

proach. Targeting protein kinase C $\beta$  (PKC $\beta$ ) may serve as an attractive candidate because activation of PKC $\beta$  has been implicated in tumor proliferation, survival, invasion, and angiogenesis in model systems. Enzastaurin is a selective PKC $\beta$  inhibitor. There is evidence for its efficacy in both preclinical and clinical trials. The objective of this study was to assess the effect of enzastaurin on proliferation and phosphoprotein signaling in lung cancer.

**Methods:** Four non small cell lung cancer (non-SCLC) cell lines (H125, H322, H23, A549) and four SCLC cell lines (H69, SW210.5, DMS-79, H211) were tested. Cells were exposed to different doses of enzastaurin for 4 days, and the anti-proliferation effect was assessed with the CellTiter-Glo Luminescent Cell Viability Assay. The efficiency of enzastaurin in suppressing intracellular phosphoprotein signaling in these cells was tested at 1  $\mu$ mol/L concentrations at difference time points (0.5, 1, 2, 4 hours). PKC $\beta$ II and phospho-PKC $\beta$ II(ser660), GSK3 $\beta$  and phospho-GSK3 $\beta$ (ser9), ribosomal protein S6 and phospho-ribosomal protein S6(ser240/244) were measured by western blotting.

**Results:** Enzastaurin suppressed cell proliferation of all NSCLC and SCLC cell lines in the low micromolar range (0.1, 1, 5  $\mu$ M). Western blot analysis of these cells showed that phosphorylation of PKC $\beta$ II(ser660), GSK3 $\beta$ (ser9) and ribosomal protein S6(ser240/244) were suppressed in a time-dependent manner after enzastaurin treatment.

**Conclusions:** These data indicate that enzastaurin has a direct anti-cancer effect through suppression of proliferation, and they suggest that this effect is mediated by inhibition of intracellular phosphoprotein signaling.

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**A novel Bayesian dose-escalation Phase Ib study design investigating combination of the mammalian target of rapamycin (mTOR) inhibitor RAD001 with standard chemotherapy in patients with lung cancer**

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**Background:** RAD001C (everolimus) is an oral inhibitor of mammalian target of rapamycin (mTOR) with anti-tumor activity. As single-agent and in combination with other anticancer agents, RAD001C showed efficacy in *in vitro* and *in vivo* NSCLC models. RAD001C is currently being investigated in clinical trials as an anticancer agent in patients with advanced lung cancer. In a phase I study, 4 disease stabilizations (SD) and 1 partial response (PR), as per RECIST, were reported from 14 NSCLC patients treated with RAD001C monotherapy. Further phase Ib studies have been designed to investigate the feasibility of different regimens combining RAD001 doses and schedules with selected standard chemotherapeutic agents in patients with advanced non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). A novel statistical design was tailored to optimize the regimen selection in this combination phase Ib setting.

**Methods:** Three phase I, open-label, multi-center studies of RAD001 with standard chemotherapy in patients with advanced NSCLC or SCLC are designed as an adaptive Bayesian dose-escalation scheme. This is based on a Time-to-DLT (Dose Limiting Toxicity) model estimating the risk that patients experience a DLT within their first cycle of treatment (as primary endpoint) or at any other time-point during the

study. This novel statistical design improves on the traditional phase I designs. The adaptive nature of the Bayesian methodology allows the DLT rate to be re-evaluated at any time during the study, allowing quicker intervention following toxicity events, without the constraint of fixed-size cohorts of patients. Decision-making takes into account all available information from all patients in the study thanks to the time-to-event model. All toxicities occurring at any time during treatment are incorporated. In this phase Ib combination setting, the knowledge already available on the safety profile of the individual agents allows to make full use of the Bayesian methodology to identify the optimal dose (in terms of toxicity profile) for future studies. DLT rates are provided with model-based confidence intervals. The way the design operates has been investigated under a variety of toxicity scenarios. In particular, the risk to choose too high (too toxic) or too low a dose has been calculated and discussed versus traditional designs.

**Results:** Studies are ongoing. Results will be provided as available with a discussion of advantages and disadvantages of the novel phase I study design.

**Conclusions:** This adaptive phase Ib study design may be considered as a better decision-making tool which can exploit all safety information available and ensure higher confidence in the regimen selected for later phase clinical studies.

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**Anti-tumor efficacies of liposomal honokiol combined with cisplatin in lewis lung cancer and human a 549 lung cancer models**

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**Purpose:** To fully explore the clinical possibility of honokiol, we encapsulated honokiol with long circulating liposome to improve its solubility and prolong its circulation time in body. The potential to increase the antitumor effect of liposomal honokiol by combination with cisplatin and possible mechanism have been investigated in *in vitro* and *in vivo*.

**Experimental Design:** Honokiol was encapsulated with long circulating modified liposomes. The pharmacokinetic study and biodistribution of liposomal honokiol in tumor-bearing mice was detected by high performance liquid chromatography. Inhibition of cell proliferation and induction of apoptosis by liposomal honokiol were tested *in vitro*. In *in vivo* study, LL/2 tumor model and human A549 tumor models were established in C57BL/6N mice and athymic nude mice, respectively. Mice were treated with PBS group, liposomal honokiol, free liposome, cisplatin, and liposomal honokiol plus cisplatin. Tumor volume and survival time were observed and the mechanisms underlying the anti-tumor effect was investigated by detecting the microvessel density, apoptosis in tumor tissue.

**Results:** Liposomal honokiol has shown remarkable improvement of water solubility and could be dissolved in PBS for i.p injection.