16th Annual Department of Surgery Research Conference

Agenda – Friday, June 10, 2011

Welcome and Introduction of Visiting Professor
Welcome and introduction by E. Christopher Ellison, MD, the Robert M. Zollinger Professor and Chairman of Surgery, Associate Vice President for Health Sciences, Vice Dean of Clinical Affairs, The Ohio State University College of Medicine

Introduction to the Conference
Conference purpose and format by Ginny L. Bumgardner, MD, PhD, Professor of Surgery, Division of Transplantation, Associate Dean for Research Education, Director, Master’s of Medical Science Program, The Ohio State University College of Medicine

Judges
Carlo M. Croce, MD, E. Christopher Ellison, MD, Fabrizio Michelassi, MD, R. Lawrence Moss, MD and Chandan K. Sen, PhD. Poster judges: Jaideep Banerjee, MS and Jason Zimmerer, PhD

Moderators
Session 1 moderated by Lisa D. Yee, MD, Associate Professor of Surgery, Division of Surgical Oncology, The Ohio State University College of Medicine
Session 2 moderated by Kyle A. Perry, MD, Assistant Professor of Surgery, Division of General and Gastrointestinal Surgery, The Ohio State University College of Medicine

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Visiting Professor

Fabrizio Michelassi, MD, FACS

Dr. Fabrizio Michelassi is the Lewis Atterbury Professor of Surgery and Chairman, Department of Surgery at Weill Cornell Medical College, and Surgeon-in-Chief at New York-Presbyterian Hospital, New York, New York. Born in Pisa, Italy, Dr. Michelassi graduated summa cum laude from the University of Pisa School of Medicine, and then completed his internship and surgical residency at New York University and a research fellowship at Massachusetts General Hospital, Harvard University. In 1984, he joined the faculty of the Department of Surgery at the University of Chicago. He became Section Chief of General Surgery in 1994, tenured Professor in 1995, Vice Chair of the Surgery Department in 2000 and the Thomas D. Jones Professor of Surgery in 2001. He also served as Director of the Surgical Oncology Fellowship from 1988 through 1995 and Director of the General Surgery Residency Program from 1997 through 2004. He moved to his current position at NewYork-Presbyterian Hospital/Weill Cornell Medical Center in 2004.

Internationally renowned as an outstanding clinician, researcher and teacher, Dr. Michelassi has delivered many named and keynote lectures across the country, has been invited to be a visiting professor at 32 national and international institutions, and has delivered over 180 national and international presentations. He is on the editorial board of five prestigious medical journals including: the Journal of Gastrointestinal Surgery, Surgery, The British Journal of Surgery, the World Journal of Gastroenterology, and Annals of Surgery. A recognized leader in the gastrointestinal surgical field, Dr. Michelassi has been appointed to many international and national task forces and panels.

A prolific author of more than 200 papers, book chapters and abstracts, Dr. Michelassi has contributed new insight in the surgical treatment of pancreatic and colorectal cancers, ulcerative colitis and Crohn’s disease. He has pioneered the development of important new techniques that ensure better outcomes and improved quality of life for patients with rectal cancer and ulcerative colitis. Dr. Michelassi’s recognized expertise in the surgical treatment of pancreatic cancer has led many patients to seek his counsel and in turn, he has contributed new knowledge to this field through clinical trials. His experience and expertise in treating Crohn’s disease led him to develop a novel bowel-sparing procedure, now known as the Michelassi strictureplasty, designed to avoid sacrificing large amounts of bowel at the time of surgery and facilitating quiescence of the acute disease affecting the diseased intestinal loops.

Dr. Michelassi has served as President of the Society of Surgical Oncology, the Illinois Surgical Society, the Western Surgical Society and the Central Surgical Association. Dr. Michelassi is President-elect of the Society of Surgical Chairs and of the New York Surgical Society and currently serves as Secretary of the Society of the Alimentary Tract and a Director of the American Board of Surgery. He is a member of more than 40 professional societies in the United States and internationally. He is also a member of the Advisory Council for General Surgery of the American College of Surgeons and has served as Vice-President of the International Society of Digestive Surgery.

In 2009, in recognition of his many lifetime achievements, Dr. Michelassi was honored as an Official of the Order of Merit of the Republic of Italy with the rank of Commendatore, the most prestigious and important distinction awarded by the President of the Republic of Italy to Italian citizens of particular merit.
Invited OSU College of Medicine Faculty Judge

Carlo M. Croce, MD

Dr. Carlo Croce is Professor of Internal Medicine and Chairman, Department of Molecular Virology, Immunology and Medical Genetics at The Ohio State University College of Medicine.

Dr. Croce is a graduate of a combined program in biochemistry and medicine at the School of Medicine University of Rome, Italy, earning a medical degree in 1969. He was research scientist, associate, and then professor at the Wistar Institute of Anatomy and Biology in Philadelphia, 1970-1991, and professor of pathology, medicine, and pediatrics at Temple University School of Medicine in Philadelphia, 1988-1991. He contributed to multiple research foundations and institutes before he was attracted to OSUMC in 2004 as the Wolfe Professor and Chairman of the Department of Molecular Virology, Immunology, and Medical Genetics. Since 2000 he has been Professore Ordinario di Oncologia Medica at the University of Ferrara, Italy. He also is director of the OSU Human Cancer Genetics Program.

Dr. Croce is a leader in the study of the molecular basis of cancer. His research has had an international impact. He has authored over 825 peer-reviewed journal articles in the study of DNA synthesis and regulatory genes. He recently published about gene alterations associated with lung and esophageal cancer and the role of microRNA in these gene alterations. Dr. Croce’s research has pioneered investigation of microRNA signatures and their gene targets for many human cancers.

His work has been acknowledged by awards or prizes from: the American Cancer Society, MD Anderson Cancer Center, General Motors Cancer Research Award, Herbert and Maxine Block Award, the Albert Szent-Gyorgyi Prize, Henry M. Stratton Medal, and honors from the Universities of Pittsburgh, Pennsylvania State, Uppsala in Sweden, Tel Aviv in Israel, Wurtzburg in Germany, plus recognition in Japan and France. He was granted the Honor of Merit of the Italian Republic. The United States government recognized his accomplishments with a Career Research Development Award, the NIH Outstanding Investigator Award, and selection as a member of the National Academy of Sciences. He has maintained his international contacts, and though the articles he published in his early years listed him as first author or co-author, for over a decade investigators in multiple countries and from his own busy laboratory appear in the list of over a dozen collaborators. He is one of the editors of the British Journal of Cancer, and is editor in chief of Cancer Research. Dr. Croce has patented several discoveries and co-founded three companies.
PGE$_2$ Induces Oncostatin M Expression in Human Chronic Wound Macrophages by a Axl Receptor Tyrosine Kinase Pathway

Kasturi Ganesh, MBBS$^1$, Amitava Das, MPharm$^1$, Ryan Dickerson$^1$, Savita Khanna, PhD$^1$, Lynn Lambert$^1$, Narasimham L. Parinandi, MD$^2$, Gayle M. Gordillo, MD$^3$, Chandan K. Sen, PhD$^1$ and Sashwati Roy, PhD$^1$

1. The Ohio State University Department of Surgery; 2. The Ohio State University Department of Pulmonary, Allergy, Critical Care and Sleep; 3. The Ohio State University Department of Plastic Surgery

INTRODUCTION: Macrophages (mφ) play a key role in wound repair such that both inadequate inflammatory responses to wounding as well as unresolved inflammation compromise wound closure. Although peripheral blood monocyte derived mφ (MDM) are commonly studied in the context of disease they do not resemble mφ residing in the microenvironment of diseased tissue. Thus, this study compares MDMs with mφ isolated from chronic wound of patients. Genechip™ microarray data showed substantial differences in the transcriptome of MDMs versus wound mφ from the same donor. Oncostatin M (OSM) was differentially induced in wound mφ. PGE$_2$, an inducer of OSM expression, was present in high levels in wound fluid of patients. PGE$_2$ treatment induced OSM expression in wound mφ. Consistently, induction of OSM mRNA was noted in mφ isolated from PGE$_2$–enriched PVA sponges. Treatment of human Thp-1 cell derived mφ with PGE$_2$ resulted in a dose and time dependent induction in OSM expression.

METHODS: Adult chronic wound (defined as wound present > 4 weeks) patients (25-80 years old) undergoing VAC® (negative pressure) therapies of their wounds at the Comprehensive Wound Center (CWC) were recruited (IRB approved) for the study. VAC dressing (sponges) was collected. In addition, paired peripheral blood samples were collected from each patient. High levels of PGE2 and OSM (p<0.01; n=19) were observed in chronic wound fluids as compared to plasma from the same subjects. Wound derived macrophages, but not pair-matched blood monocyte derived macrophages, produced high levels of OSM. To characterize the mechanism of OSM production in wound fluid, differentiated cultured human macrophages were treated with PGE2 for 24h. PGE2 potently induced OSM (protein and mRNA). The PGE2 induced OSM production is through the cyclic AMP dependent mechanism. This induction was completely blocked using a receptor tyrosine kinase (RTK) inhibitor. Characterization studies indicated that PGE2 strongly induces the phosphorylation of a RTK, Axl, in macrophages. To verify the findings in vivo, PGE2 treated PVA sponges were implanted in C57BL/6 mice to harvest wound macrophages. Wound macrophages harvested from PGE2 treated sponges demonstrated significantly increased OSM gene expression.

RESULTS: Characterization of the signaling pathway demonstrated involvement of EP4 and cAMP. In human mφPGE$_2$, phosphorylated Axl identifying it as a novel receptor tyrosine kinase target. cAMP also induced Axl phosphorylation demonstrating an interplay between cAMP and RTK pathways. PGE$_2$ activated AP-1 and induced OSM via a Axl dependent pathway.

CONCLUSIONS: In summary, these studies recognize an abundance of OSM in human wound mφ. Such induction was executed by PGE$_2$ present in the wound fluid via a novel Axl RTK signaling pathway.

Acknowledgments Wound healing research in the authors’ laboratory is funded by NIDDK R01 DK076566 (SR).
The dynamic microRNA profile in varying severities of sepsis

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¹. The Ohio State University Department of Surgery; 2. The Ohio State University Department of Pulmonary, Allergy, Critical Care and Sleep

INTRODUCTION: A fundamental gap exists in the understanding of the inflammatory response in sepsis. Despite advances in care, mortality remains around 30-40%. Recently, plasma microRNAs (miRNA) have been identified as novel mediators of intercellular communication and possible inflammatory mediators. Several descriptive studies have shown decreases in several microRNAs are associated with worsening mortality in sepsis. It has been suggested that plasma miRNAs are an accurate biomarker for survival in sepsis. However, the time course of these changes has not been characterized, and it is not known at what time point miRNAs can be used to predict outcome. The specific aim of the present study is to use models of varying sepsis severity to characterize the dynamic changes seen in plasma miRNA profiles.

MATERIALS AND METHODS: C57 bl/6 mice were subjected to cecal ligation and puncture (CLP) and sham operations as a model of intra-abdominal sepsis. Two models of severity were used based on the size of cecal puncture, 27 gauge (less severe) and 22 gauge (more severe). Blood was drawn at 6 hours, 18 hours and 7 days and subjected to miRNA profiling with RT-PCR to characterize the dynamic miRNA changes during the development of sepsis. Profiling data were analyzed using mean normalization and ΔCT comparisons.

RESULTS: The sepsis models demonstrated significant differences in mortality at 7 days (22ga 85% v. 27ga 30%). MiRNA analysis demonstrated significant decreases in miR-146a, miR-150, miR-155, and miR-223 expression in the severe sepsis model as early as 6 hour after insult when compared to the less severe sepsis model. The mild severity sepsis model demonstrated significant increases in miR-146a, miR-150, miR-155 and miR-223 expression compared to sham controls and the severe sepsis model.

CONCLUSIONS: The models in the present study exhibit plasma miRNA changes that correlate with human sepsis. This provides a tailored, reproducible model for studying the role of plasma miRNAs in sepsis and inflammation. This study is the first to characterize the dynamic profile of miRNA in sepsis and demonstrates significant changes as early as 6 hours into the septic process. The translational significance of the present study may allow earlier detection and predictive capabilities in patients with sepsis. Based on predicted outcomes from miRNA profiles, clinicians could tailor their therapies to be more or less aggressive as needed.
Tocotrienol vitamin E improves collateral circulation during focal cerebral ischemia attenuating stroke injury

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INTRODUCTION: Vitamin E is a generic term for tocopherols and tocotrienols. To date, the vast majority of scientific literature has focused on the characterization of α-tocopherol (TP), an isoform found in great abundance in Western diets. The lesser characterized isoform α-tocotrienol (T3) exhibits unique neuroprotective properties not shared by TP and has a long history of safe human consumption in Eastern diets. The current work is based on our striking previous finding that oral supplementation of T3, not TP, attenuated stroke-mediated brain injury and neurodegeneration in spontaneously hypertensive rats. This work pointed to 12-lipoxygenase as a molecular target for T3 neuroprotection. As small animal studies have historically failed to predict success for neuroprotective agents in clinical trials, we now seek to test the efficacy of T3 in a randomized, double-blind placebo controlled pre-clinical study using a large animal stroke model that more closely approximates the human stroke condition.

METHODS: In this work, mongrel canines (mean weight=26.3±3.2kg, N=20) were supplemented with 200mg of T3 or vehicle placebo for ten weeks prior to inducing transient middle cerebral artery occlusion using a minimally invasive, neuroradiological approach. Canines receiving oral T3 supplementation demonstrated significantly higher concentration of T3 in cortical brain tissue. Clinical 3T magnetic resonance imaging (MRI) was performed 1h and 24h post-reperfusion to measure stroke-induced lesion volume.

RESULTS: Animals supplemented with T3 had significantly attenuated ischemic stroke-induced lesion volume and neurodegeneration as compared to placebo controls.

CONCLUSIONS: In the clinical setting, cerebrovascular collaterals have been documented to perfuse, via retrograde flow, the distal branches of the proximally occluded parent vessel (i.e. MCA) and improve stroke-associated outcomes. While improving collateral circulation represents a therapeutic target of recognized value for stroke patients, strategies to increase collateral recruitment during stroke remain unknown. Post-hoc analysis of cerebral angiograms during 60min of middle cerebral artery (MCA) occlusion revealed canines supplemented with T3 had improved collateral circulation to the ischemic MCA territory as compared to placebo controls.
Laparoscopic Ventral Hernia Repair – Does Primary Repair In Addition to Placement of Mesh Decrease Recurrence?

Ambar Banerjee, MD, Vimal Narula, MD, John Linn, MD, Sabrena Noria, MD, Bradley Zagol, MD, Dean Mikami, MD

The Ohio State University Department of Surgery

INTRODUCTION: The advent of laparoscopic ventral hernia repair (LVHR) not only reduced the morbidity associated with open repairs but also led to a decrease in the hernia recurrence rate. However, the rate continues to remain significant.

METHODS: A retrospective observational study was conducted on 193 patients who were treated with LVHR by two minimally invasive surgeons in a 24 month period. The patient population was broadly divided into two groups based on the laparoscopic repair of the fascial defect with mesh underlay, or with primary suture repair and mesh underlay. Patient demographics, rates of hernia recurrence, and other associated complications were compared among the two groups. Patient variables and the clinical outcomes were analyzed with descriptive statistics and chi-square test.

RESULTS: One hundred and ninety three consecutive patients underwent LVHR for incisional (n=136), umbilical (n=44), epigastric (n=9) and parastomal (n=4) hernias. Hernia recurrence was documented in 8 patients (4.1%). The mean follow-up period was 10.5 months (range 1-36 months). Incisional hernias accounted for all the recurrences while umbilical, epigastric and parastomal hernias were not associated with any recurrences. The rate of recurrence in those treated with primary suture repair along with mesh underlay was 3% (2 of 67 cases) in comparison with 4.8% (6 of 126 patients) associated with mesh alone. Three of six recurrences associated with mesh repair alone were further treated with primary repair with mesh without occurrence of re-recurrence to date.

CONCLUSIONS: Primary laparoscopic repair along with mesh placement for the management of ventral hernia was found to be effective in selected cases as evidenced by the low rate of recurrence when compared to conventional repair with a mesh alone. Further retrospective and prospective studies, with larger patient enrollment, are warranted to confirm the benefit of this technique over the traditional repair.
Pancreatic Stellate Cells Induce Overexpression of MicroRNA-21 and MicroRNA-210 in Pancreatic Cancer Cells in Hypoxia

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1. The Ohio State University Department of Surgery, 2. The Ohio State University Comprehensive Cancer Center, 3. The Ohio State University Department of Molecular Virology, Immunology and Medical Genetics

INTRODUCTION: Pancreatic cancer cells are able to survive in a hypoxic environment. This survival may rely on input from nearby stromal elements such as pancreatic stellate cells (PSCs), perhaps through induction of microRNAs (miRs). We hypothesized that PSCs could induce the expression of two common oncomiRs, miR-21 and miR-210, in pancreatic cancer cells.

METHODS: PSCs were harvested from resected human pancreatic tumors and grown in culture. Pancreatic cancer cells (PANC-1 and AsPC-1) were grown in normoxia (21% O2) or in hypoxia (1% O2) in the presence or absence of varying concentrations of PSC conditioned media (PSC-CM). Cell proliferation was determined using alamarBlue cell viability assay. Migration was assessed using transwell assays. MiR-21 and -210 levels were determined by stem-loop RT-PCR.

RESULTS: PSC-CM significantly increased PANC-1 and AsPC-1 proliferation in normoxia and hypoxia in a dose-dependent fashion and significantly increased migration in PANC-1 (p<0.01). In hypoxia, PSC-CM increased miR-21 and miR-210 in AsPC-1 (p<0.01).

CONCLUSIONS: PSCs induce proliferation and migration in pancreatic cancer cells. In hypoxia, an essential component of the neoplastic microenvironment, PSCs induce the upregulation of two of the most commonly dysregulated microRNAs in cancer, miR-21 and miR-210 in pancreatic cancer cells.
Trends in Productivity and Work Effort of Today’s Vascular Surgeon

Mika Matthews, MD, Bhagwan Satiani, MD, MBA

On behalf of the Community Practice Committee of the Society for Vascular Surgery

Background: Based on a concept created by Hsiao and his staff at Harvard School of Public Health, the relative value unit (RVU) was developed to quantify the amount of resources utilized to provide a specific service. The relative value scale was then used to generate a common scale of reimbursement for all physician services within and between specialties, as well as to assign payments more appropriately between surgeons and non-surgeons. While RVU’s were only designed to measure consumption of resources, compensation plans are now being designed based purely on RVU ‘production’. Through a nationwide survey questionnaire regarding demographics, practice structures, employment trends, productivity trends, measurement of work effort including RVUs and work RVUs, we sought to understand the current trends within the Vascular Surgery Community. The results will be useful in developing educational offerings particularly related to practice management, measurement of productivity, employment and workforce planning issues.

Methods: Through the Society for Vascular Surgery (SVS) Community Practice Committee, we developed a brief survey questionnaire regarding the physician’s demographics, location, type of practice, hours worked, work and total RVU’s produced in 2010, the affects of productivity on compensation, and anticipated changes in their practice in the next 3 years. We utilized SurveyMonkey and distributed the survey to Vascular Surgeon members of the SVS. Responses were collected between February and April 2011.

Results: The survey questionnaire was sent electronically to 2,230 SVS members of whom 207 responded (10.7% response rate). Sixty-eight (32.7%) of the physicians were between 50-59 years old, 186 (90.3%) were men, and 192 (92.8%) worked full-time (>36 hours of patient care per week) while 7 (3.4%) worked part-time (<36 hours of patient care per week) and 8 (3.9%) were retired. Of the 175 physicians who answered, 88 (50.3%) said they or their group kept record of RVU’s/Work RVU’s. Also, 125 of 179 (69.8%) stated some of their compensation was based on productivity, with 56 of 161 (34.8%) stating their productivity determined 76-100% of their compensation.

Conclusion: The current workforce is predominantly male, full-time and 1/3 are between 50-59 years old. Almost 70% of SVS members’ compensation was at least partially based on productivity. Only half of the members or practices tracked their RVU’s. While a complete analysis is pending, we expect to have a comprehensive understanding of the trends in the work habits, productivity and employment status compared to previous surveys. The information will allow the SVS to tailor its educational, training and governmental activities to best serve its members and their patients.
Engulfment of apoptotic cells induces miR-21 expression and switches human macrophages from a pro-inflammatory to anti-inflammatory phenotype

Amitava Das, MPharm, Kasturi Ganesh, MSSB, Ryan Dickerson, Savita Khanna, PhD, Bhakthi Deshpande, Chandan K. Sen, PhD and Sashwati Roy, PhD

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INTRODUCTION: Engulfment of apoptotic cells (also known as efferocytosis) by macrophages is the final removal step in the cell-death program and plays a critical role in resolution of wound inflammation. Engulfment of apoptotic cells by macrophages is one of the cues that drive the switching of macrophages towards an anti-inflammatory state facilitating resolution of inflammatory response. A critical role of microRNA (miRNA) in regulation of wound inflammation has been recently suggested. Emerging studies indicate that miRNAs, especially miR-21, miR-146a/b and miR-155, play a key role in regulating several hubs that orchestrate the inflammatory process. The objective of the current study was to investigate whether miR-21 plays a role in the efferocytosis-induced switching of macrophages to anti-inflammatory state.

METHODS: Peripheral Blood Monocytes (PBMCs) were isolated from buffy coats (source leukocytes) obtained from the American Red Cross using Ficoll-Hypaque. The cells were further purified using magnetic cell isolation system and CD14 microbeads (Miltenyi Biotec, Auburn, CA). After isolation, monocytes were resuspended in RPMI 1640 medium supplemented with 10% FBS (heat inactivated) and seeded in 6-well plates. After 2h, the non-adherent cells were removed and cells were washed with warm RPMI 1640 medium. The cells were cultured in RPMI supplemented with 10% FBS, 1% PSA (penicillin G sodium, streptomycin sulfate, and amphotericin B), 10 ìg/ml of polymyxin B, and 20 ng/ml of M-CSF for 5 days in 37°C with 5% CO2. For the Phagocytosis assay, apoptotic Jurkat cells were added to culture plates containing human macrophages at a 1:5 macrophage to Jurkat cell ratio. Prior to co-culture with macrophage, the Jurkat cells were labeled with a red fluorescence cell-tracker reagent. Phagocytosis assay was performed for 1 h at 37°C. Macrophages were then washed to remove non-phagocytosed cells.

RESULTS: LPS treatment of human macrophages resulted in a time dependent increase in miR-21 expression. Such increase was further potentiated (~1.5 fold, 6 hours and ~2 folds, 24 hours post LPS stimulation; p<0.05; n=4) by efferocytosis. The tumor suppressor Programmed Cell Death Protein (PDCD4) is a direct target of miR-21. PDCD4 expression was significantly downregulated (p<0.05; n=3) following engulfment of apoptotic cells compared to controls (macrophages co-cultured with viable cells) demonstrating that increased miR-21 expression following efferocytosis results in PDCD4 silencing. Efferocytosis stimulated the production of IL-10, an anti-inflammatory cytokine, by macrophages. Delivery of miR-21 (via miR-21 mimic) to human primary macrophages silenced PDCD4 and induced IL-10 expression. Knockdown of PDCD4 using siRNA resulted in increased IL-10 production (p<0.05; n=3).

CONCLUSIONS: This study demonstrates a novel miR-21-PDCD4 dependent mechanism of IL-10 production following engulfment of apoptotic cells by macrophages.
Development of biofilm in a full thickness porcine burn model

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INTRODUCTION: A biofilm is an aggregate of microbial cells that are attached to a surface and embedded within a self-produced extracellular polymeric substance. Once in biofilms, bacteria become resistant to both host defenses and antimicrobials. There is growing acceptance in the wound care community that biofilms are a major cause of wound chronicity. Biofilms seem to play an important role in maintaining the persistent inflammatory state of many types of chronic wounds such as diabetic foot ulcers or venous leg ulcers. The polymicrobial nature of biofilm with phenotypic diversity among its populations of cells renders the clinical management of biofilm quite challenging. The opportunistic pathogen Pseudomonas aeruginosa is an adept biofilm-forming organism commonly found in chronic wounds, and it has become a model organism to study the pathological importance of biofilm formation. Little is known about the host immune response to biofilm infection. The aim of this study is developing a biofilm infection model in full thickness burn wounds in pigs, which can be used to study the role of biofilm in wound healing impairment and to develop novel therapeutics against this type of infection.

METHODS: Third degree burn wounds were established on the backs of pigs by using a microprocessor controlled electrically heated device. The wounds were inoculated with P. aeruginosa strain PAO1 either by topical application or intra-dermal injection of a suspension of either $5 \times 10^3$ or $5 \times 10^7$ bacteria. At specific time intervals (1, 3, 7 days) 6 mm punch biopsies were taken from the center of the wounds. Samples were analyzed for the presence of P. aeruginosa and tissue damage as well as biofilm formation.

RESULTS: Visual inspection of the wounds revealed yellowish green discoloration with discharge that increased by time. Infected animals maintained localized infections in the wound site, no evidence of systemic infection was observed. Homogenized tissue biopsies of both doses of inoculated topical and sub-dermal wounds stabilized bacterial counts to approximately $10^3$ and $10^6$ CFU/g tissue, respectively, after day 1 and through day 7. Gram staining of tissue biopsies showed aggregations of gram negative bacilli on the surface of the burn wounds. Histological analysis via fluorescent immunohistochemistry staining using an antibody reactive against P. aeruginosa confirmed the presence of P. aeruginosa in the wound bed.

CONCLUSIONS: While detection of biofilm specific growth is challenging, the histology suggest that aggregates of P. aeruginosa are growing in a biofilm state in the wound. Future experiments will confirm the existence of the biofilm community and explore the role of biofilm in wound healing impairment. This model will also be employed to evaluate the efficacy of specific therapeutic strategies against biofilm related infections.
The Impact of Early Initiation of Methadone in Trauma Patients Requiring Mechanical Ventilation

G. Morgan Jones, PharmD, Kyle Porter, BS, Rebecca Coffey, CNP, Sidney Miller, MD, FACS, Melissa Whitmill, MD, FACS, Claire V. Murphy, PharmD, BCPS

INTRODUCTION: Numerous studies have identified strategies to reduce mechanical ventilation duration by targeting appropriate sedation levels. However, applicability of these strategies to trauma patients has not been established. At our medical center, methadone is used in trauma patients to treat acute pain and limit the development of opioid tolerance. The purpose of this study is to evaluate the impact of early methadone initiation in trauma patients requiring mechanical ventilation.

METHODS: This retrospective pilot study compared clinical outcomes of trauma patients who received early methadone to patients who did not receive methadone while mechanically ventilated. The primary outcome was the number of ventilator-free days in a 28-day period. Patients who received methadone within 4 days of intubation and remained ventilated for 2 days after the first dose were included in the methadone group. Propensity scores were used to match up to three control patients to each methadone patient.

RESULTS: The study included 118 patients: 30 methadone and 88 control patients. Patients in the methadone group had 15.2 ventilator-free days compared to 12.6 in the control group (p=0.18). In the burn subgroup, the number of ventilator-free days was significantly higher in the methadone group (16.5 vs. 11.5 days; p=0.03). Among all patients, the incidence of ventilator-associated pneumonia was 20.0% in the methadone group and 39.8% in the control group (p=0.05).

CONCLUSIONS: Our results suggest that early methadone may result in more ventilator-free days and a lower incidence of ventilator-associated pneumonia in trauma patients requiring mechanical ventilation. Further randomized trials are warranted.
Comparison of Two Burn Resuscitation Formulas: Fresh Frozen Plasma (FFP) based vs. the Parkland formula, a single-center review

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BACKGROUND: Traditionally resuscitation of major burns has been by the Parkland formula. Although this crystalloid-based formula decreased mortality from burn-induced shock, many continue to investigate the use of colloids and blood-products to confront the challenge of interstitial edema that is unique to burns. To address the challenge of fluid overload with the resuscitation of major burns, we instituted a FFP-based resuscitation formula previously reported by Slater et al (2 L LR/day; FFP starting at 75 ml/hr titrated to urine output). Some authors have challenged the use of colloid resuscitation and cited increased sepsis and infection complications.

HYPOTHESIS: The use of FFP during the initial resuscitation of major burns decreases volume of resuscitation, without increased morbidity and mortality.

METHODS: An IRB approved retrospective review of major burn (greater than 40% TBSA or greater than 30% TBSA with inhalation injury) resuscitations during two separate time periods was undertaken. The first time period represents crystalloid resuscitation (PR) and the second colloid based resuscitation (CR).

RESULTS: The records of 58 patients were evaluated (31 PR; 27 CR). All patients in both groups were appropriately resuscitated. The two groups were comparable for age, gender, TBSA burn, and survival. PR received more than double the amount of fluids, at 24 (31 L vs. 15 L, p < 0.001) and 48 hours (48.5 L vs. 22.5 L, p < 0.001) than the CP group. The PR group received, on average, 100% more volume in the first 24 hours than was estimated by the Parkland formula. For both survivors and non-survivors, there was no statistically significant difference in rates of wound infection, ventilator-associated pneumonia, bacteremia, ventilator days, ICU LOS or hospital LOS.

CONCLUSIONS: The implementation of FFP resuscitation for major burns significantly reduced resuscitation volumes in the first 48 hours post-burn without increased incidence of infections. Our results support the safety and efficacy of colloids resuscitation in major burns and suggest the need for prospective comparison of various approaches to resuscitation.
A Novel Model for Deep Vein Thrombosis

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INTRODUCTION: In the United States over 2 million individuals have a deep vein thrombosis (DVT) annually. DVT alone leads to 600,000 annual hospitalizations and places patients at increased risk of pulmonary embolus and post thrombotic syndrome. While the clinical sequelae is well documented, the timeframe of clot resolution is poorly understood. At present the literature examining venous thrombus in preclinical studies focuses on the utilization of an inferior vena cava ligation or stenosis models which do not closely mimic clinical disease. Objective: To develop a model of femoral deep vein thrombosis that allows for noninvasive determination of clot resolution and more closely mimics clinical pathology.

METHODS: Male rats underwent anesthesia with isoflurane. A longitudinal incision was made in the left thigh along anatomic planes. 0.1cc of blood was removed from the tail vein and allowed to sit within a nonheparinized syringe. The femoral vein was isolated and ligated with a silk suture. The 0.1cc autologous clot was injected distal to the ligation. The animal was recovered for 48 hours and returned to surgery to remove the ligation suture. The clot and its resolution were determined by both noninvasive ultrasound as well as immunohistochemistry.

RESULTS: Clot area was calculated in pixels² via Image J software. The average area of thrombus was 2112.17 pixels² with a standard deviation of 44.18.

CONCLUSIONS: A rat model of femoral deep vein thrombosis provides reproducible clot burden and allows for noninvasive measurements of clot resolution.
PTEN is a key mediator of vascular remodelling in pulmonary hypertension.

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INTRODUCTION: Pulmonary hypertension (PH) induces vascular remodeling and impairs cardiac function. In addition, PH, which is secondary to CHF, is a major hurdle in the management of cardiac failure. PTEN, a phosphatase-and-tensin homolog deleted on chromosome 10 and implicated in multiple advanced cancers, has been shown to have a causative role in vascular restenosis. The objective of the present study was to test our hypothesis that PTEN may be a key mediator of vascular remodeling in PH.

METHODS: PH was induced in rats (male Sprague Dawley; N=6 per group) by three different methods: (i) Monocrotaline administration (60 mg/kg sc) - pharmacological; (ii) Hypoxic exposure (10% O2) - physiological; (iii) Ligation of left-anterior-descending (LAD) coronary artery - clinical. High-frequency ultrasound (ECHO) and micro-MRI were used to monitor cardiac function during the development of PH. Western-blot analysis was used to measure PTEN and other key downstream proteins involved in PH.

RESULTS: All three methods resulted in the development of PH in 2–4 weeks as verified by RV systolic pressure monitoring. Noninvasive serial imaging studies showed a significant deterioration of cardiac function, particularly LV-EF (37% vs 75% in control). Phosphorylated PTEN was markedly decreased to 45.1% in lung, 59.5% in LV, 37.2% in RV when compared to respective tissues in control. In contrast, pAkt levels were significantly upregulated in the tissues of the PH group (341% lung, 157% LV, 128% in RV).

CONCLUSIONS: The study showed that PH was associated with reduction of PTEN levels in the cardiac and pulmonary tissues. The study suggests that PTEN may be a potential target for the management of PH.
Heparin-Binding EGF-Like Growth Factor Preserves Gut Barrier Function by Blocking Neutrophil-Endothelial Cell Adhesion after Hemorrhagic Shock and Resuscitation in Mice

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INTRODUCTION: The gut is susceptible to injury after hemorrhagic shock and resuscitation (HS/R) due to progressive mesenteric hypoperfusion. We have previously shown that HB-EGF improves gut microcirculation after HS/R in mice. The current study was designed to explore the mechanisms underlying the ability of HB-EGF to preserve gut barrier function after HS/R.

METHODS: In vivo, mice were made neutropenic with anti-polymorphonuclear leukocyte (PMN) antibodies. Neutropenic and non-neutropenic mice were randomly assigned to: (1) sham surgery; (2) HS/R (35-40 mmHg for 90 min and resuscitation for 3 h); or (3) HS/R + HB-EGF (1200 mg/kg/dose administered intra-arterially at the end of HS), and gut barrier function quantified. In vitro, human umbilical vein endothelial cells (HUVEC) were cultured with or without HB-EGF (100 ng/mL) and subjected to anoxia (60 min) and reoxygenation (4 h or 12 h) (A/R). Simultaneously, freshly isolated human PMN were treated with or without HB-EGF, and then co-incubated with HUVEC to determine PMN-EC adherence. Signaling pathway inhibitors were used to determine which signaling pathways were involved. Adhesion molecules expressed in HUVEC were determined by Western blotting.

RESULTS: In vivo, all mice subjected to HS/R had significantly increased mucosal permeability compared to sham operated mice. HB-EGF treatment of non-neutropenic mice led to significantly decreased mucosal permeability. Neutropenic mice had significantly decreased mucosal permeability. HB-EGF treatment of neutropenic mice did not lead to further improvement in intestinal permeability. In vitro, subjecting HUVEC to A/R led to significantly increased PMN-EC adherence. Pretreatment of PMN with HB-EGF significantly decreased PMN-EC adherence 4 h after A/R, and this effect was reversed with EGFR inhibition. Pretreatment of HUVEC with HB-EGF significantly decreased PMN-EC adherence 12 h after A/R, and this effect was reversed in the presence of EGFR or PI3K inhibitors. The expression of the adhesion molecules ICAM-1, E-selectin and PECAM in HUVEC after A/R were significantly decreased by HB-EGF.

CONCLUSIONS: These results show that the ability of HB-EGF to preserve gut barrier function is dependent upon the presence of neutrophils. These findings support the clinical use of HB-EGF for protection of the intestines from disease states associated with intestinal hypoperfusion injury.
Mitochondrial Uncoupling by Increased Proton Leak is Not a Mechanism of Decreasing Reactive Oxygen Species Production After Ischemia-Reperfusion

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BACKGROUND: Cardiac ischemia-reperfusion (IR) injury leads to increased production of reactive oxygen species (ROS). Increased ROS production causes further damage to cardiac myocytes leading to apoptosis. Complex III of the mitochondrial electron transport chain has been identified as the major producer of mitochondrial ROS after IR. Ischemic preconditioning (IPC) decreases mitochondrial ROS production and preserves cardiac function after IR, however the mechanism is unknown. It has previously been demonstrated that mitochondrial proton (H+) leak decreases ROS production; therefore H+ leak has been proposed as a possible mechanism of decreasing ROS formation after cardiac IR. The pyridine nucleotide NADPH is a reducing agent that is involved in scavenging excess ROS. We hypothesized that increased H+ leak is a part of the mechanism of IPC and that pharmacologically increasing the rate of H+ leak would lead to decreased ROS production. We further hypothesized that IPC would preserve the levels of NADPH after IR.

METHODS: Excised rat hearts (n=10/group) were subjected to either Control or IPC. Rate pressure product (RPP) was recorded as an index of myocardial function. Mitochondria were isolated at end-equilibration, end-ischemia and end-reperfusion. Mitochondrial membrane potential (mΔΨ) was measured using a tetraphenylphosphonium electrode. H+ leak was measured as the respiratory rate required to maintain membrane potential at -130 mV in the presence of oligomycin and was titrated with increasing concentrations of carbonyl cyanide p-(tri-fluromethoxy)phenyl-hydrazone (FCCP, 0-80nM). Mitochondrial complex III ROS production was measured by fluorometry using Amplex-Red. Pyridine nucleotide levels were measured by HPLC.

RESULTS: IPC led to decreased ROS production after an episode of IR (13±1 vs. 23±3 nmol H2O2/mg protein/minute, p<0.05). In IPC mitochondria, H+ leak remained unchanged throughout an episode of IR, while Control mitochondria had an initial increase at the end of ischemia and a return to baseline at end-reperfusion. Prior to IR, mild increases in H+ leak led to decreased ROS production; however after IR, neither Control nor IPC mitochondria responded to changes in H+ leak. mΔΨ changed throughout an episode of IR, but these changes were not due to increased H+ leak. Ischemia caused a 61% decline in the levels of NADPH in both IPC and Control mitochondria. These levels did not recover following reperfusion.

CONCLUSIONS: We demonstrate that hearts subjected to IPC prior to the induction of IR do not have a significant change in the rate of H+ leak; therefore increased H+ leak is not the mechanism responsible for decreased ROS formation in IPC. The decline in NADPH levels indicate that preservation of the ROS scavenging apparatus is also not the mechanism responsible for decreased ROS in IPC. Pharmacologic manipulation of mitochondrial H+ leak does not appear to be a method of attenuating ROS production after cardiac IR. However, replenishing the NADPH system may still offer another means of pharmacologically altering the levels of ROS after IR.
miR-199a-3p targets CD44 and reduces proliferation of CD44 positive hepatocellular carcinoma cell lines

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INTRODUCTION: Hepatocellular carcinoma (HCC) is the second most common cause of worldwide cancer death (Jemel, 2011). microRNA (miRNA) are a family of non-coding RNAs that regulate gene expression through inhibition of mRNA translation (DeSano,J.T. 2009). miRNAs are differentially expressed in essentially all solid tumors including hepatocellular carcinoma (HCC)(Gramantieri,L.2008). We have shown miR-199a-3p to be down-regulated in HCC (Jiang,J. 2008). miR-199a-3p’s deregulation has also been noted in Hepatitis B and C along with alcohol induced steatohepatitis all of which are precursors to HCC(Dolganiuc,A. 2009; Murakami,Y . 2009;Zhang,G .L. 2010). In this project we demonstrate that miR-199a-3p targets CD44 and hence reduce the HCC cell lines malignant phenotype through this interaction.

METHODS: Nine pairs of HCC tumors and adjacent benign liver tissues were used for the study. Using Trizol (Invitrogen) RNA was extracted from a portion of the pulverized tissue and RT-PCR was conducted to obtain the mature miR-199a-3p levels. Protein was also isolated from the same tissues and western blots were performed to measure the CD44 protein expression in the tissues. Luciferase analysis was conducted with the full length CD44 3’UTR. Seven HCC cell lines were transfected with miR-199a-3p oligonucleotide or a scrambled control. Seven HCC cell lines had RT-PCR conducted to measure the miR-199a-3p levels and western blots performed for the CD44 level. Proliferation assays were conducted on these cells. Western blot analysis was conducted on a subset of these cells after transfection to determine the change in CD44 protein expression. On a subset of these cell lines Mitragel (BD biosciences, San Jose, CA) invasion assays were conducted along with chemosensitivity tests. Finally a stable cell line was created from SNU449 cells (a CD44+ HCC cell line) that were transfected with a miR-199a-3p expressing plasmid or empty-control plasmid. Proliferation assays and colony formation trials were conducted as well as RT-PCR confirmation of the miR-199a-3p expression. Invasion assays, western-blots for CD44, and in vivo tumor growth studies will be conducted with these cells.

RESULTS: In the human tissue HCC specimens we demonstrated an inverse correlation between the expression of miR-199a-3p and CD44 protein was noted in primary HCC specimens. We report here a significant reduction of miR-199a-3p expression in 7 HCC cell lines. To determine if miR-199a-3p has a tumor suppressive role, pre-miR-199a-3p oligonucleotides were transfected into the HCC cell lines. Pre-miR-199a-3p oligonucleotide reduced cell proliferation by approximately 60% compared to control oligonucleotide in only two cell lines (SNU449 and SNU423); the proliferation of the other 5 treated cell lines was similar to control oligonucleotide. Proliferation assays and colony formation trials were conducted as well as RT-PCR confirmation of the miR-199a-3p expression. Invasion assays, western-blots for CD44, and in vivo tumor growth studies will be conducted with these cells.

CONCLUSIONS: We have shown here that miR-199a-3p mimic targets CD44 and reduces the malignant phenotype in HCC cell lines. This is significant as CD44 has been found to be correlated with more poorly differentiated forms of HCC, the more metastatic forms of the disease, a greater chance for reoccurrence, and poorer prognosis for patients (Endo,K. 2000;Yang,G.H. 2008; Hirohashi,K. 2004}. Therefore miR-based therapy directed at this target may be beneficial to the subset of patients with HCC who have the more aggressive form of the disease.
Cytotoxic CD8+ T cells utilize FasL and perforin to downregulate post-transplant alloantibody production

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INTRODUCTION: We have previously reported that CD8+ T cells downregulate graft specific alloantibody responses following murine hepatocellular transplantation but the exact mechanism(s) of regulation remain unclear. We hypothesize that CD8+ T cells utilize cytotoxic effector molecules to eliminate immune cells that support the alloantibody response since recipients deficient in FasL and perforin have enhanced post-transplant alloantibody levels.

METHODS: CD8 KO mice reconstituted with column purified CD8+ T cells derived from C57BL/6 (wild-type, WT), FasL KO or perforin KO splenocytes were transplanted with allogeneic hepatocytes. Serum alloantibody levels were measured by flow cytometry. ELISPOT was used to quantify IgG1+ antibody producing cells following transplantation. In vitro cytotoxicity was analyzed using a two-color fluorescence assay with B cell apoptosis indicated by propidium iodine (PI) uptake. An in vivo cytotoxicity assay measured the elimination of CFSE-labeled syngeneic naive and alloactivated (IgG1+) B cells following adoptive transfer into WT or CD8 KO recipient.

RESULTS: CD8-deficient recipients reconstituted with FasL (61.2 ± 33%, p=0.03) or perforin (60.3 ± 25.4%, p=0.005) deficient CD8+ T cells had significantly greater alloantibody levels as compared to recipients receiving WT CD8+ T cells (24.5 ± 15%). CD8 KO recipients were discovered to have significantly more antibody producing cells than WT recipients. An in vitro cytotoxicity assay revealed that alloactivated B cells were induced to undergo apoptosis by alloactivated CD8+ T cells. This finding was also observed in vivo as alloactivated B cells are preferentially killed in WT (CD8-replete) as opposed to CD8 KO recipients.

CONCLUSIONS: CD8+ T cells utilize specific cytotoxic effector molecules to downregulate the post-transplant alloantibody response by the direct elimination of alloactivated B cells. Further elucidation of the mechanism(s) by which cytotoxic CD8+ T cells influence antibody production will contribute to the development of therapies to regulate post-transplant humoral responses.
Portable Chest X-Rays Add No Predictive Value in Diagnosis of Ventilator Associated Pneumonia

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INTRODUCTION: Diagnosis of ventilator associated pneumonia (VAP) in the Surgical ICU is problematic. In our unit, patients with high suspicion based on clinical pulmonary infection scores (CPIS) and concomitant infiltrates on portable chest x-ray (pCXR) undergo diagnostic bronchoalveolar lavage (BAL) with quantitative cultures followed by empiric antibiotic therapy. We became skeptical of the predictive value of pCXR for VAP, and hypothesized that pCXR findings on the day of BAL add little to the diagnosis of VAP in the ICU setting.

METHODS: Intubated patients with suspected VAP undergoing concomitant pCXR and BAL testing (n=295) were included. Blinded pCXR were evaluated by surgical intensivists, fellows, residents, and radiologists and rated as (0) not, (1) possibly, or (2) definitely suspicious for pneumonia. These results were compared to BAL results for patients with and without culture confirmed VAP. Analyses included random effects logistic regression to determine the predictive value of the pCXR.

RESULTS: Regardless of interpreter specialty or level of training, pCXR had no predictive value for VAP. Positive predictive value, negative predictive value, and ROC curve all had values below 50%. The inter-rater agreement (Rho) was 0.965, showing little to no discrepancy between raters. Knowing the rater or the score assigned yielded no diagnostic value to determining VAP (p=0.9995).

CONCLUSIONS: pCXR appears to add no predictive value in elucidating which patients should be evaluated for VAP. Clinical suspicion and diagnostic decision making for VAP should therefore not be influenced by pCXR findings.
Pelvic Malignancies Undergoing Exenteration

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INTRODUCTION: In patients with locally advanced and recurrent pelvic malignancies, total pelvic exenteration may be necessary for curative treatment. Early studies reported high morbidity and mortality rates associated with the procedure due to the extensive resection and the aggressiveness of the tumors. Although mortality rates have improved since total pelvic exenteration was first described, morbidity rates remain high. At our institution, a large volume of pelvic exenterations have been undertaken and their outcomes have been studied.

METHODS: The medical records of fifty-three patients with various pelvic pathologies who underwent total pelvic exenteration between 2004 and 2010 were examined. Demographics, operative reports, pathology reports, periprocedural events, and outcomes were analyzed. Comparison of the two groups was performed with a student’s T-test and Fisher’s exact test. Survival curves were constructed using the Kaplan Meier method and compared using the log rank test.

RESULTS: Patients were divided into colorectal (n=36) and non-colorectal (n=17) groups for analysis based on histology. The two groups were similar in demographics, operative time, perioperative events, and length of stay. Chemotherapy use was increased in the colorectal group compared to the non-colorectal group (55.6% vs. 23.5%, p <.05). Preoperative radiation was administered to 77.8% of patients in the colorectal group and 64.7% of patients in the non-colorectal group (p=.34). There was a trend towards increased intraoperative radiation therapy use within the colorectal group. Between the groups, complications rates were similar – 86% in the colorectal group and 76% in the non-colorectal group. In the colorectal group 27.8% of patients developed perineal abscesses, while no patients developed these in the non-colorectal group (p<.05). No survival difference was seen in primary versus recurrent colorectal tumors; however, within the colorectal group there was a survival advantage when comparing R0 resection to R1 and R2 resection. Median survival rates were 27.3 months for R0 resection, 11.8 months for R1 resection, and 8.3 months for R2 resection. The median survival was 21.4 months for the colorectal group and 6.9 months for the non-colorectal group.

CONCLUSIONS: Patients undergoing total pelvic exenteration for colorectal tumors have improved survival when compared to patients undergoing exenteration for pelvic malignancies of other origins. The greatest effect on long-term survival is seen in the colorectal group undergoing an R0 resection. Despite pelvic exenterations carrying a high morbidity, mortality rates have improved and careful patient selection can optimize outcomes.
Previous Award Winners

2010

Intern Award
Mary Dillhoff, MD, MS - Inactivation of p16 in Patients with Esophageal Adenocarcinoma Portends Poor Prognosis in Esophageal Adenocarcinoma

Poster Award
Peter Nau, MD, MS - Novel Reconstruction Techniques of the Extrahepatic Biliary Trees with a Biosynthetic Absorbable Graft

Best Post Doctoral Presentation Award
Alyssa Charrier - Connective Tissue Growth Factor Production by Activated Pancreatic Stellate Cells in Mouse Alcoholic Chronic Pancreatitis

1st Place Resident Award
Kristan Guenterberg, MD, MS - Natural Killer Cells are Activated by IL-21 and Induce ADCC and IFN-Gamma Release in Pancreatic Cancer Cell Lines

2nd Place Resident Award
Thomas Pham, MD - IL-4 Dependent, IgG1-Dominant Post-Transplant Alloantibody Production is Regulated by CD8+ T Cells