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ORIGINAL ARTICLE

Clinical outcomes of oral metronomic vinorelbine in advanced non-small cell lung cancer: correlations with pharmacokinetics and MDR1 polymorphisms

Milena Gusella[1] · Felice Pasini[2] · Donatella Caruso[3] · Carmen Barile[1] · Yasmina Modena[1] · Anna Paola Fraccon[2] · Laura Bertolaso[1] · Daniela Menon[1] · Giorgio Crepaldi[1] · Antonio Bononi[1] · Roberto Spezzano[3] · Giorgia Anna Telatin[1] · Giuseppe Corona[4] · Roberto Padrini[5]

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Abstract

Purpose This study investigated correlations of the clinical outcomes of oral metronomic vinorelbine (VNR) with VNR pharmacokinetics and MDR1 polymorphisms.

Methods Eighty-two patients with metastatic non-small cell lung cancer (NSCLC) unfit for standard chemotherapy were treated with VNR at the oral doses of 20–30 mg every other day or 50 mg three times a week. They had a performance status (PS) ≤ 3, were > 70-year-old and drug-naïve or cisplatin-pretreated. MDR1 2677G > T and 3435C > T polymorphisms were analysed and blood concentrations of VNR and desacetyl-VNR (dVNR: active metabolite) assayed. Overall survival (OS), treatment duration and drug-related toxicity were the main endpoints.

Results Median OS and treatment duration were 27 weeks (range 1.3–183) and 15 weeks (range 1.3–144), respectively. OS was directly correlated with the duration of VNR treatment and number of therapy lines after VNR treatment (multiple linear regression: adjusted $r^2 = 0.71$; $p < 0.00001$). Neither MDR1 genotypes nor VNR/dVNR concentrations predicted OS. VNR blood levels were positively correlated with platelet counts ($r^2 = 0.12$; $p = 0.0036$). Patients who had long-term benefit (treated for ≥ 6 month without toxicity) showed lower VNR concentrations than those who had not. Twelve patients stopped therapy due to grade 3–4 toxicity. Toxicity was associated with blood concentrations of VNR ≥ 1.57 ng/mL and dVNR ≥ 3.04 ng/mL, but not with MDR1 polymorphisms.

Conclusions Neither pharmacokinetic nor pharmacogenetic monitoring seem useful to predict OS. On the other hand, high VNR and dVNR blood levels were associated with severe toxicity.

Keywords Vinorelbine · Metronomics · Lung cancer · Pharmacokinetics · MDR1 polymorphisms.

Introduction

Metronomic chemotherapy is characterised by frequent, continuous administration of low oral doses of a single drug, which are able to inhibit angiogenesis and promote antitumor immunity by several mechanisms (increased activity of cytotoxic T-cells, antigen-presenting cells, dendritic cells and ablation of immunosuppressive regulatory T cells) [1]. These features are expected to translate at clinical level into delayed tumour progression, avoidance of drug resistance and improved tolerability [2, 3]. For this reason, metronomic oral chemotherapy appears particularly suitable for palliative purpose in elderly patients or patients exposed to several therapy lines.

✉ Milena Gusella
milenagusella@libero.it

1 AULSS5 Polesana, UOC Oncologia, Ospedale Santa Maria della Misericordia, viale Tre Martiri, 140, 45100 Rovigo, Italy

2 Servizio di Oncologia, Ospedale Pederzoli, Peschiera del Garda, Verona, Italy

3 Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy

4 Dipartimento di Ricerca Traslazionale, IRCCS Centro di Riferimento Oncologico, Aviano, Italy

5 Servizio di Farmacologia Clinica, Dipartimento di Medicina, Università di Padova, Padua, Italy

Low doses of vinorelbine (VNR) and its active metabolite 4-desacetyl vinorelbine (dVNR) have demonstrated anti-angiogenetic activity in vitro [4]. Phase I–II clinical trials adopting metronomic oral VNR schedules in various types of advanced cancer reported acceptable clinical efficacy and manageable toxicity [5–11] in the same range of VNR blood concentrations which proved to be anti-angiogenetic in preclinical studies [8]. Oral doses between 20 and 70 mg thrice a week are linearly correlated with VNR steady-state blood levels, without evidence of blood accumulation [9]. VNR is eliminated mainly by the liver through drug metabolism (CYP3A4/5) and biliary excretion (P-glycoprotein) and partly by renal excretion, with a wide interpatient variability. Wong et al. [12] reported that vinorelbine clearance is predicted by creatinine clearance and by ^{99m}Tc-MIBI clearance (a marker of hepatic P-gp activity) [13], but not by midazolam clearance (a marker of CYP3A activity). Part of P-gp variability has been associated with two single nucleotide polymorphisms of the MDR1 gene, MDR1-2677G > T (rs2032582) and MDR1-3435C > T (rs1045642), expressing a reduced P-gp activity [12–14]. A role of MDR1 polymorphisms in the clinical response to VNR has been suggested by two studies in lung cancer patients receiving intravenous VNR and cisplatin. In one of them the haplotype MDR1 2677G–3435C showed a significantly better response rate [15] and in the other the MDR1 3435CC polymorphism was associated with a significantly reduced risk of disease progression (but not survival) [16].

The aim of the present study was to investigate the correlation between clinical response/toxicity to metronomic oral VNR and drug exposure, as assessed by pharmacokinetic and pharmacogenetic parameters.

Patients and methods

Eligibility criteria

Patients were eligible if they had documented metastatic, cytologically/histologically proven, non-small cell lung cancer (NSCLC). Other inclusion criteria were: treatment-naïve patients aged ≥ 70 years clinically unfit for cisplatin-based chemotherapy; patients who received at least one line of cisplatin-based chemotherapy and not suitable for further conventional chemotherapy; ECOG performance status (PS) score ≤ 3 according to ECOG/WHO scale; adequate bone marrow, hepatic and renal functions.

Exclusion criteria were: oncogene-dependant tumour; active brain metastases; severe cardiac diseases (i.e., myocardial infarction within 3 months, severe/unstable angina, congestive heart failure); concurrent chemotherapy. Limited field radiation therapy (RT) was allowed.

Baseline evaluation included medical history, CT scan of the thorax and upper abdomen, hemogram and blood chemistry.

Dose schedule

In consideration of patients' frailty and long half-lives of VNR and dVNR (about 40 h for VNR and > 100 h for dVNR), a low dose every other day was administered (without weekend break), instead of the standard three-times-a-week schedule. In the first 8 subjects, the dose was as low as 20 mg every other day. Since this dose was associated with good tolerance, next patients received 30 mg (63 patients) every other day or 50 mg (11 patients) three times a week.

After initiation of OMV, patients were monitored weekly for 1 month, fortnightly for 2 months and then monthly with clinical visit, haemogram, blood chemistry and pharmacokinetics. The drug was administered until progression, unacceptable toxicity, intercurrent diseases or patient's refusal. Informed consent was obtained according to the Italian law.

Study endpoints

The study aimed at evaluating whether any clinical, pharmacokinetic or pharmacogenetic variable can predict the clinical outcomes of the metronomic OMV treatment, in a real-life palliative setting.

The clinical outcomes considered were: (1) overall survival (OS), defined as the time elapsed between initiation of OMV therapy and death from any cause; (2) treatment duration, defined as the time from treatment start to disease progression, ECOG performance status (PS) deterioration, unacceptable toxicity, intercurrent diseases or patient's refusal; (3) long-term benefit, achieved when VNR treatment lasted more than the mean treatment duration without severe toxicity; (4) number of therapy lines after stopping VNR treatment; (5) drug-related toxicity, evaluated according to National Cancer Institute Common Toxicity Criteria V3.

Instrumental tumour assessment (chest-X rays, CT scan, ultrasound, clinical measurements) was generally performed every 8–12 weeks or when clinically required according to physicians' judgment.

Pharmacokinetics

On occasion of routine follow-up visits 2-mL whole blood samples were collected into EDTA tubes at 8 a.m., 2 h before VNR oral administration (46 h after last administration). The first sample was obtained at least 7 days from starting VNR treatment. The number of samples was not predefined but depended on follow-up duration (6.75 samples/patient, on average).

Samples were stored at − 20 °C until analysis. Briefly, the assay of VNR and its major 4-*O*-deacetyl metabolite (dVRN) consisted in deproteinizing blood samples (250 µL) with two volumes of acetonitrile containing deuterized vinorelbine as an internal standard, followed by evaporation to dryness. Concentrations of VNR and dVNR were quantified by LC–MS/MS using an Agilent 1200 series binary pump connected to PAL HTS system autosampler (CTC Analytics) and an API 4000 triple quadrupole instrument (AB SCIEX) in multiple reaction monitoring (MRM) mode to monitor precursor-to-product ion transition *m*/*z*; an electrospray ionization (ESI) interface in positive ionization mode was used and data processing was performed with Analyst 1.6.1 software. Chromatographic separation was carried out on Monolithic Column Onyx C18 (50 × 2 mm, Phenomenex) maintained at 25 °C. The mobile phase was: 10 mM NH_4 with 0.1% formic acid (phase A) and acetonitrile (phase B). The flow was 0.5 mL/min and overall chromatographic run time was 7.00 min and the gradient was: T_{0min} 95% A, T_{5min} 5% A, $T_{5.1min}$ 95%, T_{7min} 95%. The injection volume was 50 µL and the temperature of auto-sampler was maintained at 4 °C. The lower limit of quantification was about 0.05 ng/mL for both compounds and response was linear in the range of 0.05–25 ng/mL ($R^2 \geq 0.994$). Inaccuracy and imprecision were < 16.1% and < 11.2% for VNR, and < 14.5% and < 12.8% for dVNR.

Mean VNR and dVNR concentrations during long-term period were calculated by dividing the area under the plasma concentration–time curve (calculated by the trapezoidal rule) from the first to the last concentration available (AUC: ng/mL × day) by the time elapsed from the first to the last concentration (days). Since the parent drug and its metabolite have similar antitumoral [9] and antiangiogenetic activity [4], both blood concentrations were used for PK/PD correlations.

Pharmacogenetics

Genomic DNA was extracted from peripheral blood white cells using a commercial kit (Pomega). MDR1 2677 PCR was performed in a final solution of 50 µl with the following primers:

Fw. 5′-TGC AGG CTA TAG GTT CCA GG-3′;

Rv: 5′-TTT AGT TTG ACT CAC CTT CCC G-3′.

The cyclothermal program consisted of 35 cycles with 60 °C as annealing temperature; genotyping was performed through RFLP with BanI enzyme that cut the wild-type allele in two fragments of 198 and 26 bps.

MDR1 3435 amplification was performed using the following primers:

Fw 5′-GAT CTG TGA ACT CTT GTT TTC A-3′

Rv 5′-GAA GAG AGA CTT ACA TTA GGC-3′

The cyclothermal program consisted of 30 cycles with 60 °C as annealing temperature; genotyping was performed through RFLP with DpnII enzyme that cut the wild-type allele in two fragments of 172 and 72 bps.

Genotype-phenotype score

To assess the combined influence of MDR1 2677 and MDR1 3435 genotypes on MDR1 phenotype, different activity scores have been assigned to homozygous or heterozygous genotypes, as follows: 0, to the homozygous TT2677 and TT3435 defective genotypes, 0.5 to any heterozygous genotypes, and 1 to the homozygous GG2677 and CC3435 wild-type genotypes. Accordingly, the sum of scores of the combined MDR1-2677G>T and MDR1-3435C>T haplotypes could range from 0 to 2.

Statistical analysis

Data are generally presented as means ± SD and distribution normality was assessed by D'Agostino-Pearson test. The two-tailed unpaired *t* test (with Welch's correction if needed) was applied to compare normally distributed variables. In the case of skewed distribution, the nonparametric Mann–Whitney test was used. OS was expressed as median with range.

Correlations of OS and blood concentrations with continuous variables was first assessed by univariate regression analysis. The continuous variables considered were: age; ECOG PS; number of previous therapy lines; number of additional therapy lines after stopping VNR; number of metastatic sites; blood concentrations (ng/mL) of VNR and dVNR; leukocyte and platelet counts; haemoglobin, creatinine and ALT values. The variables which passed the significance level $p < 0.10$ were included in a forward and backward stepwise regression analysis.

The OS probability over time were estimated using the Kaplan–Meier method and survival curves of patients with different characteristics were compared by the log-rank test and hazard ratio (HR).

Mean blood concentrations of VNR and dVNR of patients with different MDR1 haplotypes and dose regimens were compared by Kruskal–Wallis nonparametric analysis of variance.

The receiver operating characteristic (ROC) curve was used to identify the concentration values separating patients with and without grade 3–4 toxicity.

For all tests, the level of significance was set at < 0.05.

Table 1 Patients' demographic characteristics

	Mean ± SD	Range
Age (years)	71.6 ± 9.9	29–88
Male/female	65/17	–
Body weight (kg)	72.3 ± 13.7	47–98
Body surface area (m^2)	1.83 ± 0.20	1.40–2.19
Body mass index	26.2 ± 3.6	19.3–32.0
Haemoglobin (g/dL)	11.1 ± 1.8	7.2–14.7
Platelets ($\times 10^3/mm^3$)	244 ± 109	56–643
Leukocytes ($\times 10^3/mm^3$)	4.20 ± 2.69	0.11–14.65
ALT (U/L)	31.3 ± 30.3	12–215
Creatinine (mg/dL)	1.14 ± 0.40	0.63–2.71

Table 2 Patients' oncological characteristics

	Number of patients	%
PS ECOG		
0	22	26.8
1	40	48.8
2	18	21.9
3	2	2.4
Histology		
Adenocarcinoma	48	58.5
Squamous	33	40.2
NOS	1	1.2
Metastatic sites		
1	12	14.6
2	33	40.2
3	25	30.5
4–5	12	14.6
Prior chemotherapy lines		
0	23	28.0
1	28	34.1
2	20	24.4
3–4	11	13.4

Results

Clinical outcomes

Eighty-two patients were the study population. Their baseline characteristics are outlined in Tables 1 and 2. Most of them were males (79.3%), aged over 70 (62.2%) and had a PS < 2 (75.6%). Forty nine patients had adenocarcinoma histology (58.5%) and 37 had ≥3 metastatic sites (45.1%). Twenty-three patients were chemotherapy naïve (28.0%).

The mean treatment duration was 24 weeks (median:15 weeks; range 1.3–144). Twenty-three patients continued the treatment beyond 24 weeks (6 months) without severe toxicity and were considered to have long-term benefit. Median OS was 27 weeks (range 1.3–183) and survival rates at 3, 6 and 12 months were 80%, 51% and 25%, respectively. The median OS was 32 weeks in chemotherapy-naïve patients and 27 weeks in pre-treated patients.

Treatment was stopped due to disease progression ($n = 41$), PS deterioration ($n = 20$), toxicity ($n = 12$), patient refusal (n = 2) or other reasons unrelated to treatment (4 intercurrent diseases, 1 emergency surgery); at time of writing, treatment is ongoing in 2 patients. The adverse events leading to drug withdrawal were: G4 neutropenia (8), G3 neutropenia (1), G3 asthenia (2) and G3 constipation (1).

Twelve patients, who stopped VNR for disease progression or toxicity and were considered clinically fit for additional treatments, received 1–3 more chemotherapy lines.

Pharmacogenetics

Genotype distributions were in Hardy–Weinberg equilibrium, as follows: MDR1 2677GG 34%, GT 53% and TT 13% and MDR1 3435 CC 26%, CT 55% and TT 19%. Haplotype 2677GG-3435CC (associated with a completely efficient P-gp) and 2677TT-3435TT (associated with deficient P-gp) represented 26% and 11% of all subjects, respectively; the remaining 63% had various combinations of both SNPs. Minor allele frequencies were 0.40 for MDR1-2677G > T (expected MAF (T) = 0.45 in Caucasians) and 0,45 for MDR1-3435C > T (expected MAF (T) = 0.47 in Caucasians).

To assess the influence of the two MDR1 polymorphisms on OS, we used a genotype-based score (see "Patients and methods") to classify patients in three phenotype groups: defective (score 0–0.5), intermediate (score 1), and wild-type (score 1.5–2). With this approach OS did not differ among the three groups (Fig. 1). Likewise, the mean geno-phenotype score did not differ between the 12 patients who developed severe toxicity and those who were drug-tolerant (1.07 ± 0.63 vs 1.17 ± 0.58; n.s.).

Pharmacokinetics

Mean and median blood levels of VNR and dVNR were highly variable among patients, as inferred by SDs and ranges (Table 3). Although mean blood levels tended to be lower in patients taking the lowest dose (20 mg) the difference with the other two dose groups was not significant.

None of the clinical, haemato-chemical (ALT, sCr), and genetic variables correlated with VNR or dVNR blood levels except for platelet count, which was significantly ($p = 0.014$) and positively correlated with VNR blood levels (Fig. 2). However, after removing an out-layer data (indicated by a closed symbol in Fig. 2) the correlation was no more significant.

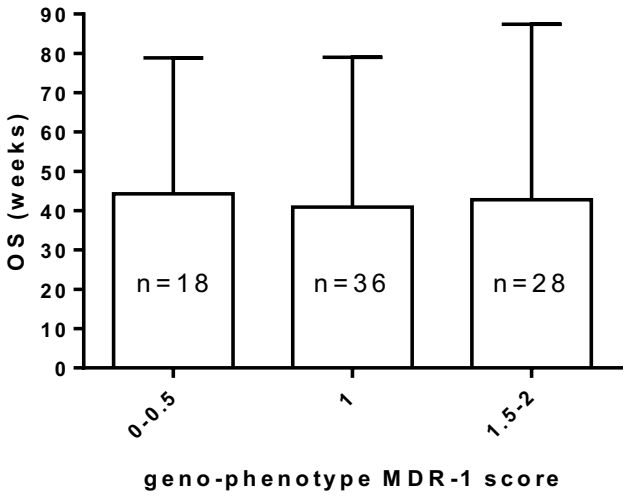

Fig. 1 Overall survival (means ± SDs) in the three MDR1 haplotypes. Scores < 1 indicate low MDR activity, > 1 high MDR activity

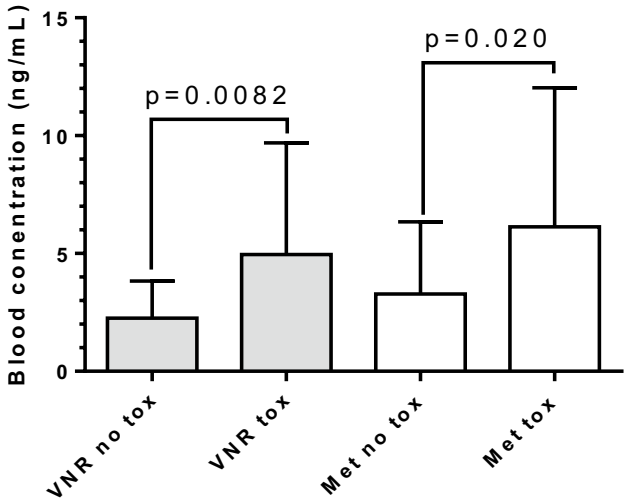

Fig. 3 Blood concentrations (means ± SDs) of VNR and dVNR in patients with and without severe toxicity (grade 3–4)

Table 3 Mean blood concentrations ± SD (range) of VNR and dVNR for each dose level

	Dose levels		
	20 mg ($n=8$)	30 mg ($n=63$)	50 mg ($n=11$)
Blood concentrations (ng/mL)			
VNR	1.73 ± 1.19 (0.37–4.29)	2.77 ± 2.66 (0.17–18.00)	2.43 ± 1.66 (0.64–5.17)
dVNR	2.12 ± s1.52 (0.41–4.78)	3.84 ± 3.99 (0.13–21.94)	3.70 ± 1.85 (2.07–8.04)

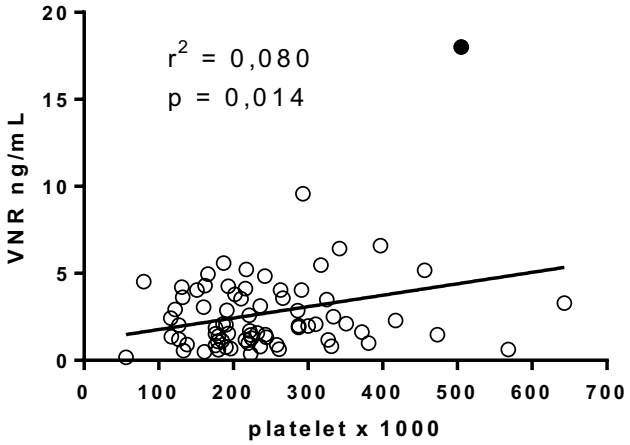

Fig. 2 Linear correlation between platelet count and VNR blood concentration. After removing an out-layer data (indicated with a closed symbol) the correlation was no more significant

No correlation was found between the blood concentration of VNR or dVNR and OS. However, blood levels of both compounds were significantly higher (about twofold) in patients with severe (grade 3–4) toxicity than in those who were drug-tolerant (Fig. 3). ROC curve analysis yielded toxicity cut-off concentrations of 1.57 ng/mL for VNR (area: 0.74; $p=0.0093$) and 3.04 ng/mL (area: 0.71; $p=0.022$) for dVNR.

Mean VNR concentrations of patients who had clinical benefit (i.e., continued VNR treatment for ≥ 6 months without severe toxicity) were lower than those of patients who stopped treatment prematurely because of tumour progression or PS deterioration (1.73 ± 1.27 ng/mL vs 2.50 ± 1.65 ng/mL, $p=0.047$; Fig. 4), suggesting that high exposure to VNR may negatively impact also on therapeutic outcomes. dVNR concentrations showed the same trend as VNR, without reaching the significance level ($p=0.14$).

Univariate regression analyses

OS was positively associated with duration of VNR therapy and number of additional chemotherapy lines ($p<0001$ and $p=0.0002$, respectively) and negatively associated with PS and number of metastatic sites ($p=0002$ and $p=0.0126$, respectively), whereas patients' age, number of previous therapy lines, hemato-chemical data and blood concentration of VNR and dVNR were not.

Multiple regression analyses

A multiple regression analysis was then performed, using the variables significantly correlated with OS by univariate analyses, namely: duration of VNR therapy (Tdur:weeks), number of additional chemotherapy lines ($n°$ lines), ECOG PS and number of metastatic sites ($n°$ sites). The only variables still significant after the forward and backward stepwise

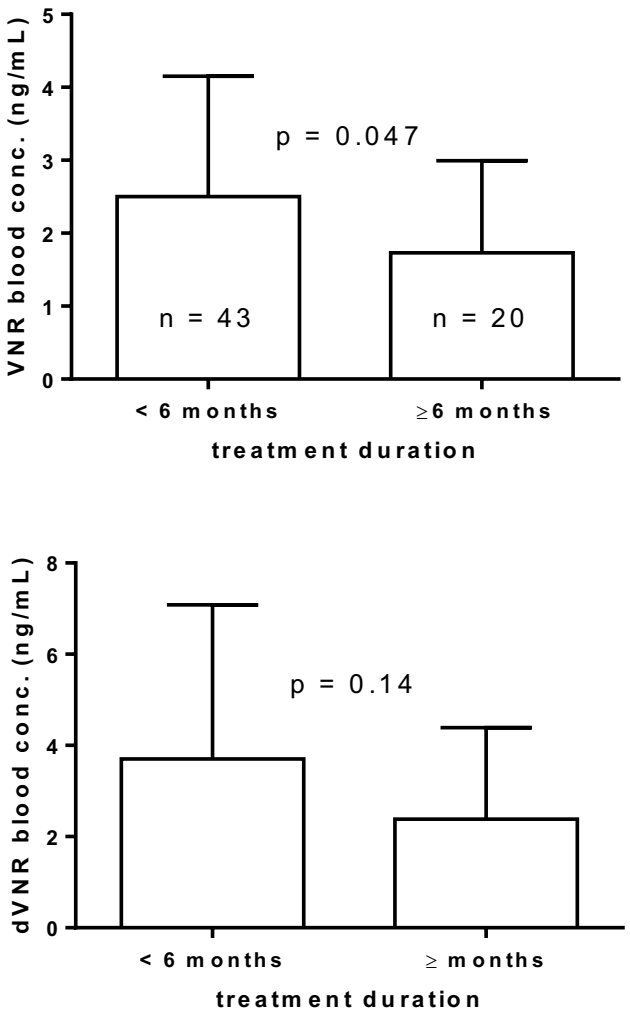

Fig. 4 Blood concentrations (means ± SDs) of VNR and dVNR in patients without severe toxicity, who had clinical benefit (treatment duration ≥ 6 month) or no clinical benefit (treatment duration < 6 months)

procedures were $n°$ lines and Tdur, and yielded the following equation:

$$\text{OS (weeks)} = 11.8 + 1.09 \times (\text{Tdur}) + 22.8 \times (n°\text{lines}) \quad \text{adjusted } r^2 = 0.71; p < 0.000001.$$

No redundancy between variables was found (tolerance = 0.99). Partial significance levels were: $p = 0.00033$ for intercept, $p < 0.00001$ for Tdur and $p < 0.00001$ for $n°$ lines.

Discussion

This study aimed at evaluating the clinical outcomes of OMV therapy in a real-world palliative setting of metastatic NSCLC and at identifying possible demographic, pharmacokinetic and pharmacogenetic predictors of efficacy and/or toxicity. Our patients were considered unfit for standard chemotherapy due to advanced age or previous cisplatin-based treatment lines. The median OS was 27 weeks (6.75 months) in the whole population and 32 weeks (8 months) in previously untreated patients. The latter finding is notable in view of the advanced age of these patients (mean 80 ± 5.4 years) and it compares favourably with the median OS previously reported in elderly, naïve, patients with NSCLC (8–9 months) treated with metronomic VNR doses (50 mg three time a week) [5–7]. Interestingly, median OS of our patients was similar to that of another study (7 months) in which VNR was given intravenously—on days 1 and 8, every 21 days for up to six cycles—to an elderly NSCLC population [17].

In our study, OS was predicted by duration of VNR treatment and number of additional chemotherapy lines after stopping VNR, without any significant contribution by other clinically relevant variables, included MDR1 haplotypes and VNR/dVNR blood levels. At a first glance, these findings seem to indicate that prolonging chemotherapy, with either VNR or other agents, translates in a survival benefit for the patients. Although this explanation may have clinical sense, it is also conceivable that prolongation of therapy was permitted by better patients' clinical conditions. Actually, PS and number of metastatic sites were significantly correlated with OS by univariate analysis but were considered redundant variables by multivariate analysis. Discriminating between the two hypotheses is not possible without a control group.

Excluding from analysis patients who stopped therapy because of severe toxicity or intercurrent diseases, it appeared that those who continued VNR treatment beyond 6 months (i.e., obtained a clinical benefit) had lower VNR blood levels than those who stopped therapy earlier. These findings seem to contradict the standard concentration–effect relationship and suggest that a ceiling VNR concentration exists above which the clinical benefit is lost.

While further clinical confirmations of our results are needed, some recent experimental studies [1, 4] support the view that low, persistent VNR concentrations—like those attained by metronomic schedules—can improve the activity of cytotoxic lymphocytes, decrease the number of immunosuppressive cells in the tumour and inhibit immune checkpoints, thus promoting anti-tumour immunity. In contrast, high, pulsating concentrations—like those attained by standard intravenous schedules—depress the immune system, thus promoting tumour growth and the onset of adverse drug reactions.

Toxicity requiring drug withdrawal occurred in 12 of our patients (14.6%) and mainly consisted in grade 3–4 neutropenia. ROC analysis identified concentrations > 1.57 ng/mL for VNR and > 3.04 ng/mL for dVNR associated with severe toxicity. Other studies found a correlation between VNR AUC and neutrophil decrease [12, 18–21] but none of them establish an AUC cut-off to predict haematological toxicity. Regrettably, the VNR cut-off we found in the whole population (> 1.57 ng/mL) was in the range of the concentrations measured in the subpopulation with clinical benefit and no severe toxicity (1.73 ± 1.27 ng/mL), making it impossible to propose a therapeutic concentration interval.

Our results on VNR pharmacokinetics and pharmacogenetics need some comments. We confirmed previous results of a wide inter-patient variability of VNR blood concentrations and a correlation between VNR clearance/blood concentration and platelet count [20, 22, 23]. This latter correlation is not to be intended as an effect of VNR on platelet count but as an influence of platelets on VNR blood concentrations. Higher VNR blood levels in patients with higher platelet count can be explained by the extensive VNR binding to platelets (> 70% of blood content) [24]. Although the correlation we found was weak and disappeared after removing an outlier value, our results are in agreement with other experimental and clinical data [22, 24].

Conversely, we were unable to correlate VNR pharmacokinetic variability with MDR1 polymorphisms implicated in P-pg efflux protein expression. The involvement of P-gp in VNR biliary excretion is supported by two pieces of evidence: (a) a correlation exists between ^{99m}Tc-MBI clearance (a purported marker of P-gp hepatic activity) and VNR clearance [12]; (b) zosuquidar (a potent and specific inhibitor of P-gp activity) decreases VNR clearance by 24% [25]. In spite of this—and in agreement with our results—Wong et al. [12] failed to demonstrate an association between VNR clearance and three MDR1 polymorphisms (2677G > T, 3435C > T, and 1236T > C) and hypothesised that other drug clearance mechanisms (such as CYP3A-mediated metabolism) may have weakened the genotype–phenotype relationship.

Further uncertainty is added to this puzzling picture by two clinical studies which demonstrated [15, 16] that the most active MDR1 variants (3435CC and 2677GG) predicted a better response to VNR chemotherapy, when the opposite should be expected. To justify these counterintuitive results Vignolas et al. [16] advocated the possibility of a linkage with other mutations elsewhere within the MDR1 gene which are responsible for improved response.

At the present state of knowledge, it should be admitted that the relationships between MDR1 genotypes and VNR pharmacokinetics or clinical response are still poorly understood.

Conclusions and limitations

Sticking to the results of the present study, the following conclusions can be drawn:

1. Oral metronomic VNR in elderly and/or pretreated NSCLC patients is associated with a median OS similar to that of previous studies which adopted a metronomic approach. OS was correlated with duration of VNR treatment and number of additional chemotherapy lines but not with VNR and dVNR blood levels or MDR1 polymorphisms.
2. MDR1 polymorphisms did not predict VNR blood levels.
3. Severe toxicity occurred in 14% of patients and was associated with blood concentrations > 1.57 ng/mL of VNR and > 3.04 ng/mL of dVNR.
4. Patients who benefitted more from treatment had also lower VNR blood concentrations (1.73 ± 1.27 ng/mL) than those who did not, thus envisaging an inverse concentration/effect relationship which makes it difficult to define a therapeutic concentration range.

The main limitation of our study lies in its observational design. In the real life, drug response is evaluated openly according to clinicians' experience and adherence to international guidelines. However, the main outcome measures adopted (OS and severe toxicity) are hard endpoints, therefore, we feel confident enough about the reliability of our data. In addition, our aim was not to prove the clinical efficacy of VNR but to identify which biological variables were associated with therapeutically relevant outcomes. These findings, if confirmed by adequately powered studies, may improve therapy management of NSCLC patients.

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Compliance with ethical standards

Conflict of interest All Authors declares that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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