Ege Journal of Medicine / *Ege Tıp Dergisi* 2014;53(1):13-18

# C-Met immunoreactivity in operated stage I non-small cell lung cancer patients: Is it a prognostic marker?

## Opere edilmiş evre I küçük hücreli dışı akciğer karsinomlu olgularda c-Met immünreaktivitesi: Prognostik bir belirteç mi?

Ozdil A[1] Cakan A[1] Cagirici U[1] Turhan K[1] Ergonul A G[1] Veral A[2]

[1]Ege University Faculty of Medicine, Department of Thoracic Surgery, Izmir, Turkey
[2]Ege University Faculty of Medicine, Department of Pathology, Izmir, Turkey

**Summary**

**Aim:** In this study we evaluated the correlation between c-Met immunoreactivity and survival, thus the effect of hepatocyte growth factor (HGF) on prognosis, in operated early-stage non-small cell lung carcinoma (NSCLC) patients.

**Materials and Methods:** Seventy patients who underwent resection for stage IA or IB NSCLC were examined retrospectively. Pathological preparations were evaluated to investigate c-Met immunoreactivity. Those with normal bronchial epithelium in the sections were used as positive control group and the sections of those who had not been stained by primary antibody were regarded as the negative control group. C-Met immunoreactivities were determined according to scores of staining.

**Results:** There were no significant differences between c-Met immunoreactivity and clinicopathological factors. The analysis after exclusion of large cell carcinoma cases showed that c-Met immunoreactivity was higher in adenocarcinomas than in squamous cell carcinomas and the difference was statistically significant. Survival analysis to estimate the prognostic significance of c-Met immunoreactivity showed no significant differences for both disease-free (p=0.499) and long-term survival (p=0.261). The difference between c-Met immunoreactivity and survival for the adenocarcinoma and squamous cell carcinoma groups was not significant.

**Conclusion:** It seems that HGF expression and serum HGF levels appeared to be stronger prognostic markers than c-Met immunoreactivity for NSCLC, therefore detailed studies were needed regarding these prognostic factors.

**Key Words:** Lung cancer, c-Met immunoreactivity, prognosis, survival.

***Özet***

***Amaç:*** *Bu çalışmada opere edilmiş erken evre küçük hücreli dışı akciğer karsinomlu (KHDAK) hastalarda, c-Met immünreaktivitesi ile sağkalım arasındaki korelasyon ve hepatosit büyüme faktörünün (HGF) prognoz üzerindeki etkisi araştırılmıştır.*

***Gereç ve Yöntem:*** *Evre IA veya IB KHDAK tanısıyla opere edilen 70 hasta retrospektif olarak incelendi. Patoloji preparatları c-Met immünreaktivitesinin belirlenmesi için tekrar değerlendirildi. Kesitlerdeki normal bronş epiteli pozitif kontrol grubu, primer antikor tarafından boyanmayan kesitler ise negatif kontrol grup olarak kabul edildi. C-met immünreaktivitesi boyanma skorlarına göre belirlendi.*

***Bulgular:*** *C-met immünreaktivitesi ile klinik ve patolojik faktörler arasında istatistiksel olarak anlamlı fark bulunmadı. Büyük hücreli karsinomlar çalışma dışında bırakılarak yapılan analizler adenokarsinomlarda skuamöz hücreli karsinomlara göre c-Met immünreaktivitesinin daha yüksek olduğunu gösterdi ve fark istatistiksel olarak anlamlı idi. C-met immünreaktivitesinin prognostik etkisini tahmin etmek için yapılan analizlerde gerek hastalıksız sağkalım (p=0.499) gerekse uzun dönem sağkalım (p=0.261) açısından anlamlı fark saptanmadı. C-met immünreaktivitesi ile adenokarsinom ve skuamöz hücreli karsinom gruplarının sağkalımı arasındaki fark da istatistiksel olarak anlamlı değildi.*

***Sonuç:*** *KHDAK'da HGF ekspresyonu ve serum seviyeleri c-Met immünreaktivitesine göre daha güçlü prognostik marker olarak gözükmektedir. Bu nedenle bu prognostik faktörler ile ilgili daha kapsamlı çalışmalara gereksinim vardır.*

***Anahtar Sözcükler:*** *Akciğer kanseri, c-Met immünreaktivitesi, prognoz, sağkalım.*

Corresponding Author: Ali OZDIL
Ege University Faculty of Medicine, Department of Thoracic Surgery, Izmir, Turkey
Received: 03.10.2013 Accepted: 31.10.2013

## Introduction

Although the survival rates after surgery can be low, the best curative treatment of early stage lung cancer is surgical resection. Local recurrence and distant metastasis rates can be decreased by postoperative adjuvant treatments such as chemotherapy, radiotherapy and chemoradiotherapy. To estimate prognosis, patient factors like age, smoking history, nutrition, physical properties, histopathological factors like stage and tumor cell differentiation, and molecular tumor markers can be used. The studies about prognostic factors have shown that tumor stage is the most important predictor of survival and it can be determined only by surgical procedure. For determination of prognosis, molecular studies on factors like oncogenes, growth factors and chromosomal abnormalities have started in recent years (1,2).

Growth factors that are effective on formation of carcinoma are also used in finding new therapeutic agents and methods (3). The studies with the "Western Blotting" technique have shown that levels of immunoreactive hepatocyte growth factor (HGF) are significantly higher in lung cancer tissues. The difference is more specific for non-small cell lung cancer (NSCLC) compared with other types (4). The HGF level is a good indicator for patient survival, and therefore it can be used as a prognostic marker (5). The expression and amplification of hepatocyte growth factor receptor (c-Met) also increased in lung cancer as well as in stomach, colon, liver, prostate and ovarian cancers (6,7). As reported in studies, the relationship between c-Met immunoreactivity and prognosis is stronger than HGF (5).

The aim of this study is to investigate the interaction between c-Met immunoreactivity and prognosis, thus the effect of HGF on prognosis in operated early-stage NSCLC patients.

## Materials and Methods

207 patients who underwent pulmonary resection for primary lung cancer in the Department of Thoracic Surgery of Ege University School of Medicine between January 2000 and December 2007 were analyzed retrospectively. Due to the short follow up duration, patients operated after December 2007 were not included, so that the shortest follow-up period was 24 months. Ninety-four patients who underwent resection for stage IA or IB NSCLC were enrolled in the study. Since survival data could not be accessed or a pathological examination could not be completed, 24 patients excluded from the study. Pathological preparations of patients were evaluated again and c-Met immunoreactivity was investigated.

### *Protocol of Immunohistochemical (IHC) Evaluation*

Sections (5 µm thick) which were obtained from parafin blocks with sufficient tumor tissue and normal lung parenchyma were taken on the electrostatical slides (X-traTM, Surgipath Medical Industries, Richmond, Illinois, USA) and dried at 60 $^{o}$C for at least two hours. All IHC staining procedures including deparafinization and antigen revealling were fully performed in the BenchMark XT automated immunohistochemistry staining instrument. Multimeric HRP based, without biotin, containing hydrogen peroxide substrate and 3,3'-diaminobenzidine tetrahydrochylorite (DAB) chromogene kit (*ultra*View™ Universal DAB Detection Kit, Catalog number 760–500, Ventana Medical Systems, Tucson, AZ) and a full automatic immunohistochemistry staining instrument (Ventana BenchMark XT, Ventana Medical Systems, Tucson, AZ) were used as IHC staining system. Only the c-Met primary antibody (clone 8F11, Novocastra, 1:500 dilution) was administered manually and samples were incubated at 37°C for 32 minutes. After completion of opposite staining wtih hematoxylin and bluing solution, steps including dehydration, transparenting of sections with xylen, closure of lamels were performed by hand and then the process was terminated.

Normal bronchial epithelium in the sections were used as positive control. The sections which were prepared for all cases and had not been stained by primary antibody were regarded as negative control. c-Met immunoreactivities were evaluated with the Nakamura et al. method (8). Accordingly, the following scores were assigned: staining (-), absence of staining or only focal weak staining, 1+: weak to moderate staining in less than 40% of cancer cells, 2+: weak to moderate staining in at least 40% of cancer cells, 3+: strong staining in at least 10% of cancer cells, among the specimens with weak to moderate staining in at least 40% of cancer cells. Weak to moderate staining was accepted as same staining or weaker staining than the staining of surrounding bronchial epithelium and strong staining was accepted as more intense staining than the surrounding bronchial epithelium. Cases were divided into two groups as low c-Met (-/1+) and high c-Met (2+/3+).

Staging of cancer was performed according to the International TNM Classification System for Lung Cancer (9). Survival data were obtained from our own Thoracic Oncology Departments' records where postoperative follow up of patients have been recorded.

### *Statistical Analysis*

Data analysis was performed using SPSS version 15.0 for Windows. Associations between c-Met immunoreactivity and the other factors were determined

by the $\chi^2$-test and Fisher's Exact Test. The Kaplan-Meier method was used for the analysis of long term survival and disease free survival. The Log-Rank Test was used to evaluate statistical significance of the factors affecting long term survival and disease free survival. *p* values of less than 0.05 were considered to be statistically significant.

**Results**

The patients included 62 (88.6%) males and 8 (11.4%) females and the mean age of cases was 60.19 ± 9.90 years (range, 35-86 years; median: 60.50). Sixty-two (88.6%) cases had a smoking history ranging from 15-120 packages per year (mean: 41.51 ± 25.51 package per year; median: 40). 31 (44.3%) right superior lobectomy, 15 (21.4%) right inferior lobectomy, 12 (17.1%) left superior lobectomy, 9 (12.9%) left inferior lobectomy and 3 (4.3%) left pneumonectomy were performed. The histopathological diagnosis were adenocarcinoma in 46 (65.7%), squamous cell carcinoma in 18 (25.7%) and large cell carcinoma in 6 (8.6%) patients. Thirty-three (47.1%) patients were classified as stage IA and 37 (52.9%) were classified as stage IB. Tumor sizes were greater than 3 cm in 26 (70.27%) of the stage IB cases, the remaining 11 (29.73%) were staged as IB because of other factors. The mean tumor size was 32.06 ± 16.22 mm (range 5 - 80 mm; median: 30).

In respect of c-Met immunoreactivity, the score of staining was "-" in 18 (25.7%), "1+" in 16 (22.9%), "2+" in 30 (42.9%) and "3+" in 6 (8.6%). Thirty-four (48.6%) were in the "low c-Met" group and 36 (51.4%) were in "high c-Met" group.

After the analysis of survival data, recurrence was seen in 22 (31.4%) patients and 18 (25.7%) patients were exitus. The means of disease-free survival and long-term survival were 42.76 ± 26.79 months (range 4 - 106 months; median: 43.5) and 46.79 ± 26.50 months (range 5 - 106 months; median: 44.5), respectively. The mean of recurrence time after surgery was 20.77 ± 16.48 months (range 4 - 64 months; median: 16). Average life time of exitus was 25.67 ± 18.78 months (range 5-78 months; median: 24) (Table-1).

**Table-1.** Disease-Free and Long Term Survival of Patients.

| | **n** | **%** |
|---|---|---|
| Recurrence (+) | 22 | 31.4 |
| Recurrence (-) | 48 | 68.6 |
| Exitus | 18 | 25.7 |
| Alive | 52 | 74.3 |
| Mean of disease-free survival | 42.76 ± 26.79 (4–106) months | |
| Mean of long-term survival | 46.79 ± 26.50 (5–106) months | |

There were no significant differences between c-Met immunoreactivity and the other factors. The analysis after the exclusion of large cell carcinomas (because the number of large cell carcinomas was low) showed that the c-Met immunoreactivity was higher in adenocarcinomas than squamous cell carcinomas and the difference was significant ($p<0.05$) (Table-2). There were no significant differences between c-Met immunoreactivity, disease-free survival and long-term survival (Table-3).

**Table-2.** Correlation of c-Met Immunoreactivity with Clinical and Pathological Parameters.

| | **c-Met Immunoreactivity** | | *p* |
|---|---|---|---|
| | Low | High | |
| **Sex** | | | |
| Male | 32 | 30 | 0.261 |
| Female | 2 | 6 | |
| **Age (years)** | | | |
| < 65 | 23 | 22 | 0.624 |
| ≥ 65 | 11 | 14 | |
| **Smoking History** | | | |
| (+) | 32 | 30 | 0.261 |
| (-) | 2 | 6 | |
| **Histopathology** | | | |
| Adenocarcinoma | 19 | 27 | 0.048* |
| Squamous cell carcinoma | 13 | 5 | |
| Large cell carcinoma | 2 | 4 | 0.058** |
| **Stage** | | | |
| IA | 18 | 15 | 0.473 |
| IB | 16 | 21 | |
| **Tumor size (cm)** | | | |
| ≤ 3 | 21 | 23 | 0.99 |
| > 3 | 13 | 13 | |

* *p* value after the exclusion of large cell carcinoma cases from analysis.
** *p* value after the analysis of all cases.

**Table-3.** Correlations of c-Met Immunoreactivity with Disease-Free Survival and Long-Term Survival**.**

| | **c-Met Immunoreactivity** | | *p* |
|---|---|---|---|
| | Low | High | |
| **Disease-Free Survival** | | | |
| Recurrence (+) | 24 | 24 | 0.800 |
| Recurrence (-) | 10 | 12 | |
| **Long-Term Survival** | | | |
| Alive | 27 | 25 | 0.417 |
| Exitus | 7 | 11 | |

Survival analysis using the Kaplan-Meier method was carried out to estimate the prognostic significance of c-Met immunoreactivity and there were no significant differences for both disease-free survival (p=0.499) and long-term survival (p=0.261) (Figure 1 and 2).

The analysis among the groups of adenocarcinoma and squamous cell carcinoma showed no significant differences between c-Met immunoreactivity and survival for both groups.

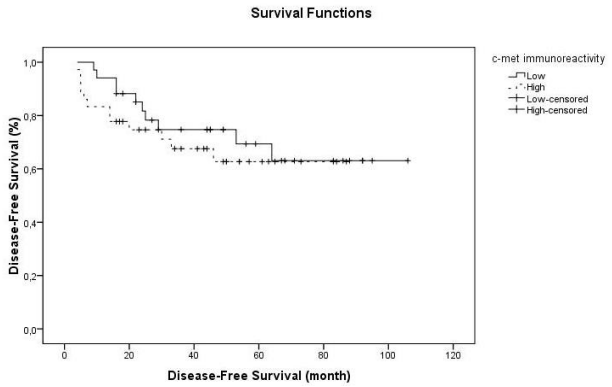


**Figure-1**. Disease-free survival according to the c-Met immunoreactivity.

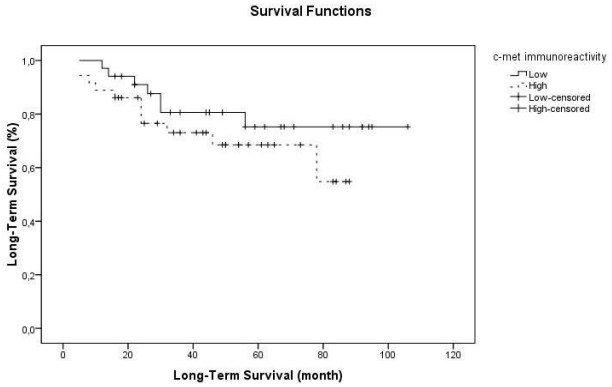


**Figure-2.** Long-term survival according to the c-Met immunoreactivity.

## Discussion

Many studies have been conducted on the prognostic factors and markers of NSCLC. Tumor size, lymph node involvement and distant metastases have also been used in staging. Several studies have been conducted on research markers that could be identified in patient's serum and used as a prognostic factor. However, D'Amico et al. (10) examined the hepatocyte growth factor, vascular endothelial growth factor and e-selectine as a prognostic factors and they could not find any significant differences. Although many factors like carcinoembryonic antigen, epithelial growth factor and hepatocyte growth factor were found to be used as a prognostic factor, none of them are recognized as a powerful marker for prognosis (11,12).

In this study, we examined the relationship between c-Met immunoreactivity and survival in operated stage I NSCLC patients and also we investigated whether hepatocyte growth factor could be used as a prognostic factor. While most of the studies about hepatocyte growth factor and c-Met included all stages of NSCLC, this report differs from these studies in that only stage I NSCLC cases were included in our study.

Firstly we investigated the correlation between c-Met immunoreactivity and the other clinical variables and we could not find any significant relation. In similar studies it was reported that there had been no significant relationship between c-Met immunoreactivity and clinical variables such as age, sex, smoking history (4,8,13,14). Chen et al. (15), studied the relationship between smoking history and the production of hepatocyte growth factor and reported increased expression of hepatocyte growth factor in type II pneumocytes both in healthy smokers and smokers with NSCLC. The researchers indicated that the patients in the healthy group with increased expression of growth factor had a higher risk of developing lung cancer.

There were no significant differences between c-Met immunoreactivity and the pathological parameters such as histopathological diagnosis, stage and tumor size. The analysis after the exclusion of large cell carcinoma showed that c-Met immunoreactivity was significantly higher in adenocarcinoma than squamous cell carcinoma. Ichimura et al. (16), reported that c-Met immunoreactivity had been 77% in adenocarcinoma and 37% in squamous cell carcinoma. Harvey et al. (17) reported that almost all squamous cell carcinoma cells have shown c-Met immunoreactivity but the reactivity has been more powerful and higher in adenocarcinoma cells. Tsao et al. (18), reported that adenocarcinoma cells' c-Met expression had been three times higher than the normal bronchial epithelium and more than 90% of squamous cell carcinomas had shown lower c-Met protein levels than normal epithelium. The high c-Met immunoreactivity in adenocarcinoma can be related to the positive effect of hepatocyte growth factor on glandular differentiation of lung and development of adeocarcinoma from the differentiation of these neoplastic glandular cells.

The high levels of c-Met immunoreactivity in adenocarcinoma brought about the studies on hepatocyte growth factor to be conducted in this tumor group. Takanami et al. (5), suggested there was significant evidence on how this situation caused that the activation of autocrine HGF-c-Met signal pathways and as a result of this the activation of tumorigenesis and

differentiation in adenocarcinoma. In addition, Yi et al. (19) announced that mechanisms and regulators that make up this autocrine effect should be investigated.

Analysis conducted in order to evaluate hepatocyte growth factor receptor immunoreactivity as a prognostic factor in early stage NSCLC, which was the basic purpose of our study, showed no significant relationship for both disease-free survival and long-term survival. Nakamura et al. (20), stated that no significant difference between c-Met immunoreactivity and disease-free survival and long-term survival in cases with stage I-III adenocarcinoma, which were similar in our study. On the other hand, as a result of their study on adeoncarcinoma cases Takanami et al. (5), reported a significant relationship between overexpression of c-Met and short survival. Siegfried et al. (21), investigated c-Met receptor and its ligand hepatocyte growth factor in their study and reported a significant negative correlation between increased HGF expression and survival. They emphasized that the significance was valid for both two groups including early staged cases and all cases. In the study published in 1998 and resumed by the same researchers (22), a significant relationship between c-Met immunoreactivity and survival was reported. A negative correlation between increased serum HGF levels and prognosis in various organ carcinomas such as breast and pancreas was reported in several studies (23,24). As a result of these studies and our study, it is understood that HGF expression and serum HGF level are stronger and more significant prognostic markers compared to c-Met immunoreactivity.

## Conclusion

The majority of the studies about HGF and c-Met included groups which were formed from patients of all stages. The difference in survival between stage I and other stages can be determined according to tumor size and lymph node metastasis, but the number of prognostic factors to determine the difference in survival between the patients in the same stage is very low. This is because why we examined the patients only in stage I. We could not find any significant difference between c-Met immunoreactivity and survival, neither in the patient groups of the same stage nor in the patient groups of the same histopathology. We suggest that studies about HGF expression and serum HGF levels be planned because it seems that these factors appeared to be stronger prognostic markers than c-Met immunoreactivity for NSCLC patients.

**References**


1. Reed CE, Silvestri GA. Diagnosis and staging of lung cancer. In: Shields TW, LoCicero III J, Ponn RB, Rusch VW (eds). General Thoracic Surgery, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2005;1534-47.
2. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: Epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc 2008;83(5):584-94.
3. Brambilla E, Gazdar A. Pathogenesis of lung cancer signalling pathways: Roadmap for therapies. Eur Respir J 2009;33(6):1485-97.
4. Siegfried JM, Weissfeld LA, Singh Kaw P, Weyant RJ, Testa JR, Landreneau RJ. Association of immunoreactive hepatocyte growth factor with poor survival in resectable non-small cell lung cancer. Cancer Res 1997;57(3):433-9.
5. Takanami I, Tanana F, Hashizume T, et al. Hepatocyte growth-factor and *c-met*: hepatocyte growth-factor receptor in pulmonary adenocarcinomas: An evaluation of their expression as prognostic markers. Onco 1996;53(5):392-7.
6. Ma PC, Maulik G, Christensen J, et al. c-Met: structure, functions and potential for therapeutic inhibition. Cancer Metastasis Rev 2003;22(4):309-25.
7. Christensen JG, Burrows J, Salgia R. c-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. Cancer Lett 2005;225(1):1-26
8. Nakamura Y, Niki T, Goto A, et al. c-Met activation in lung adenocarcinoma tissues: An immunohistochemical analysis. Cancer Sci 2007;98(7):1006-13.
9. Mountain CF. Revisions in the international system for staging lung cancer. Chest 1997;111(6):1710-7.
10. D'Amico TA, Brooks KR, Joshi MBM, et al. Serum protein recurrence in patients with early-stage lung cancer after resection. Ann Thorac Surg 2006;81(6):1982-7.
11. Sawabata N, Ohta M, Takeda S, et al. Serum carcinoembryonic antigen level in surgically resected clinical stage I patients with non-small cell lung cancer. Ann Thorac Surg 2003;74(1):174-9.
12. Sadoff L. The usefulness of carcinoembryonic antigen testing in the overall management of patients with non-small cell lung cancer. Am J Clin Oncol 1998;21(3):284-6.
13. Okuda K, Sasaki H, Yukie H, Yano M, Fuji Y. Met gene copy number predicts the prognosis for completely resected non-small cell lung cancer. Cancer Sci 2008;99(11):2280-5.
14. Olivero M, Rizzo M, Madeddu R, et al. Overexpression and activation of hepatocyte growth factor/scatter factor in human non-small cell lung carcinomas. Br J Cancer 1996;74(12):1862-8.
15. Chen J, Lin T, Chow K, et al. Cigarette smoking induces overexpression of hepatocyte growth factor in type II pneumocytes and lung cancer cells. Am J Respir Cell Mol Biol 2006;34(3):264-73.
16. Ichimura E, Maeshima A, Nakajima T, Nakamura T. Expression of c-met/HGF receptor in human non-small cell lung carcinomas in vitro and in vivo and its prognostic significance. Jpn J Cancer Res 1996;87(10):1063-9.
17. Harvey P, Warn A, Newman P, Perry LJ, Ball RY, Warn RM. Immunoreactivity for hepatocyte growth factor scatter factor and its receptor, met, in human lung carcinomas and malignant mesotheliomas. J Pathol 1996;180(4):389-94.

18. Tsao M, Liu N, Chen J, et al. Differential expression of c-met/hepatocyte growth factor receptor in subtypes of non-small cell lung cancers. Lung Cancer 1998;20(1):1-16.
19. Yi S, Tsao M. Activation of hepatocyte growth factor-met autocrine loop enhances tumorigenicity in a human lung adenocarcinoma cell line. Neoplasia 2000;2(3):226-34.
20. Siegfried JM, Luketich JD, Stabile LP, Christie N, Land SR. Elevated hepatocyte growth factor level correlates with poor outcome in early-stage and late-stage adenocarcinoma of the lung. Chest 2004;125(5Suppl):116-9.
21. Siegfried JM, Weissfeld LA, Luketich JD, Weyant RJ, Gubish CT, Landreneau RJ. The clinical significance of hepatocyte growth factor for non-small cell lung cancer. Ann Thorac Surg 1998;66(6):1915-8.
22. Taniguchi T, Kitamura M, Arai K, et al. Increase in the circulating level of hepatocyte growth factor in gastric cancer patients. Br J Cancer 1997;75(5):673-7.
23. Yamashita J, Ogawa M, Beppu T. Immunoreactive hepatocyte growth factor is present in tissue extracts from human breast cancer but not in conditioned medium of human breast cancer cell lines. Res Commun Chem Pathol Pharmacol 1993;82(2):249-52.
24. Harpole DH Jr, Herndon JE II, Young WG Jr, Wolfe WG, Sabiston DC Jr. Stage I non-small cell lung cancer: A multivariate analysis of treatment methods and patterns of recurrence. Cancer 1995;76(5):787-96.