Preclinical assessment of cisplatin-based therapy versus docetaxel-based therapy on a panel of human non-small-cell lung cancer xenografts

Fariba Némati[a], Rui Bras-Gonçalves[g], Jean-Jacques Fontaine[h], Gonzague de Pinieux[i], Patricia De Cremoux[b], Alain Chapelier[j], Catherine Daniel[c], Pierre Laurent-Puig[f], Alain Livartowski[c], Jean-Gabriel Judde[k], Vincent Bordier[e], Marie-France Poupon[a] and Didier Decaudin[a,d]

The success of treatment of advanced non-small-cell lung cancer (NSCLC) remains very poor. The aim of this study was, on a series of NSCLC xenografts, to compare the efficacy of standard cisplatin-based or docetaxel-based chemotherapy. Seven human xenografts were obtained from six patients (two xenografts were derived from primary or metastatic tumors of the same patient). Three xenografts were adenocarcinomas and four were squamous cell carcinomas. All xenografts reproduced the same histology as that of the patient's original tumor. Docetaxel, administered as single-agent chemotherapy, induced a significant response in five of the seven NSCLC xenografts (71%), without significant increase after combination with cisplatin, vinorelbine, or gemcitabine. Relative expression of genes putatively involved in drug response was also studied in all xenografts and did not explain the variability of drug sensitivity. In conclusion, this panel of human NSCLC xenografts reliably reproduces the data obtained in patient tumors and the relative sensitivity to docetaxel reported in NSCLC patients. *Anti-Cancer Drugs* 20:932–940 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Preclinical Investigation Laboratory, Departments of [a]Translational Research, [b]Tumor Biology, [c]Clinical Oncology, [d]Clinical Hematology, [e]Animal Experiments Unit, Research Section, Institut Curie, [f]Inserm U775, Hôpital Européen Georges Pompidou, Paris, [g]Institut de Recherche en Cancérologie de Montpellier, Parc Euromédecine, Montpellier, [h]Department of Pathology, Alfort Veterinary School, Maisons-Alfort, [i]Department of Pathology, Hôpital Trousseau, Tours, [j]Department of Chest Surgery, Hôpital Foch, Suresnes and [k]XenTech, Evry, France

Correspondence to Dr Didier Decaudin, MD, PhD, Laboratoire d'Investigation Pré-clinique/Service d'Hématologie Clinique, Institut Curie, 26 rue d'Ulm, 75248 Paris Cedex 05, France
Tel: +33 1 44 32 46 90; fax: +33 1 53 10 40 11;
e-mail: didier.decaudin@curie.net

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Introduction

Lung cancer is the leading cause of death from malignant disease in Western countries. Non-small-cell lung cancers (NSCLC) represent 80% of all lung cancers; they constitute a heterogeneous entity of tumors comprising similar proportions of adenocarcinomas and squamous cell carcinomas, and 10% of large cell carcinomas. They are characterized by a high metastatic potential, a fairly low proliferation index, and drug resistance. At diagnosis, 40–50% of patients with NSCLC have metastatic disease, requiring systemic treatment.

Although the efficacy of chemotherapy remains debated, several publications over the last decade have reported a real clinical benefit of chemotherapy in advanced forms with response rates of 20–40% and a median survival of 8 months [1,2]. Many meta-analyses have defined cisplatin-based chemotherapy as the standard treatment for NSCLC. Most studies comparing new chemotherapeutic agents including paclitaxel, docetaxel, gemcitabine, vinorelbine, alone or combined with cisplatin have shown the superiority of platinum-based doublets in terms of response rate and survival duration. In particular, the meta-analysis by Hotta *et al.* [3] compared platinum-based doublets with single new agent therapy alone in eight trials including 2374 patients conducted between 1994 and 2003: platinum-based doublets produced an approximately two-fold higher overall response rate (odds ratio = 2.32; 95% confidence interval = 1.68–3.20) and increased the overall survival rate by 13% (odds ratio = 0.87; 95% confidence interval = 0.80–0.94, $P < 0.001$). The Eastern Cooperative Oncology Group E1594 trial randomized 1157 patients with advanced NSCLC to four platinum-based doublets: paclitaxel/cisplatin, cisplatin/gemcitabine, cisplatin/docetaxel, and carboplatin/paclitaxel. All four combinations showed

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comparable efficacy in terms of response (17–21%) and survival (31–36% at 1 year and 10–13% at 2 years) rates [4]. At this time, the choice of drug combined with cisplatin was therefore based on the patient's comorbidity, safety profile, and the modalities of administration of chemotherapy. No biomarkers are currently available to select the best cisplatin-based chemotherapy.

Selection of the most active combinations could be guided by preclinical experimental assays. These assays must be conducted on valid preclinical models, that is, models providing information predictive of the clinical efficacy of new drugs or combinations. Human tumor xenografts are widely used to define the efficacy of new compounds as single agents, but less frequently to evaluate the efficacy of combination therapy. As NSCLC are largely chemoresistant, the panel of xenografts must include not only resistant tumors, but also sensitive tumors which could help identify the parameters of response, as drug response is determined by numerous biological parameters such as p53 mutations and expression of genes involved in drug metabolism and cell survival. These biological features can be evaluated by various methods. For example, extended profiles of mRNA expression levels of multiple genes can be obtained by cDNA microarrays or, alternatively, the level of selected genes putatively involved in drug response, that is, genes encoding enzymes involved in drug metabolism, or drug resistance proteins, or proteins involved in DNA repair, can be measured by quantitative real-time PCR. The definition of a chemotherapy resistance gene expression profile therefore seems to be a useful tool in the preclinical evaluation of new compounds and new combination therapies.

In this study, we present a panel of human NSCLC xenografts, obtained from patients with known clinical characteristics and outcome, which were tested for their sensitivity to docetaxel-based chemotherapies. The aims of this study were to (i) constitute a representative panel of NSCLC, which could be further used to test new compounds and new combinations *in vivo*, (ii) identify biological markers of drug resistance, and (iii) compare the preclinical efficacy of docetaxel-based chemotherapies on NSCLC xenografts with clinical data derived from NSCLC patients.

Materials and methods

Histological examination of xenografts

Histological examination was performed on each xenograft and compared with the histological findings of the corresponding patient's tumor. For light microscopy examination, 4-μm-thick formalin-fixed paraffin-embedded sections were stained with hematoxylin and eosin safran.

Non-small-cell lung cancer xenografts and in-vivo tumor growth

Tumor specimens were obtained from NSCLC patients during surgery according to ethical rules. Tumor samples were established as xenografts by subcutaneous implantation of a tumor fragment into the scapular area of 'nude' mice and sequentially transplanted. Swiss nu/nu male or female mice were bred in the Institut Curie Animal Facilities, Paris, France. The animals were maintained under specific pathogen-free conditions. Their care and housing were performed in accordance with the institutional guidelines of the French Ethical Committee (Ministère de l'Agriculture, Paris, France) and under the supervision of authorized investigators.

For therapeutic experimental assays, female mice aged 6–10 weeks received a subcutaneous graft of tumor fragments with a volume of approximately 15 mm^3. Tumors developed at the graft site 2–5 weeks later. Mice bearing growing tumors with a volume of 63–400 mm^3 were individually identified and randomly assigned to the control or treatment groups (6–10 animals in each group, as detailed in the tables and legends of figures) and treatment was started on day 1. Mice were weighed weekly. Tumor-bearing mice were killed when the tumor volume reached 2500 mm^3, defined as the ethical limit.

Tumor volumes were calculated by measuring two perpendicular diameters using a caliper. Each tumor volume (V) was calculated according to the following formula: $V = a \times b^2/2$, where a and b are the largest and smallest perpendicular tumor diameters. Relative tumor volumes (RTV) were calculated from the formula: $RTV = (V_x/V_1)$, where V_x is the tumor volume on day x and V_1 is the tumor volume on initiation of therapy (day 1). Growth curves were obtained by plotting mean RTV on the y-axis against time (x-axis, expressed as days after initiation of treatment). Antitumor activity was evaluated according to tumor growth inhibition, which was calculated according to the following formula: percentage of growth inhibition $= 100 - (RTVt/RTVc \times 100)$, where RTVt is the mean RTV of treated mice and RTVc is the mean RTV of controls at a given time when the antitumor effect was optimal. Fifty percent tumor growth inhibition was considered to be the limit of a meaningful biological effect. Statistical significance of differences observed between individual RTVs corresponding to the group of treated mice and the control groups were calculated by a paired Student's t-test.

Drug formulation and administration

Docetaxel (DOC, Taxotere, clinical preparation kindly provided by Sanofi-Aventis, France) was reconstituted in the appropriate solution according to the manufacturer's instructions and administered intraperitoneally (i.p.) at a dose of 20 mg/kg, every 3 weeks. Cisplatin (CDDP, Cisplatyl, Sanofi-Aventis, France) was reconstituted with

water and diluted in 0.9% sodium chloride solution and administered through i.p. injection in 0.2 ml volume to tumor-bearing mice on day 1, at a dose of 6 mg/kg/day, every 3 weeks, when administered alone or 1 mg/kg/day when combined. Vinorelbine (Navelbine, kindly provided by Pierre Fabre Laboratories, France) was administered through i.p. injection in 0.2 ml volume to tumor-bearing mice every 10 days at 1.25 mg/kg/day dose. Gemcitabine (Gemzar, Lilly, France) was diluted in 0.9% sodium chloride solution and administered through i.p. injection in 0.2 ml volume to tumor-bearing mice, weekly, at a dosage of 80 mg/kg/day. All drugs were prepared extemporaneously; in the case of combined treatments, each drug was injected separately into the animals. Mice in the control groups received 0.2 ml of the drug-formulating vehicle with the same schedule as the treated animals.

Determination of p53 mutations and gene expression

p53 mutations in NSCLC panel were determined as described earlier [5]. For gene expression, total RNA was isolated from xenografts using the RNA plus kit reagent (Qbiogen, Illkirch, France) according to the manufacturer's instructions, and purified RNA was stored in RNAse-free water at −80°C. RNA was quantified by the nanodrop technique (NaNoDrop, Wilmington, Delaware, USA). RNA was reverse transcribed using the Cloned AMV First-Strand cDNA synthesis kit according to the manufacturer's instructions (Invitrogen, Cergy Pontoise, France). Real-time PCR was performed with SYBR green (Eurogentec, Angers, France) on a 7500 Sequence Detector System (Applied Biosystems, Courtaboeuf, France). PCR primers used (MWG Biotech, Courtaboeuf, France) are as follows: β2 microglobulin: forward primer: 5′-ACC CCC ACT GAA AAA GAT GAG TAT-3′; reverse primer: 5′-GCT TAC ATG TCT CGA TCC CAC TTA-3′; amplicon length: 92 bp. GSTP1: forward primer: 5′-CCC TGG TGG ACA TGG TGA AT-3′; reverse primer: 5′-CCC GCC TCA TAG TTG GTG TAG A-3′; amplicon length: 82 bp. CYP3A5: forward primer: 5′-CCT GAA AGG TTC AGT AAG AAG AAG GA-3′; reverse primer: 5′-AAC CTC ATG CCA ATG CAG TTT-3′; amplicon length: 92 bp. CYP3A4: forward primer: 5′-CAG ATC CCC CTG AAA TTA AGC TT-3′; reverse primer: 5′-TCA GGC TCC ACT TAC GGT GC-3′; amplicon length: 99 bp. CYP3A7: forward primer: 5′-ACA GAT CCC CCT GAA ATT ACG CTT T-3′; Reverse primer: 5′-GGA AAT CAG GCT CCA CTT A CG GTC T-3′; amplicon length: 105 bp. CYP2B6: Forward primer: 5′-TCC ATG ACC CAC ACT ACT TTG AAA-3′; reverse primer: 5′-CAA GAC AAA TCC GCT TCC CTA A-3′; amplicon length: 126 bp. XRCC1: forward primer: 5′-TGA GAA CAC GGA CAG TGA GGA A-3′; reverse primer: 5′-TGG AAG AAA TCT GGG AGC TCA G-3′; amplicon length: 72 bp. XRCC2: forward primer: 5′-GTG TAG TGC CTT CCA TAG GGC T-3′; Reverse primer: 5′-TGG TTC TAT TTC TTT CAA GGA ACT TCT A-3′; amplicon length: 85 bp. XRCC3: Forward primer: 5′-TGC TTC AGA AGC TCC GAT TTG-3′; Reverse primer: 5′-CAC TCC AAC AAG GTG TCC ACA-3′; amplicon length: 73 bp. XRCC4: forward primer: 5′-GGT TGG CTT CAG CTG CTG TAA-3′; reverse primer: 5′-TTG CAT TCG CTG TCT CCT TTT-3′; amplicon length: 98 bp. XRCC5: forward primer: 5′-GGA AGT TCT GTC ACA GCT GAG GA-3′; reverse primer: 5′-CAA ATA CAG CTG CTG TGT CTC CAC-3′; amplicon length: 82 bp. ABCG2/BCRP: forward primer: 5′-CCT GCA GAC TTC TTC TTG GAC A-3′; reverse primer: 5′-CTA ATT TTT CTA TGA GTG GCT TAT CCT G-3′; amplicon length: 128 bp. LRP/MVP: forward primer: 5′-GGG TGA GAA GGA CAC AGC TAA GAG-3′; reverse primer: 5′-ACT GCT CCT CAG GAC CCA GC-3′; amplicon length: 171 bp. ABCC1/MRP1: forward primer: 5′-GGA CTC AGG AGC ACA CGA AAG-3′; reverse primer: 5′-ACG GCG ATC CCT TGT GAA-3′; amplicon length: 68 bp. ABCB1/MDR1ex3-4: forward primer: 5′-CAT GAT GCT GGT GTT TGG AGA A-3′; reverse primer: 5′-GGT CAT GTC TTC CTC CAG ATT CA-3′; amplicon length: 134 bp.

The reaction was then performed with the following protocol: 60°C for 2 min, 95°C for 10 min then 40 cycles of 95°C for 15 s, 60°C for 30 s. For normalization, eight housekeeping genes were tested and the β2 microglobulin gene was identified as the most stable reference gene using GenNorm software [6]. A standard curve was generated for each primer pair, to which gene expression levels were normalized by a standard curve method. Finally, a ratio was calculated comparing normalized gene expression values in treated mice versus control for each xenograft.

Results

Clinical characteristics of non-small-cell lung cancer patients

All tumors were derived from male patients aged 54–74 years who had given their consent to the use of their tumor samples in experimental studies. Tumors were obtained after surgical excision of the primary lesion. The characteristics of the patients from whom the xenografts were derived are shown in Table 1. Seven xenografts were obtained from six NSCLC patients, including three adenocarcinomas and four squamous cell carcinomas. Five xenografts were derived from metastatic lesions, mainly skin metastases, and two were derived from lung tumors (one primary tumor and one recurrence). Two xenografts were derived from the same patient, but from the initial primary tumor and adrenal metastases, namely ML5 and IC14, respectively. All xenografts, except for IC131 and IC9, were obtained before administration of chemotherapy to the patient for corresponding tumor. Patient outcome was variable: short survival for four patients (overall survival ranged between 2 and 12 months), and longer for two patients (overall survival of 67 and 78 months corresponding to IC8 and ML5-IC14 xenografts, respectively).

Table 1 Clinical characteristics of xenograft-originated NSCLC patients

Patients	Tumor site	Xenografts	Histology and initial staging	Patients' treatment before graft	Age (years) and sex	Patient's treatment(s)	Response	Status (months)
1	Brain metastasis	IC8	ADC T1N0M1	None	71/M	CT + RT for primary tumor surgery + RT[a]	Stabilization	Survival (67)[b]
2	Skin metastasis	IC1	Squamous cell T3N1	None	71/M	Surgery of primary tumor NVB	Progression	Survival (6)
3	Skin metastasis	IC131	Squamous cell T2N2	CT[c]	54/M	CT[c] surgery	Progression	Survival (12)
4	Primary tumor	ML5	ADC T2N0M0	None	68/M	Surgery of primary tumor + CT (CDDP + 5FU + NVB)	Biological response[d]	Survival (78)
	Adrenal metastasis	IC14		None		Surgery + CT (docetaxel + gemcitabin) + gefitinib		
5	Lung recurrence	IC11	Squamous cell T2N1	None	72/M	Surgery of primary tumor	None	Survival (2)
6	Skin metastasis	IC9	Squamous cell T2N2M+	NVB	66/M	Surgery + mitomycin C	Progression	Survival (2)

ADC, adenocarcinoma; CDDP, cisplatin; CT, chemotherapy; 5-FU, 5-fluorouracil; M, male; NSCLC, non-small-cell lung cancer; NVB, vinorelbine; RT, radiotherapy.
[a]The patient received two courses of chemotherapy, consisting of CDDP combined with 5-FU and NVB, followed by doxorubicin plus cyclophosphamide.
[b]Death was not because of cancer.
[c]The patient received three courses of chemotherapy, first consisting of CDDP combined with 5-FU and NVB, second consisting etoposide plus cyclophosphamide, followed by mitomycin C and NVB.
[d]CEA (carcinoembryonic antigen) assay.

Histological examination of non-small-cell lung cancer xenografts

The histological features of xenografts reproduced those of the patient's original tumor. IC8 was a pseudopapillary adenocarcinoma composed of false papillae without fibrovascular cores and sparse glandular lumina (Fig. 1a). IC1 was a well-differentiated squamous cell carcinoma composed of pink cells with large irregular nuclei, sometimes with keratin pearls (Fig. 1b). IC131 was a squamous cell carcinoma with a compact pattern (Fig. 1c). ML5, derived from the primary tumor of patient 4, was a papillary adenocarcinoma composed of neoplastic columnar cells covering the fibrovascular cores of the papillae, with elongated and hyperchromatic nuclei (Fig. 1d). IC14 xenograft, derived from an adrenal metastasis of the same patient, presented a very similar histology (Fig. 1e). IC11 was a more poorly differentiated squamous cell carcinoma composed of cells with hyperchromatic and pleomorphic nuclei (Fig. 1f). Finally, IC9 was very similar to IC1, corresponding to a well-differentiated squamous cell carcinoma (Fig. 1g).

Antitumor efficacy of chemotherapy

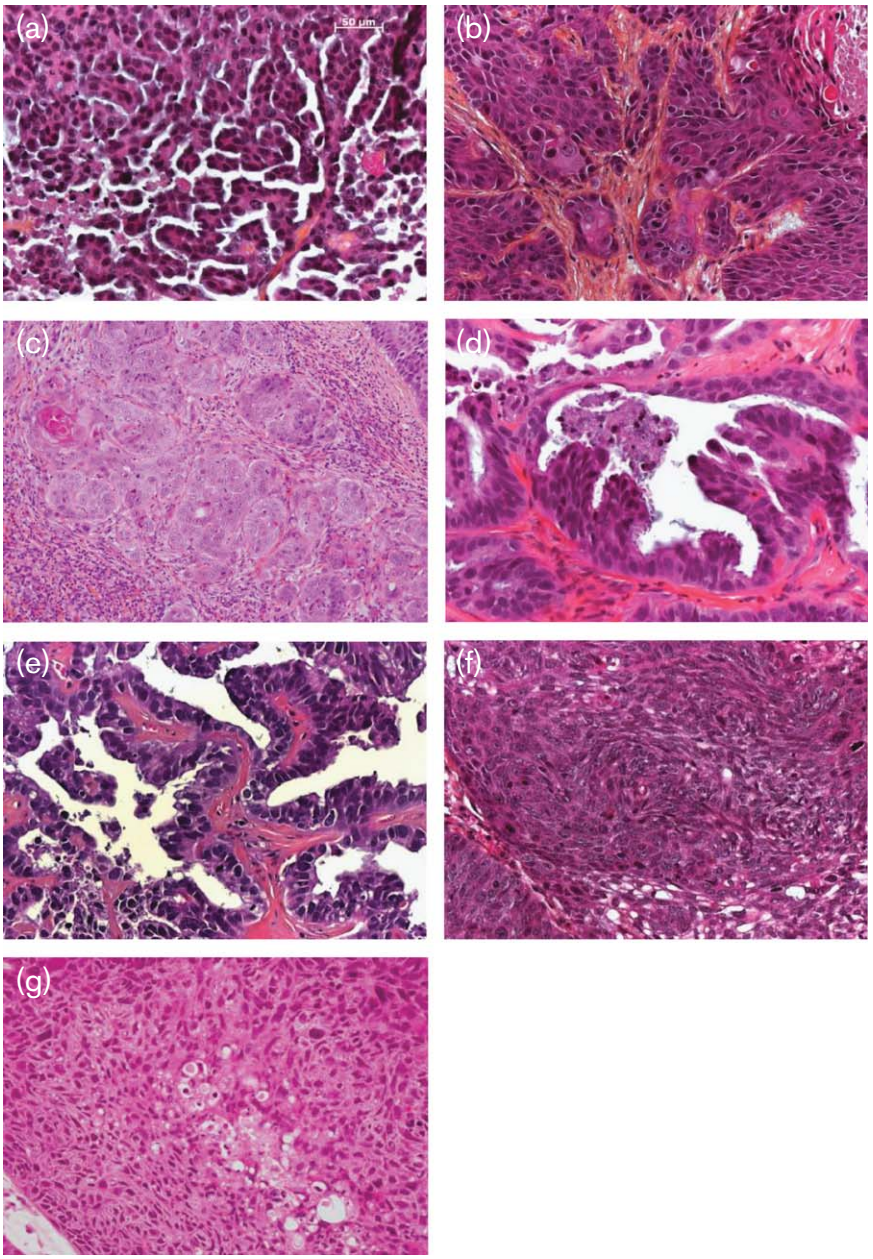
Tumor growth characteristics of each xenograft were initially recorded, including tumor growth rate, growth latency, and survival time after transplantation. The growth rate of NSCLC xenografts ranged between 70 and 100%. Growth latency after transplantation, corresponding to the time to reach a minimum volume of 63 mm^3, ranged between 18 and 46 days. Survival time, corresponding to the time to reach a size of 2500 mm^3, ranged between 53 and 141 days. No spontaneous regression of the NSCLC xenograft was observed.

Tumor-bearing mice were treated when their tumor reached a volume between 63 and 400 mm^3. All compounds were administered at tolerated dosages, according to an optimal dosage and sequence of administration as defined by previous experiments. In the case of combination therapy, drugs were injected as the same dosage of single-agent treatment, excepted for cisplatin, for which the dosage was reduced to decrease toxicity. Treatments were repeated as long as the mice survived. As shown in Table 2, antitumor efficacy of cisplatin combined with vinorelbine or gemcitabine was evaluated using four xenografts. Tumor growth of all tested xenografts was slightly inhibited by these treatments and these NSCLC xenografts were therefore considered to be models not responding to cisplatin-based chemotherapy. Docetaxel, administered as single-agent chemotherapy, induced a significant response in five of the seven NSCLC xenografts (71%) with optimal tumor growth inhibition ranging between 44% (ML5) and 91% (IC14) (Table 2). Similar results are shown in Fig. 2. Surprisingly, for the two grafts derived from the same patient, IC14 was two times more sensitive than ML5 to docetaxel. Docetaxel combinations were tested using the IC1 xenograft (Fig. 3). No significant increase of docetaxel-induced growth inhibition was observed after docetaxel was combined with cisplatin, vinorelbine, or gemcitabine. Same results were observed with IC8, ML5, and IC14 xenografts (Table 2).

Biological characteristics of non-small-cell lung cancer xenografts

p53 gene was inactivated by various alterations in almost all NSCLC. Moreover, real-time PCR analyses of the mRNA level of the various genes putatively involved in drug response are presented in Table 3. The mRNA level of genes encoding CYP450 (CYP3A4, 3A5, 3A7, and 2B6) did not vary significantly between xenografts and were not correlated with in-vivo docetaxel-induced tumor

Fig. 1



Histological features of the patients' non-small-cell lung cancers corresponding to xenografts. IC8 (a), IC1 (b), IC131(c), ML5 (d), IC14 (e), IC11 (f), IC9 (g). Hematoxylin and eosin sections, ×200.

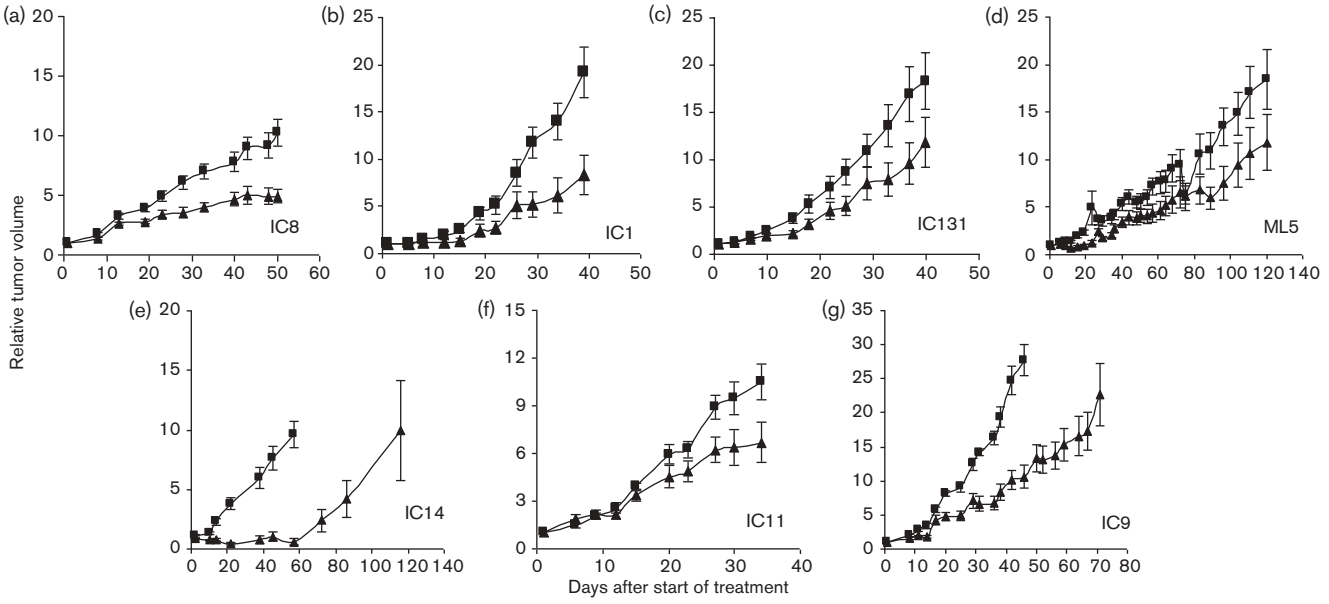
growth inhibition. Median relative breast cancer resistance protein expression was increased in three xenografts (IC131, IC1, and IC11), but with no correlation with in-vivo efficacy of docetaxel. The relative level of expression of multidrug resistance-associated protein (MRP1) gene was low in all xenografts except for ML5. The level of lung resistance-related protein and multidrug resistance 1 (MDR1) genes did not significantly vary between xenografts and were not correlated with tumor growth inhibition. The relative level of expression of glutathione *S*-transferase π-1 (GSTP1) gene was very variable and ranged from 0.02 (IC1) to 62.4 (ML5) with no correlation with in-vivo drug response. The expression of genes involved in DNA repair, such as X-ray cross-complementing 1 (XRCC1), XRCC2, XRCC3, XRCC4, and XRCC5, did not vary significantly between the various xenografts studied, except for XRCC1, which was highly expressed in ML5 tumor, and XRCC5 which was highly expressed in IC131 and ML5 tumors. As before, no correlation was observed

Table 2 **Response[a] of human NSCLC xenografts to docetaxel-based chemotherapy**

Xenografts	DOC	CDDP	VNR	GZ	CDDP + VNR	CDDP + GZ	DOC + CDDP	DOC + VNR	DOC + GZ
IC8	53	37	45	72	39	62	55	70	79
IC1	55	49	33	54	7	9	62	56	54
IC131	58				42	65			
ML5	44		51					32	
IC14	91				11	27			81
IC11	49								
IC9	62			60					

DOC was administered at a dosage of 20 mg/kg/day, every 3 weeks; CDDP was administered at a dosage of 6 mg/kg/day, every 3 weeks, when administered alone or 1 mg/kg/day when combined, every 3 weeks; VNR, 1.25 mg/kg/day, every 10 days; GZ, 80 mg/kg/day, weekly; all drugs were prepared extemporaneously and administered separately.
CDDP, cisplatin; DOC, docetaxel; GZ, gemcitabine; NSCLC, non-small-cell lung cancer; VNR, vinorelbine.
[a]Optimal tumor growth inhibition calculated from the curves of mean tumor growth at the optimal antitumor effect.

Fig. 2



Effects of docetaxel in the panel of non-small-cell lung cancer xenografts listed in Table 2. Docetaxel (▲) was administered at a dose of 20 mg/kg every 3 weeks. Mice in the control groups (■) received 0.2 ml of the drug-formulating vehicle with the same schedule as the treated animals. Treatment started when subcutaneous growing tumor volumes were 63–400 mm^3; tumor growth was calculated by measuring two perpendicular diameters with a caliper; tumor volume and relative tumor volume (RTV) were calculated as described in Materials and methods; growth curves were obtained by plotting mean RTV against time. Error bars represent calculated standard deviation. All drugs were prepared extemporaneously.

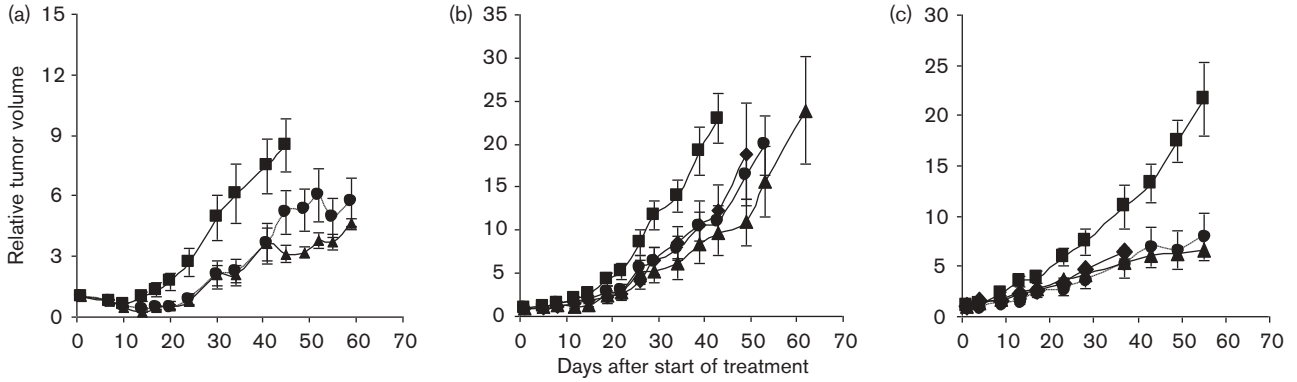
between the relative level of expression of XRCC genes and in-vivo tumor growth inhibition. Finally, genes encoding topoisomerases I and IIA showed a homogeneous high relative level of expression in all xenografts, and particularly in the least docetaxel-sensitive ML5 tumor. Altogether, ML5 xenograft was characterized by various relative levels of expression of genes putatively involved in drug response, suggesting a possible relationship between its expression profile and its relative drug resistance.

Discussion

In this study, we present a panel of human NSCLC xenografts treated by docetaxel-based chemotherapies. In every case, the histological features of the xenografts reproduced those of the patient's original tumor. Although docetaxel administered alone induced significant tumor growth inhibition in five of the seven xenografts studied, its preclinical efficacy was not increased in combination with vinorelbine or gemcitabine, as well as with cisplatin for the two xenografts that have been assessed. Finally, no significant correlations were observed between relative level of expression of genes putatively involved in drug response and in-vivo docetaxel sensitivity.

To evaluate the predictive value for response to docetaxel administered alone or in combination in our panel, we reviewed the clinical studies of docetaxel-based regimens. The standard treatment for previously untreated

Fig. 3



Effects of docetaxel combined with cisplatin, vinorelbine, and gemcitabine on IC1 xenograft. All drugs were administered by intraperitoneal injection. Docetaxel (▲) was administered at a dose of 20 mg/kg every 3 weeks. Mice in the control groups (■) received 0.2 ml of the drug-formulating vehicle with the same schedule as the treated animals. (a) Cisplatin was combined with docetaxel at a dose of 1 mg/kg/day every 3 weeks (●). (b) Vinorelbine was administered alone (♦) or with docetaxel (●) at a dose of 1.25 mg/kg/day every 10 days. (c) Gemcitabine was administered alone (♦) or with docetaxel (●) at a dose of 80 mg/kg/day weekly. Treatment was initiated when the tumors reached 63–400 mm^3. Growth curves were obtained by plotting mean relative tumor volume against time. Error bars represent calculated standard deviation. All drugs were prepared extemporaneously; for combination therapy, each drug was injected separately.

Table 3 Relative expression of genes putatively involved in drug sensitivity

Genes	Human liver	Med.	IC8	IC1	IC131	ML5	IC14	IC11	IC9
Docetaxel-induced TGI	–	–	53	55	58	44	91	49	62
B2M	120	6.90	7.60	5.10	6.90	23.8	34.5	2.80	6.80
3A4	235	0.08	0.06	0.02	0.08	0.50	0.02	0.15	0.43
3A5	13.0	0.08	0.03	0.01	0.08	0.13	0.01	0.09	0.15
3A7	0.60	0.22	0.06	**1.40**	0.37	**0.80**	0.02	0.22	0.12
2B6	0.80	0.03	0.00	**0.81**	0.00	**35.3**	0.03	**0.56**	0.01
BCRP	0.04	0.20	0.09	**1.83**	**0.49**	0.07	0.09	0.29	0.20
LRP	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MRP1	0.25	0.25	0.20	0.05	0.25	**0.80**	0.30	0.02	0.04
MDR1	0.12	0.00	0.00	0.00	0.00	0.00	0.01	0.04	0.08
GSTP1	150	2.87	**1.45**	0.02	**2.87**	**62.4**	0.08	**8.50**	**12.2**
XRCC1	0.16	0.10	0.09	0.10	0.10	**1.28**	0.19	0.22	0.05
XRCC2	0.00	0.02	0.02	0.01	0.03	0.01	0.01	0.03	0.03
XRCC3	0.00	0.02	0.01	0.02	0.02	0.04	0.01	0.01	0.02
XRCC4	0.06	0.05	0.04	0.04	0.11	0.05	0.06	0.05	0.01
XRCC5	3.20	0.84	0.62	0.72	**1.45**	**1.77**	0.99	0.84	0.72

Bold characters indicate significant values.
Med., established on the series of all NSCLC xenografts studied; NSCLC, non-small-cell lung cancer; TGI, tumor growth inhibition.

NSCLC includes platinum-based chemotherapy for a maximum of six cycles in the absence of major toxicity or progressive disease [1]. Docetaxel, a microtubule stabilizing agent, induces arrest of the cell cycle and apoptosis [7]. It has also been shown to possess antiangiogenic properties *in vitro* [8]. As shown in Table 4, docetaxel has been widely used alone or in combined chemotherapies in NSCLC patients. Docetaxel administered alone induces an overall response rate between 12 and 23% in first-line treatment [10,11,16] and between 14 and 21% in second-line treatment [9,10], with no significant difference between one injection every 3 weeks and once-weekly injection. Docetaxel combined with cisplatin has shown various results with an overall response rate ranging between 23 and 44% in first-line treatment [13,14]. Similarly, except in the neoadjuvant setting [15], docetaxel combined with gemcitabine induces similar overall response rates of approximately 25% [11,14, 16,18]. Contrary to the results of our preclinical assays, the overall response rates were significantly increased when docetaxel was combined with another cytotoxic agent in chemotherapy in naive patients with NSCLC. In contrast, the results on overall survival were variable from a study to the other one. One randomized multicenter phase III trial showed that docetaxel + cisplatin was significantly more active in terms of objective response rate compared with docetaxel alone in newly diagnosed NSCLC patients. However, the difference in overall survival between patients treated with docetaxel + cisplatin and patients treated with docetaxel alone did not reach statistical significance and the 1-year overall survival of the patients in both groups was similar [17].

Table 4 Docetaxel alone or combined in the treatment of NSCLC patients

Reference	Line of treatment	No. of patients	Treatment	RR (%)	TTP (month)	mOS (month)	OS_1 (%)
[9]	2	36	Docetaxel weekly: 37.5 mg/m^2, day 1, 8, cycle 3 weeks	16.7	3	13.3	–
			Docetaxel 75 mg/m^2, day 1, cycle 3 weeks	21.1	2.8	10.7	
[10]	1	115	Docetaxel 100 mg/m^2, day 1, cycle 3 weeks	22.6	3	8.7	38
	2	58		13.8	2.4	7.2	27
[11]	1	345	Docetaxel weekly: 36 mg/m^2, day 1, 8, 15, cycle 28 days	17	2.9	2.9[a]	–
			Docetaxel 30 mg/m^2 + gemcitabine 800 mg/m^2, day 1, 8, 15, cycle 28 days	25	4.8[b]	3.8	
[12]	1	414	Cisplatin HD 100 mg/m^2	17[a]	2.7[a]	8.1	–
			Paclitaxel 175 mg/m^2 + cisplatin 80 mg/m^2 cycle 21 days	26	4.1	8.6	
[13]	1	50	Docetaxel 75 mg/m^2 + cisplatin 75 mg/m^2 day 1, cycle 3 weeks	44	4	16	–
[14]	1	150	Docetaxel 60 mg/m^2, day 8 + gemcitabine 800 mg/m^2, day 1, 8, cycle 21 days	27	–	13.7	56.6
			Docetaxel 60 mg/m^2, day 1 + cisplatin 80 mg/m^2, day 1, cycle 21 days	23.5		11.4	47.7
[15]	1[c]	38	Docetaxel 80 mg/m^2 + gemcitabine 1000 mg/m^2, day 1, 14, cycle 28 days	58.8	3	7	–
[16]	1	155	Docetaxel 100 mg/m^2 on day 1, every 3 weeks	11.6	2.3	8.3	
		157	Docetaxel 75 mg/m^2, day 8 + gemcitabine 1100 mg/m^2, day 1, 8	26.8[b]	3.5	9.4[b]	
[17]	1	152	Docetaxel 100 mg/m^2 on day 1, every 3 weeks	21.7	2.5	8.0	43
		167	Docetaxel 100 mg/m^2, day 1 + cisplatine 80 mg/m^2, day 2	36.5[b]	4.0	10.5	44

HD cisplatin, high-dose cisplatin; mOS, median overall survival; NSCLC, non-small-cell lung cancer; RR, response rate; TTP, time to progression; 1 year-OS_1, 1-year overall survival.
[a]Not significant.
[b]Significant.
[c]Neoadjuvant chemotherapy.

In contrast, another randomized multicenter phase III trial showed that docetaxel + gemcitabin was significantly superior in terms of objective response rate and overall survival compared with docetaxel alone in chemotherapy-naive patients with advanced NSCLC [16].

In our seven human NSCLC xenografts, docetaxel induced tumor growth inhibition ranging between 44 and 91%. Surprisingly, the least (ML5) and most (IC14) sensitive xenografts were obtained from primary or metastatic tumor derived from the same patient, respectively. To understand this variability of the response to docetaxel, the relative expression of genes putatively involved in drug resistance was analyzed. Three main differences were observed between the two xenografts: higher expression of the 2B6 CYP450 gene, GSTP1 gene, and XRCC1, and XRCC5 genes involved in DNA repair.

The relative expression of genes putatively involved in drug resistance was analyzed in all xenografts studied to define biological parameters influencing response to docetaxel. No significant correlation was observed between the relative level of expression of genes encoding CYP450, BCRP, LRP, MRP1, MDR1, and GSTP1 and genes involved in DNA repair. Moreover, the p53 gene was inactivated by various alterations in all NSCLC, and only IC14 was sensitive to docetaxel. Docetaxel is mainly metabolized in the liver by CYP3A4 [19], with a pharmacokinetic profile dependent on the CYP3A4/MDR1 ratio [20]. MDR1 expression was not detected or was very low in all xenografts studied, whereas CYP3A4 expression was homogeneously low, except for ML5 and IC9 xenografts that were resistant and sensitive to docetaxel, respectively. The most marked difference in CYP expression was observed for the 2B6 gene that was not involved in docetaxel metabolism. In-vitro experiments have shown that GSTP1 can decrease docetaxel cytotoxicity [21], like our in-vivo data for the ML5 xenograft. It can therefore be proposed that an increase of both CYP3A4 and GSTP1 could explain the relative resistance of ML5 to docetaxel. Other genes such as the high mobility group 1, the breast cancer gene 1, and ribonucleotide reductase M1 and M2, which are involved in DNA replication and repair, or class III β-tubulin, could play a role in resistance in these drugs [22–26].

In conclusion, our panel of human NSCLC xenografts is representative of human NSCLC observed in patients, in term of histological characteristics and in-vivo response to docetaxel and could therefore be used for further preclinical evaluations. In view of the numerous new targeted therapies, this preclinical model could be useful for discrimination of effective molecules that can be administered alone or in combination.

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