Clinical Trial

1E10 Anti-Idiotype Vaccine in Non-Small Cell Lung Cancer

Experience in Stage IIIb/IV Patients

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ABSTRACT

Conventional treatment of non-small cell lung cancer (NSCLC) has apparently reached a plateau of effectiveness in improving the survival of the patients. For that reason the search for new therapeutic strategies in this type of tumor is justified. 1E10 is an anti-idiotype murine monoclonal antibody (Ab2 MAb) specific to P3 Ab1 MAb, which reacts with NeuGc-containing gangliosides, sulfatides and with antigens expressed in some tumors, including those from the lung. We report the treatment with aluminum hydroxide-precipitated 1E10 MAb of 34 stage IIIb and 37 stage IV NSCLC patients. These patients were treated with the anti-idiotype vaccine, after received standard chemotherapy and radiotherapy, in a compassionate-use basis study. Patients received five bi-weekly injections of 1 mg of 1E10/Alum, other 10 doses at 28-day intervals and later the patients who maintained a good performance status continued to be immunized at this same time interval. No evidence of unexpected or serious adverse effects was reported. The median survival time of the 56 patients who entered the study with partial response or disease stabilization and with a PS 1 after the first line of chemo/radiotherapy, was 11.50 months from starting vaccination. In contrast, the median survival time calculated for patients who started vaccination with progressive disease and/or a PS2 was 6.50 months.

INTRODUCTION

Lung tumors constitute the first cause of death among the neoplasias and every year 1.2 million new cases are diagnosed worldwide. Nonsmall cell lung tumors (NSCLC) constitute the predominant histological type among the lung neoplasias, representing between 75 and 80% of all tumors. Approximately 50 to 70% of the patients are diagnosed with unresectable metastatic disease (stage IIIb and IV).[1,2]

International practice in the treatment of stage IIIb tumors consists in the administration of chemotherapy associated or not with thoracic radiotherapy while chemotherapy is proposed as the treatment of choice for the patients in stage IV (NCI treatment guidelines for unresectable NSCLC, version 2, 2006).

The combinations of two agents: platinum based (cisplatin or carboplatin) together with one or two of the new drugs (paclitaxel, docetaxel, gemcitabine, vinorelbine, premetrexed) have shown an additional improvement in survival in comparison to the therapy with a single agent or with older regimens.[3-6]

Nevertheless, the median survival time even after the best available oncospecific therapy is limited to approximately 8–9 months and a 1-year survival rate of 30–36%.[4,6-8] The benefits of these therapies were observed mostly in patients with good performance status (PS). The median survival among patients with a PS0 is 9–17 months, as compared with 6–7 months for PS1 and 3–4 months for PS2.[4,9,10]

In consequence, new strategies should be tested to achieve a greater therapeutic impact in the patients bearing NSCLC.

Among the treatment strategies to develop an effective immune response against tumor-associated antigens is the use of anti-idiotype (Ab2) MAb as antigen surrogates.[11-17] The use of Ab2 as vaccines arose as a consequence of Jerne's theory,[18] that postulates the existence of Ab2 that carry the "internal image" of antigenic epitopes.

Attractive targets for immunotherapy with anti-idiotype MAbs are Neu-glycolyl (NeuGc)-containing gangliosides, because these glycolipids are not normal components of the cytoplasmatic membrane in humans. The absence of NeuGc-neuraminic acid in
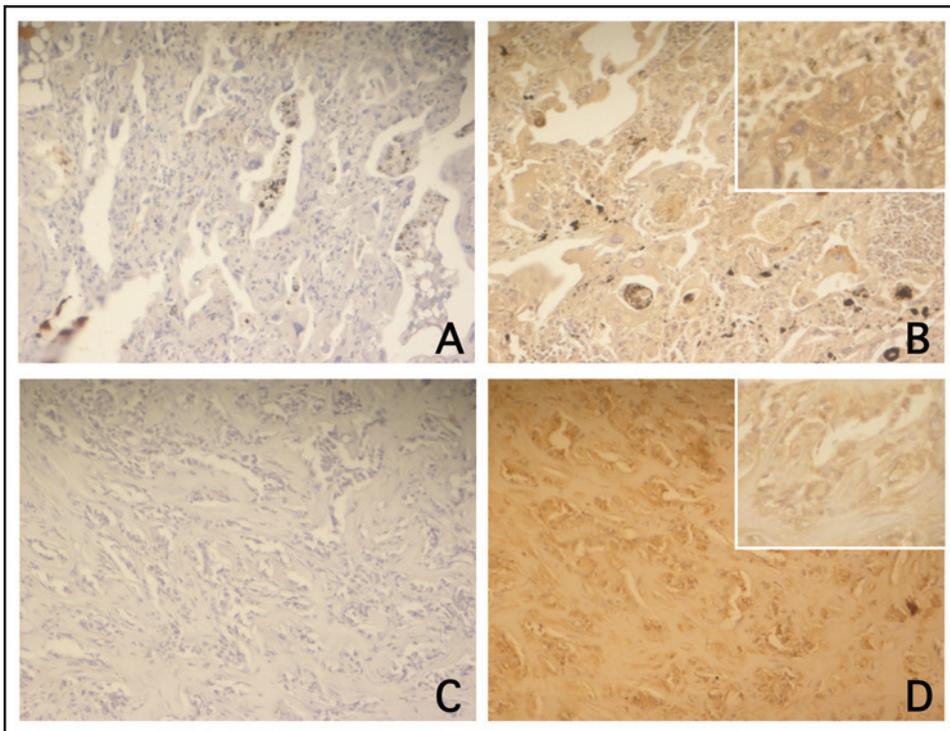
Figure 1. Immunohistochemical staining of lung cancer cells using the P3 MAb (Ab1). 5 μm formalin fixed nonsmall cell lung tumor sections were incubated with the P3 MAb at a concentration of 20 μg/mL in phosphate-buffer saline. Slides were then incubated with biotinylated anti-mouse IgM, followed peroxidase-conjugated avidin, using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA). Bound antibodies were detected by incubation with diaminobenzidine and tumor sections were counterstained with hematoxylin. Results obtained with adenocarcinoma (A, B) or squamous cell carcinoma (C, D) tumor sections are depicted. Staining with IgM isotype control (A, C) or P3 anti-ganglioside MAb (B, D). Original magnification X200; insets X400.

humans is due to the inactivation of the gene for the enzyme responsible for NeuGc biosyntesis.[19-21]

The presence of small amounts of this kind of sialic acid was reported few years ago in some human normal tissues, probably originated by the ingestion of food from mammalian origin.[22] Nevertheless, it is noteworthy that there is a differential pattern of expression of NeuGc in normal and malignant human tissues.[23-28]

We previously reported a vaccine preparation that contains a murine anti-idiotype MAb related to NeuGc-containing ganglioside antigen model. This Ab2, named 1E10,[29] was generated from the immunization of BALB/c mice with the P3 MAb, an Ab1 antibody that recognizes gangliosides having the NeuGc sialic acid, sulphated glycolipids and antigens present in different human tumors, including those from the lung[26,30-33] (see also Fig. 1).

1E10 anti-idiotype MAb was able to induce specific antibody response against NeuGc-containing gangliosides in chickens;[34] where like in humans these gangliosides are not expressed in normal tissues.[35,36] Preparations containing 1E10 MAb were able to induce anti-tumor effects against lung metastases in murine models.[37]

Different phase I clinical trials have been conducted in patients with advanced melanoma, breast cancer and small-cell-lung cancer (SCLC) using aluminum hydroxide precipitated-1E10 MAb. The results of these clinical trials demonstrated the low toxicity and the high immunogenicity of this anti-idiotype vaccine.[32,33,38,39] In particular, a prolonged survival was observed in several patients with

SCLC treated with the vaccine, and in one patient a complete regression of the tumor was reported.[33]

Here, we report the outcome of the treatment with 1E10 anti-idiotype vaccine in stage IIIb/IV NSCLC patients included in a compassionate-use study, after completion oncospecific first line treatment.

MATERIAL AND METHODS

Anti-idiotype 1E10 MAb for the clinical trial. 1E10 MAb was purified from mouse ascites in the Good Manufacturing Practice (GMP) facilities of the Center of Molecular Immunology. Purification of 1E10 MAb was performed by DEAE-exchange chromatography followed by affinity chromatography on Protein A-CL Sepharose 4B column and size exclusion chromatography on Sephadex G-25 column (Amersham Pharmacia Biotech, Uppsala, Sweden). The purity of the isolated immunoglobulin was more than 97% as determined by SDS-PAGE, high-pressure liquid chromatography, and isoelectric focusing. The vaccine was produced in accordance with the Good Manufacturing Practice guidelines and certified by the Quality Control Department of the Center of Molecular Immunology. Briefly, sterile purified 1E10 MAb was aseptically mixed at a final concentration of 1 mg/mL with 5 mg/mL of aluminum hydroxide as adjuvant (Superfos Biosector, Frederikssund, Denmark). The mixture was gently stirred for three hours at room temperature. The aluminum hydroxide-precipitated MAb was aliquoted into pyrogen-free, sterile glass vials and stored at 4°C until use. The final product was tested for sterility, pyrogenicity, and general safety in mice and guinea pigs before use, according to United States Pharmacopoeia[40,41] and to British Pharmacopoeia.[42]

Patients. Patients with histo or cytological confirmed advanced NSCLC were eligible for enrollment in the compassionate-use study, after providing written, informed consent. Staging was performed according to the criteria established for NSCLC by AJCC (2003). Prior radiotherapy and chemotherapy had to be completed 4 weeks before enter the study. Other eligibility criteria included WHO performance status ≤2, age ≥18 years, normal haematopoietic, hepatic and renal functions, and life expectancy higher than 3 months. The most important exclusion criteria included the presence of brain metastases, pregnancy or lactation, serious chronic diseases and active infections. Patients who had received treatment with MAbs or other biological modifier of the immune response were not included.

Treatment Schedule. Patients were injected intradermally with 15 doses of 1 mg of aluminum hydroxide-precipitated 1E10 MAb, as base treatment. The first five doses were administered every 14 days and the remaining ten doses were administered every 28 days. After 15 doses, reinmunizations were administered at 28 day intervals, if the patients maintained a favorable clinical status.

Table 1 **Characteristics of patients included in the study**

Characteristic	No of Patients
Sex	
Male	42
Female	29
Age (years)	
Median (range)	59 (34–80)
>60 years	32
≤60 years	39
Disease stage	
IIIb	34
IV	37
Who performance status	
PS1	61
PS2	10
Anatomopathological classification	
Adenocarcinoma	15
Squamous cell carcinoma	45
Large cell carcinoma	11
Toxic habits	
Smokers/ex-smokers	65
Nonsmokers	6
Chemo/Radiotherapy Response	
PR	11
SD	53
PD	7
No doses of 1E10 MAb	
<5	4
5–14	53
>15	14

Table 2 **Summary of the adverse events**

Event	Grade	
	1	2
Injection site pain	60	
Local erythema	60	
Injection site induration	71	
Fever	15	6
Cephalea	6	
Arthralgias	11	
Pruritus	38	
High blood pressure	3	

Evaluation before and during treatment. Baseline evaluation included medical and smoking history, physical examination, vital signs, performance status, complete blood cell count and blood biochemistry, and tumor assessment by X-rays as well as computer tomography scans. Follow-up evaluation (every month) included physical exam, imaging studies, complete blood count and biochemistry. Adverse events were evaluated according to the NCI Common Toxicity Criteria (version 3.0).

Patients who received one or more vaccine doses were evaluable for toxicity and clinical results.

Nonrandomized control. As a provisional reference control, data from 163 patients who were treated with the same radio-chemotherapy were used. This information, already published,[43] was kindly supply by Dr. E. Neninger (Department of Medical Oncology, Hermanos Ameijeiras Hospital, Havana City). Among these patients, 109 were in stage III and 54 were in stage IV. Ninety patients had PS0-1 and 73 had a PS2.

Statistical analysis. Survival times were estimated using the Kaplan-Meier method using the SPSS Program (version 10.0) and were compared using the Log-rank test.

RESULTS

Patient characteristics. Seventy one patients with histological or cytological confirmed advanced NSCLC were included in the study. Patient's characteristics are presented in Table 1. Forty-two patients were men and 29 were women. Median age was 59 years (range 34–80 years). All the patients have previously received the oncospecific treatment established in the Oncological Therapeutic Standards, according to their stage at the moment of the diagnosis by the NCCN guidelines (version 2.0, 2006). Chemotherapy consisted mainly on cisplatin/vinblastin as first line. After finishing standard therapy, 11 patients reached partial response (PR), 53 patients had stable disease (SD) while in seven patients their disease was in progression (PD). Thirty four and thirty seven patients had stage IIIb and IV, respectively. Fifhteen patients had adenocarcinoma, 45 squamous cell carcinoma and 11 large cell carcinoma. The sites of metastases were bones, liver, contralateral lung, axillary and supraclavicular lymph nodes. Sixty-one patients had a PS1 and 10 had a PS2. Most of the patients were smokers or ex-smokers (65).

Vaccine administration. Of the 71 patients included in the study 14 received more than 15 doses of the anti-idiotype vaccines, 53 received between 5–15 doses and only four received less than five doses.
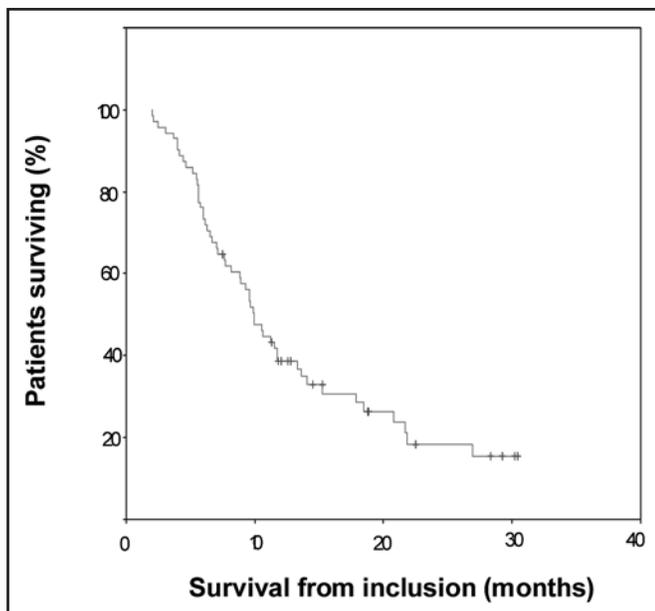

Figure 2. Kaplan-Meier overall survival curve. On the y-axis, the percentage of surviving is reported; on the x-axis, the time from entrance the study (months) is reported.

Toxicity. All patients were assessed for toxicity and the adverse events observed are shown in Table 2. The overall toxicity of 1E10 anti-idiotype vaccine was classified as grade 1 and 2, according to the NCI Common Toxicity Criteria (version 3.0). The most common side-effects were local reaction at the injection site with erythema and induration occasionally associated to mild pain that disappeared in a few days (1–3 days). Some patients had fever, pruritus, arthralgias and headache. All the symptoms were independent on the number of doses administered and they lasted between 1–3 days. An increase in blood pressure was reported in three patients, but these patients had a previous history of hypertension. Neither biochemical nor haematological abnormalities were reported.

Clinical outcome. At the time of analysis 53 patients have died and 18 patients remain alive. Among survivors, fourteen are alive after 12 months or longer of being included in the study. The overall survival time of the patients from starting vaccination was 9.93 months (95% CI, 8.61–11.25); 1-year survival rate was 34% (Fig. 2).

Although this was a compassionate-use study, a preliminary evaluation of the effect of vaccination on survival was performed by comparing survival of vaccinated patients with a nonrandomized control group. The median survival time of these controls was significantly lower (median 4.53 months; (95% CI, 3.70–5.56) (Log-rank test, p < 0,0001) than the one of the patients treated with 1E10 anti-idiotype MAb.

In Table 3, the analysis of patient's survival according to different variables is shown after entering in the study. By disease stage, the median survivals were 9.93 (95% CI, 7.44–12.43) and 9.53 (95% CI, 6.12–12.95) for stages IIIb and IV, respectively. No statistically significant difference was detected among these two patient's strata (Table 3). There were no significant differences in survival between vaccinated patients according to their sex, age or tumor histology (Table 3).

When the comparison in survival was performed between patients with different clinical responses to the first line of chemotherapy and with different performance status (PS1 vs. PS2), significant differences were observed between them (Table 3 and Fig. 3).

It is noteworthy that the median survival time of the 56 patients (79%) who entered the study with partial response or disease stabilization and with a PS 1 after the first line of chemotherapy, was 11.50 months (95% CI, 7.97–15.03 months) and 1-year survival rate was 39%. This survival was significantly longer (Log-rank test, p = 0.002) as compared with the one calculated for patients who started vaccination with progressive disease and/or a PS2 (median survival 6.50 months; 95% CI, 4.31–8.69 months; 1-year survival rate 13%) (Fig. 3).

There was also a significant difference (Log-rank test, p < 0.0001) when the comparison in survival was performed between the above mentioned 56 vaccinated patients and a subgroup of 50 control patients who reached partial response or disease stabilization after the oncospecific therapy and had a PS 1 (median 5.70 months; 95% CI, 3.27–8.13).

Table 3 **Survival analysis of patients treated with 1E10 anti-idiotype vaccine from inclusion time**

Variable	Number of Patients	Median Survival (Months)	95% CI	p
Sex				
Female	29	7.73	1.99–13.48	0.526
Male	42	9.93	7.90–11.96	
Age				
>60	32	9.93	8.68–11.19	0.644
≤60	39	8.17	4.69–11.64	
Disease stage				
IIIB	34	9.93	7.44–12.43	0.535
IV	37	9.53	6.12–12.95	
Performance status				
1	61	10.60	8.41–12.79	0.0486
2	10	6.50	2.06–10.94	
Response to first-line therapy				
PR	11	15.27	–	
SD	53	9.93	8.17–11.70	0.0064
PD	7	6.27	3.44–9.09	
Histological classification				
Adenocarcinoma	15	8.80	6.82–10.78	
Squamous cell carcinoma	45	10.57	6.48–14.65	0.1387
Large cell carcinoma	11	8.87	3.36–14.37	

DISCUSSION

NSCLC patients diagnosed in the late stages have a very poor prognosis and the life expectancy, even with the best oncospecific therapies, is short. Cytotoxic drugs that are registered at present as a second line of chemotherapy for the treatment of advanced NSCLC, as docetaxel (taxotere) and pemetrexed (alimta), are associated with a significant toxicity, mainly neutropenia grade 3–4 and febrile neutropenia. The median survival after the use of these drugs is approximately 8–9 months.[3-6]

Thus, the search for new therapeutic strategies that improve the survival of the patients suffering from advanced NSCLC, preserving their quality of life, is a priority. Among these new therapeutic approaches is the use of inhibitors of EGF-receptor signalization. Two of these molecules were registered for this indication by FDA (Food and Drug Administration, USA). Iressa (gefitinib) was approved as third line treatment of NSCLC, while Tarceva (erlotinib) received approval as second or third line therapy.[44,45] Iressa failed to demonstrate benefit in survival in a postmarketing clinical study while Tarceva evidenced only a modest increase in survival when compared with a concurrent control treated with placebo (6.7 vs. 4.7 months).[46-48]

Either the addition of trastuzumab, a MAb directed against the human epidermal growth factor receptor 2 (HER2) to platinum- or taxane- based chemotherapies did not improve survival of advanced NSCLC patients compared with chemotherapy alone.[49,50]

On the other hand, the addition of bevacizumab (anti-VEGF MAb) to standard platinum-based two-agent chemotherapy had a significant survival benefit in nonsquamous-cell carcinoma patients
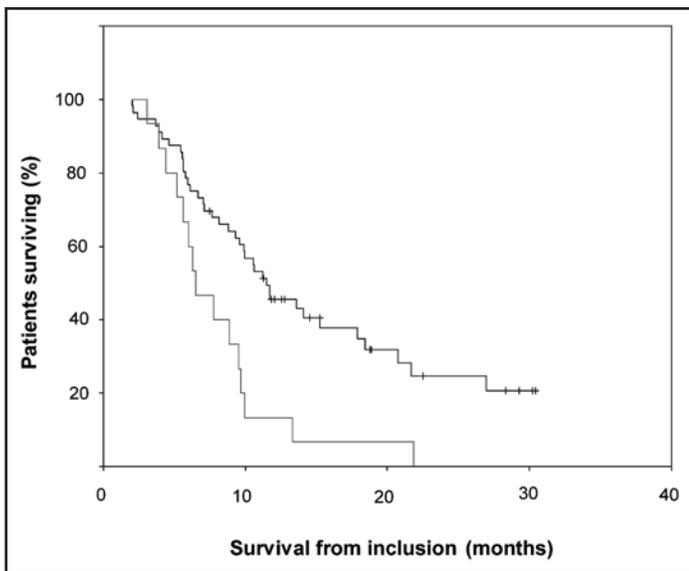
Figure 3. Kaplan-Meier survival curves of patients who entered the study with partial response or disease stabilization and with a PS 1 after the first line of chemotherapy (black) (median 11.50 months) or with progressive disease and/or a PS2 (grey) (median 6.50 months) (Log-rank test, p = 0.002). On the y-axis, the percentage of surviving is reported; on the x-axis, the time from entrance the study is reported.

(stage IIIB or IV), but accompanied of the risk of increased treatment-related deaths.[51-53]

Another focus of target therapy in NSCLC is the glycoprotein MUC1 that is overexpressed and aberrantly glycosylated in NSCLC.[54] The results of a randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV NSCLC showed that there was not statistical survival difference between the L-BLP25 arm and the one that did not receive the vaccine. Nevertheless, the results suggested that MUC1 vaccination could have an effect on survival in a selected subgroup of NSCLC patients.[55] Other vaccine strategies for advanced NSCLC are being now in clinical evaluation.[43,56-58]

This is the first report in the use of an anti-idiotype MAb related to NeuGc-containing gangliosides for the active immunotherapy of advanced NSCLC.

Although the patients were repeatly injected with 1E10/Alum and even when some of them received more than 15 doses of this vaccine, only a low rate of side effects was observed, confirming previous reports about the safety of the treatment with this anti-Id vaccine.[32,33,38,39]

The overall survival of the patients who entered the study was superior to the one reported recently[43] for a group of more than one hundred advanced NSCLC patients receiving standard oncospecific treatment in our country (9.93 vs. 4.53 months).

In particular, it is of interest that there are not statistical differences between 1E10/Alum vaccinated patients who had lung tumors of different histological classification. It is noteworthy that most of the patients who entered the study had squamous cell carcinomas, histological type tumor of the patients who did not showed benefit from bevacizumab treatment.[51-53]

Other encourage results of the present study was the fact that there was not survival differences between patients younger or older than 60 years, and that the effect of the anti-Id vaccination was similar for NSCLC patients in stages IIIb and IV.

The benefits that targeted agents have added to the standard oncospecific therapies in advanced NSCLC have been restricted to patients' subgroups.[43,51,52,55] In this study, the survival of the patients who started 1E10 MAb treatment with at least disease stabilization after the end of standard therapy and with a PS1, was significant greater compared with those patients with progressive disease and/or a PS2. To assess whether this advantage in survival could be due to the vaccination with 1E10 MAb, a comparison was performed with the median survival time calculated for a subgroup of the control patients with a PS 1 who achieved partial response or stable disease after the oncospecific therapy, and there was a statistical significant difference between the two groups (11.50 vs. 5.70 months). Thus, the treatment with the anti-Id vaccine seems to prolong survival of this group of patients.

The results reported here are promising and a Phase II randomized trial is now ongoing in NSCLC patients with the characteristics mentioned above, to confirm the clinical effect of 1E10 MAb vaccine and to evaluate the correlation between the immune responses that induce this anti-idiotype antibody preparation and patients' survival.

References

1. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ, Thun MJ. Cancer statistics, 2005. CA Cancer J Clin 2005; 55:10-30.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global Cancer statistics, 2002. CA Cancer J Clin 2005; 55:74-108.
3. Bunn Jr PA, Kelly K. Combinations of three chemotherapeutic agents and two chemotherapeutic agents plus a targeted biologic agent in the treatment of advanced non small-cell lung cancer. Clin Lung Cancer 2000; 2(Suppl 1):S23-28.
4. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH, Eastern Cooperative Oncology Group. Comparison of four chemotherapy regimens for advanced nonsmall cell lung cancer. N Engl J Med 2002; 346:92-98.
5. Cohen MH, Johnson JR, Chen YF, Sridhara R, Pazdur R. FDA drug approval summary: Pemetrexed for injection (Alimta) for the treatment of nonsmall cell lung cancer. Oncologist 2005; 10:363-368.
6. Reck M. Current approaches in chemotherapy of advanced and metastatic nonsmall cell lung (NSCLC). Anticancer Res 2005; 25:1501-1503.
7. D'Addario G, Pintilie M, Leighl NB, Feld R, Cerny T, Shepherd FA. Platinum-based versus nonplatinum-based chemotherapy in advanced nonsmall-cell lung cancer: A meta-analysis of the published literature. J Clin Oncol 2005; 23:2926-2936.
8. Cobo M, Villar E, Ales I, Gil S, Alcalde J, Gutierrez V, Carabantes F, Montesa A, Breton JJ, Benavides M. Gemcitabine and vinorelbine followed by weekly docetaxel in patients with advanced nonsmall-cell lung cancer: A phase II trial of sequential chemotherapy. Clin Transl Oncol 2006; 8:742-749.
9. Langer CJ. Dilemmas in management: The controvertial role of chemotherapy in PS2 advanced NSCLC and the potential role of CT-2103 (Xyotax TM). The Oncologist 2004; 9:398-405.
10. Cobo M, Gutierrez V, Alcaide J, Ales I, Villar E, Gil S, Duran G, Martinez J, Carabantes F, Breton JJ, Benavides M. A phase II study of days 1 and 8 combination of docetaxel plus gemcitabine for the second-line treatment of patients with advanced nonsmall-cell lung cancer and good performance status. Lung Cancer 2007.
11. Chakraborty M, Foon KA, Kohler H, Bhattacharya-Chatterjee M. Preclinical evaluation in nonhuman primates of an anti-idiotypic antibody that mimicks the carcinoembryonic antigen. J Immunother Emphasis Tumor Immunol 1995; 18:95-103.
12. Durrant LG, Buckley DJ, Spendlove I, Robins RA. Low doses of 105AD7 cancer vaccine preferentially stimulate anti-tumor T-cell immunity. Hybridoma 1997; 16:23-26.
13. Foon KA, Sen G, Hutchins L, Kashala OL, Baral R, Banerjee M, Chakraborty M, Garrison J, Reisfeld RA, Bhattacharya-Chatterjee M. Antibody responses in melanoma patients immunized with an anti-idiotype mimicking disialoganglioside GD2. Clin Cancer Res 1998; 4:1117-1124.
14. Foon KA. Immunotherapy for colorectal cancer. Curr Oncol Rep 2001; 3:116-126.
15. Reinartz S, Kohler S, Schlebusch H, Krista K, Giffels P, Renke K, Huober J, Mobus V, Kreienberg R, DuBois A, Sabbatini P, Wagner U. Vaccination of patients with advanced ovarian carcinoma with the anti-idiotype ACA125: Immunological response and survival (phase Ib/II). Clin Cancer Res 2004; 10:1580-1587.

16. Riemer A, Kurz H, Klinger M, Scheiner O, Zielinski C, Jensen-Jarolim E. Vaccination with cetuxiMAb mimotopes and biological properties of induced anti-epidermal growth factor receptor antibodies. J Natl Cancer Inst 2005; 97:1663-1670.

17. Chong G, Bhatnagar A, Cunningham D, Cosgriff T, Harper P, Steward W, Bridgewater J, Moore M, Cassidy J, Coleman R, Coxon F, Redfern C, Jones J, Hawkins R, Northfelt D, Sreedharan S, Valone F, Carmichael J. Phase III trial of 5-fluorouracil and leucovorin plus either 3H1 anti-idiotype monoclonal antibody or placebo in patients with advanced colorectal cancer. Ann Oncol 2006; 17:437-442.

18. Jerne NK. Toward a network theory of the immune system. Ann Immunol 1974; 125C:373-389.

19. Irie A, Suzuki A. CMP-Neu-acetylneuraminic acid hydroxylase is exclusively inactive in humans. Biochem Biophys Res Commun 1998; 248:330-333.

20. Chou H, Takematsu H, Díaz S, Iber J, Nickerson E, Wright KL, Muchmore E, Nelson DL, Warren ST, Varki A. A mutation in human CMP-sialic acid hydroxylase occurred after the Homo- Pan divergence. PNAS 1998; 95:11751-11756.

21. Olson MV, Varki A. Sequencing the chimpanzee genome: Insights into human evolution and disease. Nat Rev Genet 2003; 4:20-28.

22. Tangvoranuntakul P, Ganneux P, Díaz S, Bardor M, Varki N, Varki A, Muchmore E. Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. PNAS 2003; 100:12045-12050.

23. Higashi H, Nishi Y, Kukui Y, Ikuta K, Ueda S, Kato S, Fujita M, Nakano Y, Taguchi T, Sakai S, Sako M, Naiki M. Tumor-associated expresion of glycosphingolipid Hanganutziu-Deicher antigen in human cancers. Gann 1984; 75:1025-1029.

24. Higashi H, Sasabe T, Fukui Y, Maru M, Kato S. Detection of ganglioside as N-glycolil neuraminic acid-specific tumor-associated Hanganutziu-Deicher antigen in human retino-blastoma cells. Jpn J Cancer Res 1988; 79:952-956.

25. Devine PL, Clark BA, Birrel GW, Layton GT, Ward BG, Alewood PF. The breast tumor-associated epitope defined by monoclonal antibody 3E1.2 is an O-linked mucin carbohydrate containing N-glycolylneuraminic acid. Cancer Res 1991; 51:5826-5936.

26. Marquina G, Waki H, Fernández LE, Kon K, Carr A, Valiente O, Pérez R, Ando S. Gangliosides expressed in human breast cancer. Cancer Res 1996; 56:5165-5171.

27. Malykh Y, Schauer R, Shaw L. Neu-glycolylneuraminic acid in human tumors. Biochimie 2001; 83:623-634.

28. Yin J. Hypoxic culture induces expression of sialin, a sialic acid transporter, and cancer-associated gangliosides containing nonhuman sialic acid on human cancer cells. Cancer Res 2006; 15:2937.

29. Vázquez AM, Perez A, Hernandez AM, Macias A, Alfonso M, Bombino G, Perez R. Syngeneic anti-idiotypic monoclonal antibodies to an anti-NeuGc-containing ganglioside monoclonal antibody. Hybridoma 1998; 17:527-534.

30. Vázquez AM, Alfonso M, Lanne B, et al. Generation of a murine monoclonal antibody specific for N-glycolylneuraminic acid-containing gangliosides that also recognizes sulfated glycolipids. Hybridoma 1995; 14:551-556.

31. Moreno E, Lanne B, Vazquez AM, Kawashima I, Tai T, Fernandez LE, Karlsson KA, Angstrom J, Perez R. Delineation of the epitope recognized by an antibody specific for N-glycolylneuraminic acid-containing gangliosides. Glycobiology 1998; 8:695-705.

32. Alfonso M, Diaz A, Hernandez AM, Perez A, Rodriguez E, Bitton R, Perez R, Vazquez AM. An anti-idiotype vaccine elicits a specific response to N-glycolyl sialic acid residues of glycoconjugates in melanoma patients. J Immunol 2002; 168:2523-2529.

33. Neninger E, Diaz RM, de la Torre A, Saurez G, Gabri MR, Alonso DS, Wilkinson B, Alfonso AM, Crombet T, Perez R, Vázquez AM. Active immunotherapy with 1E10 anti-Id-iotype vaccine in patients with small cell lung cancer. Report of a Phase I Trial Cancer Biol Ther 2007; 6:145-150.

34. Hernández AM, Rodriguez M, López-Requena A, Beausoleil I, Pérez R, Vázquez AM. Generation of anti-Neu-glycolyl-ganglioside antibodies by immunization with an anti-idio-type monoclonal antibody: A self versus nonself-matter. Immunobiology 2005; 210:11-21.

35. Fujii Y, Higashi H, Ikuta K, Kato S, Naiki M. Specificities of human heterophilic Hanganutziu and Deicher (H-D) antibodies and avian antisera against H-D antigen-active glycosphingolipids. Mol Immunol 1982; 19:87-94.

36. Ledeen RW, Yu RK. Gangliosides: Structure, isolation, and analysis. Methods Enzymol 1982; 83:139-191.

37. Vazquez AM, Gabri MR, Hernandez AM, Alonso DF, Beausoleil I, Gomez DE, Perez R. Antitumor properties of an anti-idiotypic monoclonal antibody in relation to N-glycolyl-containing gangliosides. Oncol Rep 2000; 7:751-756.

38. Diaz A, Alfonso M, Alonso R, Saurez G, Troche M, Catala M, Diaz RM, Perez R, Vázquez AM. Immune responses in breast cancer patients immunized with an anti-idiotype antibody mimicking NeuGc-containing gangliosides. Clin Immunol 2003; 107:80-89.

39. Guthmann MD, Castro MA, Cinat G, Venier C, Koliren L, Bitton RJ, Vázquez AM, Fainboim L. Cellular and humoral immune response to N-Glycolyl-GM3 elicited by pro-longed immunotherapy with an anti-idiotypic vaccine in high-risk and metastatic breast cancer patients. J Immunother 2006; 29:215-223.

40. United States Pharmacopeia USP 24, 2000.

41. United States Pharmacopeia USP 26, 2002.

42. British Pharmacopeia BP 98, 1998.

43. González G, Crombet T, Neninger E, Viada C, Lage A. Therapeutic vaccination with epidermal growth factor (EGF) in advanced lung cancer: Analysis of pooled data from three clinical trials. Hum Vaccin 2007; 3:8-13.

44. Cohen MH, Johnson JR, Wang YC, Sridhara R, Pazdur R. FDA drug approval summary: Erlotinib (Tarceva) tablets. Oncologist 2005; 10:461-466.

45. Dyer O. FDA announces fast track approval of new drug for lung cancer. BMJ 2003; 326:1004.

46. Barlesi F, Tchouhadjian C, Doddoli C, Villani P, Greillier L, Kleisbauer JP, Thomas P, Astoul P. Gefitinib (ZD1839, Iressa) in nonsmall-cell lung cancer: A review of clinical trials from a daily practice perspective. Fundam Clin Pharmacol 2005; 19:385-393.

47. Giaccone G. Epidermal growth factor receptor inhibitors in the treatment of nonsmall-cell lung cancer. J Clin Oncol 2005; 23:3235-3242.

48. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabarbara P, Seymour L. National cancer institute of canada clinical trials group: Erlotinib in previously treated nonsmall-cell lung cancer. N Engl J Med 2005; 353:123-132.

49. Gatzemeier U, Groth G, Butts C, Van Zandwijk N, Shepherd F, Ardizzoni A, Barton, Ghahramani P, Hirsh V. Randomized phase II trial of gemcitabine-cisplatin with or without trastuzuMAb in HER2-positive nonsmall-cell lung cancer. Ann Oncol 2004; 15:19-27.

50. Krug LM, Miller VA, Patel J, Crapanzano J, Azzoli CG, Gomez J, Kris MG, Heelan RT, Pizzo B, Tyson L, Sheehan C, Ross JS, Venkatraman E. Randomized phase II study of weekly docetaxel plus trastuzuMAb versus weekly paclitaxel plus trastuzuMAb in patients with previously untreated advanced nonsmall cell lung carcinoma. Cancer 2005; 104:2149-2155.

51. Herbst RS. Toxicities of antiangiogenic therapy in nonsmall-cell lung cancer. Clin Lung Cancer 2006; 8(Suppl 1):S23-30.

52. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH. Paclitaxel-carboplatin alone or with bevacizuMAb for nonsmall-cell lung cancer. N Engl J Med 2006; 355:2542-2550.

53. Giaccone G. The potential of antiangiogenic therapy in nonsmall cell lung cancer. Clin Cancer Res 2007; 13:1961-1970.

54. Ho SB, Niehans GA, Lyftogt C, Yan PS, Cherwitz DL, Gum ET, Dahiya R, Kim YS. Heterogeneity of mucin gene expression in normal and neoplastic tissues. Cancer Res 1993; 53:641-651.

55. Butts C, Murray N, Maksymiuk A, Goss G, Marshall E, Soulieres D, Cormier Y, Ellis P, Price A, Sawhney R, Davis M, Mansi J, Smith C, Vergidis D, Ellis P, MacNeil M, Palmer M. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV nonsmall-cell lung cancer. J Clin Oncol 2005; 23:6674-6681.

56. Raez LE, Rosenblatt JD, Podack ER. Present and future of lung cancer vaccines. Review Expert Opin Emerging Drugs 2006; 11:445-459.

57. Toloza EM. Gene therapy for lung cancer. Review Thorac Surg Clin 2006; 16:397-419.

58. van Zandwijk N, Mathy A, Boerrigter L, Ruijter H, Tielen I, de Jong D, Baas P, Burgers S, Nederlof P. EGFR and KRAS mutations as criteria for treatment with tyrosine kinase inhibitors: Retro- and prospective observations in nonsmall-cell lung cancer. Ann Oncol 2007; 18:99-103.