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MYC Family DNA Amplification in 126 Tumor Cell Lines From Patients With Small Cell Lung Cancer

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Abstract We identified 126 tumor cell lines established from patients with small cell cancer at the NCI-Navy Medical Oncology Branch from 1977 through 1992. Extensive clinical information was available on 96 patients from whom these cell lines were established. These patients comprised approximately one fourth of the 407 patients treated on prospective therapeutic clinical trials during the same time period. The proportion of tumor cell lines established from previously untreated patients with both limited and extensive stage small cell lung cancer increased during the 16 years of the study ($P = 0.008$). *MYC* family DNA amplification was present in 16 of 44 (36%) tumor cell lines established from previously treated patients compared to 7 of 52 (11%) of tumor cell lines established from untreated patients ($P = 0.009$). *MYC* DNA amplification in tumor cell lines established from patients previously treated with chemotherapy continued to be associated with shortened survival ($P = 0.001$). The initiation of a policy to obtain tumor tissue for the purpose of selecting chemotherapeutic agents given to individual patients was associated with an increase in the proportion of patients from whom tumor cell lines could be established for both extensive and limited stage patients ($P = 0.0001$ and 0.05, respectively).

Key words: lung neoplasms, oncogenes, drug therapy, mortality, pathology

Three of the members of the *MYC* family, *MYC*, *NMYC*, and *LMYC*, have been shown to be amplified in tumors and tumor cell lines from patients with small cell lung cancer [1–18]. *MYC* DNA amplification has been the most frequently observed and is associated with a variant form of small cell lung cancer cell lines that have a more rapid growth rate than the classic type [2,19,20]. We have previously shown that *MYC* family DNA amplification is more common in tumors and tumor cell lines derived from small cell lung cancer patients previously treated with combination chemotherapy [7,10,14,21]. Furthermore, DNA amplification of *MYC* in tumor cell lines established from chemotherapy-treated patients is associated with a shortened survival time [7,14].

This supplement has attempted to provide a comprehensive list of all cell lines established at the NCI-Navy and NCI-VA Medical Oncology Branches. We have collected extensive clinical information on nearly all the patients with small cell lung cancer from whom cell lines were established. However, many of our cell lines have appeared in the literature (and included in this supplement) which have not been part of our analysis of *MYC* family DNA amplification and its association with small cell lung cancer patients' clinical status and course. Tumor cell lines established from patients treated on prospective clinical protocols and patients who have comprehensive data about their treatment have been included in this patient treatment analysis. Patients from whom tumor cell lines were established but not included in this analysis have footnotes explaining why they have been excluded. In addition, we have included references from our previous articles to show where previous reports of the cell lines which have had the

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MYC family DNA amplification information were published. We hope this information will be of use to other investigators undertaking analyses of the tumor cell lines.

MATERIALS AND METHODS

All small cell lung cancer cell lines established at the National Cancer Institute-Navy Medical Oncology Branch were noted. The patients with small cell lung cancer evaluated for primary treatment protocols during the same time period were identified. The patients underwent staging evaluations as previously described [22–24]. The patients were classified as having limited stage disease, defined as tumor limited to one hemithorax and bilateral hilar, mediastinal, and supraclavicular nodes, or extensive stage disease, defined as tumor spread beyond these areas. The limited stage patients were treated with cyclophosphamide based combination chemotherapy with or without chest irradiation [22] or etoposide cisplatin with concurrent chest radiotherapy followed by either an empiric cyclophosphamide, doxorubicin, and vincristine or an in vitro selected regimen [24]. The induction chemotherapy for the patients with extensive stage small cell lung cancer consisted of either etoposide plus cisplatin [23] or a cyclophosphamide based combination regimen as previously described [7]. Attempts to establish cell lines from the patients' tumor specimens were made as previously detailed [19,25,26]. Small cell lung cancer cell lines established in athymic nude mice were designated with the prefix NCI-N while those established in cell cuture media were designated NCI-H. Small cell lung cancer cell lines were identified as having been established from patients prior to the initiation of chemotherapy, or after one or more courses of combination chemotherapy. If more than one cell line was established from the same patient, only a single cell line was studied for *MYC* family DNA amplification so each patient was reported only once.

DNA was prepared from small cell lung cancer cell lines and *MYC, NMYC,* and *LMYC* DNA copy number of the tumor cell line determined as previously described [7]. The presence of high molecular weight DNA from the tumors and tumor cell lines was confirmed by agarose gel electrophoresis of the undigested DNA. The copy number of the *MYC* family genes was considered to be amplified if the signals were fourfold greater than the single copy gene controls.

The survival time of the patients was calculated from the date of initial chemotherapy to the date of the last follow-up or death. The differences in frequency of amplification between the totals of the treated and untreated groups, and between the totals of the two treated groups, were statistically compared using the chi-square test for comparing proportions. When sample numbers were small, Fisher's exact test was used. Mantel's test for linear trend in proportions was conducted to determine whether there were increasing or decreasing trends over time. Data were grouped when needed to achieve valid results [27]. The Kaplan-Meier survival curves for patients with family DNA amplified vs. non-amplified for each of the three *MYC* family members were statistically compared using the Mantel-Haenszel test [28,29]. All P values in this report are of the two-sided type.

RESULTS

Four hundred seven previously untreated patients with small cell lung cancer were entered into therapeutic protocols from 1977 to 1992

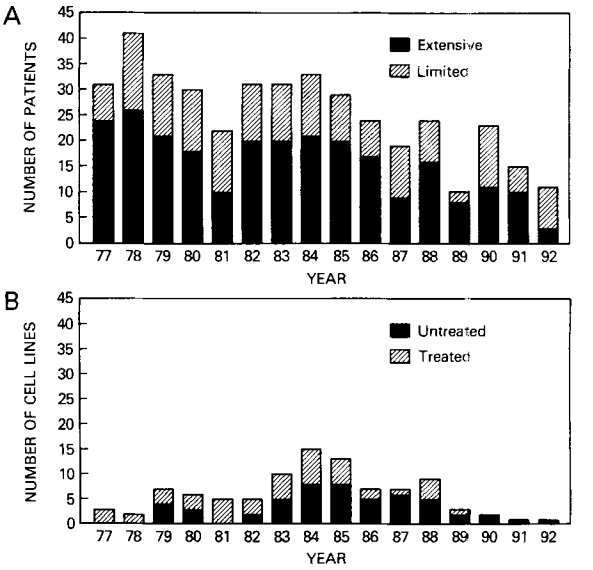

Fig. 1. **A:** Small cell lung cancer patients entered onto studies from 1977 to 1992. The number of extensive stage and limited stage patients entered onto study each year is depicted by the solid black and hatched bars, respectively. **B:** Small cell lung cancer patients who had tumor cell lines established from 1977 to 1992. The number of chemotherapy-treated and untreated patients who had tumor cell lines established entered onto study each year is depicted by the hatched and solid black bars, respectively. There was an increase in the proportion of tumor cell lines established from untreated small cell lung cancer patients in the latter years of the study period.

TABLE I. *MYC* Family DNA Amplification of Small Cell Lung Cancer Cell Lines*

Cell line number	*MYC* family amplification	Chemotherapy regimen	Reference	Cell line number	*MYC* family amplification	Chemotherapy regimen	Reference
1. NCI-H60	***MYC***	**CBC**	**[2,7,14]**	**56. NCI-H735**	**None**	**VP/PT**	**[7,14]**
2. NCI-H64	**None**	**CBC**	**[7,14]**	**57. NCI-H738**	**None**	**None**	**[7]**
3. NCI-H69	***NMYC***	**CBC**	**[7,14,17]**	**58. NCI-H740**	**None**	**None**	**[7,14]**
4. NCI-H82	***MYC***	**CBC**	**[2,7,14]**	**59. NCI-H748[m]**	**None**	**CBC**	**[7]**
5. NCI-H123[a]	**None**	**CBC**	**[7,14]**	**60. NCI-H774**	**None**	**None**	**[7]**
6. NCI-H128	**None**	**CBC**	**[2,7,14]**	61. NCI-H792[m]	Not done	CBC	
7. NCI-H146	**None**	**CBC**	**[2,7,14]**	**62. NCI-H841**	**None**	**CBC**	**[7]**
8. NCI-N177[b]	*MYC*	CBC	[2]	**63. NCI-H847**	***MYC***	**CBC**	**[7]**
9. NCI-H182	**None**	**CBC**	**[7,14]**	**64. NCI-H862**	**None**	**VP/PT**	**[7]**
10. NCI-H187	**None**	**None**	**[7,14,15,17]**	**65. NCI-H865**	**None**	**CBC**	**[7]**
11. NCI-H196	**None**	**CBC**	**[7,14]**	**66. NCI-H889**	***LMYC***	**None**	**[7]**
12. NCI-H209	**None**	**None**	**[7,14,15]**	**67. NCI-H890**	**None**	**None**	**[7]**
13. NCI-H211	***MYC***	**CBC**	**[7,14,19]**	**68. NCI-H930**	**None**	**CBC**	**[7]**
14. NCI-H220	**None**	**None**	**[7,14]**	69. NCI-H962[n]	Not done	Unknown	
15. NCI-H249[c]	*NMYC*	None	[15,17]	**70. NCI-H1008**	**None**	**CBC**	**[7]**
16. NCI-H250	**None**	**None**	**[7,14]**	**71. NCI-H1045**	**None**	**VP/PT**	**[7]**
17. NCI-H285	Not Done	None		**72. NCI-H1048[o]**	**Not done**	**None**	**[30]**
18. NCI-H289	**None**	**None**	**[7,14]**	73. NCI-H1059	*NMYC*	None	
19. NCI-H298	***LMYC***	**CBC**	**[7,14]**	**74. NCI-H1061[p]**	**None**	**None**	**[7]**
20. NCI-H345	**None**	**CBC**	**[7,14]**	75. NCI-H1062[p]	Not done	None	
21. NCI-H360[d]	*MYC*	Unknown	[19]	**76. NCI-H1086**	**None**	**CBC**	
22. NCI-H369	**None**	**CBC**	**[7,14]**	**77. NCI-H1092**	**None**	**None**	**[7]**
23. NCI-H372	***NMYC***	**CBC**	**[7,14,15,17]**	**78. NCI-H1105**	**None**	**None**	**[7]**
24. NCI-H378[e]	***LMYC***	**CBC**	**[7,14,15]**	**79. NCI-H1173**	**None**	**None**	**[7]**
25. NCI-H379[e]	Not done	CBC		**80. NCI-H1184**	**None**	**None**	**[7]**
26. NCI-N390	**None**	**None**	**[2,7,14]**	**81. NCI-H1185**	**None**	**CBC**	**[7]**
27. NCI-N408	**None**	**None**	**[7,14]**	**82. NCI-H1238**	**None**	**None**	
28. NCI-N417[f]	*MYC*	None	[2,7]	**83. NCI-H1284**	**None**	**None**	**[7]**
29. NCI-H432[g]	Not done	Unknown		**84. NCI-H1304**	***MYC***	**VP/PT**	**[7]**
30. NCI-H433[g]	Not done	Unknown		**85. NCI-H1339**	**None**	**None**	**[7]**
31. NCI-H446	***MYC***	**CBC**	**[2,7,14, 15,17]**	86. NCI-H1341[q]	Not done	None	[30]
32. NCI-H449[h]	**None**	**CBC**	**[7,14]**	**87. NCI-H1417**	**None**	**None**	**[7]**
33. NCI-H450[h]	Not done	CBC		**88. NCI-H1436**	**None**	**None**	**[7]**
34. NCI-H462[i]	***LMYC***	**None**	**[7,14,19]**	89. NCI-H1450[r]	Not done	None	
35. NCI-H463[i]	Not done	None		**90. NCI-H1451[r]**	**None**	**None**	**[7]**
36. NCI-H478[j]	None	Unknown	[2]	**91. NCI-H1514**	**None**	**None**	**[7]**
37. NCI-H510[k]	*LMYC*	CBC	[15,30]	**92. NCI-H1522**	**None**	**VP/PT**	
38. NCI-H524	***MYC***	**CBC**	**[7,14,19]**	**93. NCI-H1607**	**None**	**None**	
39. NCI-H526	***NMYC***	**None**	**[7,14,15,17]**	94. NCI-H1618[s]	Not done	None	
40. NCI-H568	**None**	**None**	**[7,14]**	**95. NCI-H1622[s]**	**None**	**None**	
41. NCI-H571	**None**	**CBC**	**[7,14]**	**96. NCI-H1628**	**None**	**None**	
42. NCI-H578	**None**	**CBC**	**[7,14,19]**	**97. NCI-H1672**	**None**	**None**	
43. NCI-H580	**None**	**CBC**	**[7,14]**	**98. NCI-H1688**	**None**	**None**	
44. NCI-N592[c]	*NMYC*	None	[17]	99. NCI-H1694[t]	*LMYC*	None	
45.NCI-H606	**None**	**CBC**	**[7,14,19]**	**100. NCI-H1769**	**None**	**None**	
46. NCI-H615	**None**	**None**	**[7,14]**	**101. NCI-H1788**	**None**	**VP/PT**	
47. NCI-H618	**None**	**None**	**[7,14]**	**102. NCI-H1836**	***LMYC***	**CBC**	
48. NCI-H620	**None**	**CBC**	**[7,14]**	103. NCI-H1870[u]	MYC	None	
49. NCI-H660	**None**	**None**	**[7,14]**	104. NCI-H1876[v]	Not done	None	
50. NCI-H678	**None**	**None**	**[7,14]**	105. NCI-H1881[v]	Not done	None	
51. NCI-H686[l]	**None**	**None**	**[7,14]**	**106. NCI-H1882[v]**	**None**	**None**	
52. NCI-H689	***NMYC***	**CBC**	**[7,14,17]**	**107. NCI-H1926**	**None**	**None**	
53. NCI-N691	***LMYC***	**CBC**	**[7,14,15]**	108. NCI-H1930[w]	None	None	
54. NCI-H711	**None**	**None**	**[7,14]**	**109. NCI-H1963**	**None**	**None**	
55. NCI-H719	**None**	**None**	**[7,14]**	**110. NCI-H1994[x]**	***LMYC***	**CBC**	

TABLE I. (continued)

Cell line number	*MYC* family amplification	Chemotherapy regimen	Reference	Cell line number	*MYC* family amplification	Chemotherapy regimen	Reference
111. NCI-H2028	**None**	**None**		120. NCI-H2196[aa]	Not done	None	
112. NCI-H2029[x]	None	CBC		121. NCI-H2198[aa]	Not done	None	
113. NCI-H2059[y]	**None**	**VP/PT**		**122. NCI-H2227**	**None**	**None**	
114. NCI-H2081	**None**	**VP/PT**		**123. NCI-H2330**	**None**	**None**	
115. NCI-H2107[z]	***LMYC***	**None**		**124. NCI-H2332**	**None**	**None**	
116. NCI-H2108[z]	Not done	None		**125. NCI-H2552**	***LMYC, NMYC***	**None**	
117. NCI-H2141[y]	None	VP/PT					
118. NCI-H2171	***MYC***	**VP/PT**		**126. NCI-H2679**	***MYC***	**None**	
119. NCI-H2195[aa]	**None**	**None**					

*Those cell lines designated NCI-N were established in athymic nude mice while those initially established in cell culture media were designated as NCI-H.

[a]NCI-H123 had been previously identified as a tumor cell line which came from a patient who had not received chemotherapy because the tumor specimen from which the tumor cell line was established was obtained only 8 days after starting combination chemotherapy. Therefore, the cell line is now listed as coming from a patient who has received chemotherapy.

[b]NCI-N177 was established from the same biopsy specimen which gave rise to NCI-H60.

[c]NCI-H249 and N592 were established from the same tumor specimen from a patient who had been previously treated with methotrexate for non-small cell lung cancer and was not treated on a protocol because of metachronous cancers.

[d]NCI-H360 was established from a patient identified as having extraosseous Ewing's sarcoma and was treated at another institution.

[e]NCI-H378 and NCI-379 were established from the same pleural effusion from the same patient and therefore were not evaluated separately.

[f]NCI-N417 was established from a patient's tumor specimen who was not treated at the National Cancer Institute and therefore is not included in the patient analysis.

[g]NCI-H432 and NCI-H433 were established simultaneously from different soft tissue lesions from the same patient. The patient was not treated at the National Cancer Institute so no clinical information was available and the cell lines were not studied for MYC family DNA amplification.

[h]NCI-H449 and NCI-H450 were established simultaneously from a bone marrow biopsy and from a lymph node biopsy of the same patient and therefore were not evaluated separately.

[i]NCI-H462 and NCI-463 were established simultaneously from bilateral bone marrow biopsies from the same patient and therefore were not evaluated separately.

[j]NCI-H478 was established from a patient's tumor specimen who was not treated at the National Cancer Institute and there is inadequate information to include this patient in the analysis.

[k]NCI-H510 was established from a patient with extrapulmonary small cell cancer which started in the brain and is therefore excluded from the patient analysis.

[l]NCI-H686 was reported in references [7,14] but did not become and permanent cell line so it is reported here but is not in the NCI-Navy Medical Oncology Branch Cell Line Data Base.

[m]NCI-H748 and NCI-H792 were established from the same patient who had previously received chemotherapy before both biopsies so is included only once in the patient analysis.

[n]NCI-H962 was established from a patient's tumor specimen who was not treated at the National Cancer Institute and therefore is not included in the patient analysis.

[o]NCI-H1048 was established from a patient with extrapulmonary small cell cancer which started in the uterus and is therefore excluded from the patient analysis.

[p]NCI-H1061 and NCI-1062 were established simultaneously from bilateral bone marrow biopsies from the same patient and therefore were not evaluated separately.

[q]NCI-H1341 was established from a patient with extrapulmonary small cell cancer which started in the cervix and is therefore excluded from the patient analysis.

[r]NCI-H1450 and NCI-1451 were established simultaneously from bilateral bone marrow biopsies from the same patient and therefore were not evaluated separately.

[s]NCI-H1618 and NCI-1622 were established simultaneously from a lymph node and bone marrow biopsy from the same patient and therefore were not evaluated separately.

[t]NCI-H1694 was established from a patient's tumor specimen who was not treated at the National Cancer Institute and therefore is not included in the patient analysis.

[u]NCI-H1870 was established from a small cell carcinoma of the cervix and is therefore not analyzed with the other small cell lung cancers.

[v]NCI-H1876, NCI-H1881, and NCI-1882 were established simultaneously from a lymph node biopsy and bilateral bone marrow biopsies from the same patient and therefore were not evaluated separately.

[w]NCI-H1930 was established from a patient's tumor specimen who was not treated at the National Cancer Institute and therefore is not included in the patient analysis.

[x]NCI-H1994 and NCI-H2029 were established from the same patient who had previously received chemotherapy before both biopsies so it is included only once in the patient analysis.

[y]NCI-H2059 and NCI-2141 were established from the same patient who had previously received chemotherapy before both biopsies so is included only once in the patient analysis.

[z]NCI-H2107 and NCI-2108 were established simultaneously from bilateral bone marrow biopsies from the same patient and therefore were not evaluated separately.

[aa]NCI-H2195, NCI-H2196, and NCI-2198 were established simultaneously from bilateral bone marrow biopsies and a lymph node biopsy from the same patient and therefore were not evaluated separately.

(Fig. 1A). Two hundred fifty-four patients (62%) had extensive stage disease and 153 (38%) had limited stage disease. There has been a gradual decline in the number of patients entered annually on prospective studies. There has been an increase in the proportion of patients with limited stage disease entered onto therapeutic studies but this did not achieve standard statistical significance ($P = 0.148$).

One hundred twenty-six small cell cancer cell lines were identified (Table I). Of these 124 cell lines, the information on clinical presentation, treatment, and clinical course was available on 96 patients from whom cell lines could be established. The 96 small cell cancer cell lines which form the basis of this report are printed in bold type in Table I.

Small cell cancer cell lines were established from 73 patients with extensive stage and 23 patients with limited stage disease. There has been an increase in the proportion of tumor cell lines established from previously untreated patients with small cell lung cancer from 1977 through 1992 (Fig. 1B; $P = 0.008$). In 1983 the extensive stage small cell lung cancer study [23] and in 1986 the limited stage study [24] were initiated. Both trials included systematic biopsy of tumors prior to the initiation of chemotherapy. The biopsies were performed in order to obtain tumor cells to provide drug sensitivity testing information which was utilized to select the second 12 weeks of chemotherapy. As a result of this policy, the percentage of untreated patients with extensive stage small cell lung cancer who had tumor cell lines established increased from 9 of 119 (8%) during 1977–1982 to 36 of 135 (27%) after the start of the new study in 1983 ($P < 0.0001$). Similarly, the percentage of untreated patients with limited stage small cell lung cancer who had tumor cell lines established increased from 2/101 (2%) during 1977–1985 to 5/52 (10%) during 1986–1992 ($P = 0.05$).

Forty-four tumor cell lines were established from patients previously treated with combination chemotherapy, 35 from patients given cyclophosphamide based regimens, and 9 from patients given the etoposide cisplatin induction regimen (Table I). Fifty-two tumor cell lines were established from patients who had not received chemotherapy.

NCI-H1870, NCI-H2171, and NCI-H2679 had *MYC* DNA amplification (Fig. 2). NCI-H1870 was excluded from the analysis because she had small cell carcinoma of the cervix and was treated by gynecologic oncologists. NCI-H1059 and NCI-H2552 had *NMYC* DNA amplification (Fig. 3). NCI-H1694, NCI-H1836, NCI-H1994, NCI-H2107, and NCI-H2552 had *LMYC* DNA amplification (Fig. 4). NCI-H1694 was not included in the analysis because the patient was not treated by us and insufficient clinical information was available. The other cell lines did not have *MYC* family DNA amplification or have been previously reported.

Sixteen of 44 (36%) cell lines established from patients who had previously received chemotherapy had *MYC* family DNA amplification compared to 7/52 (11%) cell lines established from patients who had not received any therapy ($P < 0.01$). Eleven of 28 (39%) tumor cell lines established from patients with extensive stage disease previously treated with chemotherapy had *MYC* family DNA amplification compared to 7 of 45 (15%) tumor cell lines established from

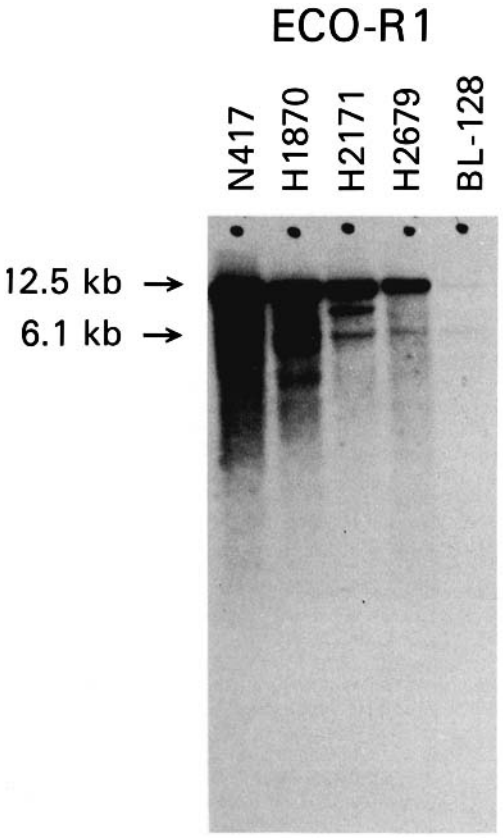

Fig. 2. Autoradiograms of DNA from small cell lung cancer cell lines hybridized to *MYC* and gastrin-releasing peptide (GRP) fragments. Ten micrograms of DNA prepared from small cell lung cancer and lymphoblastoid cell lines, digested with *Eco R1* restriction endonuclease, Southern blots prepared, and hybridized to a *MYC* and gastrin releasing peptide fragment. NCI-N417 is a cell line previously shown to have *MYC* DNA amplification. The *MYC* signal appears at 12.5 kb and the single copy 6.1-kb gastrin releasing peptide fragment signal appears below the *MYC* signal.

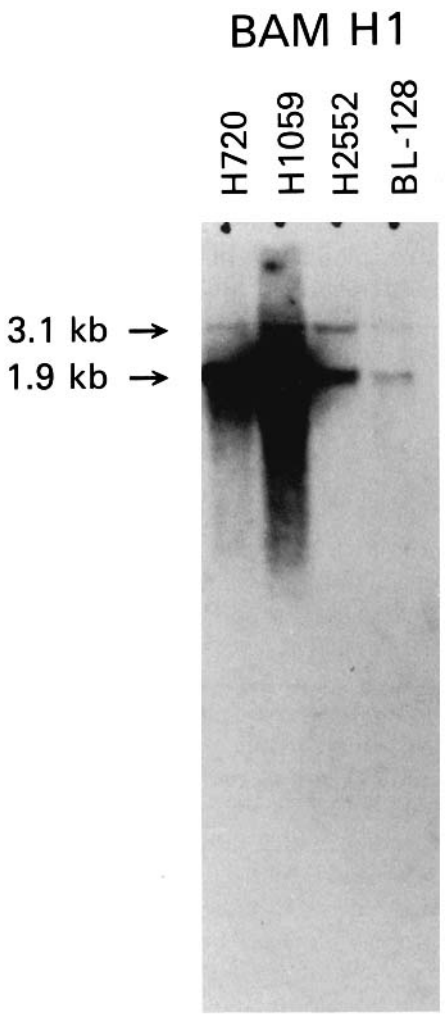

Fig. 3. Autoradiograms of DNA from small cell lung cancer and lymphoblastoid lines hybridized to *NMYC* and a gastrin releasing peptide probe. Ten micrograms of DNA prepared from small cell lung cancer and lymphoblastoid cell lines, digested with *Bam H1* restriction endonuclease, Southern blot prepared, and hybridized to a *NMYC* and gastrin releasing peptide fragment. NCI-H720 which has previously been shown to be amplified for *NMYC* was used as a control for an amplified tumor cell lines. The *NMYC* signal (1.9 kb) of small cell lung cancer cell lines are more intense than the lymphoblastoid cell line BL-128. The single copy gene control (gastrin releasing peptide) signal appears at 3.1 kb and the similar intensity of the signals in all lanes show similar amounts of DNA have been added to each lane.

patients who had not received any therapy ($P = 0.02$). Five of the 16 (31%) tumor cell lines established from patients with limited stage disease previously treated with chemotherapy had *MYC* family DNA amplification compared to none of 7 tumor cell lines established from similar patients who had not received any therapy. Therefore, *MYC* family DNA amplification appears more commonly in tumor cell lines established from patients with both limited and extensive stage small cell lung cancer after they have been treated with chemotherapy. Two of 9 (22%) patients treated initially with etoposide cisplatin prior to obtaining tumor for establishment of a cell line had *MYC* family DNA amplification compared to 14 of 35 (40%) tumor cell lines established from patients previously treated with cyclophosphamide based regimens ($P = 0.45$). Unfortunately, no patients had matched tumor cell lines established before and after treatment with chemotherapy.

Nine additional tumor cell lines established from patients with small cell lung cancer previously treated with chemotherapy have been studied since our last report [7]. Three of the nine tumor cell lines (NCI-H1836, NCI-H1994, and NCI-H2171) had *MYC* family DNA amplifica-

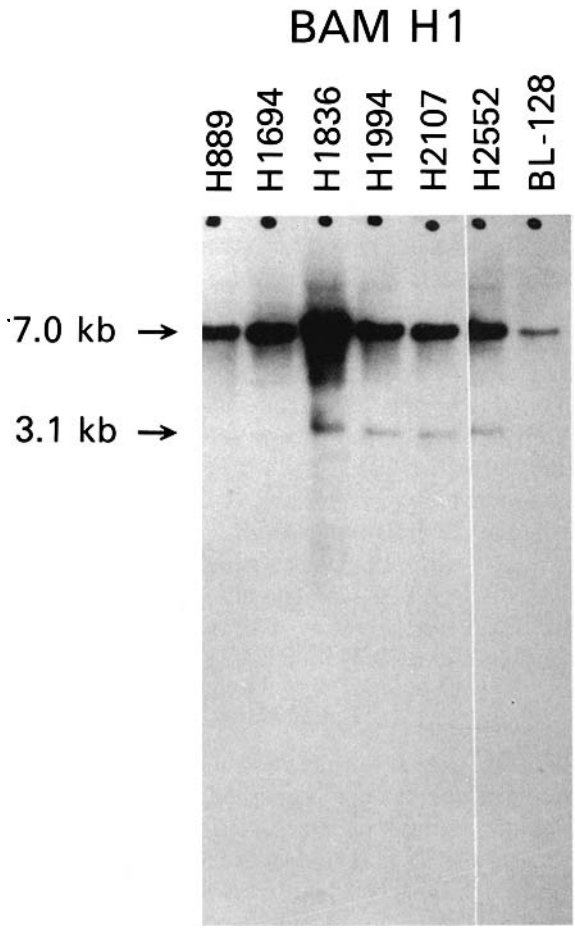

Fig. 4. Autoradiograms of DNA from small cell lung cancer cell lines hybridized to *LMYC* and gastrin releasing peptide fragments. The tumor cell line DNA was digested with BamHI and hybridized to an *LMYC* fragment and a gastrin-releasing peptide fragment. NCI-H889 is a tumor cell line previously shown to be amplified for *LMYC*. BL-128 is a lymphoblastoid cell line which has been previously examined and does not have *LMYC* DNA amplification. The single copy 3.1-kb Bam-HI gastrin-releasing peptide fragment signal appears below the *LMYC* fragment signal.

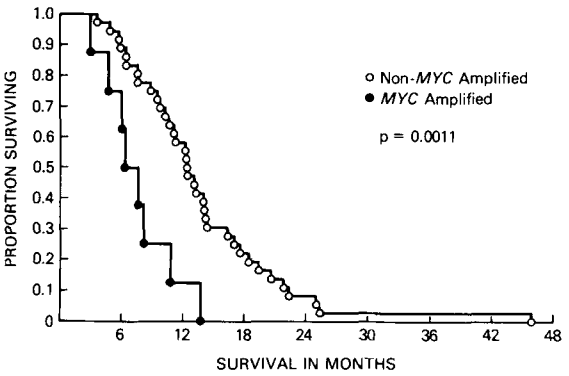

Fig. 5. Survival from the initiation of chemotherapy of small cell lung cancer patients whose cell lines established at relapse had *MYC* DNA amplification compared with patients whose cell lines did not. Patients whose cell lines had DNA amplification of *MYC* lived a shorter time than patients whose did not ($P = 0.0011$).

tion. The survival from the time of initiation of treatment for patients who had been previously treated with chemotherapy when their cell lines were established revealed with *MYC* DNA amplification survival was shorter (median of 25 weeks) than the survival of patients whose tumor cell lines did not have *MYC* DNA amplification (median of 54 weeks), similar to our previous findings (Fig. 5; $P = 0.001$).

Eighteen additional tumor cell lines have been established from previously untreated patients with small cell lung cancer since our last report [7]. Four of the 18 tumor cell lines have *MYC* family DNA amplification (NCI-H1059, NCI-H2107, NCI-H2552, and NCI-H2679). This included the first cell line (NCI-H2679) established at the NCI-Navy MOB from an untreated patient with *MYC* DNA amplification. In addition, NCI-H2552 was the first cell line which had DNA amplification of multiple *MYC* family members, both *LMYC* and *NMYC* (Figs. 3 and 4).

DISCUSSION

In this paper we have extended our previous observations of *MYC* family DNA amplification in tumor cell lines from patients with small cell lung cancer. In addition to the 69 small cell lung cancer cell lines [7,14] that have been previously reported, we have studied the *MYC* family DNA copy number from an additional 27 tumor cell lines and now analyze a total of 96 different patients with small cell lung cancer from whom tumor cell lines were established. We have continued to observe that *MYC* family DNA amplification is more frequently present after treatment with different combination chemotherapy regimens and that DNA amplification of *MYC* in tumor cell lines established from chemotherapy-treated patients is associated with a shortened survival time, similar to our previous reports.

We have again attempted to study the possible effect of different regimens of induction chemotherapy on *MYC* family DNA amplification. Only 2 of 9 (22%) tumor cell lines which were established from patients with small cell lung cancer previously treated with etoposide and cisplatin induction chemotherapy had *MYC* family DNA amplification. Although this is not statistically different from the cyclophosphamide treated group ($P = 0.45$), this is approximately one-half the rate of *MYC* family DNA amplification observed in tumor cell lines established from patients treated with the latter combinations (40%). Additional studies will be needed before this issue can be resolved.

An increasing proportion of our small cell lung cancer patients have had a cell line established from 1977 through 1992 ($P = 0.008$). This has been associated with the introduction of the current extensive stage small cell lung cancer study in 1983 and of the current limited stage small cell lung cancer therapeutic study in 1986. These studies which link obtaining patients' tumors for individualized chemotherapy determinations have been associated with an increase in the proportion of cell lines established from a defined cohort of both untreated limited and extensive stage patients entering a study ($P = 0.05$ and < 0.0001, respectively).

The proportion of *MYC* family DNA amplified cell lines which are established from chemotherapy treated patients with small cell lung cancer has decreased since 1983. Additional studies will need to be done to determine if specific chemotherapy regimens can actually induce *MYC* family DNA amplification in tumor and/or tumor cell line specimens obtained before and after cytotoxic therapy. We believe the studies presented here show that the presence of *MYC* family DNA amplification in tumor cell lines established from patients with small cell lung cancer can be affected by the patients' treatment status, and the initial observations 7 years ago have remained the same after study of another 52 cell lines.

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