored |       | 29    |
| HCC         | 132            | OS, DFS | No correlation | denest lymphocytic infiltrates scored |       | 22    |
| Nasopharyngeal carcinoma | 106 | OS | No correlation | |       | 53    |
| Epithelial ovarian carcinoma | 104 | OS | No correlation | |       | 23    |
| Giant cell tumors of bone | 74 | DFS | No correlation | |       | 54    |
| Esophageal SCC | 215         | OS      | High IL-17\textsuperscript{+} cells in tumor muscularis propria correlated with improved OS | Yes correlation between IL-17\textsuperscript{+} cells in tumor nests and survival not studied | 39*  |
| CRC         | 78             | OS, DFS | Improved | Yes mainly tumor cells positive | 55*  |
| Stage IV glioblastoma | 41 | OS | Improved | Yes mainly tumor cells positive | 56*  |
study analyzed IL-17 expression in tumor adjacent normal appearing biopsies (∼10 cm from the tumor center) from 19 colorectal cancer patients. The other study in 17 ovarian cancer patients only measured the presence of PCR products on agarose gel. Insufficient data were available to conclude on an association between IL-17 RNA expression in fresh frozen tissue and survival.

A high number of Th17 cells in tissue is correlated with improved survival

The total number of Th17 cells can be quantified using a combination of a T-cell marker and IL-17 in FFPE slides. Using immunohistochemistry, our group has shown that a high number of Th17 cells was correlated with improved prognosis in squamous cervical cancer, while another study found a trend toward improved DFS (p = 0.06) in differentiated thyroid cancer (Table 1.3). We did not include analyses on the ratio of the number of IL-17+ cells over the number of CD3+ or CD4+ T cells, because we do not regard this as a measure for Th17 cells since IL-17 is also produced by other cell types.

A high number of Th17 cells in peripheral blood is correlated with improved survival

Flow cytometry was used to quantify the Th17 cell frequency among PBMCs, usually defined as CD4+IL-17+ cells (see Table 1.3 for details). A high number of Th17 cells was correlated with improved survival in four studies. Two studies found a correlation with poor survival, while three studies did not find a significant correlation. Notably, while two studies reported on a correlation between a high number of Th17 cells and poor prognosis, twice as many studies (n = 4) reported on a correlation with improved prognosis (see Table 2).

A high number of Th17 cells in tumor-associated fluids is correlated with improved survival

Tumor-associated fluids have infrequently been studied for the correlation between IL-17 or Th17 numbers and survival (Table 1.4). Two studies have analyzed the number of Th17 cells in lung cancer malignant pleural effusion by flow cytometry. Both found a significant correlation between a high number of Th17 cells and improved overall survival. One group has studied the correlation between high IL-17 protein levels in lung cancer malignant pleural effusion measured by ELISA and described a correlation with poor survival. Another study found a correlation between high IL-17 protein levels in ovarian carcinoma ascites and improved survival. Finally, a study in gastric cancer patients showed a significant correlation between high IL-17 RNA expression measured by qRT-PCR and improved survival in patients treated with curative resection.

Collectively, of the five studies on tumor-associated fluids, the studies quantifying Th17 cells using flow cytometry (n = 2) and

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**Figure 2.** Forest plots for IL-17+ cells in tissue. Schematic quantitative analyses of the studies on the number of IL-17+ cells in FFPE tissue are shown by forest plots. Cox regression hazard ratios and 95% confidence intervals for the correlation between a high number of IL-17+ cells and overall survival (A) and disease-free survival (B) were obtained from the articles or via personal communication with the authors. An asterisk (*) indicates that a multivariate Cox regression analysis was used because a univariate analysis was not provided. A dagger (†) indicates that part of the data were obtained via e-mail. The cut-off value used to divide the IL-17+ cell frequency in a high and low group is indicated for comparison. The center of the random effects model represents the pooled hazard ratio, while the 95% confidence interval is represented by the diamond horizontal borders.
Table 1.3. Correlation between Th17 cells and survival. Analyses of Th17 quantification on FFPE tissue, peripheral blood PBMCs and fresh frozen samples. Bref = brefeldin A; FC = flow cytometry; GVHD = graft-versus-host disease; Iono = ionomycin; Mon = monensin; PMA = phorbol 12-myristate 13-acetate

| Cancer type                                      | Sample                          | Sample size (n) | Measurement                          | Outcome | Correlation | Multivariate Cox p < 0.05? | Notes                          | Ref # |
|-------------------------------------------------|---------------------------------|-----------------|--------------------------------------|---------|-------------|---------------------------|--------------------------------|-------|
| Cervical SCC                                    | FFPE                            | 51              | IHC CD3+ IL-17+ cells                | OS      | Improved    | Yes                       |                                 | 37    |
| Differentiated thyroid carcinoma                | FFPE                            | 266             | IHC CD4+ IL-17+ cells                | DFS     | Trend toward improved |                         |                                 | 59    |
| HCC                                             | PBMC                            | 150             | FC CD4+ IL-17+ cells                 | DFS, OS | Poor        | Yes                       |                                 | 41    |
| Gastric carcinoma                               | PBMC + PMA/iono/mon 1h         | 32              | FC CD4+ IL-17+ IFNG+                | OS      | Improved    | Yes                       |                                 | 62    |
| Acute leukemia                                  | PBMC + PMA/iono/mon 5h         | 93              | FC CD3+CD4+ IL-17+ cells            | OS      | Improved    | Yes                       |                                 | 32    |
| CLL                                             | PBMC + 5h PMA/iono/mon/mon 1h  | 66              | FC CD3+CD4+ IL-17+ cells            | OS      | Improved    | Yes                       | Same group as ref²³          | 38    |
| End-stage melanoma treated with ipilimumab (aCTLA4) | PBMC + activation mix at 5h and 5d | 30              | FC CD4+ IL-17+ cells                 | OS      | Improved (+30d measurement) | Yes                       | NS for -5h measurement          | 60    |
| HCC treated with transarterial chemoembolization | PBMC + PMA/iono/mon 1h         | 150             | FC CD3+CD4+ IL-17+ cells            | OS      | No correlation |                          |                                 | 63    |
| CLL hematological malignancies treated with allogeneic HSCT | PBMC + 5h PMA/iono/mon/mon 5h | 30              | FC CD3+CD4+ CD8 IL-17+ cells         | DFS     | No correlation | NA                        | 60% of patients got GVHD        | 64    |
| HCC                                             | PBMC                            | 26              | FC CD4+ IL-17+ cells                 | DFS     | No correlation | IL-17 ELISA <detection limit |                                 | 65    |
| Stage IV melanoma with anti-tumor antigen T cell response | Melan-A reactive CD3+CD4+ PBMC + 12d peptide mix + 12h antigen/mon | 38              | FC IL-17 present/absent             | OS      | Poor        | Yes                       | IL-17 present in n = 3 ; CD4 related with poor OS | 65    |
| Acute myeloid leukemia                           | BMMC                            | 98              | FC CD3+CD8-IL17+ cells               | OS      | Poor        | NA                        | no correlation for PBMCs (n = 30) | 66    |
| CRC                                             | normal biopsies ~10 cm from tumor center | 19              | qRT-PCR IL-17                        | OS      | No correlation |                          |                                 | 57    |
| Ovarian carcinoma                               | fresh frozen tumor tissue       | 17              | agarose gel RT-PCRIL-17 present/absent | OS      | No correlation |                          |                                 | 58    |
qRT-PCR ($n = 1$) found a correlation with improved prognosis. Of the two studies quantifying IL-17 using ELISA, one found a correlation with improved, and one with poor prognosis.

**Differences between cancer types**

While functional differences between IL-17 and Th17 cells may be due to the cellular source of IL-17 and the accompanying immune response, this might also depend on the cancer type. In studies on liver cancer ($n = 13$), a negative ($n = 9$), or no significant ($n = 3$) correlation was found between high IL-17 or Th17 cells and prognosis, except for the study of hepatocellular cancer treated with transarterial chemoembolization. All studies on colorectal cancer ($n = 6$) also found a correlation between high IL-17 and poor prognosis ($n = 4$) or no significant correlation ($n = 1$), except for one study that reported on IL-17 being expressed mainly by tumor rather than tumor infiltrating immune cells. The studies on non-small cell lung cancer ($n = 3$) reported a significant correlation between IL-17 and poor prognosis as well.

In contrast, all analyses described in six leukemia studies ($n = 8$) showed a significant correlation between PBMC Th17 cells or serum IL-17 and improved prognosis ($n = 3$) or no effect ($n = 4$), except for one study of serum IL-17 in patients that received stem cell transplantation after myeloablative conditioning. This might indicate that the immune response in haematological malignancies may differ from solid tumors. Of the studies on ovarian cancer ($n = 4$), two described a correlation between high IL-17 and improved survival. The other two groups did not find a significant correlation with disease-specific survival, but one of the studies described a correlation between high IL-17 and improved progression-free survival.

These findings indicate that there may be context-specific effects on the IL-17 or Th17 cell immune response, although the number of studies per cancer type was too limited to determine whether the cancer type or sample type is more important for the effect on survival.

**Discussion**

The clinical impact of Th17 cells has remained unresolved in cancer. The aim of this review was to identify the correlations between a high amount of IL-17 protein or high number of Th17 cells in human cancer and patient survival. Following an extensive electronic database search, publications were manually selected without format or language restrictions. Survival analyses were studied in the full article if any analysis regarding prognosis was mentioned in the abstract, minimizing the risk of publication bias. Although the risk of bias in included studies was limited, all studies used different cut-off levels to divide IL-17 or Th17 expression in a high and low expression group due to a lack of established cut-off levels. This study limitation makes it difficult to compare different studies directly.

The sample type studied proved to be crucial for the correlation with clinical outcome. This may partly be explained by a difference in cell source. Some tumor microenvironments may be more favorable for Th17 cells, while others may be more readily infiltrated by IL-17 producing neutrophils. Additionally, the method used determines whether Th17 cells, IL-17 protein, or all IL-17 producing cells are measured. A high amount of IL-17 protein, predominantly produced by neutrophils and mast cells in cancer and measured in serum, FFPE tissue and tumor-associated fluids, was over three times more frequently correlated with poor than with improved prognosis. A meta-analysis could only be performed for IL-17 in FFPE tissue due to the limited number of studies on the other sample types. The forest plots clearly showed that a high number of IL-17$^+$ cells was correlated with an increased hazard ratio, despite the use of a range of cut-off values, which might depend on the type of cancer and analysis. In contrast, a high number of Th17 cells measured in FFPE tissue, peripheral blood or tumor-associated fluids was four times more frequently correlated with improved than with poor prognosis. Since, IL-17 RNA can generally not be quantified in neutrophils and the data obtained by RT-PCR analyses most likely represent IL-17 produced by Th17 cells. The PCR measurements in tumor-associated fluids and fresh frozen tissue are thus regarded as an indicator of the Th17 cell frequency. Because of limited data available, we could not conclude on an association between IL-17 RNA expression and survival.

Th17 cells seemed to primarily have a tumor-suppressing effect, whereas IL-17 was generally associated with poor outcome. IL-17 has been shown to be produced by only a small Th17 cell population. The tumor-promoting function can be explained by the role of IL-17 in inducing angiogenesis and recruiting neutrophils. Neutrophils have been reported to convert to a tumor-promoting phenotype and to induce angiogenesis. The immune cells capable of producing IL-17 include...
neutrophils as well as other cell types,7-9 which may determine an important part of the clinical outcome. The tumor suppression by Th17 cells is probably due to different properties than the secretion of IL-17. Th17 cells might stimulate the Th1 and cytotoxic T cell tumor targeting immune responses. 76 Additionally, Th17 cells have been shown to have memory stem cell-like properties and the ability to differentiate to Th1/Th17 cells that produce interferon-gamma.77 Th17 cells may thus either directly or indirectly suppress tumorogenesis.

The type of IL-17 response is thus likely to be context dependent. Liver cancer, colorectal cancer, and non-small cell lung cancer seem to be correlated with an unfavorable IL-17 response.15,16,18–20,24–26,40–46 Leukemia on the other hand might provide an environment favorable for Th17 cells to suppress tumor growth.32,38 Similarly, ovarian cancer might attract a tumor suppressing IL-17 response, as the majority of studies found a correlation with a favorable outcome.28,66 A possible explanation might be that different microenvironments favor infiltration or differentiation toward more or less tumor-promoting immune cell phenotypes. Not only Th17 cells, but also innate cell types capable of producing IL-17 may be correlated with improved prognosis, as we and others have shown for mast cells.37,39 Although we cannot discriminate whether the cancer type or method used is more important for the correlations found, it is likely that both are important for the cell source studied and thus clinical outcome.

Based on the findings described in the current review, cancer patients with high total IL-17 protein levels might benefit from anti-IL-17 treatment, blocking the tumor promoting response. Adoptive transfer of Th17 cells might be another promising treatment. The feasibility of both approaches needs to be investigated further. Human Th17 cells can be induced by a combination of IL-1β, IL-6, and IL-23, although the exact conditions required are still under debate.75 The functions of differentially obtained Th17 cell populations should be determined by functional studies. Animal models may be helpful to clarify this, as the induction of Th17 cells by IL-6 and TGF-β is clearer in mice than in humans, although the described effects on survival are still contradictory.12,79

We conclude that while IL-17 primarily promotes tumorigenesis, the subpopulation of IL-17 producing Th17 cells seems to have a tumor-suppressing effect. Future research should use methodology that makes a distinction between soluble IL-17 protein, Th17 cells, and other IL-17+ cells. This will help to determine whether IL-17 and Th17 cells should be targeted or used in a clinical setting.

Methods

Study design

A systematic search in the NCBI PubMed, Ovid Embase, and Web of Science bibliographic databases was conducted without language restriction in collaboration with information specialist JML following the PRISMA guidelines.80 Since IL-17 is frequently studied in autoimmune diseases and together with tumor necrosis factor, these terms were excluded as major topic. The full search terms are provided in Table S9.

Selection criteria

All original research studies reporting on an IL-17A or Th17 cell measurement and OS or DFS in human cancer published until 10 September 2014 were included. Articles that did not meet all inclusion criteria (e.g., conference abstracts; studies on IL-17 producing γδ TCR or CD8+ cytotoxic T cells or only describing remission or progression-free survival) were excluded. All relevant references in reviews were manually checked for presence in the systematic search.

Data collection and analysis

Survival analyses, generally Kaplan–Meier survival curves, a log rank test and Cox regression analyses, but in some cases a Spearman’s rank correlation,33,68 Wilcoxon signed-rank test29 or Satterwaite t-test61 were obtained. All included articles were reviewed by both SP and ESJ. If extracted data did not match, the data were discussed until a consensus was reached. Cox regression hazard ratios and confidence intervals were used to perform a meta-analysis. The authors of all articles that did not provide sufficient information on study details, clinico-pathological data or Cox regression analyses were contacted via e-mail. Due to the number of studies with sufficient data per sample type studied, only the analyses of the number of IL-17+ cells in FFPE tissue were suitable for meta-analysis. To keep the data as comparable as possible, scores in the tumor center were used in the cases where data were reported on different scores (e.g., invasive margin, peritumor).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. J. Cao for extracting data from the Chinese articles. We also acknowledge Dr. I.T.A. Peters for providing critical feedback on the manuscript. We are very grateful for the data provided by authors via e-mail.

Funding

This work was supported by grant UL2010–4801 from the Dutch Cancer Society.
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