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Review paper

Recent advances in the treatment of non-small cell lung cancer

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Non-small cell lung cancer (NSCLC), which represents the bulk of primary carcinomas of the lung, is an aggressive malignancy. The majority of patients with NSCLC present with advanced disease, not curable by surgery, at the time of diagnosis. Recent randomized trials have shown an improvement in survival for patients with loco-regional disease treated with combination, platinum-based, chemotherapy and curative irradiation. Similarly, randomized studies of good performance status patients with metastatic disease have documented a survival advantage, albeit a modest advantage, for those receiving chemotherapy. New chemotherapy agents with activity in NSCLC have been studied in phase II trials. These agents need to be evaluated, in loco-regional and metastatic disease, in large randomized phase III trials before conclusions can be drawn about their role in treatment. Novel treatments which among others include gene therapy, anti-angiogenic and anti-metastatic agents are currently being assessed in early phase I and II studies. Gene therapy will likely be combined with standard chemotherapy and radiation in the treatment of NSCLC, whereas anti-angiogenic and anti-metastatic agents may play a role in prevention and maintenance therapy. Finally, regardless of the approach or modality, new interventions will need to be assessed for their impact on overall survival and the quality of life of patients with NSCLC.

Key words: Angiogenesis, chemotherapy, gene therapy, metalloproteinase inhibitors, new drugs, NSCLC, radiation therapy.

Introduction

The treatment of lung cancer, the leading cause of cancer death in men and women in the western world, has recently been the subject of multiple comprehensive reviews.[1–6] Non-small cell lung cancer (NSCLC) represents 75–80% of all primary cancer of the lung and therefore the bulk of lung cancers.[1] Pathologically NSCLC can be sub-divided into four cell types: adenocarcinoma, squamous cell carcinoma, adeno-squamous carcinoma and large cell anaplastic carcinoma.[7] It is staged according to the TNM classification into four stages (stage I–IV) but for practical and therapeutic purposes may be grouped into three: localized disease (stage I and II), loco-regional disease (stage IIIA and IIIB) and metastatic disease (stage IV).[8] Twenty percent, 35% and 45% of patients present with localized (I and II), loco-regional (IIIA and IIIB) and metastatic (IV) NSCLC, respectively, at the time of diagnosis.[9] Therefore at initial presentation 80% of patients present with advanced disease which in this review will be considered as stage III and IV NSCLC. For patients with resectable stage I and II disease the 5 year survival rate is 50–60%, for stage IIIA 15–20%, and very few patients with stage IIIB and stage IV survive 5 years, giving an overall 5 year survival for all patients with NSCLC of 10–15%.[8]

The past 20 years has seen an improvement in peri-operative mortality due to improvements in anesthetic and surgical techniques, antibiotic therapy, and post-operative care. However, this improved peri-operative mortality has had no impact on the 80% of patients who are inoperable at diagnosis and has not changed the overall survival of patients with NSCLC significantly. Despite a lack of improvement in the field and dismal treatment results, there has been a flourish of reviews in the literature. This, at least in part, is due to a new optimism among the medical and scientific community. This optimism stems from multiple sources including: a number of randomized studies in advanced NSCLC that have demonstrated a survival advantage for patients receiving chemotherapy versus those receiving best supportive care (BSC);[10–17]

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the result of a recent large meta-analysis reporting a survival advantage for patients receiving platinum-based chemotherapy;[18] and four major randomized studies comparing radiation alone to combination chemotherapy and radiation (combined modality) showing a statistically significant survival advantage at 2 and 3 years for patients receiving combined modality therapy.[19–22] Furthermore, for the first time in 15 years, there are at least four new agents with single-agent response rates over 15%.[23] It is hoped that these new agents will provide a survival advantage when tested either alone or in combination in double-blind, randomized, controlled studies. In addition to these novel chemotherapy agents we appear to be on the brink of an era of new treatment modalities. These include new biological agents, differentiating agents, antioxidants, and inhibitors of angiogenesis and metastatic invasion. Some of these agents will be combined with chemotherapy resulting in a net additive effect in the treatment of lung cancer patients. Their use may be further exploited by combining them with three-dimensional radiation, an evolving area in this disease. Lastly, gene therapy offers the hope of tumor selectivity and a reduction of treatment related toxicity in this debilitated patient population.

As the current treatment for patients with NSCLC has been extensively summarized elsewhere it will only be covered briefly in the review.[3–6] The main focus of this article will be to provide an overview of new principles and techniques in radiation oncology, and review the chemotherapy agents and new technologies which are either on the brink of entering or have entered early clinical trials in humans.

Chemotherapy for advanced NSCLC

Despite more than 30 years of clinical evaluation, the role of chemotherapy in the treatment of NSCLC remains controversial. In the 1960s and 1970s the major question was 'whether chemotherapy provided a survival advantage for patients?'. This question has been answered by the publication of a number of randomized clinical trials,[10–17] and a large meta-analysis in the 1980s and early 1990s.[18] Why then does chemotherapy remain controversial? The answer is the small survival advantage in the majority of patients, the absence of a cure rate and the toxicity of current modalities of treatment. The discussion below will more clearly elucidate the problem and provide hope for its resolution.

Single-agent chemotherapy

Before discussing the response rate of single-agent chemotherapy in NSCLC it is necessary to point out that the response to chemotherapy in loco-regional disease (30–60%) is 50% greater than in metastatic disease (20–40%).[24,25] Therefore any study that includes more patients with loco-regional (stage III) rather than metastatic disease (stage IV) would have a higher response and overall survival. This is an important confounding variable when trying to compare the response and survival of patients receiving different single-agent and combination chemotherapy regimens in a historical fashion. Most phase II studies assessing response have included a preponderance of stage IV but some stage III patients.

Although many agents have been screened over the past 30 years, less than a dozen have shown activity adequate to justify continued use. These drugs are listed in Table 1. As can be seen from this table, only five drugs when administered as single agents have a response rate over 15%. These are cisplatin, epirubicin, ifosphamide, mitomycin and vinblastine. Response rates of 13–28% have been documented in phase II studies.[26] Of these agents, cisplatin remains one of the most important agents. The optimal dose and schedule of cisplatin, however, is controversial. Some studies have shown that higher doses are superior with respect to response and survival,[24,27] whereas other randomized studies looking at different doses of cisplatin in combination regimens demonstrated a similar overall survival at doses varying from 75 to 120 mg/m^2.[28] As cisplatin is associated with only modest myelosuppression it has been successfully combined with a number of other chemotherapy agents including newer agents, e.g. taxol and gemcitabine.[29–31] In summary, despite some activity,

Table 1. Active chemotherapy agents against NSCLC

Response > 15%
ifosphamide
cisplatin
epirubicin
vinblastine
mitomycin C
Response < 15%
etoposide
carboplatin
cyclophosphamide
5-flurouracil
methotrexate
doxorubicin

Table 2. Factors affecting treatment outcome

Patient factors
performance status
sex
history of pre-treatment weight loss
significant co-morbid disease
Tumor factors
stage
bulk of disease
elevated plasma LDH

these older agents confer what must be regarded as a dismal response and survival benefit.

Combination chemotherapy

Response to combination chemotherapy is higher than single-agent therapy in all studies, apart from one study.[24,32] In phase II trials response rates of 20–40% in metastatic disease (stage IV) and 30–60% in loco-regional disease (stage III), respectively, have been reported in the literature. There have been at least eight prospectively randomized trials of chemotherapy versus BSC in advanced NSCLC. These studies have shown a survival advantage of 2–5 months for the group receiving chemotherapy but in only four of the studies was this advantage statistically significant ($p \geq 0.05$).[10–17]

In general complete responses to combination chemotherapy remains low with only 10–15% of patients with advanced loco-regional disease and less than 5% of patients with metastatic disease achieving a complete remission. Despite the low complete and overall response rate in metastatic disease it is interesting to note that amelioration of symptoms occurs in a much higher percentage of patients. One British study found 75% of patients had disappearance or improvement in one major tumor-related symptom.[33] This suggests the benefit from chemotherapy that has often been claimed by proponents, is greater than that measured by response and survival. In order to substantiate this claim, future prospective randomized trials need to include indices of palliation and quality of life as part of the study objectives and design.

Radiotherapy in locally advanced NSCLC

Patients with stage III disease represent 30–40% of all cases of NSCLC. Only a small proportion of these are potentially resectable and, traditionally, most patients receive thoracic irradiation at some time during their disease. Median survival is 9–10 months with less than 10% surviving beyond 5 years. Death is usually attributed to progressive intra-thoracic disease and distant failure, with 70–80% of patients developing distant metastases during the course of their illness.[9] It is noteworthy, that even for the same stage and disease status there are variations in management depending on the center, country or continent, reflecting different patterns of practice.

Stage III incorporates a heterogenous group of patients which accounts for the variable patterns of management. Some patients are treated with palliative radiotherapy, others with a 'watch and wait' policy, with intervention occurring only when symptoms arise, and others with an aggressive approach upfront using combined modality therapy incorporating combinations of surgery, chemotherapy and thoracic irradiation. A number of issues are addressed in reaching these decisions including: (i) patient factors, (ii) tumor factors, and (iii) availability of surgical, medical and radiation oncology expertise (see Table 2).

Furthermore, there are many controversies surrounding the role of radiation in the treatment of stage III NSCLC: (i) dose (high versus low), (ii) timing (immediate versus when symptoms develop), (iii) fractionation scheme (standard, hypofractionation or hyperfractionation) and (iv) alone versus combination with surgery or chemotherapy (sequential, concomitant or alternating).

Based on a number of single-arm studies and two small randomized studies, a small sub-group of stage III patients, mainly stage IIIA, can be considered for neoadjuvant chemotherapy followed by definitive surgery plus or minus thoracic irradiation. However, further discussion of this topic is beyond the scope of this review.

Dose of thoracic irradiation

Although the overall prognosis for stage III NSCLC patients is poor, there is a small group who will survive greater than 5 years after definitive thoracic irradiation. This is usually in a selected population who have received high-dose irradiation delivered with curative intent. The RTOG[34] conducted two large randomized studies (protocols 73-01 and 73-02) to determine the optimal dose of radiation and fractionation schedule in patients with inoperable or unresectable NSCLC—40 Gy in 10 fractions with a 2-week split, 40 Gy/20 fractions/4 weeks, 50

Gy/25 fractions/5 weeks and 60 Gy/30 fractions/6 weeks. The incidence of tumor recurrence in the irradiated volume for patients enrolled in protocol 73-01, i.e. T1,2,3–N0,1,2 tumors, varied inversely with dose, i.e. 58% for 40 Gy in 20 fractions (continuous), 53% for 40 Gy in 10 fractions (split), 49% for 50 Gy in 25 fractions and only 35% for 60 Gy in 30 fractions. For patients with T4 or N3 tumors (protocol 73-02), overall intrathoracic failure rate was approximately 70% and not significantly influenced by dose variation. The 3 year survival, however, was slightly better for those treated with 60 Gy versus lower doses, but this survival advantage was not sustained over a longer period of time. Based on these studies, 60 Gy in 30 fractions became the North American standard for curative thoracic irradiation in unresectable or inoperable NSCLC.

The argument for low-dose palliative irradiation in stage III patients is based on the poor 5 year survival rates of less than 10%. Many of these patients are in poor general condition due to symptoms related to the primary malignancy or other medical problems. Low-dose irradiation effectively palliates symptoms in over 70% of patients with subsequent improvement in quality of life.[35] The optimal regimen, to maximize palliation of symptoms while minimizing patient inconvenience and maintaining quality of life, is unknown although a number of recent studies have addressed this issue. The MRC[36] conducted a randomized trial in 369 patients with advanced NSCLC comparing 30 Gy in 10 fractions (or 27 Gy in six fractions—a biologically equivalent dose) (FM) to 17 Gy in two fractions (F2). Palliation of the main symptoms, i.e. cough, hemoptysis, chest pain and anorexia, was achieved in a high proportion of the patients (56–86%) and was similar in the two treatment groups. The median duration of palliation was 50% or more of remaining survival. There was no difference in survival between the two groups (179 days in F2 and 177 days in FM group). A second trial, confined to patients with poor performance status, randomized 117 patients to 17 Gy in two fractions (F2) and 118 patients to a single fraction of 10 Gy (F1).[37] The results were not statistically different in the two arms, i.e. median duration of symptom palliation was 50% or more of survival with median survival being 100 days in F2 and 122 days in the F1 group. Interestingly, dysphagia was substantially more common in the F2 group than the F1 group, 56 versus 23%. These studies suggest that one or two fractions of radiation may be sufficient to palliate symptomatic patients with poor performance status and advanced disease.

Fractionation

The goal of irradiation delivered with curative intent is to eradicate tumor cells while minimizing damage to healthy normal tissues within the irradiated volume. A number of factors influence this including total tumor dose, dose per fraction, inter-fraction interval and overall duration of treatment. In recent years, a number of novel fractionation schemes have been used to try and achieve the above endpoints. The RTOG (protocol 83-11) conducted a prospective randomized trial of hyperfractionated thoracic irradiation; total doses of 60, 64.8, 69.6, 74.4 and 72.9 Gy, given at a dose of 1.2 Gy b.i.d. with a minimum 4 h interfraction interval.[38] Survival was compared among the five arms and with the standard fractionation arms (60 Gy/30 fractions/6 weeks) of earlier RTOG studies. Patients with favorable prognostic features, i.e. performance status (PS) 70–100% and less than 5% weight loss, had a significantly better survival ($p = 0.001$) than those with PS 50–69% or weight loss greater than 5%. In favorable stage III patients, the 69.6 Gy arm was significantly better than all other arms: 1 year survival and 3 year survival were 58 and 20%, respectively. These results were significantly better than results with standard fractionation in comparable patients from earlier RTOG trials: 1 and 3 year survival being 30 and 7%, respectively.

CHART[39] (Continuous Hyperfractionated Accelerated Radiation Therapy) is a unique accelerated fractionation regimen developed by Saunders, Dische and their colleagues in the Gray Laboratory. This scheme delivers 54 Gy, in 1.5 Gy fractions t.i.d. with a 6 h inter-fraction interval. Treatment is delivered over a 12 day continuous period with no break for the intervening weekend. The rationale for completing the treatment in such a short period is an attempt to overcome the potential for repopulation of clonogenic tumor cells during a course of prolonged fractionation.[40] By giving a lower dose per fraction, the risk of late radiation damage should be reduced. A pilot study conducted between January 1985 and December 1988, in Mount Vernon Hospital, England, enrolled 62 patients with locally advanced NSCLC of the bronchus. The results of this pilot study were compared with a group of historical controls from the same institution and preliminary results were promising with a 1 year probability of survival of 64% (CHART) compared with 44% (historical control) and a 2 year survival of 34 versus 12%, respectively.

A randomized prospective controlled trial was subsequently conducted by the MRC comparing

CHART to conventional radiotherapy (60 Gy in 30 fractions) for locally advanced NSCLC. Between 1990 and 1995, 563 patients were enrolled and preliminary results were recently presented at ECCO, Paris, November 1995. The endpoints were survival, local tumor control and morbidity. Patient survival in the CHART arm was superior to the conventional arm at 1 and 2 years. Improved survival was attributed to improved local control, confirming the need to optimize local tumor control for long-term survival.

As opposed to hyperfractionation, hypofractionation schemes are thought to increase the potential for late normal tissue toxicity.[41] Hypofractionation is therefore generally employed in palliative situations where life expectancy is short and the risks of late toxicity minimal.

Combination of chemotherapy and thoracic irradiation

At some stage in their disease 70–80% of patients with stage III NSCLC will develop distant metastases.[34] The risk of developing systemic disease could potentially be reduced by the administration of effective systemic chemotherapy at initial diagnosis, when the metastatic burden is small. In addition, certain chemotherapeutic agents such as cisplatin, taxol and gemcitabine have radiosensitizing properties which enhance the efficacy of irradiation. Another example, vinblastine alters the cell cycle and hence gates tumor cells into a potentially radiosensitive phase complimenting the effect of irradiation. The timing of chemotherapy and irradiation is still controversial, and a number of studies have used combined modality regimens delivering the two types of treatment in either a sequential, concurrent or alternating schedule.

Sequential chemotherapy and radiotherapy

Table 3 summarizes the main randomized trials with *sequential* chemotherapy and irradiation, for stages IIIA and IIIB NSCLC.

These randomized trials comparing radiation alone versus radiation in combination with systemic chemotherapy for stage III NSCLC have had conflicting results. The Finnish Group[42] and North Central Cancer Treatment Group[43] showed no benefit in terms of either median or long-term survival for combined modality therapy over standard radiation alone.

In contrast, Dillman *et al.*[19,44] reported improved survival sustained to 5 years with the administration of two cycles of platinum and vinblastine prior to the definitive thoracic irradiation. Median survival was 9.7 months in the radiotherapy alone arm versus 13.8 months in the combined modality arm ($p=0.01$) and 1, 2 and 5 year survival were 40, 13 and 7% versus 54, 26 and 19%, respectively, favoring the addition of chemotherapy. Le Chevalier[20,45] also demonstrated a benefit for chemotherapy delivered sequentially to definitive thoracic irradiation with prolongation of survival at 1, 2 and 3 years, i.e. 51, 21 and 12% for combined modality therapy versus 41, 14 and 4% for radiotherapy alone. The improvement was attributable to a reduction in the rate of distant metastases rather than an improvement in the primary local tumor control.

These positive results were confirmed by a joint study done by the RTOG and ECOG groups. The RTOG and ECOG conducted a phase III trial comparing three regimens of treatment for locally advanced NSCLC.[22] The three arms were (i) standard radiation therapy alone (60 Gy/30 fractions/6 weeks), (ii) induction chemotherapy with cisplatin and vinblastine followed by standard radiation as above according to the CALGB regimen,[44] and (iii) hyperfractionated thoracic irradiation (69.6 Gy/1.2 Gy per fraction b.i.d./5 days per week) according to the RTOG regimen.[38] Toxicity was acceptable with four treatment related deaths. One year survival (%) and median survival (months) were as follows: standard radiation therapy 46%, 11.4 months; induction chemotherapy followed by standard radiation 60%, 13.8 months; and hyperfractionated thoracic irradiation 51%; 12.3 months. The combined chemotherapy plus radiotherapy arm was statistically superior to the other two treatment arms ($p=0.03$). While these early results suggest a survival benefit for combined modality therapy, the long-term results are eagerly awaited.

A smaller European randomized trial has also supported the findings of the above three studies. Fifty-six patients were evaluable and treated with radiotherapy alone (56 Gy alone) or two cycles of induction chemotherapy (cisplatin and etoposide) followed by the same radiotherapy. Median survival was 9 months in the RT alone arm versus 14 months in the combined treatment arm ($p=0.0559$). Survival at 1, 2 and 5 years was 56, 30 and 10%, respectively, for the chemo/radiotherapy arm versus 37, 14 and 0% for the radiotherapy alone arm. Toxicity was acceptable in both arms.[46]

Table 3. Randomized trials of radiation therapy + chemotherapy administered sequentially in locally advanced NSCLC

Source	Radiotherapy (Gy)	Chemo therapy	No. of patients	Median survival (months)	Survival (%)			
					1 year	2 years	3 years	5 years
Mattson *et al.*[42]	55 (S)	CAP	119	10.4	41	17		
	55 (S)		119	11.1	42	19		
Morton *et al.*[43]	60		58	10.4	45	16		7
	60	MACC	56	10.6	46	21		5
Le Chavalier *et al.*[20,45]	65		177	10	41	14	4	
	65	VCPC	176	12	51	21	12	
Dillman *et al.*[19,44]	60		77	9.7	40	13	10	7
	60	PV	78	13.8	54	26	24	19
Crino *et al.*[46]	56		56	9	37	14		0
	56	EC	total for both arms	14	56	30		10
Sause *et al.*[22]	60		149	11.4	46			
	60	PV	151	13.8	60			
	69.6 HFX		152	12.3	51			

Abbreviations: S, split course; CAP, cyclophosphamide, doxorubicin, cisplatin; MACC, methotrexate, doxorubicin, cyclophosphamide, lomustine; VCPC, vindesine, lomustine, cisplatin, cyclophosphamide; PV, cisplatin, vinblastine; EC, etoposide, cisplatin. HFX, hyperfractionated 1.2 Gy b.i.d.

Concurrent chemotherapy and radiotherapy

A number of groups have investigated the administration of chemotherapy and radiation concurrently. This is based on experimental data which has indicated that some chemotherapeutic agents, such as cisplatin, enhance radiation cell kill *in vitro*.[47] This effect of radiation enhancement has led to the development of multiple clinical trials incorporating single- or multi-agent chemotherapy delivered concurrently with irradiation. The major randomized trials are summarized in Table 4. All of the trials were phase III studies with the control arm delivering radiotherapy alone.[48–50] The chemotherapy in almost all of the trials included cisplatin (Jeremic used carboplatin) and was delivered on a variable basis from daily to 3 weekly. The trials by Soresi[48] and Blanke[49] suggest an improved survival for combined modality therapy but this did not reach significance.

The three-arm EORTC trial[21] randomized patients to: radiotherapy alone (30 Gy/10 fractions, followed by a 3 week rest, then an additional 25 Gy/10 fractions); identical RT combined with cisplatin 30 mg/m^2 weekly on day 1 on each treatment week; or RT with daily cisplatin, i.e. 6 mg/m^2 given every day prior to radiation during the treatment period. Survival was significantly improved in the radiotherapy/daily platinum group when compared with the radiotherapy alone group ($p=0.009$); 1, 2 and 3 year survival being 54, 26 and 16%, respectively, for combined therapy versus 46, 13 and 2% for RT alone. The survival benefit was attributed to improved local control. Survival in the radiotherapy/weekly cisplatin group was intermediate and not significantly different from the other two arms.

In a German multicenter trial,[51] 85 patients were randomized to radiotherapy alone (30 Gy in 15 fractions, 2 week rest interval followed by an additional 20 Gy in 10 fractions) versus similar radiation preceded by two cycles of ifosfamide and vindesine. In addition, cisplatin was given as a radiosensitiser (20 mg/m^2 weekly) during radiotherapy. Seventy-eight patients were evaluable and showed a statistically significant advantage for combined modality therapy. The median survival was 9 versus 13.7 months and the 2 year survival was 12 versus 24%, both in favor of the group receiving chemotherapy.

Finally, Jeremic *et al.*[52] conducted a three-arm randomized study between January 1988 and January 1989. Group 1 received hyperfractionated radiotherapy (HFXRT) with 1.2 Gy b.i.d. to a total dose of 64.8 Gy; group 2 received the same HFXRT with chemotherapy 1 (100 mg of carboplatin on days 1 and 2, and 100 mg of etoposide on days 1–3 of each week during RT); and group 3 was given the same HFXRT with chemotherapy 2 (200 mg of carboplatin on days 1 and 2, and 100 mg of VP16 on days 1 to 5 of first, third and fifth weeks of the RT course). The median survival time was 8 months for

Table 4. Randomized trials of radiation therapy ± chemotherapy administered concurrently in locally advanced NSCLC

Source	Radiotherapy (Gy)	Chemotherapy	No. of patients	Median survival (months)	Survival time (%)			
					1 year	2 years	3 years	5 years
Soresi *et al.*[48]	50		50	11	48	25		
	50	P weekly	45	16	73	40		
Trovo *et al.*[50]	45		83	10.3		?15		
	45	P daily	84	9.97		?15		
Blanke *et al.*[49]	60		111	11.5	45	13	3	2
	60	P q 3 weeks	104	10.75	43	18	9	5
EORTC[21]	55 (S)		108		46	13	2	
	55 (S)	P weekly	98		44	19	13	
	55 (S)	P daily	102		54	26	16	
Wolf *et al.*[51]	50 (S)		41	9		12		
	50 (S)	IVP	37	13.7		24		
Jeremic *et al.*[52]	64.8 (HFX)		61	8	39	25	6.6	4.9
	64.8 (HFX)	CE weekly	52	18	73	35	23	21
	64.8 (HFX)	CE alt week	56	13	50	27	16	16

P, cisplatin; S, split course; IVP, ifosphamide, vindesine, cisplatin; HFX, hyperfractionated RT; 1.2 Gy b.i.d.; CE, carboplatin, etoposide.

group 1, 18 months for group 2 and 13 months for group 3. The 3 year survival rate was 6.6, 23 and 16%, respectively, with a significant difference favoring group 2. The relapse-free survival was also higher in this group and this was attributed to improved local control. The trend in these studies favored combined modality therapy but the price of combination therapy is increased acute toxicity.

Although these studies suggest a benefit for combined modality therapy with high-dose platinum-based chemotherapy and high-dose thoracic irradiation, one must be cautious in recommending this as standard treatment. Most of these studies have demonstrated increased toxicity and an increase in the duration of treatment during which the patient's quality of life may be diminished. This must be weighed against the possible survival gain. Quality of life, an important factor in determining the overall benefits of treatment has not been adequately evaluated in these studies. Moreover, the optimal sequencing of chemotherapy with radiation has not been determined and remains the focus of many ongoing studies using multiple radiation enhancing agents. In summary, further studies are clearly indicated. These should be large phase III studies with stringent eligibility criteria, accurate documentation of tumor response, patterns of failure and survival. These data should be accompanied by a quality of life analysis which will document the benefit of this combined approach in this debilitated patient population.

New chemotherapy agents

As alluded to above, in the past 5 years, a number of new chemotherapy agents have become available for clinical evaluation. These include the taxanes, campothecins, vinorelbine and gemcitabine. These agents will be discussed below (Tables 5–7).

Taxanes

Taxanes are a group of chemicals that confer their cytotoxic effect by promoting polymerization of tubulin and inhibiting its depolymerization, thereby stabilizing the mitotic spindle. Taxol, a compound derived from the bark of the Pacific yew tree, was the first in the group to enter clinical trials in the late 1980s. It was found active in the salvage treatment of ovarian and breast cancer. Information about its benefits in the treatment of NSCLC became available in 1990. Early clinical trials documented concerns about a high incidence of severe hypersensitivity reactions, but the subsequent use of premedication with antihistamines and steroids markedly improved tolerance. Also, the more recent use of a protracted infusion (24 h instead of 1–3 h) is thought to have helped reduce the incidence of allergic reactions and possibly have conferred improved activity.[53] The dose-limiting toxicity of current schedules is myelosuppression and neuropathy. Two major studies established the activity of

Table 5. Phase I/II trials of single agents

Agent	Patients evaluable	Population	Dose/schedule	Response (%)	Median survival (weeks)	Reference
Taxol	24/25	IV	250 mg/m^2/24 h, q 3 weeks	21	24	55
	25/27	III, IV	200 mg/m^2/24 h, q 3 weeks	24	40	54
	60/61	III, IV	210 mg/m^2/3 h, q 3 weeks	32		58
	25/25	IIIb, IV	200 mg/m^2/3 h, q 3 weeks	28		57
	19/23	advanced	200 mg/m^2/24 h, q 3 weeks	26		56
	12/13	IV	200 mg/m^2/3 h, q 3 weeks	42		59
Taxotere	35/43	III, IV	100 mg/m^2/1 h, q 3 weeks	23	36	78
	39/41	IIIb, IV	100 mg/m^2/1 h, q 3 weeks	33	47	79
	29/29	III, IV	100 mg/m^2/1 h, q 3 weeks	38	27	80
	32/38	III, IV	100 mg/m^2/1 h, q 3 weeks	28		81
	20/20	IIIb, IV	75 mg/m^2/1 h, q 3 weeks	25	39.4+	83
	84/93	III, IV	60 mg/m^2/1–2 h, q 3–4 weeks	21.4		82
Gemcitabine	76/84	III, IV	1000–1250 mg/m^2 × 3 weeks, escal	20		150
	332/360*	III, IV	800–1250 mg/m^2 × 3 weeks, escal	20		151
	93/116	III, IV	1250 mg/m^2 × 3 weeks, escal	20.4		152
	31/33	IIIb, IV	1000 mg/m^2 × 3 weeks, escal	26	46	149
	29/36	III, IV	1250 mg/m^2 × 3 weeks, escal	21	30	153
Vinorelbine	70/78	I–IV	30 mg/m^2 weekly	33	33	109
	79/80	III, IV	25 mg/m^2 weekly	29	40+	110
	44/50	III, IV	30 mg/m^2 weekly	32		111
CPT-11	73/73	III, IV	100 mg/m^2 weekly	32	42	91
Topotecan	20/20	IIIb, IV	2 mg/m^2/d × 5, q 3 weeks	0	33	102
	37/43	IV	1.5 mg/m^2/d × 5, q ? weeks	13.5		103
	38/38	advanced	1.5 mg/m^2/d × 5, q 3 weeks	18.4	36	105
	37/40	advanced	1.3 mg/m^2/d conti i.v. × 3, q 4 weeks	8.1	26	105
	17/21	IV, squa mous cell type	1.5 mg/m^2/d × 5, q ? weeks	29		104

[a] Four studies; escal, dose escalation permitted; pts, patients.

taxol in first line therapy in advanced NSCLC. In one study conducted at MD Anderson Cancer Center, taxol was administered over 24 h at 200 mg/m^2 every 3 weeks. The overall response was 24% in the 25 evaluable patients and the median survival duration was 40 weeks.[54] The other study conducted by ECOG compared taxol to piroxantrone and merbarone. Taxol administered at 250 mg/m^2 every 3 weeks produced a 21% objective response. The median survival time and 1 year percent survival were 24 weeks and 42%, respectively.[55] This level of activity (26–42%) has been confirmed in more recently published studies.[56–59] Clinical trials of taxol in refractory NSCLC have yielded conflicting results. While most studies report response rates less than 15%,[60–62] one study found objective responses in 38% of patients previously treated with a cisplatin-based regimen.[63]

Taxol has also been evaluated with other cytostatic drugs active in NSCLC. Because of taxol's potential to induce neuropathy, carboplatin is the platinum agent of choice for combination therapy. Studies investigating this combination report myelosuppression[64–70] and occasionally, peripheral neuropathy[71] to be dose limiting. Since investigators have reported a dose–response relationship,[66–68] many recent studies include strategies to dose escalate at least one of the two agents. The overall response rates of 12–63%[64–71] in these phase I combination studies indicate a significant level of activity which justifies further investigation in phase II or phase III comparative trials.

Dose finding studies of taxol + cisplatin ± etoposide also report significant levels of activity with response rates ranging between 38 and 56%. However, because of cisplatin and taxol's combined toxicity profiles, significant neuropathy becomes the limiting factor.[29,30,72,73] Phase I studies of taxol in combination with ifosfamide or adriamycin have been reported and these combinations are now being tested in the phase II setting.[74–76]

To date only one comparative trial has been reported on taxol in NSCLC. The EORTC recently published the results of a phase II study evaluating

Table 6. Phase I/II trials of combination chemotherapy

Agents		Patients evaluable	Population	Response (%)	Median survival (weeks)	Reference
Taxol						
	cisplatin	16/19	III, IV	56		72
	cisplatin	29/32	IIIb, IV	38		30
	cisplatin	17/17	IIIb, IV	47		29
	carboplatin	53/54	IIIb, IV	63	54	69
	carboplatin	50/60	III, IV	12		66
	carboplatin	47/51	IIIb, IV	24	25.5	67
	carboplatin	28/42	IIIb, IV	25		68
	carboplatin	27/40	III, IV	63		71
	carboplatin	26/26	IV	50		64
	carboplatin	11/17	IIIb, IV	36		70
	carboplatin	19/24	IV	37+		65
	VP + P	24/24	IIIb, IV	45		73
Taxotere						
	cisplatin	36/47	III, IV	33		86
	cisplatin	24/24	III, IV	25		87
	cisplatin	22/22	IIIb, IV	46		88
Gemcitabine						
	cisplatin	47/50	III, IV	30		154
	cisplatin	52/60	III, IV	38		31
	cisplatin	35/38	III, IV	46		156
	cisplatin	46/48	IIIb, IV	58		155
	cisplatin	26/30	IIIb, IV	42		157
Vinorelbine						
	cisplatin	30/32	III, IV	33	48	117
	cisplatin	30/30	IIIb, IV	46		118
	cisplatin	111/115	III, IV	52	43	119
	ifosfamide	18/18	IIIb, IV	56	47	125
	fosfamide	20/20	III, IV	40		126
	ifosfamide	34/39	IIIB, IV	44		128
	ifosfamide	37/41	IIIB, IV	32	48	127
	ifos + P	19/22	IIIB, IV	47		129
	ifos + P	19/23	IV	42		131
	ifos + P	35/45	IIIb, IV	60	52	130
	5-FU + P	76/76	II–IV	31–57		133
	5-FU + P	32/33	III, IV	55–58		136
	5-FU + FA + P	16/40	advanced	25		134
	5-FU + FAP	92/92	I, IV	41–65		135
	other multidrug	29/35	III, IV	45		137
		19/19	III, IV	42	25	138
		20/21	IV	30	26	139
CPT-11						
	cisplatin	26	IIIb–IV	54		93
	cisplatin	70	IIIb–IV	48		94
	P + G-CSF	20	IIIb–IV	50		95

VP, etoposide; P, cisplatin; ifos, ifosfamide; FA, folinic acid.

taxol/cisplatin (TaP) against teniposide/cisplatin (TeP).[77] Both arms had acceptable levels of toxicity. Febrile neutropenia was seen exclusively and myelosuppression was more prominent, in the TeP arm (25% of patients). Peripheral neuropathy (grade 2–3) appeared similar in the two arms [15% (TaP) and 20% (TeP)], whereas myalgia (grade 2–3) was reported only in the TaP arm. Response rates were 7/31 and 3/28 in the TaP and TeP arms, respectively. Survival information was not available, but will become the primary end point of a phase III extension of this study.

Although the activity of taxol in the treatment of NSCLC has been clearly demonstrated, data on its impact on survival remains sparse. ECOG is presently conducting a phase III comparative cisplatin/taxol versus cisplatin/etoposide. Results of this trial are expected to be presented at the 1996 ASCO

Table 7. Phase II/III comparative trials

Sample size	Population	Dose/schedule	Response (%)	Median survival (weeks)	One year survival (weeks)	Reference
Taxol						
69	III, IV	Ta 175 mg/m^2 + P 80 mg/m^2, q 3 weeks	23			77
		P 80 mg/m^2 d1 + Te 100 mg/m^2 d1,3,5, q 3 weeks	11			
Vinorelbine						
574/612	inoperable	V 30 mg/m^2 weekly	14	31	30	123
		V as above + P 120 mg/m^2, q 4–6 weeks	30	40	35	
		D 3 mg/m^2 weekly or biweekly + P as above	19	32	27	
208/240	III, IV	V 30 mg/m^2 weekly	16	32		122
		V as above + P 80 mg/m^2, q 3 weeks	43	33		
204/210	IIIB, IV	V 25 mg/m^2 weekly	31	54		115
		D 3 mg/m^2 weekly	9	52		
132	IIIB, IV	P 100 mg/m^2 d1 + D 3 mg/m^2 d1, 15 + M 6 mg/m^2 d1, q 4 weeks	13			132
		P 80 mg/m^2 d1 + I 3 gm/m^2 d1 + V 25 mg/m^2 d1, 8, q 3 weeks 15	15			
		B 350 mg/m^2 d1 + V 25 mg/m^2 d1, 8, q 28	10			
52/70	IIIB, IV	V 30 mg/m^2 weekly	32			124
		V as above + P 100 mg/m^2/4 weeks	33			

P, cisplatin; V, vinorelbine; D, vindesine; LV, leucovorin; M, mitomycin C; B, carboplatin; Ta, taxol; Te, teniposide; I, ifosfamide.

meeting.[53] Further phase III studies with survival rates and quality of life as endpoints are required to completely assess the benefits of this agent.

Several other taxane analogs have been studied in the pre-clinical setting but the only other one tested in clinical trials is taxotere. Taxotere is a semi-synthetic compound synthesized from the needles of the European yew tree. Phase I studies using various administration schedules were conducted in Europe, North America and Asia. Following the results of the North American and European studies, a dose schedule of 100 mg/m^2 i.v. over 1 h, repeated every 3 weeks has been adopted for phase II and III western studies. Japanese studies have used a reduced dose of 60–70 mg/m^2 i.v. over 1–2 h, repeated every 3–4 weeks. Early results of its efficacy in the treatment of NSCLC became available in 1993 and continue to be confirmed in the literature. Response rates of 23–38% were reported from phase II studies of 100 mg/m^2 conducted by the EORTC,[78] the MD Anderson Cancer Center,[79] Memorial Sloan-Kettering Cancer Center[80] and a Canadian group.[81] A Japanese report of three phase II studies using a lower dose of taxotere (60 mg/m^2) found an overall response of 21%[82] and an American study utilizing 75 mg/m^2 reported a 25% objective response.[83] The principal form of toxicity observed in all these studies was leukopenia, but infusion-related hypersensitivity, fluid retention, skin rash and sensory neuropathy were also significant.[78–82,84] The prophylactic use of corticosteroid and antihistamines markedly reduces the dermatologic toxicity and hypersensitivity, but appears to have little impact on fluid retention.[83,85] The use of 8 mg dexamethasone given twice a day for 5 days, starting 24 h prior to intravenous taxotere, has become the standard pre-medication.

Taxotere is also one of the few agents active as second line therapy in NSCLC. The MD Anderson Cancer Center reported response rates of 21% in 42 evaluable platinum-refractory patients.[84] A total of 88 patients with recurrent NSCLC treated with taxotere as second line therapy have been reported in the literature with a 19% response rate.

Studies combining taxotere with other agents active in NSCLC are ongoing. The results of the cisplatin combination are only now becoming available but, unfortunately, reported response rates

between 25 and 46%[86–88] suggest that the benefit of adding cisplatin, if any, is limited. The response to single-agent taxotere is comparable to that of taxol. It remains to be determined, however, whether it will be as active as taxol in combination with other agents and whether either drug in combination will be superior when compared to more standard regimens.

Campothecins

Campothecin is a plant alkaloid which acts by inhibiting the enzyme topoisomerase I. Recently, two of its derivatives, irinotecan (CPT-11) and topotecan, have been investigated for their anti-tumor activity in a variety of tumors, including NSCLC. A phase I study of 50–180 mg/m^2 irinotecan given weekly for 4 weeks, followed by a 2 week rest, reported diarrhea (grade 4) as the dose-limiting toxicity (DLT). The maximum tolerated dose (MTD) was established as 150 mg/m^2.[89] A Japanese phase II study using weekly infusion of 50–180 mg/m^2 irinotecan reported leukopenia and diarrhea as the DLT, and 100 mg/m^2 as the MTD.[90] In a follow-up phase II study, this agent yielded a 32% response rate in previously untreated patients with NSCLC.[91] Other phase II studies of single-agent CPT-11 are ongoing and report encouraging results.[92] CPT-11 has been combined with cisplatin or etoposide. Two groups have evaluated the combination of monthly cisplatin (80 mg/m^2) with weekly CPT-11 for 3 weeks followed by 1 week rest, in stage IIIB and stage IV NSCLC. Response rates of 48–54% were observed,[93,94] with leukopenia and diarrhea being of significant severity. At a weekly CPT-11 dose of 60 mg/m^2, 46% of patients experienced grade 3 or 4 leukopenia, and 42% reported diarrhea of grades 3 or 4. One study reported two deaths related to paralytic ileus following severe diarrhea.[94] The CPT-11 dose could be increased by 33% when the above regimen was supported with granulocyte colony stimulating factor (G-CSF), but diarrhea continued to be dose limiting.[95] Regimens utilizing other cisplatin schedules also appear feasible and active.[96] Combinations of etoposide/CPT-11 and vindesine/CPT-11 are feasible, and warrant further investigation.[97,98]

Topotecan has been evaluated in phase I studies in a variety of schedules, from daily i.v. bolus injections to continuous infusions. These studies suggest some activity in NSCLC.[99–101] However, in a follow-up study when topotecan, 2 mg/m^2 daily × 5 every 3 weeks, was administered to chemo-naive stage IIIB and IV NSCLC patients no objective responses were observed in the first 20 patients causing the premature closure of the study.[102] Another study conducted at MD Anderson utilizing a similar regimen reported moderate activity of topotecan (13%) in NSCLC. Interestingly, a high response rate (36%) was observed in the subset of patients with squamous carcinomas.[103] This study was expanded to include more patients with squamous carcinomas of the lung and preliminary results confirm the activity of topotecan in this group (29%).[104] It is noteworthy that the negative trial had entered only three patients with squamous cell type. In an attempt to determine the most appropriate schedule for topotecan administration in the treatment of NSCLC, the NCCTG conducted a comparative trial of daily bolus and continuous administrations. The study demonstrated an advantage for the daily i.v. bolus injection schedule, but reported only modest activity in either arm (8 and 18%).[105] The experience of topotecan combinations in NSCLC is limited. Phase I studies with cisplatin suggest that it may have some activity and the DLT of this regimen is reported to be leukopenia.[106,107] Topotecan is expected to undergo trials in combination with the topoisomerase II inhibitor, etoposide. Finally, because of its apparent radiosensitizing property, it is currently being investigated in combination with radiotherapy in the treatment of locally advanced disease.[108]

Vinorelbine

Vinorelbine is a semi-synthetic vinca alkaloid with demonstrated activity ranging between 29 and 33% in chemo-naive patients with NSCLC.[109–111] This level of activity makes it one of the most active single agents in NSCLC. However, few or no objective responses have been seen in advanced NSCLC when vinorelbine has been used as second line therapy.[112,113] The DLT associated with vinorelbine therapy is hematologic, consisting primarily of granulocytopenia. The incidence of neurotoxicity associated with vinorelbine therapy is significantly less than that observed with other vinca alkaloids.[114] A recent randomized phase II trial comparing single-agent vinorelbine to vindesine in 210 previously untreated patients demonstrated better tolerance and improved efficacy (response rate 31 versus 9%) in the vinorelbine arm.[115]

In view of its significant single-agent activity and manageable toxicity, vinorelbine has been combined with several other agents active in NSCLC. The experience with vinorelbine in NSCLC has

recently been reviewed by Goa and Faulds.[116] Combination vinorebine with cisplatin or carboplatin in previously untreated patients results in response rates ranging between 33 and 52%.[117–119] Myelosuppression, nausea and vomiting are the primary toxicities.[117–121] A French group compared vinorelbine + cisplatin to vinorelbine alone.[122] Patients were randomized to receive vinorelbine 30 mg/m^2 weekly or vinorelbine 30 mg/m^2 weekly + cisplatin 80 mg/m^2 on day 1, every 3 weeks. The response rates in the 208 evaluable patients were significantly different (43 versus 16%), in favor of the combination arm. However, the median survival was the same in both arms (33 versus 32 weeks). Le Chevalier *et al.* evaluated this combination in a three-arm model.[123] Six hundred and twelve patients were randomized to receive weekly vinorelbine 30 mg/m^2 (V); vinorelbine 30 mg/m^2 weekly + cisplatin 120 mg/m^2 on days 1 and 29 and then every 6 weeks (VP); or vindesine 3 mg/m^2 weekly for 6 weeks and then every 2 weeks + cisplatin 120 mg/m^2 on days 1 and 29 and then every 6 weeks (DP). The vinorelbine + cisplatin arm was demonstrated to be significantly superior with respect to response [30% (VP), 19% (DP) and 14% (V)] and survival. The median survival was 40 (VP), 32 (DP) and 31 weeks (V), and the 1 year survival was 35 (VP), 27 (DP) and 30% (V), respectively. Another smaller comparative trial, of cisplatin + vinorelbine versus vinorelbine alone with response rates as their primary end point demonstrated significant activity in both arms, but failed to detect a survival difference between the arms.[124]

Studies of combination vinorelbine and ifosfamide have yielded very encouraging response rates (32–56%),[125–128] with one study reporting a complete response rate of 33% and a median survival of 47 weeks.[125] The independent success of vinorelbine with cisplatin and ifosfamide prompted the investigation of their combination in advanced disease. Results of phase II studies with these three drugs suggest significant levels of activity (42–60%) with hematological toxicity continuing to be limiting.[129–131] This combination was recently compared to cisplatin + vindesine + mitomycin and carboplatin + vinorelbine in a three-arm randomized Italian clinical trial. No arm demonstrated any superiority in tumor control and all response rates were moderate to poor.[132]

The combination of vinorelbine + 5-fluorouracil (5-FU) + cisplatin has also been reported active (responses between 25 and 65%),[133–136] but randomized studies are required to determine whether the addition of 5-FU imparts a significant advantage. Other multi-drug trials have incorporated vinorelbine with various success rates,[137–139] and the comparative study of vinorelbine-containing regimens remains ongoing.[140,141]

The encouraging activity seen in stage IV disease has prompted its incorporation into multi-modality approaches for the treatment of locally advanced unresectable NSCLC. Preliminary results suggest that the use of combination vinorelbine and cisplatin as induction therapy prior to radiotherapy is promising.[142,143] Finally, since granulocytopenia has been consistently demonstrated to be dose limiting in vinorelbine-based combinations, some trials have incorporated G-CSF into the treatment regimen.[144–146] Recently, treatment with oral vinorelbine has been proven feasible and active in the palliative setting.[147,148]

Gemcitabine

Gemcitabine is a pyrimidine antimetabolite with a broad spectrum of activity. Phase I/II studies of gemcitabine have demonstrated that it can be administered safely at doses up to 2800 mg/m^2.[149] It appears active in NSCLC when given by short infusion weekly for 3 out of 4 weeks. The dose-limiting toxicity is hepatocellular and myelosuppression. While an early US study of gemcitabine reported poor activity, two large phase II studies and a review of four phase II studies conducted in patients with locally advanced or metastatic disease have confirmed the activity of gemcitabine in NSCLC. In all the studies the weekly × 3, q4 weekly regimen has been used with starting doses of gemcitabine ranging from 800 to 1250 mg/m^2, and allowing dose escalation.[150–152] Overall, 20% of patients achieved a response and most of these were partial responses. Toxicity was generally mild and reversible. When significant toxicity occurred the following were the most frequent: myelosuppression, transaminase elevation, nausea and vomiting, albuminuria, flu-like symptoms and rashes. Evidence of activity of single-agent gemcitabine within that range continues to emerge.[149,153]

Response rates with single-agent gemcitabine are similar to those of other active agents in NSCLC. This response and its modest toxicity profile make it a good candidate for combination therapy. Results of these studies are only now becoming available. In the phase I/II setting, combination cisplatin + gemcitabine has been well tolerated and offers encouraging response rates ranging between 30 and 58%.[31,154–157] The study of combination gemcita-

bine and ifosfamide is underway, and preliminary results suggest a significant level of activity.[158]

Gemcitabine has a short half-life and, because of this, optimal tumor control may be afforded by the continuous infusion of the drug. Phase I studies of 24 h continuous infusion gemcitabine have already shown it to be feasible and tolerable,[159,160] but comparative studies are required to establish its activity.

Finally, *in vitro* data of gemcitabine and concurrent radiation has demonstrated that gemcitabine causes synergistic cell kill when combined with radiation. This has led our group to embark upon a phase I study of gemcitabine and cisplatin given weekly with concurrent curative thoracic irradiation in stage III NSCLC.

Gene therapy

Despite the introduction of new chemotherapy agents and combined modality approaches for the treatment of advanced NSCLC, the gains in response, quality of life and overall survival are at best modest. This has led to a search for new approaches to this devastating disease. The recent development of new technologies in molecular biology has allowed molecular geneticists to establish cancer as a genetic disease. Common tumors develop through a multi-stage process involving multiple genetic and epigenetic events in oncogenes, tumor suppressor genes and anti-metastatic genes.[161] Tumor growth and burden is a consequence of abnormalities of genes that control proliferation, differentiation, apoptosis and the metastatic process.[162] It is the study of the genes governing these processes and the development of gene or anti-gene manipulation technologies that promoted the concept of 'the gene therapy of cancer'. Gene therapy involves a variety of new approaches such as gene transfer, gene repair, gene deletion or gene replacement. A number of gene delivery techniques have been developed and can be grouped into non-viral[163–165] (e.g. direct DNA injection, liposome-mediated gene transfer and ligand-mediated gene transfer) and recombinant virus-mediated gene transfer systems [e.g. adenovirus, retrovirus, adeno-associated virus, herpes simplex virus (see Table 8)].[166–170] Each of these systems has advantages and disadvantages.

Therapeutic strategies can be broadly divided into two groups: those that involve genetic modification of immunoeffector cells making them more potent tumor killers and those that involve genetic modification of tumor cells. The later approach at present appears to hold the most hope for improved treatment of NSCLC. Therefore genetic modification of tumor cells by gene therapy techniques will be briefly reviewed. This strategy involves anti-oncogene therapy which will suppress or modulate active oncogenes or the replacement of normal wild-type genes where tumor suppressor genes have been inactivated. Both strategies aim at restoration of normal control of growth and differentiation.

Various techniques have been used to suppress oncogenes. These strategies have attempted to block the pathway at different stages from DNA to the protein product. The techniques include the use of anti-sense oligonucleotides, anti-sense RNA, anti-gene oligonucleotides and ribozymes. The latter two approaches, anti-gene oligonucleotides and ribozymes, although conceptually exciting are in early stages of development and require multiple technical problems to be addressed.[171–173] On the other hand, anti-sense oligonucleotides have been shown to inhibit the activity of multiple proto-oncogenes including *ras*, c-*fos*, c-*abl*, c-*fes*, c-*fms*, c-*kit*, c-*myb*, c-*myc*, c-*raf* and c-*src* *in vitro* in a large number of different cancer cell lines.[174–176] Furthermore in several tumor and animal models gene expression and the cancer phenotype can be inhibited.[177] Although there have been a number of postulates, the exact mechanism by which anti-sense oligonucleotides inhibit gene expression is not known. Despite initial success in cancer cell lines and in animal models there remain considerable limitations to this approach in humans. These include rapid catabolism, preferential accumulation in liver and kidney rather than the tumor target, lack of specificity, and low cellular uptake efficiency. Ongoing development to improve the *in vivo* stability and delivery of anti-sense oligonucleotides ranges from modification of the nucleotides to the synthesis of oligonucleotide–liposome complexes.[178]

Table 8. Delivery systems for gene transfer

Non-viral systems
direct DNA injection
liposome mediated
ligand mediated
Viral-mediated systems
retroviruses
adenoviruses
herpes viruses
adeno-associated viruses

Anti-sense RNAs, on the other hand, have an advantage over anti-sense oligonucleotides since their template can be efficiently delivered into target cells by viral vectors and active forms can be produced within cells in a controlled manner by different promoters. Successful suppression of transcription or translation of oncogenes by anti-sense RNA has been demonstrated for c-*fos*, c-*myc* and K-*ras* leading to a less malignant phenotype in the target cell lines. The problems associated with anti-sense RNA therapy include degradation by nucleases, variation in the expression levels and self-inhibitory secondary structure.[179–181] Zhang *et al.* designed an anti-sense K-*ras* RNA and demonstrated that the synthesis of the K-*ras* protein could be successfully inhibited in the H460a cells.[178] Colony formation in soft agar was dramatically lower in transduced cells than in non-transduced or vector only transduced cells.[182] This anti-K-*ras* retrovirus was then further evaluated in a mouse model of human lung cancer. Irradiated nude mice were given H460a cells by intratracheal installation. Following this, the mice were given daily anti-K-*ras* retrovirus on days 4, 5 and 6. After 30 days mediastinal blocks were harvested from the mice and assessed for tumor growth. Anti-K-*ras* retrovirus prevented H460a tumor cell growth in 86–90% of mice in three independent experiments. The control mice that received the sense K-*ras* construct, vector or medium alone showed no significant difference in tumor growth. The tumors seen in the mice treated with anti-K-*ras* were significantly smaller than those seen in control mice. As a result of these studies a clinical trial of intra-tumor injection of anti-K-*ras* retrovirus for the treatment of unresectable NSCLC is in progress at the MD Anderson Cancer Center.[178]

Tumor suppressor genes are normally occurring genes which regulate proliferation, differentiation and apoptosis. In order for the cell to escape from control both alleles need to be defective. This can occur in a number of ways including deletion of one allele and a mutation in the other, deletion of both alleles or a mutation in both alleles. The strategy of tumor suppressor gene therapy is to restore normal gene function by inserting the wild-type gene. Human tumor suppressor genes that have been cloned and characterized include p53, Rb, WT1, APC and DCC. Mutations or deletions of these genes in animal models leads to an increased susceptibility to cancer.[162,183] Mutations of the p53 gene located on chromosome 17p is among the commonest abnormalities identified in human cancers.[184] Wild-type p53 plays a role in transcriptional regulation, DNA replication, control of the cell cycle and induction of apoptosis.[185–187] Insertion of a single copy of wild-type p53 into a number of human tumor cell lines that lacked p53 or express mutant p53 results in suppression of proliferation.[188–190] Experiments in nude mice have shown that human tumor cells in which wild-type p53 has been transinfected are no longer tumorigenic or less tumorigenic than parental cells.[188–190] Roth *et al.* have shown that the intratracheal installation of retrovirus containing wild-type P53 prevented the growth of established orthotropic human lung cancer in nude mice. In order to improve the delivery efficiency of the wild-type p53 gene into lung cancer cells *in vivo* a replication-defective and helper-independent recombinant p53 adenovirus (Ad5CMV-p53) has been generated by this group.[191] When evaluated against lung cancer cell lines those lines with a deleted or mutated p53 were 72–79% inhibited by the Ad5CMV-p53 whereas cell lines containing wild-type p53 were less inhibited (28%). The optimal dose for growth rate inhibition was somewhere between 10 and 50 p.f.u./cell.[178,192] At dose rates of 10 p.f.u./cell no evidence of apoptosis was found but these cells were found to be more sensitive to cisplatin and irradiation.[193] This Ad5CMV-p53 was further evaluated in a mouse orthoptic human lung cancer model. The mice were inoculated with human lung cancer cells and 3 days later were treated with intra-tracheal installation of Ad5CMV-p53. At the end of a 6 week period tumor formation was evaluated by dissecting the lung and mediastinal tissues. Twenty-five per cent of Ad5CMV-p53-treated mice formed tumors, whereas 70–80% of mice in the control group formed tumors. In addition to there being fewer tumors, the lung and mediastinum tumors were also smaller in the Ad5CMV-p53 group. Based on these findings the group at the MD Anderson are preparing to embark upon a phase I clinical trial of a combination regimen of Ad5CMV-p53 and cisplatin in patients with NSCLC.[178] In addition to the Ad5CMV-p53 study, this group has also commenced a phase I study of p53–retrovirus in NSCLC.

Other new strategies

The clinical manifestation and initial presentation of the common human cancers usually occurs at a late stage in the disease process when the potential for invasion has already been realized. At the time of diagnosis a high percentage of patients already have clinically occult or detectable metastatic disease.[194]

For multiple reasons it is difficult for currently available chemotherapy agents to control and cure these advanced cancers and consequently the time from diagnosis to death of the individual is relatively short. In contradistinction there is accumulating data that the period from the initiation of carcinogenesis to the development of an invasive cancer is much longer. This long pre-invasive period may afford an opportunity for intervention and the consequent prevention of life-threatening metastatic disease.[195,196]

There are two events which initiate the metastatic process. These two events occur in parallel. The first is the initiation of local cellular invasion and the second is tumor-induced neovascularization. Agents that either blocked angiogenesis or retard invasion could arrest neoplastic progression at the non-invasive stage.[197–199] Cellular invasion is thought to involve the formation of pseudopodia which have proteins on their surface coordinating sensing, protrusion, burrowing and traction.[200,201] In order for a cell to invade it has to achieve forward locomotion which couples local proteolysis with coordinated, limited, attachment and detachment.[200–202] Localized to the tip of invading pseudopodia are proteases bound to activated proteins and receptors. These protease form a zone of proteolysis which is followed by adhesion of pseudopodia and dissociation of the rear of the cell from adjacent cells in order that the cell may move forward and be released from its initial position.[203,204] However, a major barrier to cellular invasion is the basement membrane. In addition to being a physical barrier the basement membrane is also a depot for latent proteases and cytokines including angiogenesis factors which may be released by invading cell pseudopodia.[205,206] The aggressiveness of tumors is positively correlated with protease levels.[205,207–209] There are four classes of protease including serine, aspartyl, cysteinyl and metal atom dependent proteases. These proteases may all be equally important at various stages of invasion but it is now well established that the integrity of the basement membrane is regulated by a balance between metalloproteinases and metalloproteinase inhibitors.[205–209]

Matrix metalloproteinases (MMPs)

The gene family MMPs is implicated in tumor invasion and metastases formation. The enzymes are divided into three sub-classes: stromelysins, interstitial collagenases and gelatinases. They are secreted as pro-enzymes and require activation. Their actions on tissues are largely irreversible and therefore inhibitors of these enzymes are important in the study of the physiological role of MMPs and their role in malignant invasion.[210–212] Three strategies are currently under study. The first is to use tissue inhibitor of metalloproteinases (TIMPs) or TIMP fragments as directly selective inhibitors of MMP activation or activity.[208–214] The second is to use peptide inhibitors which mimic the amino acid terminal of MMP that maintains the latent enzyme state[215,216] and the third strategy is synthetic compounds which compete for the substrate or bind to the active site.[217,218] All three groups of metalloproteinases inhibitors have been shown to inhibit invasion in physiological models *in vitro* and animal tumor models *in vivo*.[207,219,220] The collagen substrate analogs are the largest group of synthetic metalloproteinases inhibitors. They are less than six amino acids long and thought to act by binding to the site of active metalloproteinases.[221] One of these inhibitors, BB94 (batimastat), has in pre-clinical studies inhibited the growth of primary tumor or delayed the growth and size of established metastases of colon cancer, ovarian cancer and melanoma in animal models. Inhibition of growth may be indirectly linked to the effect on angiogenesis and the inhibition of local tumor invasion.[217] A number of MMP inhibitors have completed phase I studies and are ready for further evaluation. The National Cancer Institute of Canada is about to embark on a phase III study of chronic administration of BB2516, an oral MMP inhibitor, in patients with small cell lung cancer who have responded to first line chemotherapy. The primary objectives of the study are to measure time to disease progression, overall survival and quality of life. This is a new and exciting area of clinical research. These agents potentially could play a role in the maintenance of remission following surgery in stage I and II disease or following chemo/radiotherapy in stage III or IV NSCLC. Phase III studies are currently being planned.

Calcium homeostasis modulators

During the process of carcinogenesis multiple genetic abnormalities can lead to altered gene expression of growth factors, autocrine loops and transmembrane signal transduction resulting in an imbalance of signaling homeostatis. An important regulator of transmembrane signaling is intracellular calcium.[222,223]

An inhibitor of non-voltage-gated calcium pathways CAI has been shown *in vitro* and *in vivo* to inhibit tumor cell proliferation invasion and angiogenesis.[222,224,225] Human xenograft bearing mice given daily CAI were found to have a reduction in total tumor burden, tumor incidence and metastatic dissemination.[224] CAI-sensitive calcium influx events have been linked to downstream signaling pathways including phospholipase A2, calcium-sensitive phospholipase Cδ and protein tyrosine kinase activity. Modulation of these signaling pathways by CAI resulted in inhibition of steps in invasion, including tumor cell migration, gelatinase A production, inhibition of proliferation and inhibition of invasive potential, in a variety of malignant human cell types.[226] A phase I clinical trial of CAI has been undertaken in patients with refractory tumors and the toxicity appears to be acceptable. This sets the stage for the drug to be evaluated in patients in phase III studies including NSCLC.

Conclusion

NSCLC remains a devastating disease with only 10–15% of patients disease free at 5 years. Nevertheless recent developments are grounds for optimism. Randomized trials of combined modality therapy in loco-regional disease and chemotherapy alone in metastatic disease have demonstrated that the natural history of the disease can be positively altered, albeit to a modest degree. Further developments of innovative radiation techniques, including three-dimensional radiotherapy and hyperfractionated schedules, may result in increased cell kill with decreased normal tissue toxicity. Progress in molecular genetics suggests that 'gene therapy' will be a therapeutic reality. These techniques are likely to be used sequentially or in combination with radiotherapy, standard and new chemotherapy agents, and will ultimately lead to better local tumor control. However, better control of metastatic disease is necessary to improve survival. Together with new chemotherapy agents, it is hoped that the novel anti-angiogenic and anti-metastatic modulators will positively impact on the control of systemic disease. Furthermore, these agents may play a role in the early prevention and maintenance therapy of NSCLC.

Finally, regardless of the modality of treatment, new therapies will have to be objectively evaluated, in the setting of the phase III randomized trial, in order to assess their effect on the natural history of the disease. These studies will certainly require concomitant quality of life evaluations, so that an accurate assessment can be made of the impact of these modalities on the NSCLC patient.

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