Metronomic vinorelbine is active on EGFR-wt and EGFRL858R/T790M Non Small Cell Lung Cancer cells, alone and in combination with EGFR tyrosine kinase inhibitors

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Background. Vinorelbine (VNR) is a semi-synthetic vinca-alkaloid approved for the treatment of breast cancer and non small cell lung cancer (NSCLC) alone or in combination with cisplatin, gemcitabine, anthracyclines o taxanes (1). VNR is a microtubule-targeting agent that have shown peculiar activity in terms of angiogenesis inhibition, suppression of endothelial progenitor cells and HIF-1α pathway inhibition. These characteristics, together with oral administration, make VNR one of the most promising agents to be evaluated with metronomic regimens (2). Metronomic chemotherapy is defined as a frequent, regular administration of low dose chemotherapeutic drugs, able to maintain active drug concentrations for long periods of time without causing severe toxicities (3). Gefitinib and erlotinib, EGFR tyrosine kinase inhibitors (TKIs), are administered in patients with advanced NSCLC with EGFR activating mutations (e.g., L858R) but not in patients with NSCLC EGFR-wt or resistant mutations. Acquired resistance to TKIs has been attributed in particular to the secondary point mutation T790M in the kinase domain of EGFR, that increase the affinity of EGFR for ATP (4).

Aim of the study. To establish, for the first time, the activity of metronomic VNR (mVNR) alone or in combination with EGFR TKIs on NSCLC cells not harboring the activating mutations or with a resistant mutation. Indeed, the possible synergistic effects with TKIs might be clinically beneficial to NSCLC EGFR-wt patients who are poorly responsive to EGFR-TKIs and it might be effective to overcome the resistance caused by the T790M mutation.

Methods. Proliferation assays were performed on endothelial cell (HUVEC) and on A-549 (EGFRhigh), H-292 (EGFR-wt), H-358 (EGFR-wt), H-1975 (EGFRL858R/T790M) NSCLC cell lines exposed to mVNR, its active metabolite deacetyl-VNR (D-VNR), gefitinib and erlotinib for 144h daily treatments. The synergism between mVNR and TKIs was determined with the method by Chou (5) and quantified by the combination index (CI). Cyclin-D1 and ABCG2 genes expression was performed with Real-Time PCR. Intracellular concentrations were investigated with a LC-HRMS system.

Results. mVNR confirmed its preferential activity on endothelial cells after 144h, but it was extremely active also on NSCLC cell lines, in particular on H-1975, resistant to TKIs. The daily treatment with erlotinib and gefitinib showed a lower activity on H-1975 when compared to NSCLC EGFR-wt. The simultaneous combination of VNR and TKIs determined a synergism on NSCLC cell lines (CI<1), with the highest effect on H-1975 cells. Interestingly, mVNR decreased the expression of both cyclin-D1 and ABCG2 genes. The simultaneous combination determined the intracellular increase of drug concentrations, suggesting the possible mechanism underlying the seen synergistic effect.

Conclusions. mVNR was active on NSCLC cells not harboring EGFR activating mutations or EGFR TKIs resistant cells. The simultaneous combination of mVNR and TKIs in vitro demonstrated a strong synergistic activity on EGFR-wt and EGFRL858R/T790M NSCLC cells suggesting this inedited option for future clinical trials.

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