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REVIEW

# Gemcitabine in Non-Small Cell Lung Cancer (NSCLC): The Evidence of its Therapeutic Value in Molecular Age

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**Abstract:** Lung cancer contributes to 31% of male and 26% of female cancer-related deaths and is the largest cause of cancer-related mortality in both men and women. Evaluation of new treatment strategies is ongoing to identify more effective treatments to overcome this dismal prognosis. Numerous studies and meta-analyses have proved the superiority of cisplatin-based third generation chemotherapy in NSCLC first line treatment but no gold standard exists. Two-drug third-generation chemotherapy provides a suitable alternative for patients with contraindications to platinum. Gemcitabine is a third generation agent successfully tested in non small lung cancer, either alone or in platinum and non platinum combination, and has become a drug of choice due to its advantageous toxicity profile. It has also been successfully combined with premetrexed, a new antitoxic agent, as well as with the vascular endothelial growth factor (VEGF) inhibitor bevacizumab, for adenocarcinoma histological nonsmall cell subtype. On the other hand, the identification of potential prognostic and predictive molecular markers in the latest years, not only for novel targeted tyrosine kinase inhibitors but also for gemcitabine and other agents, may enable customized therapies for specific patient populations, allowing improved efficacy and reduced toxicity and cost. This review aims to provide an update on gemcitabine and its therapeutic value in modern molecular sitting.

**Keywords:** Nonsmall cell lung cancer, gemcitabine, chemotherapy, predictive molecular markers

*Clinical Medicine Reviews in Oncology* 2010:2 273–281

doi: 10.4137/CMRO.S5842

This article is available from http://www.la-press.com.

## Introduction

Gemcitabine is a cytotoxic drug with distinctive pharmacological properties and a wide antitumor-activity spectrum, including nonsmall cell lung cancer. Interindividual differences in gemcitabine pharmacokinetics and pharmacodynamics may account for different sensibility and resistance among patients. Recent advances in molecular oncology prompted successful development of new targeted therapy against selectively activated pathways and further association with gemcitabine with encouraging results. The validation of predictive biomarkers to tailor chemotherapy is a key issue in the development of effective treatment options against cancer.[1] Examples of how genetics and epigenetics might affect drug response are offered by gemcitabine. This review is aimed to provide an update on gemcitabine lung cancer trials with focus on recent studies of customized chemotherapy based on genetic markers as well as promising pharmacogenetic determinants, including BRCA1 (*breast cancer 1*), RRM1 (regulatory subunit of ribonucleotide reductase M1), ERCC1(excision repair cross-complementation group 1), and several else.

## Mechanism of Action, Metabolism and Pharmacokinetic Profile

Gemcitabine (2′,2′-difluorodeoxycytidine) is a broadly active cytosine analog that is incorporated into DNA causing chain termination. The pharmacological characteristics of gemcitabine are unique because two main classes of genes are essential for its antitumor effects: on the one hand, membrane transporter protein-coding genes, which products are responsible for drug intracellular uptake; on the other hand, enzyme-coding genes catalyze its intracellular activation and inactivation. Therefore, gemcitabine is transported into cells by membrane transporter proteins such as equilibrative and concentrative nucleoside transporters (mainly hENT-1 *human equilibrative nucleoside transporter 1*-, and, to lesser extent, hCNT-1 and hCNT-3, *human concentrative nucleoside transporters 1 and 3*, respectively. Then, it is firstly phosphorylated by DKC (*deoxycytidine kinase*, activation enzymes) and further phosphorylated to its active diphosphorylated and triphosphorylated forms. Gemcitabine is rapidly metabolized by CDA (*cytidine deaminase*, inactivation enzymes) to an inactive metabolite, dFdU (*2′,2′-difluorodeoxyuridine*), which is excreted into the urine.[2–4]

Gemcitabine active metabolites antitumoral effects depend on several mechanisms. They inhibit DNA synthesis by inhibition of DNA polymerase and ribonucleotide reductase, specific for the S-phase of the cycle. On the one hand, gemcitabine diphosphate inhibits DNA synthesis by inhibiting ribonucleotide reductase. On the other hand, gemcitabine triphosphate incorporates into DNA and inhibits DNA polymerase. One type of gemcitabine resistance occurs when the inactivation of ribonucleotide reductase decreases, which in turns correlates with increased expression of M1 subunit of ribonucleotide reductase. The ribonucleotide reductase M1 (RRM1) gene encodes the regulatory subunit of ribonucleotide reductase, the molecular target of gemcitabine. The overexpression of RRM1 mRNA in tumor tissues is reported to be associated with gemcitabine resistance. Thus, single nucleotide polymorphisms (SNPs) of the RRM1 gene are potential biomarkers of the response to gemcitabine chemotherapy.[5]

Since gemcitabine is only incorporated during DNA repair o replication and most solid tumors have a relatively low S-phase, single agent activity is limited although it may be cytostatic. Concomitant administration of gemcitabine with a DNA damaging agent such as a platinum compound might lead to upregulated DNA repair and could tend to prevent resistance to that platinum agent by two effects.[6] Firstly, gemcitabine will be incorporated more readily into DNA of non-dividing cells. Secondly, its ability to inhibit DNA polymerase will tend to prevent resistance to the DNA-damaging agent. Several groups have proved that gemcitabine can reverse resistance to cisplatin by its inhibition of DNA repair or replication. The effect on DNA repair seems to occur at much lower concentration of gemcitabine than direct incorporation, suggesting no need of high doses of gemcitabine in platinum combinations.[5]

Although some studies have suggested that gemcitabine delivered at fixed dose rate (FDR) infusion of 10 mg/m(2)/min could be more effective than the standard 30-min infusion, recent available pharmacokinetic data have shown that prolong infusion time over 60 minutes and administration more frequently than once a week increase toxicity. Prolonged infusion times increase the accumulation of the active metabolite gemcitabine triphosphate. Patients who receive gemcitabine FDR experience more grade 3/4 hematologic toxicity.[7]

Even a starting dose of 800 mg/m2 is recommended for serum bilirubin >1.6 mg/dl to avoid toxicity, there is limited information on gemcitabine dosing in patients with elevated bilirubin levels.[8] A recent retrospective study at the Medical University of South Carolina indicates the possibility that no initial dose reduction is necessary for patients with liver dysfunction receiving gemcitabine; however, close monitoring of these patients is required.[9]

## Clinical Studies

Whenever possible, therapy should be individualized based upon molecular and histological features of the tumor. Assessment of patients overall medical condition and prior therapy are crucial in the decision making. For locally advanced and metastatic NSCLC, gemcitabine can be use with palliative intent as a single agent or in combination with platinum and non platinum compounds. There are fewer trials in non palliative sitting as for adjuvant, neoadjuvant and chemoradiotherapy regimens.

### Palliative single agent

Gemcitabine single agent activity is limited although it may be cytostatic. As initial treatment it is generally limited to elderly patients and those with borderline performance status. Two phase studies and a phase III trial single agent gemcitabine have been reported in advanced and metastatic NSCLC (Table 1).[10–12] In the first trial, sixteen (20%; 95% confidence interval [CI], 12% to 31%) of 79 patients assessable for response had independently validated partial responses, with a median duration of 7 months. The overall median survival duration was 7 months. Gemcitabine improved disease-related symptoms (70% of patients) and increased WHO performance status (44%). Toxicity was generally mild and reversible. Patients experienced little WHO grade 3 and 4 toxicity. In the second multicenter phase II study, 161 patients with NSCLC were recruited from 10 sites in nine countries. Most patients had stage IIIb (31.3%) or IV (64.6%) disease and 93.8% had a performance status of 0 or 1 according to the WHO scale. Among 151 evaluable patients, there were 3 complete responses and 30 partial responses lasting at least 4 weeks for an objective response rate of 21.8% (95% CI 15.5%–29.3%). All responses were validated by an extramural Oncology Review Board. The mean duration of response was 8.8 months. The mean survival for all patients was 11.5 months (16.1% of patients were still alive 26 months after last patient started treatment). The phase III randomized trial, with 334 randomly assigned patients to gemcitabine-carboplatin (G-Cb) versus gemcitabine alone (G), showed significantly better overall survival (log-rank $P = 0.0205$) and 2-year survival (15% v 5%; $P = 0.009$) favoring the GC arm. Per Cox multivariate analysis, only two covariates, treatment arm (G-Cb v G) and baseline performance status (0 or 1 v 2), independently influenced survival. Per-protocol analyses showed significantly longer median time to progression (5.7 v 3.9 months; $P = 0.0001$) and significantly higher objective response rate (29.6 v 11.3%; $P < 0.0001$) in the G-Cb arm. These results reinforce the fact that combination therapy may be a better choice when toxicity is not of concern.

### Palliative combination treatment

Combinations regimens are preferred as first line treatment for younger patients with advanced disease and good performance status (PS) because of survival benefit. The same is true for adjuvant, neoadjuvant, concurrent chemoradiotherapy. In this latter sitting,

**Table 1.** Gemcitabine monotherapy non-small cell lung cancer trials.

| Regimen dose and schedule | N | RR | OS (months) | Trial/Reference |
|---|---|---|---|---|
| Gemcitabine d 0, 7, 14<br>54 p 800 mg/m$^2$<br>28 p 1000 mg/m$^2$ | 82 | 20% | 7 | Phase II trial<br>*J Clin Oncol*. 1994 Sep;12(9):1821–6. |
| Gemcitabine 1250 mg/m$^2$<br>d 1, 8, 15 | 161 | 21.8% | 11.5 | Multicenter phase II<br>*Eur J Cancer*. 1996 Feb;32A(2):243–8. |
| Gemcitabine 1250 d 1,8 with or without carboplatin AUC 5 d 1/21d | 334 | G: 11.3%<br>GC: 29.6% | G: 9.4<br>GC: 11 (S) | Swedish Phase III trial.<br>*J Clin Oncol*. 2005 Nov 20;23(33):8380–8. |

full concurrent chemotherapy doses are crucial to optimize results.

Owing their slightly better performance, platinum based combination regimens with third generation chemotherapy are usually preferred as first-line treatment for younger patients with advanced NSCLC and good performance status (PS). Given its ability to interfere with inhibition of repair of platinum induced DNA damage, gemcitabine has emerged as an excellent platinum partner. Taking into account its favorable toxicity profile, this combination is commonly used as first line treatment, especially in unselected population.

At least three phase III studies compared gemcitabine/cisplatin (GC) regimen with first generation cisplatin-based combination or cisplatin alone in the first line sitting. In the Italian Lung Cancer Project randomized phase III trial[13] the objective response rate was 38% in the GC arm compared with 26% in the mitomycin, ifosfamide, cisplatin (MIC) arm ($P = 0.029$). The median survival time was 8.6 months in the GC arm and 9.6 months in the MIC arm ($P = 0.877$, log-rank test). Grade 3 and 4 thrombocytopenia was significantly worse in the GC arm (64% v 28%, $P < 0.001$), whereas grade 3 and 4 alopecia was reported more commonly in the MIC arm (39% v 12%, $P < 0.001$). They found no differences in survival or quality of life as compared with the previous regimen.

Gemcitabine-platinum based combination has been compared with other third generation-platinum combinations. No single regimen has demonstrated superiority in patients with advanced NSCLC. At least three cooperative group trials compared multiple platinum-based doublets.[14–16] In the largest of these trials, 1155 patients were randomly assigned to one of four doublets: gemcitabine plus cisplatin, docetaxel plus cisplatin, paclitaxel plus carboplatin, or cisplatin plus paclitaxel. Overall response rates (approximately 19 percent), median survival (average 7.9 months), and one- and two-year survival rates were similar in all four groups, although there were differences in toxicity. Gemcitabine combination with nedaplatin, a new platin compound is being checked.[17] Le Chevalier et al[18] performed a meta-analysis to quantify the treatment effect of gemcitabine plus a platinum agent in the treatment of advanced NSCLC and compared the combination to other platinum-containing regimens. A significant reduction in overall mortality in favor of gemcitabine–platinum regimens was observed, hazard ratio (HR) 0.90 (95% CI: 0.84–0.96) with an absolute benefit at 1 year of 3.9%. Median survival was 9.0 months for the gemcitabine–platinum regimens and 8.2 months for the comparator regimens. Sub-group analysis of the first- and second-generation platinum-based comparator regimens also indicated a significant benefit for gemcitabine–platinum regimens, HR 0.84 (CI: 0.71–0.9985). Analysis of third-generation agent plus platinum regimens showed a non-significant trend favoring gemcitabine–platinum regimens, HR 0.93 (CI: 0.86–1.01). There was a significant decrease in the risk of disease progression in favor of gemcitabine–platinum regimens, HR 0.88 (CI: 0.82–0.93). An absolute benefit of 4.2% at 1 year was estimated. Median progression-free survival was 5.1 months for gemcitabine–platinum regimens compared with 4.4 months for the comparator regimens. Sub-group analysis indicated a statistically significant progression-free survival benefit for patients assigned to gemcitabine–platinum treatment compared to first- and second-generation platinum regimens, HR 0.85 (CI: 0.77–0.94), and third-generation agent plus platinum regimens, HR 0.89 (CI: 0.82–0.96).

In addition to the clinical research of gemcitabine-cisplatin combinations, gemcitabine has also been tested in various double and triple combinations with, paclitaxel, docetaxel, vinorelbine (Table 2) with similar response rate and overall survival among platinum combinations.[19–28] To test efficacy and tolerability of non-platinum regimens for advanced non-small-cell lung cancer, our group compared gemcitabine-docetaxel versus gemcitabine-cisplatin in a phase II randomized trial for advanced NSCLC, with similar results.[19] Non platinum combinations provide a suitable alternative when toxicity is of concern or there are contraindications to platinum.

## Novel agent combinations

Novel agents have been added to platinum and nonplatinum combinations in an attempt to improve NSCLC grim prognosis. Several gemcitabine-novel agent associations have been reported. Gefitinib in combination with gemcitabine and cisplatin in chemotherapy-naive advanced NSCLC patients did not have improved efficacy over gemcitabine and cisplatin alone.[29]

**Table 2.** Gemcitabine non-platinum combination trials.

| Regimen dose and schedule | N | RR (%) | OS (months) | References |
|---|---|---|---|---|
| **Gemcitabine-docetaxel (GD) versus** | | | | |
| - Docetaxel/CDDP (CD) | 441 | GD: 30.2; CD: 32,4 | GD: 9; CD: 9.7 (NS) | [20] |
| - CDDP-vinorelbine (CV) | 331 | GD: 31; CV: 35,9 | GD: 11.1; CV: 9.6 (NS) | [21] |
| - Docetaxel (D) | 350 | GD: 25; D: 17 | GD: 5.6; D: 5.1 (NS) | [22] |
| - CDDP-gemcitabine (CG) | 108 | GD: 40; CG: 34.5 | GD: 8.9; CG: 8.9 (NS) | [19] |
| **Paclitaxel-gemcitabine (PG) versus** | | | | |
| - CBDA-P (PCb)/CBDA-G (GCb) | 1135 | PG: 32.1; PCb: 29.8; GCb: 25.3 | PG: 8.5/PCb: 8.7/GCb: 7.9 (NS) | [23] |
| - CBDA-G (GCb) | 552 | PG: 31; GCb: 27 | PG: 9.97; GCb: 10.49 (NS) | [24] |
| - CBDA-P (PCb) | 509 | PG: 35; PCb: 28 | PG: 9.8; PCb: 10.4 (NS) | [25] |
| **Vinorelbine-gemcitabine (VG) versus** | | | | |
| - CV/CG | 501 | VG: 25; CG: 30 | VG: 9 CG/CV: 8 (NS) | [26] |
| - C-V-G | 287 | VG: 13; GVP: 28.3 | VG: 9/CVG 8 (NS) | [27] |
| - Cb-V | 316 | VG: 28; CbV: 20.8 | VG: 11.5; CbV 8.6 (S) | [28] |

Final analysis of AVAiL (AVAstin In Lung cancer) trial confirmed the efficacy of bevacizumab when combined with cisplatin-gemcitabine. Progression free survival benefit did not translate into significant overall survival benefit, possibly due to high use of efficacious second-line Bevacizumab therapies. Unlike a previous non-gemcitabine trial where several toxic deaths occurred,[30] there was no risk of increased treatment related deaths.[31]

A noninferiority, phase III, randomized study compared the overall survival between cisplatin-gemcitabine and cisplatin-premetrexed using a fixed margin method (hazard ratio [HR] <1.176) in 1,725 chemotherapy-naive patients with stage IIIB or IV NSCLC. Overall survival for cisplatin/pemetrexed was noninferior to cisplatin/gemcitabine (median survival, 10.3 *v* 10.3 months, respectively; HR = 0.94; 95% CI, 0.84 to 1.05). Overall survival was statistically superior for cisplatin/pemetrexed versus cisplatin/gemcitabine in patients with adenocarcinoma (n = 847; 12.6 *v* 10.9 months, respectively) and large-cell carcinoma histology (n = 153; 10.4 *v* 6.7 months, respectively). In contrast, in patients with squamous cell histology, there was a significant improvement in survival with cisplatin/gemcitabine versus cisplatin/pemetrexed (n = 473; 10.8 *v* 9.4 months, respectively). This is the first prospective phase III study in NSCLC to show survival differences based on histological type.[32] Pemetrexed/carboplatin provides similar quality of life and survival when compared with gemcitabine/carboplatin with less hematologic toxicity and less need for supportive care.[33]

Gemcitabine-pemetrexed combination is minimally active in late-stage NSCLC, with a high incidence of grade 3 or 4 toxic effects requiring frequent dose adjustments. Gemcitabine dose <1250 mg/m(2) might warrants consideration for future trials exploring this doublet. The administration of day 8 premetrexed immediately after gemcitabine does not appear to negatively impact therapeutic index.[34] Sequential pemetrexed/gemcitabine has shown moderate activity and is well tolerated as first-line treatments for advanced NSCLC in elderly patients or patients unsuitable for platinum-based combination chemotherapy.[35]

## Neoadjuvant chemotherapy

Preoperative chemotherapy has been assessed in several studies for patients with resectable NSCLC. It may also be useful in patients with locally extensive disease that cannot be encompassed within a reasonably safe RT portal. In this latter case, it can be followed by definitive chemoradiotherapy whenever an adequate response is achieved. In the largest of the randomized neoadjuvant trials to date, a multicenter European LU22 trial,[36] 519 patients with resectable NSCLC were randomly assigned to three cycles of neoadjuvant

platinum-based chemotherapy followed by surgery or to immediate surgery. At randomization, 93 percent of patients had clinical stage I or II disease. The chemotherapy regimen varied at different sites; the two most widely used combinations were vinorelbine plus cisplatin and gemcitabine plus cisplatin. Overall 75 percent of patients completed all three cycles of chemotherapy, and the objective response rate to chemotherapy was 47 percent. Despite the observed antitumor activity from chemotherapy, there was no improvement in progression free survival with neoadjuvant treatment (two-year PFS 53 versus 52 percent with immediate surgery; HR for recurrence 0.96, 95% CI 0.77–1.21). Similarly, there was no improvement in overall survival with preoperative chemotherapy (five-year survival 44 versus 45 percent, HR for death 1.02, 95% CI 0.80–1.31). Results were not reported as a function of stage. The negative results in this trial may be attributed to the very high percentage of patients enrolled with stage I disease.

Another neoadjuvant gemcitabine study, in the intention-to-treat population undergoing platinum/gemcitabine induction chemotherapy, there was a high resectability rate (74%); the 5-year survival rate was 25%. Median survival in resected cases was three-fold greater than in the unresected ones.[37]

## Adjuvant

The completion of large clinical trials assessing the activity of platinum-based chemotherapy for completely resected NSCLC has led to recognition of the role of adjuvant platinum therapy in improving outcome in this sitting. These results have prompted guidelines from American society of Clinical Oncology (ASCO) and from the National Comprehensive Cancer Network (NCCN) to recommend adjuvant cisplatin-based regimen in completely resected stage II and IIIA NSCLC. Nonetheless, there is no randomized trial assessing the role of adjuvant gemcitabine.

Eastern Cooperative Oncology Group 1505 trial is currently underway to investigate the value of the antiangiogenic monoclonal antibody bevacizumab. Hopefully, it will generate useful data, because three different cisplatin-based regimens are being included: combinations of cisplatin with gemcitabine, docetaxel, and vinorelbine. At present, cisplatin should be the platinum compound of choice in the adjuvant setting, unless there are medical contraindication.[38]

## Chemoradiotherapy

Concurrent chemoradiotherapy exploits the synergistic effect of radiotherapy and chemotherapy to enhance local tumor eradication and provides early treatment to micrometastatic disease. It has become the preferred approach for most patients with mediastinal lymph node involvement or T3-T4 unresectable disease after randomized trials have established the superiority of this approach compared to sequential treatment. The optimal chemotherapy regimen for use with concurrent thoracic radiotherapy is not known due to a paucity of randomized trials comparing different chemotherapy regimens in stage III setting. There are several choices available regarding which chemotherapy to use and how to optimally combine them with radiotherapy. Some regimens may be associated with increased incidence of pulmonary toxicity. Gemcitabine has activity in NSCLC, and is a potent radiosensitizer. These two characteristics make gemcitabine a potential option when treating patients with stage III NSCLC. However, there is great concert about toxicity in lung cancer. As an example, in a study of 19 patients treated with concurrent chemoradiotherapy using a gemcitabine plus carboplatin regimen, six patients (32 percent) developed high-grade radiation pneumonitis, one of whom died.[39]

Cancer and Leukemia Group B (CALGB) conducted a randomized phase II study in unresectable stage III NSCLC of two cycles of induction chemotherapy followed by two additional cycles of the same drugs with concomitant radiotherapy. Eligible patients received four cycles of cisplatin at 80 mg/m$^2$ on days 1, 22, 43, and 64 with arm 1: gemcitabine 1,250 mg/m$^2$ on days 1, 8, 22, and 29 and 600 mg/m$^2$ on days 43, 50, 64, and 71; arm 2: paclitaxel 225 mg/m$^2$ for 3 hours on days 1 and 22 and 135 mg/m$^2$ on days 43 and 64; and arm 3: vinorelbine 25 mg/m$^2$ on days 1, 8, 15, 22, and 29 and 15 mg/m$^2$ on days 43, 50, 64, and 71. Radiotherapy was initiated on day 43 at 2 Gy/d (total dose, 66 Gy). 175 eligible patients were analyzed. Toxicities during induction chemotherapy consisted primarily of grade 3 or 4 granulocytopenia. Grade 3 or 4 toxicities during concomitant chemoradiotherapy consisted of thrombocytopenia, granulocytopenia, and esophagitis. Response rates after completion of radiotherapy were 74%, 67%, and 73% for gemcitabine, paclitaxel and vinorelbine arms respectively. Median survival

for all patients was 17 months. One-, 2-, and 3-year survival rates for the patients on the three arms were 68%/37%/28%, 62%/29%/19%, and 65%/40%/23%. They concluded the survival rates exceed those of previous CALGB trials and could be attributable to the use of concomitant chemoradiotherapy.[40]

## Safety

Myelosuppression is the dose-limiting toxicity but it is usually mild. It causes less than 1% treatment discontinuation. Attention should be paid to hepatic and lung toxicities as they may be unusual severe life-threatening ones.[41] Hemolytic uremic syndrome has been reported; it is important to monitor for evidence of microangiopathic hemolysis (elevation of bilirubin or LDH, reticulocytosis, severe thrombocytopenia, and/or renal failure.[42]

Gemcitabine may have radiosensitizing activity when given less than 7 days apart from radiation therapy; optimum regimen for combination gemcitabine-radiation therapy has not been determined for all tumor types.

## Efficacy, Sensitivity and Resistance

Gemcitabine-cisplatin combination response rates range from 31% to 54%, with a median survival time between 8.4 and 15.4 months and a 1-year survival rate between 30% and 59%.[13,14,23,24,26] There are still many patients who do not benefit from this therapy. The mechanism of initial or acquired resistance to gemcitabine chemotherapy remains unknown. Massive efforts have been carried out to identify biomarkers to help clinicians to choose appropriate drugs, by identifying potentially sensitive subjects and spare toxicities in patients who are unlikely to benefit from treatment. Nearly all the available information regarding the predictive value of theses markers has been derived from retrospective studies. Initial prospective studies showed the feasibility of a customized approach based on biomarkers assessment, and phase III trials will hopefully provide further validation of this approach.

A number of studies of potential gemcitabine resistance mechanisms have been performed in lung cancer using polymerase chain reaction (PCR) or immunohistochemical methods. Multiple membrane transporters, target enzymes, enzymes involved in the metabolism of gemcitabine and alterations in the apoptotic pathways have been implicated in sensitivity and resistance to this drug in a variety of tumor types. To date there is evidence that several genes involved in gemcitabine metabolism, particularly human equilibrative nucleoside transporter 1 (hENT1) and cytosolic 5′-nucleotidase type II (cN-II) are involved in NSCLC resistance. Resistance has also been linked to the expression of DNA repair genes, particularly ERCC1 (*excision repair cross-complementation group 1*), ribonucleotide reductase subunits 1 and 2 (RRM1 and RRM2) though immunohistochemical expression of these proteins, RAD51 (ribonucleotide reductase 51) and BRCA1 could not be shown to have an effect. Recently, multidrug resistance associated protein 5 (MRP5), a membrane located pump, has been implicated in gemcitabine drug resistance. Apoptosis genes, DNA repair genes, proliferation genes, pumps/detox genes and house-keeping genes have been related to drug resistance.[43–48]

There is increased evidence on ERCC1, RRM1 and BRCA1 mRNA expression levels and clinical outcome of advanced non-small cell lung cancer and the possibility to customized cisplatin-gemcitabine therapy based on RMA o inmunohistochemical results.[49–52]

As gemcitabine remains the drug of choice for pancreatic cancer, new markers are intensively being studied in this agent for this disease. This knowledge has led to the identification of biomarkers with prognostic or predictive value and the development of novel drugs against specific abnormal targets of pancreatic tumors. In 2010 ASCO Gastrointestinal Cancers Symposium, researchers presented data showing evidence of biomarkers with prognostic value (Abstracts 166, 140, and 126) and genetic polymorphisms predicting possibly efficacy of gemcitabine treatment (Abstract 166) that could be extensive to lung cancer.

## Conclusions

Cytotoxic chemotherapy remains the primary treatment for the majority of patients with NSCLC but not all NSCLC will benefit from the same treatment. Due to patient heterogeneity, some benefit from most active chemotherapy while others do not, although they do benefit from a less active drug or combination. Patients crossing from one regimen to another in clinical trials commonly show responses, suggesting that it might be possible to optimize therapy for individual

patients if it were also possible to determine which regimen would be the most effective in a given patient. Such testing using primary tumor-derived cells are time-consuming to perform and need a large amount of tumor tissue, far more than it is generally feasible to obtain by bronchoscopic or needle biopsy in most lung cancer patient.

An increasing knowledge on the molecular mechanisms involved, still largely unknown, will allow a more accurate treatment for NSCLC where gemcitabine does certainly have a place. The validation of predictive biomarkers to tailor chemotherapy is a key issue in the development of effective treatment modalities, not only in palliative but also in definitive treatment sitting. Examples of how genetics might affect drug response are offered by gemcitabine. A substantial number of potential biomarkers for sensitivity or resistance to gemcitabine have been proposed, including ribonucleotide reductase and cytidine deaminase polymorphisms, human equilibrative transporter-1 and ribonucleotide reductase gene-expression and AKT phosphorylation status. These markers displayed a significant relationship with disease response to the drug; however, their robustness needs to be evaluated within prospective studies. Moreover, recent trials of customized chemotherapy based on genetic markers have been carried out in non-small-cell lung cancer and promising pharmacogenetic determinants are gaining momentum. Hopefully, biomarkers to select patients most likely to respond to gemcitabine will be validated in the near future.

## Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

## References


1. Adams V, Harvey D. Histological and genetic markers for non-small-cell lung cancer: Customizing treatment based on individual tumor biology. *Am J Health Syst Pharm*. 2010;67 Suppl 1:S3–9.
2. Plunkett W, Huang P, Searcy CE, Gandhi V. Gemcitabine: preclinical pharmacology and mechanisms of action. *Sem Oncol*. 1996;23(5 Suppl 10):3–15.
3. Mini E, Nobilis S, Caciagli B, Landini I, Mazzei T. Cellular pharmacology of gemcitabine. *Ann Oncol*. 2006;17 Suppl 5:v7–12.
4. Abratt RP, Bezwoda WR, Falkson G. Efficacy and safety profile of gemcitabine in non-small cell lung cancer. *J Clin Oncol*. 1994;12:1535–40.
5. Rosell R, Scagliotti G, Danenberg KD, et al. Transcripts in pretreatment biopsies from a three-arm randomized trial in metastatic non-small-cell lung cancer. *Oncogene*. 2003;22(23):3548–53.
6. van Moorsel CJ, Pinedo HM, Smid K, et al. Schedule-dependent pharmacodynamic effect of gemcitabine and cisplatin in mice bearing Lewis lung murine non-small cell lung cancer. *Eur J Cancer*. 2000;36(18):2420–9.
7. Veltkamp SA, Beijnen JH, Schellens JHM. Prolonged versus standard gemcitabine infusion: translation of molecular pharmacology to new treatment strategy. *The Oncologist*. 2008;13:261–76.
8. Floyd J, Mirza I, Sachs B, Perry MC. Hepatotoxicity of Chemotherapy. *Semin Oncol*. 2006;33(1):50–67.
9. Teusink AC, Hall PD. Toxicities of gemcitabine in patients with severe hepatic dysfunction. *Ann Pharmacother*. 2010;44(4):750–4.
10. Anderson H, Lund B, Bach F, Thatcher N, Walling J, Hansen HH. Single-agent activity of weekly gemcitabine in advanced non-small-cell lung cancer: a phase II study. *J Clin Oncol*. 1994;12(9):1821–6.
11. Gatzemeier U, Shepherd FA, Le Chevalier T, et al. Activity of gemcitabine in patients with non-small cell lung cancer: a multicentre, extended phase II study. *Eur J Cancer*. 1996;32 A(2):243–8.
12. Sederholm C, Hillerdal G, Lamberg K, et al. Phase III trial of gemcitabine plus carboplatin versus single-agent gemcitabine in the treatment of locally advanced or metastatic non-small-cell lung cancer: the Swedish Lung Cancer Study Group. *J Clin Oncol*. 2005;23(33):8380–8.
13. Crino L, Scagliotti GV, Ricci S, et al. Gemcitabine and cisplatin versus mitomycin, ifosfamide, and cisplatin in advanced non-small-cell lung cancer: A randomized phase III study of the Italian Lung Cancer Project. *J Clin Oncol*. 1999;17(11):3522–30.
14. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med*. 2002;10;346(2):92–8.
15. Scagliotti GV, de Marinis F, Rinaldi M, et al. Phase III randomized trial comparing three platinum-based doublets in advanced non-small-cell lung cancer. *J Clin Oncol*. 2002;20(21):4285–91.
16. Ohe Y, Ohashi Y, Kubota K, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. *Ann Oncol*. 2007;18(2):317–23.
17. Masago K, Fujita S, Kim YH, et al. Phase I study of the combination of nedaplatin and gemcitabine in previously untreated advanced squamous cell lung cancer. *Cancer Chemother Pharmacol*. 2010 Apr 17.
18. Le Chevalier T, Scagliotti G, Natale R, et al. Efficacy of gemcitabine plus platinum chemotherapy compared with other platinum containing regimens in advanced non-small-cell lung cancer: a meta-analysis of survival outcomes. *Lung Cancer*. 2005;47(1):69–80.
19. Casal J, Vázquez S, Vázquez F, et al. A phase II randomized trial of gemcitabine-docetaxel versus gemcitabine-cisplatin in patients with advanced non-small cell lung cancer. *Cancer Chemother Pharmacol*. 2009;64: 379–84.
20. Georgoulias V, Papadakis E, Alexopoulos, et al. Platinum-based and non-platinum-based chemotherapy in advanced non-small-cell lung cancer: a randomised multicentre trial. *Lancet*. 2001;357:1478–84.
21. Pujol J, Breton JL, Gervais R, et al. Gemcitabine-docetaxel versus cisplatin-vinorelbine in advanced or metastatic non-small-cell lung cancer: a phase III study addressing the case for cisplatin. *Ann Oncol*. 2005;16(4): 602–10.
22. Hainsworth JD, Spigel DR, Farley C, et al. Weekly docetaxel versus docetaxel/gemcitabine in the treatment of elderly or poor performance status patients with advanced nonsmall cell lung cancer: a randomized phase 3 trial of the Minnie Pearl Cancer Research Network. *Cancer*. 2007;110(9):2027–34.
23. Treat JA, Gonin R, Socinski MA, et al. A randomized, phase III multicenter trial of gemcitabine in combination with carboplatin or paclitaxel versus paclitaxel plus carboplatin in patients with advanced or metastatic non-small-cell lung cancer. *Ann Oncol*. 2010;21(3):540–7.

24. Kosmidis PA, Kalofonos HP, Christodoulou C, et al. Paclitaxel and gemcitabine versus carboplatin and gemcitabine in patients with advanced non-small cell lung cancer. A phase III study of the Hellenic Cooperative Oncology Group. *Ann Oncol*. 2008;19(1):115–22.
25. Kosmidis P, Mylonakis N, Nicolaides C, et al. Paclitaxel plus carboplatin versus gemcitabine plus paclitaxel in advanced non-small-cell lung cancer: a phase III randomized trial. *J Clin Oncol*. 2002;20(17):3578–85.
26. Gridelli C, Gallo C, Shepherd FA, et al. Gemcitabine plus vinorelbine compared with cisplatin plus vinorelbine or cisplatin plus gemcitabine for advanced non-small-cell lung cancer: a phase III trial of the Italian GEMVIN Investigators and the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol*. 2003;21(16):3025–34.
27. Laack E, Dickgreber N, Muller T, et al. Randomized phase III study of gemcitabine and vinorelbine versus gemcitabine, vinorelbine, and cisplatin in the treatment of advanced non-small-cell lung cancer: from the German and Swiss Lung Cancer Study Group. *J Clin Oncol*. 2004;22(12):2348–56.
28. Tan EH, Szczesna A, Krzakowski M, et al. Randomized study of vinorelbine—gemcitabine versus vinorelbine—carboplatin in patients with advanced non-small cell lung cancer. *Lung Cancer*. 2005;49(2):233–40.
29. Giaccone G, Herbst RS, Manegold C, et al. Gefitinib in Combination With Gemcitabine and Cisplatin in Advanced Non-Small-Cell Lung Cancer: A Phase III Trial—INTACT 1. *J Clin Oncol*. 2004;22:777–84.
30. Sandler A, Gray R, Perry M, et al. Paclitaxel–Carboplatin alone or with Bevacizumab for Non-Small-Cell Lung Cancer. *N Engl J Med*. 2006;355:2542–50.
31. Reck M, von Pawel J, Zatloukal P, et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAil. *J Clin Oncol*. 2009;27(8):1227–34.
32. Scagliotti GV, Parikh P, von Pawel J, et al. Phase III Study Comparing Cisplatin Plus Gemcitabine With Cisplatin Plus Pemetrexed in Chemotherapy-Naive Patients With Advanced-Stage Non-Small-Cell Lung Cancer. *J Clin Oncol*. 2008;26:3543–51.
33. Gronberg BH, Bremnes RM, Flotten O, et al. Phase III Study by the Norwegian Lung Cancer Study Group: Pemetrexed Plus Carboplatin Compared With Gemcitabine Plus Carboplatin As First-Line Chemotherapy in Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol*. 2009;27:3217–24.
34. West HL, Wakelee HA, Perry MC, Belt RJ, Chen R, Obasaju C. Gemcitabine and pemetrexed administered in rapid sequence as front-line chemotherapy for advanced non-small-cell lung cancer: a phase II clinical trial. *Ann Oncol*. 2009;20(5):850–6.
35. Gridelli C, Kaukel E, Gregorc V, et al. Single-agent pemetrexed or sequential pemetrexed/gemcitabine as front-line treatment of advanced non-small cell lung cancer in elderly patients or patients ineligible for platinum-based chemotherapy: a multicenter, randomized, phase II trial. *J Thorac Oncol*. 2007;2(3):221–9.
36. Gilligan D, Nicolson M, Smith I, et al. Preoperative chemotherapy in patients with resectable non-small cell lung cancer: results of the MRC LU22/NVALT 2/EORTC 08012 multicentre randomised trial and update of systematic review. *Lancet*. 2007;369(9577):1929–37.
37. Imperatori A, La Salvia D, Rotolo N, et al. Five-year survival of stage IIIA-IIIB (non-N3) non-small cell lung cancer patients after platinum/gemcitabine induction chemotherapy and surgery. *J Chemother*. 2010;22(3):191–6.
38. Benjamin Besse. Adjuvant Chemotherapy for Non-Small-Cell Lung Cancer: A Fading Effect? *J Clin Oncol*. 2008;26(31):5014–7.
39. Arrieta O, Gallardo-Rincon D, Villarreal-Garza C, et al. High frequency of radiation pneumonitis in patients with locally advanced non-small cell lung cancer treated with concurrent radiotherapy and gemcitabine after induction with gemcitabine and carboplatin. *J Thorac Oncol*. 2009;4(7):845–52.
40. Vokes EE, Herndon JE 2nd, Crawford J, et al. Randomized phase II study of cisplatin with gemcitabine or paclitaxel or vinorelbine as induction chemotherapy followed by concomitant chemoradiotherapy for stage IIIB non-small-cell lung cancer: cancer and leukemia group B study 9431. *J Clin Oncol*. 2002;20(20):4191–8.
41. Guchelaar HJ, Richel DJ, van Knapen A. Clinical, Toxicological and Pharmacological Aspects of Gemcitabine. *Cancer Treat Rev*. 1996;22(1):15–31.
42. Glezerman I, Kris MG, Miller V, Seshan S, Flombaum CD. Gemcitabine nephrotoxicity and hemolytic uremic syndrome: report of 29 cases from a single institution. *Clin Nephrol*. 2009;71(2):130–9.
43. Sève P, Mackey JR, Isaac S, et al. cN-II expression predicts survival in patients receiving gemcitabine for advanced non-small cell lung cancer. *Lung Cancer*. 2005;49(3):363–70.
44. Bepler G, Kusmartseva I, Sharma S, et al. RRM1 modulated in vitro and in vivo efficacy of gemcitabine and platinum in non small lung cancer. *J Clin Oncol*. 2006;24 (29):4731–7.
45. Watchers FM, Wong LS, Timens W, Kampinga HH, Groen HJ. ERCC1, hhRad51, and BRCA1 protein expression in relation to tumour response and survival of stage III/IV NSCLC patients treated with chemotherapy. *Lung cancer*. 2005;S0(2):211–9.
46. Reid G, Wielinga P, Zelcer N, et al. Characterization of the Transport of Nucleoside Analog Drugs by the Human Multidrug Resistance Proteins MRP4 and MRP5. *Mol Pharmacol*. 2003;63:1094–103.
47. Longley DB, Johnston PG. Molecular mechanisms of drug resistance. *J Pathol*. 2005;205(2):275–92.
48. Glaysher S, Yiannakis D, Gabriel F, et al. Resistance gene expression determines the in vitro chemosensitivity of non-small cell lung cancer (NSCLC). *BMC Cancer*. 2009;9:300.
49. Rosell R, Cobo M, Isla D, Camps C, Massuti B. Catalan Institute of Oncology, Barcelona, Spain Pharmacogenomics and gemcitabine. *Ann Oncol*. 2006;17 Suppl 5:v13–16.
50. Su C, Zhou S, Zhang L, et al. ERCC1, RRM1 and BRCA1 mRNA expression levels and clinical outcome of advanced non-small cell lung cancer. *Med Oncol*. 2010 May 14.
51. Lee JJ, Maeng CH, Baek SK, et al. The immunohistochemical overexpression of ribonucleotide reductase regulatory subunit M1 (RRM1) protein is a predictor of shorter survival to gemcitabine-based chemotherapy in advanced non-small cell lung cancer (NSCLC). *Lung Cancer*. 2010 Mar 9.
52. Dong S, Guo AL, Chen ZH, et al. RRM1 single nucleotide polymorphism –37C—>A correlates with progression-free survival in NSCLC patients after gemcitabine-based chemotherapy. *J Hematol Oncol*. 2010;13;3–10.