Chemotherapy with or without low-dose interleukin-2 in advanced non-small cell lung cancer: results from a phase III randomized multicentric trial

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Abstract. Non-small cell lung cancer (NSCLC) is associated with IL-2-dependent cell-mediated immunodeficiency. As IL-2 is the main lymphocyte growth factor, a phase III randomized multicenter trial was conducted to evaluate the impact of subcutaneous low-dose IL-2 added to standard chemotherapy (CT) on overall survival (OS) in advanced NSCLC patients. Patients (n=241) with histologically confirmed stage IIIb or IV non-operable NSCLC underwent stratified randomization on the basis of center, ECOG PS, stage of disease and percentage of weight loss. Patients received gemcitabine (1000 mg/m^2) on days 1 and 8 + cisplatin (100 mg/m^2) on day 2 every 21 days for a maximum of 6 cycles [chemotherapy (CT) arm]. In the CT+IL-2 arm, patients also received low-dose subcutaneous IL-2 3,000,000 IU/die on days 3-5, 9-11, 15-17. The study had 90% power to detect a 20% absolute increase in 1-year OS with 118 patients/arm. An overall response (OR) rate of 12.8% (14% in the CT+IL-2 arm and 11.4% in CT arm) was observed. Stable disease was 70 and 66.7%, and progressive disease 16 and 21.8% in the CT+IL-2 and CT arms, respectively. No differences in response were found in any subgroup analysis. At a median follow-up of 32 months, 1-year OS was 45% for the CT+IL-2 arm vs. 51% for the CT arm (p=0.456 log-rank). Median progression-free survival was 6.6 months in the CT+IL-2 arm vs. 6.9 months in the CT arm (p=0.573, log-rank). A higher number of grade 4 toxicities were reported with CT+IL-2. The most common grade ≥3 adverse events were gastrointestinal toxicity (mainly nausea and diarrhea) and myelosuppression. No relevant differences in clinical outcome were observed from the addition of IL-2 to CT. Future studies investigating the role of T-regulators in chemoimmunotherapeutic regimens could be performed.

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Introduction

Non-small cell lung cancer (NSCLC) accounts for 80-85% of all lung cancer cases. Approximately 90% of lung cancers in men and 80% in women are related to smoking. Incidence differs considerably across Europe, with rates varying between 22 and 63/100,000 per year for men and between 5 and 33/100,000 per year for women. In most European countries the incidence of lung cancer continues to rise in women but is decreasing in men (1). Five-year age- and area-adjusted relative survival of all lung cancer patients in Europe continues to be low at 11%. Central European countries show slightly higher survival rates compared to those of other regions. Trends in lung cancer mortality in men have decreased in numerous European countries over the last two decades, especially in North and Western Europe. Conversely, mortality rates in women are still increasing in many countries (2).

Platinum-based combination chemotherapy has been considered the gold standard treatment for advanced disease for about a decade as it prolongs survival, improves quality of life and controls symptoms in patients with good performance status (3-5). Third-generation agents recommended for combination with cisplatin or carboplatin are vinorelbine, gemcitabine, taxanes, irinotecan and pemetrexed (for non squamous histology only) (6-10). The role of maintenance treatment has not been

determined. Recently, results from phase III trials of pemetrexed and erlotinib highlighted a survival benefit for patients who obtained a response or stable disease from first-line therapy. However, there is still no firm evidence of the superiority of immediate maintenance therapy over delayed therapy (11-14).

Several immunotherapeutic approaches have been tested in NSCLC since the introduction of recombinant cytokines, in particular human recombinant interleukin-2 (IL-2), into clinical practice about 10 years ago. IL-2 (Proleukin®), a T-lymphocyte activation and growth factor, is a cytokine with cardinal functions in the physiology of cell-mediated immunity. It has been administered in a number of ways in advanced NSCLC patients in an attempt to induce a reduction in the disease; alone or in association with other treatments; by intravenous bolus or continuous infusion of the maximum tolerated dose (15-17); subcutaneously at low doses (18); and in combination with IFN-α and radiotherapy (19).

Despite low objective response rates, promising results on survival after IL-2 treatment in patients with advanced NSCLC have been reported by several authors. Ardizzoni and coworkers (17) observed a median survival of 10 months in 11 patients, without, however, obtaining objective responses. Schiller's group reported a median survival of 11 months (range 1-30+) using the combination IL-2+TNF (20), and Tummarello and corworkers obtained an improved response in 5/20 patients who showed PR after biotherapy with consolidation treatment (IL-2 + IFN-α) in a phase II trial (21). Subcutaneous administration of low-dose IL-2 (1,800,000 IU/day) in combination with weekly cisplatin (40 mg/m^2/week) and epirubicin (40 mg/m^2/week) and with medroxyprogesterone acetate (1 g/day per os) was evaluated in 30 lung cancer patients during a dose intensity phase I trial by Mantovani and coworkers, with a 30% OR and a median OS of 10 months (22). The systemic administration of IL-2 as adjuvant treatment in combination with adoptive immunotherapy was reported to improve survival after radical and non-radical surgical resection for NSCLC in two different randomized studies (23,24).

Cytokines are crucial for the regulation of the immune system, directing the response towards activation or down-regulation (25-27). In neoplastic disease a progressive imbalance in cell-mediated immunity is observed, with the following consequences: decrease in the capacity to produce endogenous IL-2; alterations in molecular systems of cell signalling for IL-2; increased secretion of immunosuppressive and pro-inflammatory cytokines produced directly by the tumor and/or by the altered immune system. In short, tumors have numerous mechanisms for evading the immune system (25,28,29). It is also known that tumor-induced immunosuppressive phenomena are reversible *in vitro* by the addition of exogenous IL-2 (30). From a systemic perspective, the decline in IL-2-dependent cell-mediated immune functionality, well characterized *in vitro*, becomes visible in the progressive reduction in peripheral circulating lymphocytes. In fact, the total peripheral lymphocyte count in advanced neoplastic disease is a significant prognostic factor for overall patient survival, independently of other clinical and biological prognostic indices of proven reliability such as performance status and extent of disease.

Since the 1970s literature data have repeatedly confirmed the prognostic significance of the baseline lymphocyte count in terms of overall survival (31-38). A decrease in the peripheral lymphocyte count is thus an expression of reduced IL-2-dependent immune competence in cancer patients and is an unfavourable prognostic factor for overall survival, independently of the extension of the disease, especially in advanced lung cancer. It represents a solid basis for the clinical interpretation of variations in lymphocyte count observed to varying degrees during IL-2 administration: rebound lymphocytosis, which occurs 36-48 h after subcutaneous administration of IL-2, shows significantly higher values in patients who obtain an objective response or disease stabilization compared to those who progress (39,40). It can thus be hypothesized that the administration of IL-2, in restoring IL-2-dependent cell-mediated immune competence, may play a significant role in the overall survival of patients with unresectable lung cancer (41-43). The action of immune restoration inducible with IL-2 can thus be considered as complementing the cytolytic action of antiblastic drugs.

In 2000 when we designed a randomized phase III trial and began recruitment, it seemed not only justifiable but also opportune to test such hypotheses in a trial model. We conjectured that subcutaneous low-dose IL-2, by increasing lymphocyte number and activity and by restoring IL-2-dependent cell-mediated immune response, would result in a significant increase in overall survival.

Patients and methods

A phase III randomized Italian multicentric trial was conducted to evaluate the impact of subcutaneous low-dose IL-2 added to standard CT on overall survival (OS) in advanced NSCLC patients. The study was approved by the local ethics committee of all participating institutions and was carried out in accordance with Italian legislation and with the ethical standards laid down in the Declaration of Helsinki.

Patient selection. Eligibility criteria were as follows: histologically/cytologically confirmed stage IIIb or IV non-operable NSCLC and measurable disease (WHO criteria); age ≥18 years; Eastern Cooperative Oncology Group performance status (ECOG PS) ≤2; life expectancy ≥12 weeks; adequate renal, hepatic and cardiac function. Written informed consent was obtained from all patients.

Exclusion criteria included brain metastasis and pre-existing autoimmune diseases. No prior chemotherapy, immunotherapy or radiotherapy were permitted.

Treatments. All patients received gemcitabine (1000 mg/m^2) on days 1 and 8 plus cisplatin (100 mg/m^2) on day 2 every 21 days for a maximum of 6 cycles (CT arm). In the CT+IL-2 arm, patients also received low-dose subcutaneous IL-2 3,000,000 IU/die on days 3-5, 9-11, and 15-17 every 21 days. After 6 cycles a re-evaluation was performed and patients who had obtained complete response, partial response or stable disease continued with maintenance IL-2 on days 1-6 and 8-13 every 28 days until progression occurred (Table I). Patients with progressive disease and ECOG PS >2 discontinued treatment, while those with ECOG PS ≤2 were considered candidates for second-line chemotherapy. Doses were adjusted on the basis of the toxicity observed in the previous cycle. Treatment was stopped earlier in the event of progressive disease, uncontrolled pain, severe systemic allergy, patient refusal or unacceptable toxicity.

Table I. Standard- and experimental-arm therapy.

Standard therapy (CT arm)	Gemcitabine 1000 mg/m^2 on days 1 and 8, and CDDP 100 mg/m^2 on day 2 for 6 cycles
Experimental therapy (CT+IL-2 arm)	Gemcitabine 1000 mg/m^2 on days 1 and 8, CDDP 100 mg/m^2 on day 2, interleukin-2 sc 3 MIU on days 3-5, 9-11 and 15-17 for 6 cycles + maintenance treatment with IL-2 sc 3 MIU on days 1-6 and 8-13 until progression occurs

Efficacy. The extent of disease was evaluated according to WHO criteria a maximum of 4 weeks before random assignment. Thereafter, disease status was evaluated every 2 cycles in both arms. The primary study end-point was overall survival. Pre-specified secondary end-points were progression-free survival, event-free survival and toxicity evaluation. Further preplanned secondary end-points were objective response rate, subgroup analysis efficacy and identification of predictive factors of clinical outcome with exploratory hypothesis-generating intent, and role of peripheral blood lymphocytes (PBLs) as prognostic or predictive factors of clinical outcome.

Toxicity. Clinical assessment was performed at baseline, before each course and every 3 months during follow-up. Toxicity was classified according to the WHO grading system.

Statistical considerations

Randomization procedure. After verifying eligibility and obtaining written informed consent, patients were randomly assigned according to a 1.1 ratio to receive either standard chemotherapy (CT arm) alone or chemotherapy plus IL-2 (CT+IL-2 arm). Randomization was performed centrally by the sponsor and stratified by investigator center, ECOG PS (0-1 vs 2), stage of disease (IIIb vs IV) and percentage of weight loss (≤5% vs >5%) during the previous 6 months. Patients started treatment within 8 days of randomization.

Sample size. It was estimated that 1-year overall survival would be 25% in the CT arm. To detect a 20% increase in overall survival in the CT+IL-2 arm (α= 0.05; β= 0.1; log-rank test) and assuming a lost to follow-up of 5%, the required sample size was 236 patients (118 per arm).

Efficacy analyses. All analyses were primarily performed on an intent-to-treat population. Overall survival was defined as the time from start of treatment until death. Progression-free survival was calculated from start of treatment until the date of objective disease progression or death. OS and PFS curves were estimated using the Kaplan-Meier method, and a number of appropriate point estimations and relative 95% CIs were calculated. The log-rank test was used to compare survival curves of treatment groups. An interim analysis, carried out to verify the safety of the CT+IL-2 combination after an enrolment of 150 patients, showed a good toxicity profile. Planned subgroup analyses in relation to pre-specified prognostic factors were performed to assess modifications in treatment efficacy in the patient subsets identified by the stratification factors.

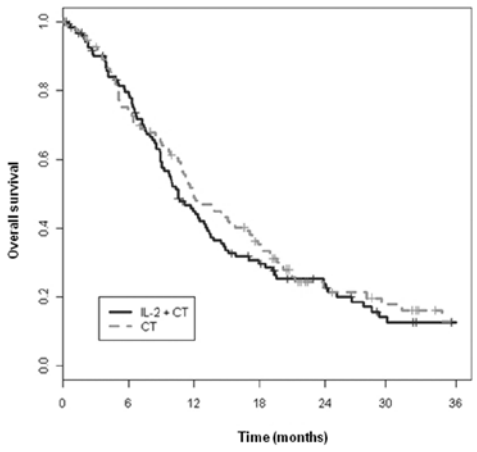
Table II. Patient characteristics.

	Treatment	
	CT n=114 n (%)	CT+IL-2 n=127 n (%)
Median age, years (range)	62 (41-76)	62 (32-75)
Gender		
Male	90 (78.9)	94 (74.0)
Female	18 (15.8)	25 (19.7)
ECOG PS		
0	47 (41.2)	59 (46.5)
1	61 (53.%)	59 (46.6)
2	6 (5.3)	9 (7.0)
Stage		
III	39 (34.2)	42 (33.1)
IV	75 (65.8)	85 (66.9)
Weight loss		
≤5%	103 (90.4)	106 (83.5)
>5%	11 (9.6)	21 (9.6)

Results

From June 2000 to October 2004, 241 patients were enrolled onto the study by 21 Italian centers and 239 were evaluable for efficacy end-points. Two patients were lost to follow-up immediately after randomization. Patient characteristics were well balanced between the arms; median age 62 years in both arms; 94 patients (74%) in the CT+IL-2 arm and 90 (78.9%) in the CT arm were males; weight loss ≤5% was 83.5% in the CT+IL-2 arm and 90.4% in the CT arm (Table II).

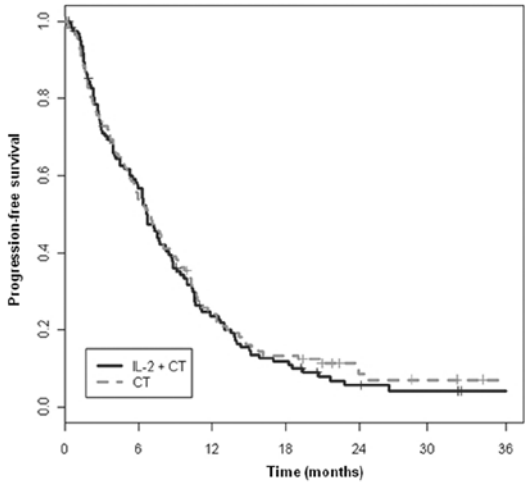
Efficacy. In the present study the addition of IL-2 to standard chemotherapy was not associated with improved outcome. Ninety-three deaths were observed among the 127 patients randomly assigned to the CT+IL-2 arm compared with 85 in the control arm. There were no statistical differences in OS or PFS between the two arms in the 239 (arm A/B 127/112) patients evaluable for efficacy end-points.



Median follow-up = 32 months

	n	Events	Median OS (months)	OS probability (%)/pts at risk at month				
				6	12	18	24	30
IL2+CT	127	93	10.5	80/93	45/49	30/30	24/20	13/9
CT	112	85	12.0	74/82	51/54	35/36	21/15	18/11
Logrank = 0.6 (1), p=0.456								

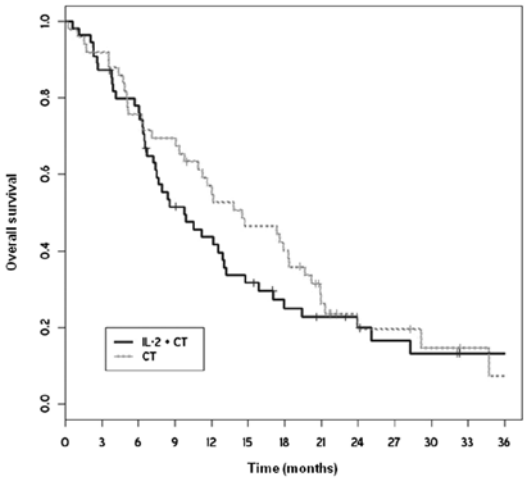
Figure 1. Overall survival.



Median follow-up = 32 months

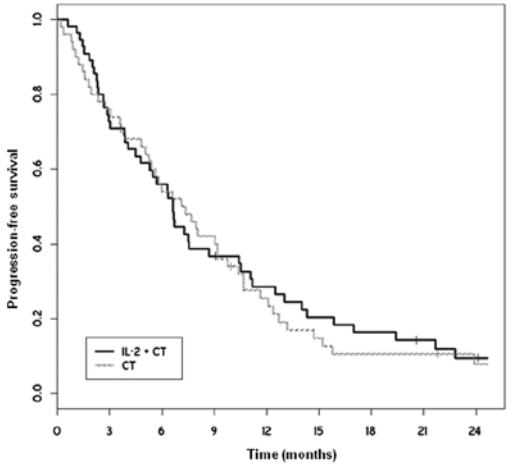
	n	Events	Median PFS (months)	PFS probability (%)/pts at risk at month			
				6	12	18	24
IL2+CT	127	111	6.6	57/68	24/27	12/14	7/6
CT	112	99	6.9	53/59	25/27	13/15	11/9
Logrank = 0.3 (1), p=0.573							

Figure 2. Progression-free survival.



	n	Events	Median OS (months)	OS probability (%)/pts at risk at month				
				6	12	18	24	30
IL2+CT	55	44	9.8	78/43	44/23	25/12	20/8	13/5
CT	50	39	14.5	76/38	57/28	35/36	20/6	15/4
Logrank = 1.0 (1), p=0.32								

Figure 3. Overall survival in patients with lymphocyte values ≤1833.



	n	Events	Median PFS (months)	PFS probability (%)/pts at risk at month			
				6	12	18	24
IL2+CT	55	48	6.6	56/31	29/15	16/9	10/5
CT	50	46	7.2	54/28	25/13	11/6	8/4
Logrank = 0.1 (1), p = 0.75							

Figure 4. Progression-free survival in patients with lymphocyte values ≤1833.

At a median follow-up of 32 months, 1-year OS was 45% for the CT+IL-2 arm vs. 51% for the CT arm. Median OS was 10.5 months in the CT+IL-2 arm and 12 months in the CT arm [log-rank = 0.6 (1), p=0.456] (Fig. 1). Median PFS was 6.6 months in the CT+IL-2 arm vs. 6.9 months in the CT arm [log-rank = 0.3 (1), p=0.573] (Fig. 2).

Overall, OR was 12.8%, subdivided by treatment arm as follows: 14% [all partial responses (PR)] for the CT+IL-2 arm and 11.4% (1.1% CR and 10.3% PR) for the control arm. Stable disease was 70 and 66.7% in the CT+IL-2 and CT arms, respectively, and progressive was 6 and 21.8%. Overall disease control was 81.2% (84.0 and 78.1% in the CT+IL-2 and CT arms, respectively). No differences were found in any of the subgroup analyses performed according to stratified factors for any efficacy end-point. PBL count was not related to either overall or progression-free survival. (Figs. 3 and 4).

Compliance to treatment and safety. Of the 127 patients randomized onto the CT+IL-2 arm, 117 received at least one

Table III. Toxicity.

Event	G3		G3	
	CT n (%)	CT+IL-2 n (%)	CT n (%)	CT+IL-2 n (%)
Leukopenia	17 (15.3)	19 (16.2)	2 (1.9)	3 (2.6)
Granulocytopenia	33 (29.7)	44 (37.6)	12 (10.8)	14 (12.0)
Thrombocytopenia	25 (22.5)	25 (21.4)	11 (9.9)	39 (25.6)
Pulmonary toxicity	7 (6.3)	4 (3.4)	1 (0.9)	0 (0)
Fever	0 (0)	1 (0.8)	0 (0)	0 (0)
Diarrhea	0 (0)	3 (2.6)	0 (0)	1 (0.8)
Nausea/vomiting	22 (19.8)	22 (18.8)	1 (0.9)	0 (0)

G3, grade 3.

dose of the study drug; 70 discontinued CT because of PD (44 patients), toxicity (16), or surgery on the residual mass (5). Of the 114 randomized onto the CT arm, 111 received at least one dose of the study drug; 63 discontinued CT due to PD (44 patients), toxicity (5) or surgery on the residual mass (5). The median number of cycles received was 5 and 6 in the CT+IL-2 and CT arms, respectively. No differences in CT dose modification were observed between the two arms.

Toxicity. Episodes (n=229) of grade ≥3 adverse events were registered. A higher number of grade 4 toxicities were reported with CT+IL-2 than with CT alone (50 vs. 27). The most frequent grade ≥3 adverse events were gastrointestinal toxicity (mainly nausea and diarrhea) and myelosuppression. Only one patient had grade 3 fever in either arm and no treatment-related deaths were reported (Table III).

Discussion

The development of tumor-targeted approaches to the treatment of lung cancer has led to the identification of new treatment combinations, some of which are still undergoing clinical evaluation. Several single-target agents used to attack angiogenic or proliferative signaling are currently being assessed as combinations e.g., cetuximab and bevacizumab together with chemotherapy in patients with non-operable NSCLC, or bevacizumab combined with erlotinib (44,45).

There is increasing evidence of crosstalk between TK signaling and the immune system. VEGF, for example, is emerging as a significant agent of immune tolerance in the tumor microenvironment. It has recently been reported that sunitinib has a positive impact on the immune system in mice and humans and appears to act in a synergistic manner with immune therapy in large tumor-bearing mice (46). Primary lung cancers are currently treated with a combination of surgery, local radiotherapy and chemotherapy. Combining cancer immunotherapy with such standard oncology treatments has been recognized as a potentially important approach (47). There is evidence that a number of chemotherapeutic agents commonly used in the treatment of NSCLC show immunostimulatory activities in both mouse and humans (48). Neninger and coworkers report that the administration of the EGF vaccine before and after first-line chemotherapy in advanced NSCLC patients is feasible, well tolerated and induces high anti-EGF antibody titers that correlate with survival (49).

In 2000 (before the era of targeted therapy) when this trial was planned and patient recruitment begun, we hypothesized that subcutaneous low-dose IL-2, by increasing lymphocyte number and activity and by restoring IL-2-dependent cell-mediated immune response, would significantly increase overall survival in patients with advanced NSCLC previously treated with standard chemotherapy. Our results, however, did not highlight any relevant differences in clinical outcome from the addition of IL-2 to standard chemotherapy, suggesting that the activity of IL-2 does not translate into a clinical benefit.

In the present study, the restoral of IL-2-induced cell-mediated immunity did not potentiate the cytolytic action of antitumor drugs. The total number of PBLs did not prove to be a prognostic factor, nor were they significantly influenced by the administration of IL-2. When the study was originally designed, there was no information available on the role of T-regulator lymphocytes (T-regs) in PBLs and tumor tissue or about their strong correlation with IL-2 (50-52). Further studies are now needed to clarify the part played by IL-2 in stimulating T-regs and/or cytotoxic-T-lymphocytes.

In the majority of cases, it has been observed that only a subset of patients respond to immunotherapeutic treatments. Genetic expression profiling of tumors is increasingly being used to predict the toxicity of new products in order to better classify heterogeneous cancer patient populations and to define subpopulations with a higher chance of benefiting from treatment. However, there are no established gene expression profiles capable of predicting response of NSCLC patients to cancer immunotherapy. The characterization of such putative predictive biomarkers was investigated to evaluate their response to MAGE-A3 ASCI in a randomized study involving 182 stage IB/II NSCLC patients (53). Gene expression profiling was carried out using microarray technology on tumor biopsies taken before immunization. A specific gene profile was identified defining a patient population in which MAGE-A3

ASCI reduced the relative risk of recurrence by 43% rather than 27% when considering the total trial population. In the near future a gene signature will be capable of identifying immune-related genes to predict probable clinical benefit in response to immunotherapy.

In conclusion, our results show that the addition of low-dose IL-2 to standard chemotherapy does not improve response rates or survival in advanced NSCLC patients. Further research, however, could be carried out to evaluate other potentially effective immunotherapeutic drugs or vaccines in this disease setting (47,54,55). Another important step would be to identify patients who could potentially benefit from an immunotherapeutic approach or from targeted therapy.

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In Memoriam

Michela Ballardini was the Data Manager for this study. She died suddenly in January 2009 at the age of 31 during a sabbatical at the University of California, San Francisco. We all miss her. Greta Di Felice was a Sponsor-Manager and, with her characteristic enthusiasm, succeeded in involving a large number of Italian researchers in this study. She died suddenly in 2010.

References

1. Karim-Kos H, De Vries E, Soerjomataram I, Lemmens V, Siesling S and Coebergh J: Recent trends of cancer in Europe: a combined approach of incidence, survival and mortality for 17 cancer sites since the 1990s. Eur J Cancer 44: 1345-1389, 2008.
2. Verdecchia A, Francisci S, Brenner H, Gatta G, Micheli A, Mangone L and Kunkler I; EUROCARE-4 Working Group: Recent cancer survival in Europe: a 2000-02 period analysis of EUROCARE-4 data. Lancet Oncol 8: 784-796, 2007.
3. Schiller J, Harrington D, Belani C, *et al*: Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 346: 92-98, 2002.
4. Kelly K, Crowley J, Bunn PA Jr, *et al*: Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non-small-cell lung cancer: a Southwest Oncology Group trial. J Clin Oncol 19: 3210-3218, 2001.
5. Scagliotti G, De Marinis F, Rinaldi M, *et al*: Phase III randomized trial comparing three platinum-based doublets in advanced non-small-cell lung cancer. J Clin Oncol 20: 4285-4291, 2002.
6. Le Chevalier T, Scagliotti G, Natale R, *et al*: Efficacy of gemcitabine plus platinum chemotherapy compared with other platinum containing regimens in advanced non-small-cell lung cancer: a meta-analysis of survival outcomes. Lung Cancer 47: 69-80, 2005.
7. Scagliotti G, Parikh P, von Pawel J, *et al*: Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 26: 3543-3551, 2008.
8. Scagliotti G, Hanna N, Fossella F, *et al*: The differential efficacy of pemetrexed according to NSCLC histology: a review of two phase III studies. Oncologist 14: 253-263, 2009.
9. Douillard J, Laporte S, Fossella F, *et al*: Comparison of docetaxel- and vinca alkaloid-based chemotherapy in the first-line treatment of advanced non-small cell lung cancer: a meta-analysis of seven randomized clinical trials. J Thorac Oncol 2: 939-946, 2007.
10. Grossi F, Aita M, Defferrari C, *et al*: Impact of third-generation drugs on the activity of first-line chemotherapy in advanced non-small cell lung cancer: a meta-analytical approach. Oncologist 14: 497-510, 2009.
11. Ciuleanu T, Brodowicz T, Zielinski C, *et al*: Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study. Lancet 374: 1432-1440, 2009.
12. Fidias P, Dakhil S, Lyss A, *et al*: Phase III study of immediate compared with delayed docetaxel after front-line therapy with gemcitabine plus carboplatin in advanced non-small-cell lung cancer. J Clin Oncol 27: 591-598, 2009.
13. Cappuzzo F, Ciuleanu T, Stelmakh L, *et al*: Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. Lancet Oncol 11: 521-529, 2010.
14. Miller VA, O'Connor P, Soh C, Kabbinavar F, for the ATLAS Investigators: A randomized, double-blind, placebo-controlled, phase IIIb trial (ATLAS) comparing bevacizumab (B) therapy with or without erlotinib (E) after completion of chemotherapy with B for first-line treatment of locally advanced, recurrent, or metastatic non-small cell lung cancer (NSCLC). Proc ASCO 27 (18S): abs. LBA80022009, 2009.
15. Lange J, Raubitschek A, Pockaj B, *et al*: A pilot study of the combination of interleukin-2-based immunotherapy and radiation therapy. J Immunother 12: 265-271, 1992.
16. Tester W, Kim K, Krigel R, *et al*: A randomized Phase II study of interleukin-2 with and without beta-interferon for patients with advanced non-small cell lung cancer: an Eastern Cooperative Oncology Group study (PZ586). Lung Cancer 25: 199-206, 1999.
17. Ardizzoni A, Bonavia M, Viale M, *et al*: Biologic and clinical effects of continuous infusion interleukin-2 in patients with non-small cell lung cancer. Cancer 73: 1353-1360, 1994.
18. Lissoni P, Barni S, Rovelli F, *et al*: Neuroimmunotherapy of advanced solid neoplasms with single evening subcutaneous injection of low-dose interleukin-2 and melatonin: preliminary results. Eur J Cancer 29: 185-189, 1993.
19. Jirillo A and Bonciarelli G: Treatment of advanced cancer with low dose radiotherapy, s.c. IL-2 and IFN-alpha. Proc ASCO: abs. 1364, 1993.
20. Schiller J, Morgan-Ihrig C and Levitt M: Concomitant administration of interleukin-2 plus tumor necrosis factor in advanced non-small cell lung cancer. Am J Clin Oncol 18: 47-51, 1995.
21. Tummarello D, Graziano F, Isidori P, *et al*: Consolidation biochemotherapy for patients with advanced non-small cell lung carcinoma responding to induction PVM (cisplatin, vinblastine, mitomycin-C) regimen. A phase II study. Cancer 77: 2251-2257, 1996.
22. Mantovani G, Macciò A, Mulas C, *et al*: Dose-intense phase II study of weekly cisplatin and epidoxorubicin plus medroxyprogesterone acetate and recombinant interleukin 2 in stage IIIB-IV non-small cell lung cancer. Oncol Rep 9: 661-670, 2002.
23. Ratto G, Zino P, Mirabelli S, *et al*: A randomized trial of adoptive immunotherapy with tumor-infiltrating lymphocytes and interleukin-2 versus standard therapy in the postoperative treatment of resected nonsmall cell lung carcinoma. Cancer 78: 244-251, 1996.
24. Kimura H and Yamaguchi Y: Adjuvant immunotherapy with interleukin 2 and lymphokine-activated killer cells after non-curative resection of primary lung cancer. Lung Cancer 13: 31-44, 1995.

25. Ribas A, Butterfield L, Glaspy J and Economou J: Current developments in cancer vaccines and cellular immunotherapy. J Clin Oncol 21: 2415-2432, 2003.
26. Rosenberg S: Development of effective immunotherapy for the treatment of patients with cancer. J Am Coll Surg 198: 685-696, 2004.
27. Szabo S, Sullivan B, Peng S and Glimcher L: Molecular mechanisms regulating Th1 immune responses. Annu Rev Immunol 21: 713-758, 2003.
28. Banchereau J and Steinman R: Dendritic cells and the control of immunity. Nature 392: 245-252, 1998.
29. Whiteside T: Immune responses to malignancies. J Allergy Clin Immunol 125 (Suppl. 2): S272-S283, 2010.
30. Yoshino I, Yano T, Murata M, *et al*: Tumor-reactive T-cells accumulate in lung cancer tissues but fail to respond due to tumor cell-derived factor. Cancer Res 52: 775-781, 1992.
31. Papatestas A and Kark A: Peripheral lymphocyte counts in breast carcinoma. An index of immune competence. Cancer 34: 2014-2017, 1974.
32. Kim U, Papatestas A and Aufses AJ: Prognostic significance of peripheral lymphocyte counts and carcinoembryonic antigens in colorectal carcinoma. J Surg Oncol 8: 257-262, 1976.
33. Lavin P, Bruckner H and Plaxe S: Studies in prognostic factors relating to chemotherapy for advanced gastric cancer. Cancer 50: 2016-2023, 1982.
34. Maltoni M, Pirovano M, Nanni O, *et al*: Biological indices predictive of survival in 519 Italian terminally ill cancer patients. Italian Multicenter Study Group on Palliative Care. J Pain Symptom Manage 13: 1-9, 1997.
35. Riesco A: Five-year cancer cure: relation to total amount of peripheral lymphocytes and neutrophils. Cancer 25: 135-140, 1970.
36. Stanley K: Prognostic factors for survival in patients with inoperable lung cancer. J Natl Cancer Inst 65: 25-32, 1980.
37. Galon J, Costes A, Sanchez-Cabo F, *et al*: Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 313: 1960-1964, 2006.
38. Galon J, Fridman W and Pagès F: The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. Cancer Res 67: 1883-1886, 2007.
39. Lissoni P, Barni S, Ardizzoia A, *et al*: Prognostic factors of the clinical response to subcutaneous immunotherapy with interleukin-2 alone in patients with metastatic renal cell carcinoma. Oncology 51: 59-62, 1994.
40. Göhring B, Riemann D, Rebmann U, Heynemann H, Schabel J and Langner J: Prognostic value of the immunomonitoring of patients with renal cell carcinoma under therapy with IL-2/IFN-alpha-2 in combination with 5-FU. Urol Res 24: 297-303, 1996.
41. Blay J, Negrier S, Combaret V, Attali S, Goillot E, Merrouche Y, *et al*: Serum level of interleukin 6 as a prognosis factor in metastatic renal cell carcinoma. Cancer Res 52: 3317-3322, 1992.
42. Tartour E, Dorval T, Mosseri V, Deneux L, Mathiot C, Brailly H, *et al*: Serum interleukin 6 and C-reactive protein levels correlate with resistance to IL-2 therapy and poor survival in melanoma patients. Br J Cancer 69: 911-913, 1994.
43. Fumagalli L, Lissoni P, Di Felice G, *et al*: Pretreatment serum markers and lymphocyte response to interleukin-2 therapy. Br J Cancer May 80: 407-411, 1999.
44. Gérard C and Debruyne C: Immunotherapy in the landscape of new targeted treatments for non-small cell lung cancer. Mol Oncol 3: 409-424, 2009.
45. Pennell N and Lynch TJ: Combined inhibition of the VEGFR and EGFR signaling pathways in the treatment of NSCLC. Oncologist 14: 399-411, 2009.
46. Ozao-Choy J, Ma G, Kao J, *et al*: The novel role of tyrosine kinase inhibitor in the reversal of immune suppression and modulation of tumor microenvironment for immune-based cancer therapies. Cancer Res 69: 2514-2522, 2009.
47. Bradbury P and Shepherd F: Immunotherapy for lung cancer. J Thorac Oncol 3 (Suppl. 2): S164-S170, 2008.
48. Zitvogel L, Apetoh L, Ghiringhelli F and Kroemer G: Immunological aspects of cancer chemotherapy. Nat Rev Immunol 8: 59-73, 2008.
49. Neninger E, Verdecia B, Crombet T, *et al*: Combining an EGF-based cancer vaccine with chemotherapy in advanced non-small cell lung cancer. J Immunother 32: 92-99, 2009.
50. Curiel T: Regulatory T-cell development: is Foxp3 the decider? Nat Med 13: 250-253, 2007.
51. Curiel T: Tregs and rethinking cancer immunotherapy. J Clin Invest 117: 1167-1174, 2007.
52. Ahmadzadeh M and Rosenberg S: IL-2 administration increases $CD4^+$ CD25(hi) $Foxp3^+$ regulatory T cells in cancer patients. Blood 107: 2409-2414, 2006.
53. Vansteenkiste JF, Zielinski M, Dahabreh IJ, *et al*: Association of gene expression signature and clinical efficacy of MAGE-A3 antigen-specific cancer immunotherapeutic (ASCI) as adjuvant therapy in resected stage IB/II non-small cell lung cancer (NSCLC). Proc ASCO 26: abs. 7501, 2008.
54. Coate L and Shepherd F: Maintenance therapy in advanced non-small cell lung cancer. J Thorac Oncol 5: 723-734, 2010.
55. Kelly R, Gulley J and Giaccone G: Targeting the immune system in non-small-cell lung cancer: bridging the gap between promising concept and therapeutic reality. Clin Lung Cancer 11: 228-237, 2010.