### Title

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### Journal

Investigative Urology, 196(5)

### ISSN

0021-0005



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### Publication Date

2016-11-01

### DOI

10.1016/j.juro.2016.05.100

Peer reviewed

HHS Public Access

Author manuscript

*J Urol*. Author manuscript; available in PMC 2017 November 01.

Published in final edited form as:

*J Urol*. 2016 November ; 196(5): 1530–1535. doi:10.1016/j.juro.2016.05.100.

# Biomarkers for Wilms Tumor: a Systematic Review

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## Abstract

**Purpose—**Wilms tumor is the most common childhood renal malignancy and the fourth most common childhood cancer. Many biomarkers have been studied but there has been no comprehensive summary. We systematically reviewed the literature on biomarkers in Wilms Tumor with the objective of quantifying the prognostic implication of the presence of individual **tumor** markers.

**Methods—**We searched for English language studies from 1980–2015 performed on children with Wilms Tumor under 18 years old with prognostic data. The protocol was conducted as per PRISMA guidelines. Two reviewers abstracted data in duplicate using a standard evaluation form. We performed descriptive statistics, then calculated relative risks and 95% confidence intervals for markers appearing in multiple level 2 or 3 studies.

**Results—**40 studies were included examining 32 biomarkers in 7381 Wilms patients. Studies had a median of 61 patients with 24 biomarker positive patients per study, and a median follow-up of 68.4 months. Median percent of patients in Stage 1, 2, 3, 4, and 5 were 28.5%, 26.4%, 24.5%, 14.1%, and 1.7%, with 10.2% anaplasia. The strongest negative prognostic association was loss of heterozygosity on 11p15, with a risk of recurrence of 5.00, although loss of heterozygosity on 1p and gain of function on 1q were also strongly linked to increased recurrence (2.93 and 2.86 respectively).

**Conclusions—**Several tumor markers are associated with an increased risk of recurrence or a decreased risk of overall survival in Wilms Tumor. These data suggest targets for development of diagnostic tests and potential therapies.

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**Conflict of Interest:** The authors have no relevant financial relationships to the article to disclose.

### Keywords

Pediatric; renal tumor; Wilms tumor; biomarker; prognosis

## INTRODUCTION

From carcinoembryonic antigen to prostate specific antigen, prognostic tumor biomarkers have a vital role in the diagnosis and treatment of cancers. Wilms tumor (WT) is the most common solid renal malignancy in children; the estimated annual incidence rate is 7–10 cases per million for children younger than 15 years. The Children's Oncology Group (COG) and the National Wilms Tumor Society (NWTS) identify approximately 600 children per year in the United States with a renal tumor, more than 90% of whom have WT. Bilateral WT is relatively uncommon, accounting for 5–7% of children with renal tumors.[1, 2]

Significant advances in multidisciplinary treatment methods have led to long-term survival rates exceeding 90% in patients with localized disease.[3] However, morbidity associated with treatment remains an issue for many patients,[4–7] and 50% of children who relapse go on to die from the disease.[8] Stratifying which children belong to which group would be an important step in improving prognosis for WT. If children with WT who are more likely to relapse based off of their tumor biology could be identified prospectively, more aggressive treatment could be administered to diminish the likelihood of relapse. On the other hand, if low risk patients could be identified prospectively, treatment producing less morbidity could be used.

Various biomarkers for WT have been identified that can be used **to** better predict the prognostic outcomes in children afflicted with the disease.[9] There have been no recent systematic reviews assessing the effectiveness of WT biomarkers at predicting prognostic factors such as relapse and mortality. The objectives of this review were to examine the accumulated literature on the use of biomarkers among children with WT, to quantify the prognostic value of biomarkers that have been adequately tested in appropriately designed studies, and to provide a useful summary for the practicing urologist.

## METHODS

### Study Selection

The initial literature search was designed and executed by a reference librarian at our institution (MVN). We searched MEDLINE, EMBASE, the Cochrane Controlled Trials Register, and Web of Science electronic databases for English language studies indexed from 1980–2015 performed on children 18 years old or younger with WT and biomarker data as per PRISMA guidelines, including grey literature. The exploded search terms were: "biological marker", "biomarker", or "prognosis", restricted to "kidney/renal neoplasm/cancer/tumor", "Wilms tumor", or "nephroblastoma". These were then restricted to articles retrieved under a second search for the exploded search terms "pediatric" or "child" or "children". Reference lists of included studies were manually screened for any additional

studies. Prior to the formal literature search, the protocol was prospectively registered at the international registry of systematic reviews, PROSPERO (registration CRD42015023928).

### Data Abstraction

Two investigators (EBC and SD) independently reviewed and abstracted data from each study. Both study reviewers collected their data separately in dedicated databases using double data entry. We then merged the studies into a single database and resolved discrepancies by consensus and third-party referee (JCR). No manuscript was excluded based on method of analysis, level of evidence, definition of success, perceived quality or susceptibility to bias. Level of evidence was assessed in accordance with the Oxford Centre for Evidence-Based Medicine guidelines as a surrogate marker of quality, and conflicts of interest were noted. In cases of ambiguity or where study reporting made evaluation difficult, we attempted to err on the side of inclusiveness.

### Analysis

One of our primary objectives was to quantify the prognostic value of biomarkers that have been adequately tested in appropriately designed studies. In addition to descriptive statistics, we calculated the relative risk (RR) and 95% confidence interval (CI) for overall survival and for disease recurrence for any biomarker that appeared in multiple studies providing level 2 or 3 evidence, with further filtering of level 3 studies to exclude those that provided insufficient data or information on their outcomes definitions. Statistical testing was 2-sided with an alpha of 0.05. As this study included all eligible papers no power calculation was performed. All studies were tracked using EndNote version X7.4 (Thomson Reuters, New York, NY) and analyzed with SAS version 9.4 (SAS Institute, Cary, NC),

## RESULTS

A total of 40 studies (Appendix) met inclusion criteria after screening of full-text papers (Figure 1), including a total of 7,381 patients with WT and 32 biomarkers. The most frequent countries of origin for the studies were the United States (n = 12), the Netherlands (n = 10), and China (n = 6). Studies were a mix of retrospective cohort (n = 21) and case control (n = 22). Studies included a median (IQR) of 61 patients (41 – 157) with 24 (19 – 40) biomarker positive patients per study and a median (IQR) follow-up of 68.4 months (51.6 – 71.9). Median percent of patients with Stage 1, 2, 3, 4, and 5 WT were 28.5%, 26.4%, 24.5%, 14.1%, and 1.7%, respectively, with 87.7% of patients displaying favorable histology and 10.2% displaying anaplasia. Quality of the included studies was variable, with 49% of studies providing Level II evidence (20/41), 29% providing Level III evidence (12/41), and 22% with level IV evidence (9/41). No significant conflicts of interest were noted.

Genetic biomarkers were the most widely studied prognostic biomarkers, with an aggregated total of 902 patients, as compared to 800 patients with prognostic protein biomarker data, and 100 patients with prognostic immune biomarker data (all biomarkers were obtained from pathologic tumor samples). The vast majority (87%) of biomarkers studied had negative prognostic implications (Table 1). Recurrence rates varied widely by study cohort,

but the majority of cohorts maintained overall survival rates in line with published averages.[2, 3]

Six biomarkers appeared in multiple studies providing level 2 or 3 evidence enabling us to calculate the RR for overall survival and/or the RR for recurrence of disease in the presence of the biomarker (Table 2). The only level 3 studies that met inclusion criteria were reports or subset analyses of NWTS[10–12] or COG[13] trials, with all other pooled trials providing level 2 evidence. The biomarker with the strongest prognostic value for risk of recurrence was loss of heterozygosity (LOH) on 11p15, with a RR (95% CI) of recurrence of 5.00 (2.8 – 7.2). LOH on 1p and gain of function on 1q were also strongly linked to recurrence with more precise CIs than LOH 11p15, with RRs (95% CIs) of 2.93 (2.6 – 3.1) and 2.86 (2.8 – 3.0) respectively.

## DISCUSSION

Current WT treatment strategies rely on attempts to predict a child's risk of progression and recurrence, with staging as well as histology generally considered the most relevant criteria to determine prognosis and guide therapy. Tumor histology could be considered the most common "biomarker" currently in use that reflects prognosis. Anaplastic histology, especially diffuse anaplasia, is associated with higher rate**s** of recurrence, metastases, and death, and the recommended NWTS/COG chemotherapy protocols differentiate patients by presence and degree of anaplasia.[14, 15]

In this study we described a wide variety of biomarkers with prognostic data, several of which could potentially be used after further study to assign patients to different treatment protocols based on their tumor characteristics. As the vast majority of biomarkers studied appeared in only one paper or in multiple papers with lower quality evidence, we focused on biomarkers that appeared in multiple papers of either Level 2 or 3 evidence.

Mutation of *p53* appeared in multiple high quality studies (four studies with Level 2 evidence) and was found in our analysis to be linked with increased recurrence and decreased overall survival. P53 is a tumor suppressor protein reported to occur more frequently in anaplastic WT and in more advanced disease stages, and there is evidence that perturbations in p53 are limited to anaplastic cells.[16, 17] Although there have been no published protocols incorporating p53 into their treatment algorithms, anaplasia has been used as a differentiator since NWTS-3 (1979–1986), and as such patients may have been receiving treatment based indirectly on their p53 status.

The only immune system biomarker that allowed for pooled risk calculation was B7-H1, a cell-surface glycoprotein of the B7 family of T-cell coregulatory molecules. Unlike anaplasia-linked p53, mutations of B7-H1 are associated with an increased risk of tumor recurrence in patients with both favorable histology and anaplastic tumors.[18] B7-H1 induces T cell apoptosis, which weakens host anti-tumor immunity and has been linked to higher risks of cancer specific death in multiple solid tumors, including clear cell renal cancer.[19, 20] Although B7-H1 blockers have shown promise in preclinical studies for renal cell,[21] their promise as WT therapy remains unclear and merits further study.

The most widely conducted research on WT biomarkers has focused on the genetic components of WT development, so it is unsurprising that several genetic markers had enough high quality evidence to enable calculation of pooled risk ratios. The strongest overall association was found for LOH at 11p15, with a RR (95% CI) for recurrence of 5.00 (2.8 – 7.2). This mutation was first discovered when examining patients with Beckwith-Wiedemann syndrome, a disease whose features include predisposition to several tumors, most commonly WT. Patients with Beckwith-Wiedemann were found to harbor mutations in the WT2 gene, located at the 11p15 region, that were linked to their risk of developing WT. LOH of 11p15 was associated with more tumor relapse in very low risk WT patients who were not treated with chemotherapy, but rather were surgically excised and then observed.[22] The precision of this increased risk is low, however, requiring further study to fully elucidate.

Although the effect size was strongest for LOH 11p15, the best quality evidence came from the report on NWTS-5 by Grundy *et al,* the largest and most rigorously structured paper reviewed in our analysis, which demonstrated the risks associated with LOH at 1p or 16q. Approximately 20% of WT patients are reported to have LOH at 16q,[23] while LOH at chromosome 1p occurs in 10% of children with WT.[24] Among patients enrolled in NWTS-5 LOH of either chromosome 16q or 1p was found to have a significantly higher rate of relapse and death independent of tumor stage or histology.[12, 25, 26]

When pooled, the evidence for increased risk of recurrence in the presence of either LOH at 1p (RR 2.93, 95% CI 2.6 – 3.1) or LOH at 16q (RR 1.95, 95% CI 1.8 – 2.4) was robust and precise. As such it is unsurprising that LOH 1p/16q is the only true biomarker that is currently included in treatment protocols is LOH for Chromosomes 1p/16q. Under NWTS/COG protocols, presence of 1p/16q LOH prompts more aggressive radiation therapy, including whole-lung therapy, as well as more aggressive chemotherapy than would otherwise be provided based on stage.[27]

A limitation of LOH at 1p/16q as a biomarker is the relatively low prevalence, as the combined LOH appeared in only 4.6% of patients in NWTS-5, and in only 9.4% of recurrences.[12] A marker that appears to have higher comparative prevalence is gain of function in 1q, best described by Gratias *et al* in a COG study in 2013.[13] They observed 1q gain of function (GOF) in 27% of prospectively enrolled patients, unassociated with stage, and calculated a RR of disease recurrence of 2.72, which correlates with our pooled RR (95% CI) of 2.86 (2.7 – 2.9). Although they were unable calculate a RR of overall survival due to a low event rate, after pooling their data with another high quality study we calculated a RR (95% CI) of overall survival of 0.91 (0.8 – 0.9).[28] With its higher prevalence and demonstrated increased risk associations, 1q GOF could be an impactful marker if incorporated into prospective trials.

Despite long-term overall survival rates exceeding 90% achieved by current WT treatment, patients with unfavorable histologic or molecular features have far worse outcomes, leaving room for progress.[29] Building on previous work showing that increased levels of B7-H1, survivin, and Ki-67 are independent predictors of poor outcome for patients with clear cell RCC, Parker *et al* created BioScore, a biomarker panel currently being evaluated as an

enhancement for established renal cell carcinoma prognostic algorithms.[30] Such biomarker panels are appealing not just as an easy translational synthesis of research on multiple biomarkers, but because they can be readily updated as new biomarker research comes to light. The data in this systematic review are an important first step in these efforts and can be built upon in future studies of biomarker-based treatment protocols and diagnostic panels.

In addition to integrating biomarker panels into the evaluation and treatment of WT, work remains to be done in further defining the clinical characteristics of these biomarkers. It remains to be seen how chemotherapy and radiation affect the level of most biomarkers, and therefore their utility in monitoring disease states is undefined. The cost-effectiveness of wide-spread biomarker testing of WT patients is another question for consideration, although were such testing to be adopted economies of scale would change the cost curve, making preliminary projections overly negative.

Most importantly, it is vital that future prospective studies incorporate biomarkers as investigative treatment differentiators, in the same way that LOH 1p/16q is currently used. Many of the biomarkers studied, however, appear in only a fraction of the population (LOH 1p/16q appears in only 5% of patients).[12] As the treatment population is subdivided into smaller and smaller categories based on biomarker positivity, the need for international collaboration only increases, as performing adequately powered trials may otherwise be impossible.

Several limitations of this study deserve mention. As with all systematic reviews, our analysis was limited by the available data from the included studies. The majority of WT biomarker studies in the literature were of highly variable quality, which limited the number of studies eligible for inclusion. Of the studies included, many lacked granular data, reported only vague prognostic data, or failed to report biomarker positivity in association with outcome, reporting instead overall outcomes and overall biomarker positivity. As such, the number of high quality studies available for pooled analysis was small.

## CONCLUSIONS

The results of this systematic review reveal that several tumor markers are strongly associated with an increased risk of recurrence or a decreased risk of overall survival in children with WT. The strongest effect size was seen with increased risk of recurrence in the presence of LOH at 11p15, but the strongest quality evidence was for increased risk of recurrence in the presence of LOH at 1p or 16q. Other biomarkers such as 1q GOF and BH-71 mutation require further prospective studies to determine their clinical utility. These data suggest several leading targets for future study and development of diagnostic tests in addition to potential therapies.

## Acknowledgments

**Funding Source:** Dr. Routh is supported in part by grant K08-DK100534 from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The funding source had no role in the collection, analysis and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

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## Appendix: Included Studies

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## LEGEND

| | |
|---|---|
| **CI** | Confidence Interval |
| **COG** | Children’s Oncology Group |
| **GOF** | Gain of Function |
| **IQR** | Interquartile Range |
| **LOH** | Loss of Heterozygosity |
| **NWTS** | National Wilms Tumor Society |
| **RR** | Relative Risk |
| **WT** | Wilms Tumor |

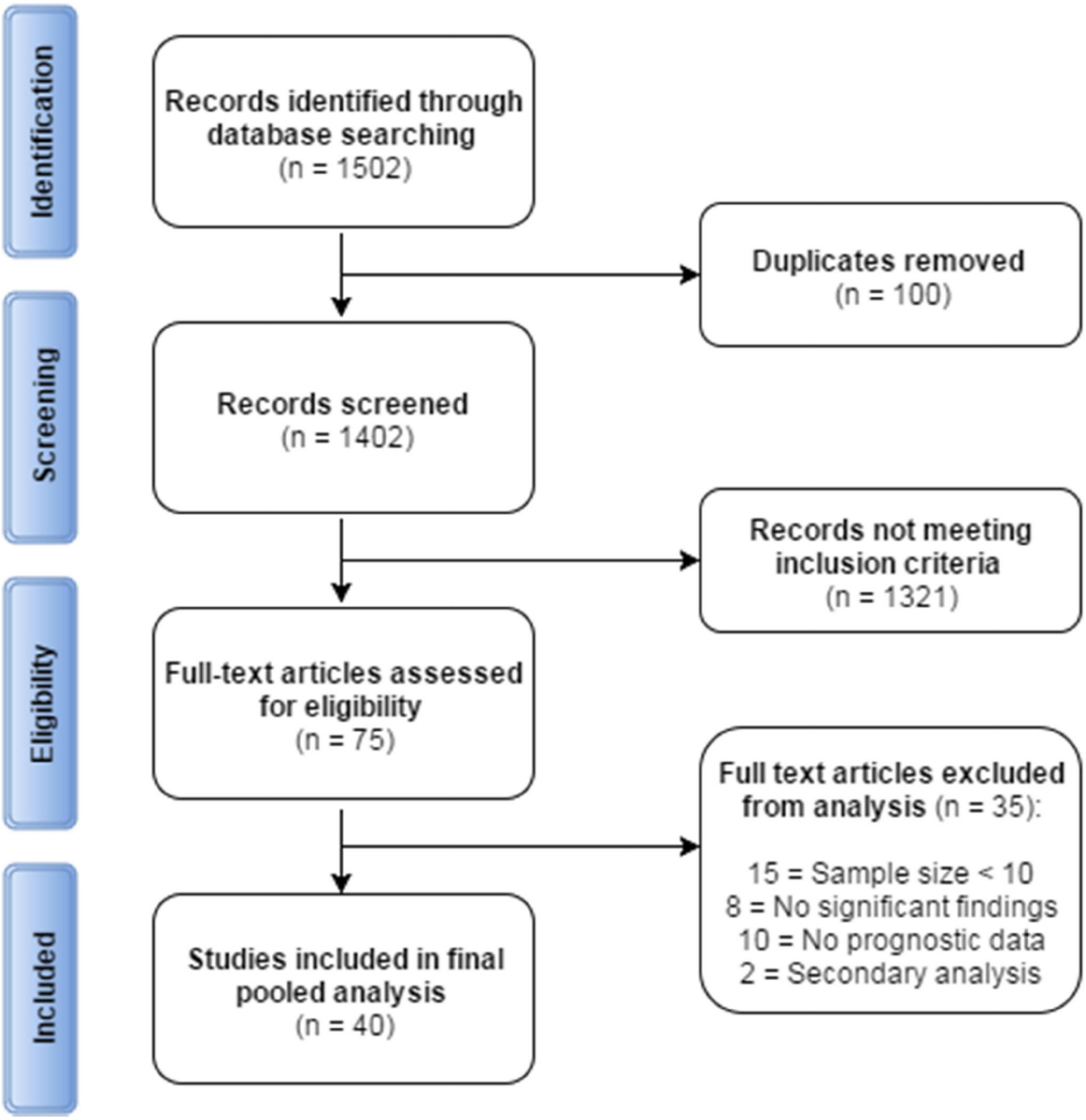


**Figure 1.**
PRISMA Flow Diagram

*J Urol*. Author manuscript; available in PMC 2017 November 01.

**Table 1**

Patient Characteristics by Biomarker

| Biomarker** | Prognostic Implication | Biomarker-Positive Patients | % Anaplastic | % Disease Recurrence | % Overall Survival |
|---|---|---|---|---|---|
| **Immune system biomarkers** | | | | | |
| B7-H1[1, 2] | Negative | 42 | 36.4% | 61.4% | * |
| CD44v5[3] | Negative | 61 | * | 9.8% | 86.9% |
| PCNA[4] | Negative | 28 | 32.1% | 24.1% | 60.7% |
| **Genetic biomarkers** | | | | | |
| 1q gain of function[5, 6] | Negative | 122 | 7.8% | 24.1% | 89.7% |
| Aneuploidy[7] | Negative | 36 | 8.3% | 38.9% | 41.7% |
| DCC_mutation[8] | Negative | 27 | 25.9% | 24.1% | 44.4% |
| Her2/neu[9] | Positive | 15 | 6.7% | 0.0% | 86.7% |
| Loss of Heterozygosity 11p15[10, 11] | Negative | 32 | * | 24.1% | * |
| Loss of Heterozygosity 16q[12–14] | Negative | 377 | 4.8% | 24.1% | 80.9% |
| Loss of Heterozygosity 1p[12, 15] | Negative | 219 | * | 24.1% | 89.9% |
| SIX2_hypomethylation[16] | Negative | 27 | 33.3% | 24.1% | * |
| Tetraploidy[17] | Negative | 23 | * | * | 69.6% |
| WT-1[18, 19] | Negative | 24 | * | 24.1% | 95.8% |
| **Protein biomarkers** | | | | | |
| AEG-1[20] | Negative | 17 | * | 47.1% | 64.7% |
| Bcl-2[21] | Negative | 61 | * | 23.0% | 86.9% |
| Overexpression of e-cadherin[22] | Positive | 20 | * | 24.1% | * |
| EGR-1[18] | Negative | 61 | * | 23.0% | 86.9% |
| FAS[23] | Negative | 49 | 20.4% | * | 73.5% |
| Flt-1[24] | Negative | 62 | * | 22.6% | 85.5% |
| HSP70[25, 26] | Positive | 23 | 8.7% | * | 82.3% |
| HSP90-alpha[26] | Positive | 23 | 0.0% | * | 87.0% |
| IL-6[27] | Negative | 23 | 39.1% | 24.1% | * |
| Ki67[28, 29] | Negative | 20 | * | * | 60.0% |

| Biomarker** | Prognostic Implication | Biomarker-Positive Patients | % Anaplastic | % Disease Recurrence | % Overall Survival |
|---|---|---|---|---|---|
| p27_Kip1[29] | Negative | 62 | * | 22.6% | 85.5% |
| p53[30–34] | Negative | 81 | 52.1% | 24.1% | 63.8% |
| PDGF-AA[35] | Positive | 62 | * | 22.6% | 85.5% |
| STAT3[27] | Negative | 17 | 41.2% | 24.1% | * |
| Telomerase[28, 36, 37] | Negative | 30 | * | * | 70.0% |
| TF[38] | Negative | 41 | 7.3% | 19.5% | 65.9% |
| TGF-alpha[39] | Negative | 62 | * | 22.6% | 87.1% |
| TrkB[40] | Negative | 24 | 37.5% | * | 66.7% |
| VEGF[24] | Negative | 62 | * | 22.6% | 85.5% |

*Not Calculable

**Citations refer to Appendix 2: Included Studies

## Table 2

Median Relative Risks for Overall Survival and Recurrence by Biomarker

| | | Relative Risk (95% CI) | |
|---|---|---|---|
| **Biomarker** | **No. Studies** | **Overall Survival** | **Recurrence** |
| **All immune markers** | | | |
| B7-H1[1, 2] | 2 | * | 2.42 (1.4 – 4.7) |
| **All genetic markers** | | | |
| 1q gain of function[5, 6] | 2 | 0.91 (0.8 – 0.9) | 2.86 (2.7 – 2.9) |
| Loss of Heterozygosity 1p[12, 15] | 2 | 0.92 (0.7 – 0.9) | 2.93 (2.6 – 3.1) |
| Loss of Heterozygosity 11p15[10, 11] | 2 | * | 5.00 (2.8 – 7.2) |
| Loss of Heterozygosity 16q 12 – 14 | 3 | 0.93 (0.7 – 1.0) | 1.95 (1.8 – 2.4) |
| **All protein markers** | | | |
| p53[31 – 34] | 4 | 0.64 (0.5 – 0.8) | 2.34 (2.2 – 2.8) |

* Not Calculable

References in Appendix 2