

attenuates the HERG inhibitory effect of Q, and when extracellular acidosis is combined with hyperkalemia (7.5 K), the effect on I<sub>Kr</sub> inhibition is similar to acidosis alone. The attenuated effect of Q at low pH may cause heterogeneity of repolarization between ischemic and normal regions, and this may set the stage for reentrant arrhythmias, contributing to Q's proarrhythmic toxicity.

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### THE EFFECT OF NARINGENIN (GRAPEFRUIT JUICE) AND ANTIARRHYTHMIC DRUGS ON I<sub>Kr</sub> INHIBITION. C. Lin, X. Ke, I. Cvetanovic, V. Ranade, J.C. Somberg, Department of Pharmacology, Rush University Medical Center, Chicago, IL.

Grapefruit juice has been reported to cause significant QT prolongation in healthy volunteers. Naringenin (N), the principal flavonoid in grapefruit juice, has been identified as the most potent HERG channel blocker among several dietary flavonoids. In light of these reports, we thought that combining naringenin with I<sub>Kr</sub>-inhibiting antiarrhythmic drugs would increase I<sub>Kr</sub> inhibition and possibly pose an increased health risk by increasing repolarization delay and ensuing arrhythmias. In this study, we investigated the effect of N combined with quinidine (Q) on I<sub>Kr</sub> inhibition. The study was performed in an oocyte system with heterogeneously expressed human-ether-a-go-go-related gene (HERG) employing two electrodes voltage clamp technique for recording. The experiments were performed at room temperature. Doses of 10 μM and 100 μM N were found to inhibit HERG channel by 15 ± 4 and 40 ± 7%. Q at 1 and 10 μM caused 9 ± 1 and 39 ± 3% inhibition of HERG current. When 10 μM N was combined with 1 μM Q, 9 ± 3% current was blocked. HERG current was blocked by 29 ± 2% when 10 μM N was combined with 10 μM Q. N 100 μM combined with 1 μM Q and N 100 μM combined with 10 μM Q caused 21 ± 2 and 36 ± 2% inhibition in HERG current, respectively. Combining naringenin and quinidine does not show an additive effect but rather a diminution in I<sub>Kr</sub> inhibition. Further studies on the interaction of N with other known I<sub>Kr</sub> channel blockers are indicated.

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### GEFITINIB RESPONSE OF ERLOTINIB-REFRACTORY LUNG CANCER WITH LEPTOMENINGEAL METASTASIS. N.W. Choong,<sup>1</sup> S. Dietrich,<sup>1</sup> T.Y. Seiwert,<sup>1</sup> M.S. Tretiakova,<sup>2</sup> V. Nallasura,<sup>1</sup> G.C. Davies,<sup>3</sup> S. Lipkowitz,<sup>3</sup> A.N. Husain,<sup>2</sup> R. Salgia,<sup>1</sup> P.C. Ma,<sup>4</sup> <sup>1</sup>Section of Hematology/Oncology, Departments of Medicine and <sup>2</sup>Pathology, The University of Chicago, Chicago, IL; <sup>3</sup>National Cancer Institute, NIH, Bethesda, MD; <sup>4</sup>Division of Hematology/Oncology, Case Western Reserve University, Case Comprehensive Cancer Center, Cleveland, OH.

Although various mutations of the epidermal growth factor receptor (*EGFR*) gene, most commonly L858R (exon 21) and short exon 19 deletions, have been identified to confer sensitivity toward EGFR tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib, it is not known if there are mutations that may result in differential activities of the two inhibitors. We describe a 70-year-old Japanese American woman diagnosed with stage IV non-small-cell lung cancer (NSCLC) with rib metastasis. While receiving treatment with the EGFR small molecule TKI erlotinib, she progressed and developed new brain metastases. She failed further chemotherapy treatments and subsequently developed symptomatic extensive leptomeningeal carcinomatosis associated with diplopia, hemiparesis, weight loss, and incontinence. Monotherapy gefitinib 250 mg daily was initiated, and she showed striking response both clinically and radiographically within the first few weeks. Using laser microdissection (LMD), we performed genomic DNA extraction and *EGFR* gene sequencing from the enriched tumor cells in her pretreatment tumor biopsy specimen and tumor cells found in her cerebrospinal fluid. Two heterozygous somatic *EGFR* mutations, L858R (exon 21) and E884K (exon 22), were identified in both specimens. In vitro transfection and biochemical studies revealed that the novel E884K mutation confers opposite effects in sensitivity to the two EGFR inhibitors. *EGFR*<sup>E884K</sup> and *EGFR*<sup>L858R-E884K</sup> enhanced the sensitivity of the mutated receptor to gefitinib inhibition. Conversely, the E884K mutation resulted in decreased responsiveness of the receptor to erlotinib, and it significantly abrogated the drug sensitivity conferred by L858R (*EGFR*<sup>L858R-E884K</sup>). This study demonstrates that it is possible to have differential response to alternative EGFR TKIs. This also represents the first report of a response of leptomeningeal metastases to EGFR inhibition by small molecule inhibitor gefitinib alone in NSCLC. Further structural studies of the mutant EGFR are warranted to improve individualized targeted therapy and small molecule inhibitors' design in lung cancer in the future.

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### SIMVASTATIN ATTENUATES ACUTE LUNG INJURY INDUCED BY ISCHEMIA-REPERFUSION IN RODENTS. L. Moreno,<sup>1</sup> J. Jacobson Jr,<sup>1</sup> P. Bonde,<sup>2</sup> J.G.N. Garcia,<sup>1</sup> <sup>1</sup>Department of Medicine, The University of Chicago, IL; <sup>2</sup>Department of Cardio-Thoracic Surgery, Royal Victoria Hospital, Belfast, United Kingdom.

Ischemia-reperfusion (IR) lung injury, a common cause of lung transplant failure, is characterized by hypoxemia, alveolar damage, inflammation, and edema. As novel lung preservation techniques could have a significant clinical impact in this setting, we employed an animal model of IR injury to investigate the potential therapeutic role of simvastatin, an agent we have previously characterized as a potent vascular barrier protectant (Jacobson JR et al, 2004 and 2005). Ischemia was induced in anesthetized Sprague-Dawley rats by ligation of the left pulmonary artery for a period of 1 hour followed by 4 hours of reperfusion. Indices of inflammation and vascular leak, including bronchoalveolar lavage (BAL) cell counts and protein content and lung tissue myeloperoxidase activity, were then assessed. Similar to the protective effects we previously observed with sphingosine 1-phosphate in this model (Moreno L, 2004), BAL from animals pretreated with simvastatin (20 mg/kg, intraperitoneal injection, 16 hours prior to ischemia) demonstrated a reduced number of total cells (48.3% decrease), neutrophils (33% decrease), and albumin concentration (20.7% decrease) compared to controls. A single dose of simvastatin also resulted in reduced (49% decrease) lung tissue MPO. These data indicate that simvastatin significantly attenuates the protein leakage and inflammation associated with IR lung injury in our animal model. Ultimately, our results could have profound clinical implications as simvastatin treatment may represent a potential agent to reduce the incidence and severity of IR injury associated with lung transplantation.

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### MAST CELLS AND EOSINOPHILS STIMULATE MYOFIBROBLAST DIFFERENTIATION BY SIGNALING THROUGH THE TRANSFORMING GROWTH FACTOR β TYPE I (ALK5) RECEPTOR. M.C. Nlend,<sup>1</sup> A. Nair,<sup>1</sup> A. Talati,<sup>1</sup> C.H. Sheen,<sup>1</sup> R. Kalhan,<sup>1</sup> K. Thavarajah,<sup>1</sup> M. Kulka,<sup>1</sup> P.H.S. Sporn,<sup>1</sup> <sup>1</sup>Feinberg School of Medicine, Northwestern University, Chicago, IL.

**Rationale:** Mast cells and eosinophils synthesize various mediators, including transforming growth factor β (TGF-β) and cysteinyl leukotrienes (cysLTs), that may promote subepithelial fibrosis in asthma. We sought to determine the roles of TGF-β and cysLTs in myofibroblast differentiation stimulated by coculture of fibroblasts with mast cells or eosinophils. **Methods:** IMR-90 human lung fibroblasts were serum starved and cocultured for 48 hours with either LAD-2 human mast cells or freshly isolated human blood eosinophils in the absence or presence of SB431542 (10 M) to block signaling through the TGF-β type I (ALK5) receptor; MK886 (1 M) to block leukotriene synthesis; MK571 (100 nM) to block the cysLT1 receptor; or Bay u9773 (3 M) to block the cysLT1 and cysLT2 receptors. α-Smooth muscle actin (α-SMA) expression was assessed as an index of myofibroblast differentiation. **Results:** Mast cells and eosinophils stimulated 2.50.2 ( $n = 5, p < .005$ )- and 1.80.2 ( $n = 5, p < .0001$ )-fold increases, respectively, in fibroblast α-SMA expression, determined by immunoblot analysis. In comparison, TGF-β (2.5 ng/mL) increased α-SMA expression by 5.50.5-fold ( $n = 6, p < .0001$ ). SB431542 inhibited the increase in α-SMA expression stimulated by mast cells, eosinophils and TGF-β by 6.019%, 8.221% and 9.210%, respectively (all  $p < .0001$ ). By contrast, none of the leukotriene pathway inhibitors significantly affected α-SMA expression. Immunofluorescence microscopy for α-SMA confirmed the immunoblot results. **Conclusion:** Our data indicate that mast cells and eosinophils stimulate differentiation of myofibroblasts by TGF-β signaling through the ALK5 receptor and that cysLTs are less important in this process. Inhibition of ALK5 receptor signaling may be a useful strategy to interrupt airway remodeling in asthma.

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### HYPERTENSION-INDUCED CONTRACTILE DYSFUNCTION, LEFT VENTRICULAR HYPERTROPHY, AND DOWN-REGULATION OF MITOCHONDRIAL METABOLIC ENZYME ACTIVITY ARE ATTENUATED BY HIGH-FAT/LOW-CARBOHYDRATE FEEDING. L.C. Okere,\* D.J. Chess,\* T.A. McElfresh,\* M.E. Young,\*\* P. Ernsberger,\* B.D. Hoit,\* M.P. Chandler,\* W.C. Stanley,\* \*Case Western Reserve University, Cleveland, OH; \*\*Baylor University, Houston, TX.

Cardiac hypertrophy and heart failure are characterized by increased expression of atria natriuretic peptide (ANP), a decrease in the activity of fatty acid oxidation (FAO) enzymes, impaired mitochondrial respiration, and LV remodeling and systolic dysfunction. Fatty acids up-regulate the expression of FAO enzymes via activation of peroxisomal proliferator activator receptors. We hypothesized that a high-fat diet would prevent down-regulation of FAO enzymes activity and slow development of cardiac dysfunction in a model of hypertension-induced hypertrophic cardiomyopathy. Three groups ( $n = 10$ ) of 11-week-old male Dahl salt-sensitive rats were fed (1) low-fat/low-salt rodent chow (10% calories from fat) (LF-LS), (2) low-fat/high-salt chow (6% NaCl) (LF-HS), or a high-fat/high-salt diet (60% calories from fat, 6% NaCl) (HF-HS) for 12 weeks. Tail cuff blood pressure and echocardiographic assessment of LV dimensions and function were performed before initiation of diets and after 11 weeks on diets. Myocardial activities of citrate synthase (CS) and medium chain acyl dehydrogenase (MCAD) activities were measured, and the mRNA expressions for ANP was measured. **Results:** There were no differences in body weight among groups. Salt feeding caused similar elevations in systolic blood pressure in LF-HS and HF-HS groups ( $\approx 190$  mm Hg compared to 120 mm Hg in LF-LS). LV mass was increased in LF-HS ( $1.30 \pm 0.06$  g) compared to LF-LS ( $0.90 \pm 0.02$  g). Hypertension down-regulated CS and MCAD activities in the LF-HS group but not in the HF-HS group. LV end-diastolic volume and ANP expression were elevated and fractional shortening reduced in HS-LF compared to LS-LF. The HF-HS group had reduced LV hypertrophy ( $1.16 \pm 0.05$  g) and ANF expression and showed no LV remodeling or systolic dysfunction compared to LF/HF fed rats. **Conclusion:** High-fat diet prevented the down-regulation of the activity of metabolic enzymes and preserved contractile function and LV chamber dimensions in this model of hypertrophic cardiomyopathy.

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### A POTENTIAL ROLE FOR 5-LIPOXYGENASE IN THE PATHOGENESIS OF CEREBELLAR DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS. O. Ostmann, T.J. Santoro, M. Tomita, Department of Medicine, University of North Dakota School of Medicine and Health Sciences, Fargo, ND.

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology associated with inflammation involving the lungs, kidneys, joints, skin, and brain. Central nervous system (CNS) involvement in human and experimental SLE is characterized, in part, by disturbances in posture, balance, and tremulousness, which map to the cerebellum (J Neurosci Res 2001;64:26). Five-lipoxygenase (5-LO) activity is increased in the setting of neurodegeneration associated with aging, ischemia, and other CNS insults, which may be accompanied by cerebellar dysfunction. To determine whether 5-LO contributes to CNS inflammation/neurodegeneration in neuropsychiatric lupus, we studied 5-LO and 5-LO activating protein (FLAP) in the cerebellum of MRL-*lpr/lpr* mice, which spontaneously develop a lupus-like illness associated with disturbances in cerebellar function. Relative to age- and sex-matched, immunologically and neurologically normal, H-2 identical CBA/CaJ mice, cerebellar homogenates from 20- to 30-week-old clinically affected MRL-*lpr/lpr* mice exhibited enhanced expression of genes encoding both 5-LO and FLAP as assessed by reverse transcriptase polymerase chain amplification. In contrast, no differences were found in the expression of genes encoding cytosolic phospholipase A2 and neuronal nitric oxide synthase in the cerebellum of autoimmune and control mice. Western blotting of homogenates indicated that MRL-*lpr/lpr* mice had a greater content of 5-LO but not of FLAP protein compared with control CBA/CaJ mice. These data suggest a potential role for 5-LO in the pathophysiology of cerebellar dysfunction in lupus.