Abstract. Aim: The objective of the present study was to determine the in vivo antitumor activity of elacytarabine, the 5'-elaidic acid ester of arabinofuranosyl cytidine, alone and in combination with bevacizumab, cetuximab and trastuzumab in Vascular endothelial growth factor (VEGF), Epidermal growth factor receptor (EGFR)- and Human epidermal growth factor receptor 2 (HER2)-expressing non-small cell lung cancer xenografts. Materials and Methods: The antitumor activity of elacytarabine was tested at the maximal tolerable dose (MTD; 50 mg/kg) and half MTD (25 mg/kg), alone and in combination with the antibodies bevacizumab (5 mg/kg), cetuximab (20 mg/kg) and trastuzumab (4 mg/kg) in two human non-small cell lung cancer xenografts. Results: Elacytarabine exhibited very high activity in the EKVX xenograft at both dose levels, but was inactive in MAKSAX. Neither of the two xenografts were sensitive to bevacizumab or trastuzumab, but the MAKSAX xenograft showed intermediate response to cetuximab. The high sensitivity of EKVX to elacytarabine precluded the assessment of a potential benefit of the combinations with the antibodies. In the elacytarabine-, bevacizumab- and trastuzumab-insensitive MAKSAX xenograft, the combination of either bevacizumab or trastuzumab with elacytarabine at the MTD or half MTD resulted in intermediate activity, suggesting a beneficial effect of the combinations, whereas for cetuximab, the effect was enhanced when combined with elacytarabine given at the MTD, but not half-MTD.

Conclusion: The results suggest that elacytarabine could be active in some cases of non-small cell lung cancer, and that the combination of elacytarabine and tyrosine kinase inhibitors may exert important additive or possibly synergistic effects of potential clinical benefit.

Elacytarabine (CP-4055; arabinofuranosyl cytidine-5'-elaidic acid ester) is a fatty acid nucleoside analog of cytarabine. Pre-clinical studies have shown that elacytarabine may overcome some of the inherent and acquired mechanisms associated with cytarabine resistance (1-4). In contrast to cytarabine, elacytarabine has shown independency of mechanisms for active transport across the cell membrane for cellular uptake and activity (5). In pre-clinical studies, elacytarabine has demonstrated activity in xenografts derived from various groups of solid tumors, in some cases superior to that of cytarabine (6). Moreover, interesting in vivo effects were observed when elacytarabine was combined with different cytotoxic compounds (7-9). Elacytarabine has undergone dose-finding studies in hematological and solid malignancies (10-14) and has demonstrated clinical activity in acute myeloid leukemia (15, 16).

Elacytarabine has not previously been combined with agents that inhibit receptor tyrosine kinase signaling in cancer. Non-small cell lung cancer (NSCLC) is characterized by rapid emergence of resistance to chemotherapy and high mortality rates. Agents that inhibit tyrosine kinase signaling have been approved or shown promising activity against this malignancy. Bevacizumab is a humanized monoclonal antibody to Vascular Endothelial Growth Factor (VEGF) that inhibits neoangiogenesis and has been approved for treatment of metastatic colorectal and breast cancer, and inoperable NSCLC of non-squamous histology. Cetuximab is a humanized monoclonal antibody against the Epidermal Growth Factor Receptor (EGFR) and is approved for the treatment of metastatic colorectal cancer and for squamous cell carcinoma of the head and neck. Clinical phase-III

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studies have suggested that cetuximab in combination with chemotherapy has beneficial effects in a subset of patients with NSCLC dependent on the chemotherapeutic drug included in the combination regimen (17, 18). Trastuzumab and other Human Epidermal Growth Factor Receptor 2 (HER2)-targeting therapies have been less successful in clinical trials, but overexpression and amplification of the HER2 gene have been reported in a small subset of patients, suggesting that targeting HER2 may be beneficial (19).

The objective of this study was to evaluate the in vivo antitumor activity of elacytarabine against NSCLC alone and in combination with bevacizumab, cetuximab and trastuzumab.

Materials and Methods

Animals. Female and male Balb/c nu/nu mice, bred at the Department of Comparative Medicine at the Norwegian Radium Hospital were used. The animals were maintained under specific pathogen-free conditions, and food and water were supplied ad libitum. Housing and all procedures involving animals were performed according to protocols approved by the National Animal Research Authority and conducted according to the regulations of the Federation of European Laboratory Animal Science Association (FELASA). The animals were 4-6 weeks of age and 20-25 g on the day of tumor implantation. Anesthesia was obtained with subcutaneous injections of 0.1 mg/kg fentanyl, 5 mg/kg fluanison and 2.5 mg/kg midazolam.

Evaluation of antitumor activity. The MAKSAX and EKVX xenografts were previously established by direct implantation of patient specimens into nude mice. For each experiment tumor fragments of 2x2x2 mm from one of the xenografts were implanted s.c. into both flanks of nude mice. The animals were randomised for treatment according to tumor size when the average tumor diameters were about 6 mm (day 0). From the first day of treatment, tumor diameters and bodyweight were measured twice weekly. Mice were monitored daily for health status and were killed by cervical dislocation if tumor volumes reached 2000 mm3. The maximal monitored daily for health status and were killed by cervical dislocation. Anesthesia was obtained with subcutaneous injections of 0.1 mg/kg fentanyl, 5 mg/kg fluanison and 2.5 mg/kg midazolam.

The antitumor activity was defined as: +, SGD >1.0 and T/C% <50%; ++, SGD >1.5 and T/C% <40%; ++++, SGD >2.0 and T/C% <25%; +++++, SGD >3.0 and T/C% <10%. Lethalities were excluded from the constructions of growth curves and calculations of SGD and T/C%. The Mann-Whitney U-test was used to test for significant differences of mean RTV on the day of maximal growth inhibition.

Drugs and treatment. Previous dose-escalation studies had shown that the MTD of the formulation of elacytarabine used here is 50 mg/kg for non-tumor bearing mice and this dose as well as the half MTD (25 mg/kg) were therefore used in the present study. Elacytarabine (Clavis Pharma, Oslo, Norway; batch KV1325/8) was administered i.p. on days 0-4 and 7-11. Bevacizumab (Avastin™; Roche, Basel, Switzerland), cetuximab (Erbitux™; Merck, Darmstadt, Germany) and trastuzumab (Herceptin™; Roche, Basel, Switzerland) were given i.v. on days 0 and 7. All drugs were stored at 4°C and diluted with saline to obtain an injection volume of 0.1 ml/10 g. Dilutions were performed immediately before injections. Control animals were treated with saline. When elacytarabine and monoclonal antibodies were given on the same day, the drugs were administered shortly after each other (days 0 and 7).

Immunohistochemistry. The EKVX and MAKSAX xenografts were characterized for receptor tyrosine kinase target expression prior to testing of antitumor activity. Tumors with diameters of around 6 mm were formalin-fixed and paraffin-embedded (FFPE). Slides from FFPE tumors were stained by immunohistochemistry (IHC) for EGFR, HER2 and VEGF expression, using the following antibodies: monoclonal mouse anti-human VEGF, clone VG1 (Dako, Glostrup, Denmark); monoclonal mouse anti-human EGFR, clone H11 (Dako); mouse anti-human HER2, NCL-CB11 (Novoceastra, Newcastle upon Tyne, UK).

Results

Expression of VEGF, EGFR and HER2 and antitumor effects of bevacizumab, cetuximab or trastuzumab. IHC staining of VEGF, EGFR and HER2 expression of the EKVX and MAKSAX s.c. xenografts are shown in Figure 1. Both types of xenografts showed a similar level of weak staining for VEGF, whereas they exhibited distinct membrane staining for EGFR and HER2, with the highest staining intensity being found for the EKVX xenograft.

Antitumor activity of bevacizumab, cetuximab or trastuzumab was initially tested at two different dose levels for each of the antibodies. The lowest doses were derived from previously published studies (20-27) and from dosing regimens recommended for clinical use (trastuzumab and bevacizumab). There were no significant gains in antitumor activity when the doses were increased from 5 to 15 mg/kg for bevacizumab (EKVX), from 20 to 40 mg/kg for cetuximab (MAKSAX), and from 4 to 12 mg/kg for trastuzumab (MAKSAX), and the lower doses were therefore chosen for the combination studies with elacytarabine (results not shown).

Effects of elacytarabine alone and combined with bevacizumab, cetuximab or trastuzumab. The treatment effects are summarized in Table I and growth curves are
shown in Figure 2. Elacytarabine alone at half MTD (25 mg/kg) and the MTD (50 mg/kg) of elacytarabine was highly effective (++++) in the EKVX model, bevacizumab was marginally-active alone (+), whereas cetuximab and trastuzumab were considered inactive. Because elacytarabine alone caused considerable tumor regression it could not be reliably concluded whether the antitumor effect was significantly enhanced by the addition of any of the monoclonal antibody tyrosine kinase inhibitors. In the MAKSAX model, however, elacytarabine was inactive at both 25 and 50 mg/kg. Neither bevacizumab nor trastuzumab displayed significant activity as monotherapies, whereas combinations with elacytarabine had intermediate activity (++). In this xenograft, cetuximab was active as monotherapy (++). In combination with 25 mg/kg elacytarabine, the effect was not significantly higher, but 50 mg/kg elacytarabine increased the efficacy (+++). Hence, the results from the MAKSAX model suggest a potential enhancement effect from combining the three kinase inhibitors with elacytarabine.

Discussion

Elacytarabine has undergone dose-finding studies in patients with hematological malignancies and solid tumors (11, 12, 14, 15), and has recently been tested in phase II (15, 16) and phase III (ClinicalTrials.gov Identifier: NCT01147939) studies for acute myeloid leukemia. In one out of the two in vivo human NSCLC models, EKVX, elacytarabine had very high activity both when administered at the MTD and at half MTD. The more resistant xenograft, MAKSAX, was sensitive to combinations of elacytarabine and the tyrosine kinase inhibitors administered at inactive or marginally active doses resulting in at least supra-additive activity. The high activity in one of our human NSCLC xenografts suggests that a subgroup of patients with this malignancy exists that has tumors sensitive to elacytarabine.

Bevacizumab has shown clinical activity in NSCLC, but the results from combination regimens with chemotherapy in clinical trials have been variable, seemingly dependent on the cytotoxic compound that was included (28). In our models, the activity of bevacizumab was limited. This may, at least in part, be attributed to the short treatment schedule used, and to the fact that the activity of this antibody may be generally underestimated in human xenografts because it only binds human VEGF, whereas it is very likely that murine VEGF also contributes to stimulating tumor vascularization in such models (29). In, EKVX, bevacizumab was weakly-active, but the tumor showed such a high sensitivity to elacytarabine that it was impossible to determine putative synergism from the combination. In the other xenograft, MAKSAX, the effect of bevacizumab, although statistically significant, did not reach the requirement for biological activity. Elacytarabine was also not active according to these criteria. The combination, however, resulted in clearly significant antitumor activity in this model.
The activity of cetuximab in combination with chemotherapy in NSCLC is somewhat debated. In two different phase III studies, contrasting results were found. In the FLEX study, it was shown that the addition of cetuximab to a chemotherapy regimen containing cisplatin and vinorelbine was beneficial (18), whereas the BMS-099 study failed to reach statistical significance when cetuximab was added to a regimen containing taxane and carboplatin (17). A plausible explanation for this discrepancy is that the FLEX study selected patients for EGFR-positivity based on IHC.

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whereas the BMS-099 study did not. Both our xenografts exhibited membrane expression of EGFR by IHC. However, whereas cetuximab was inactive in the EKVX xenograft, it had intermediate effect in the MAKSAX xenograft. The combination of elacytarabine and cetuximab in the MAKSAX xenograft was additive at the MTD of elacytarabine.

Overexpression of HER2 occurs in a subset of patients with NSCLC, with increased copy numbers in some cases. However, HER2-targeted therapies in such patients have been less successful in clinical trials (19). Both xenografts applied in this study exhibited membrane expression of HER2, as assessed by IHC, but trastuzumab was inactive in both xenografts. Although elacytarabine alone was inactive, in the MAKSAX xenograft, the combination regimens had significant activity when elacytarabine was administered at half MTD and at the MTD, suggesting a synergistic interaction between the two drugs.

Figure 2. Combination effects of elacytarabine with bevacizumab, cetuximab and trastuzumab on the human non-small cell lung cancer xenografts EKVX (left panel) and MAKSAX (right panel) in nude mice. Elacytarabine was administered i.p. on days 0-4 and 7-11 at 25 mg/kg (half MTD) and 50 mg/kg (MTD), alone, and in combination with 5 mg/kg bevacizumab (upper panel), 20 mg/kg cetuximab (middle panel) or 4 mg/kg trastuzumab (lower panel). Bevacizumab, cetuximab and trastuzumab were administered i.v. on days 0 and 7. The numbers of animals and tumors for each group are shown in Table 1.
The present study suggests that elacytarabine may be active in a subset of patients with NSCLC and that the potential activity of elacytarabine in NSCLC should be further investigated. Furthermore, the results indicate that combination of elacytarabine with tyrosine kinase inhibitors may be worthy of exploration with the possibility of developing a platform for these combinations in the treatment of NSCLC.

Conflicts of Interest

MLS is an employee at Clavis Pharma. This study was funded by Clavis Pharma.

References


