and in mice treated with bleomycin in combination with the peptide. Further, to
differentiate the crosslinking activity of LOX from other potential effects,
primary human fibroblasts were cultured with rLOX in the presence of the
inhibitor, beta-aminopropionitrile. The expression levels of ECM (collagen and
fibronec tin), pro-fibrotic factors (IL-6 and TGF-beta), and transcription factor
(c-Fos) were examined by real-time PCR. ELISA, immunoblotting, or
hydroxyproline assay. RESULTS/ANTICIPATED RESULTS: LOX mRNA was
increased in lung tissues and matching fibroblasts of SSC patients. rLOX-induced
ECM production in vitro and ex vivo in lung fibroblasts and in human lung
tissues maintained in organ culture, respectively. Additionally, TGF-beta and
bleomycin induced ECM production, LOX mRNA expression and activity.
Endostatin peptide abrogated these effects. In vivo, rLOX synergistically
exacerbated pulmonary fibrosis in bleomycin-treated mice. The inhibition of
LOX catalytic activity by beta-aminopropionitrile failed to abrogate LOX-
induced ECM production, LOX increased the production of IL-6, IL-6
neutralization blocked the effects of LOX. Further, LOX induced c-Fos
expression and its nuclear localization. DISCUSSION/SIGNIFICANCE OF
neutralization blocked the effects of LOX. Further, LOX induced c-Fos
induced ECM production. LOX increased the production of IL-6. IL-6
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neutralization blocked the effects of LOX. Further, LOX induced c-Fos
expression and its nuclear localization. DISCUSSION/SIGNIFICANCE OF
impact. LOX expression and activity were increased with fibrosis in vivo,
ex vivo, and in vivo. LOX induced fibrosis via increasing ECM, IL-6 and c-Fos
translocation to the nucleus. These effects were independent of the crosslinking
activity of LOX and mediated by IL-6. Our findings suggest that inhibition of
LOX may be a viable option for the treatment of lung fibrosis. Further, the use
of human lung in organ culture establishes the relevance of our findings to
human disease.

2045
The role of TGFβ in driving early cystic fibrosis lung disease
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OBJECTIVES/SPECIFIC AIMS: Transforming growth factor-beta (TGFβ) is a
genetic modifier of cystic fibrosis (CF) lung disease. TGFβ’s pulmonary levels in
young CF patients and its mechanism of action in CF are unknown. We examined TGFβ
levels in sputum with CF and investigated the expression of genes encoding human
airway epithelial cells (AECs) and mice to TGFβ. METHODS/STUDY
POPULATION: TGFβ levels in bronchoalveolar lavage fluid from CF patients
(n = 15) and non-CF control patients (n = 21) ≥ 6 years old were determined by
ELISA. CF mice and non-CF mice were intratracheally treated with an
adenoviral TGFβ1 vector or PBS; lungs were collected for analysis at day 7.
Human CF and non-CF AECDs were treated with TGFβ1 or PBS for 24 hours then
collected for analysis. RESULTS/ANTICIPATED RESULTS: Young CF patients
had higher bronchoalveolar lavage fluid TGFβ1 than non-CF controls (p = 0.03).
Mouse lungs exposed to TGFβ1 demonstrated inflammation, goblet cell
hyperplasia, and decreased CFTR expression. CF mice had greater TGFβ1-
induced lung mechanics abnormalities than controls; both CF human AECDs and
CF mice showed higher TGFβ1 induced MAPK and p38 signaling compared with
controls. DISCUSSION/SIGNIFICANCE OF IMPACT: For the first time, we
show increased TGFβ levels very early in CF. TGFβ1 drives CF lung abnormalities in
mice and human models; CF models are more sensitive to TGFβ1’s effects. Understanding the role of TGFβ in promoting CF lung disease is critical to
developing patient specific treatments.

2090
TL1 team approach to osteosarcoma cell detection
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OBJECTIVES/SPECIFIC AIMS: The objective of our collaboration is to develop a
strong transdisciplinary team consisting of microfluidics engineers, cancer biologists,
and clinicians, to identify cell surface markers capable of detecting circulating
osteosarcoma cells (COC) using microfluidic devices. Our goals are 3-fold: (1)
Identify cell surface markers unique to osteosarcoma (OS) for COC isolation, (2)
develop a Geometrically Enhanced Mixing (GEM) device to isolate COCs, and (3)
evaluate the efficacy of GEM device to detect COCs in OS patients under
treatment. The long-term goal is to utilize this cell detection approach to correlate the
presence of COC with metastatic incidence. METHODS/STUDY POPULA-
TION: To identify a marker to capture COCs we are utilizing flow cytometry and
microfluidic capture devices. Flow cytometry will be used to evaluate the relative
expression of epithelial cell adhesion molecule (EpCAM), CD45, cell surface
vimentin (CSV), insulin-like growth factor 2 (IGF2R), interleukin 11 receptor subunit
alpha (IL-11Rα), ganglioside 2 (GD2), and receptor activator of nuclear factor κ-B (RANK)
on a panel of OS cell lines. These cell surface markers were selected based on our
preliminary review of the literature. The OS markers will be assessed by passing a
known concentration of OS cells through a GEM microfluidic device coated with antibodies targeting the selected marker, as indicated by flow cytometry. Once captured, COCs on the device will be analyzed and
the capture efficiency for the indicated marker will be measured. ANOVA will be
used to determine any significant difference in capture efficiency between marker
types. Once an optimal marker or panel of markers has been selected, we will
conduct capture studies using OS cell spiked blood samples followed by clinical
samples obtained from OS patients. In clinical samples, COC detection will be
validated using the FDA approved triple immunocytochemistry technical definition of
a circulating tumor cell (CTC). This will enable COCs to be differentiated from
the normal whole blood cell population by selecting for CD45+, EpCAM+, and
cytokeratin+ cells. RESULTS/ANTICIPATED RESULTS. Our preliminary studies have shown that on our microfluidic device, EpCAM, a marker commonly used to
identify circulating tumor cells in other cancer settings, has a poor capture efficiency
(15.9% ± 7.7%) for HU09 OS cells while the same setup with EpCAM has a capture
efficiency of 56.9% ± 2.7% for BxPC-3 pancreatic cells. We therefore anticipate our
flow cytometry studies to show lower and COC capture rates for OS cell lines,
while showing a moderate to high expression of CSV, IGF2R, IL-11Rα, GD2, and
RANK. We expect to show a 60%-80% capture efficiency for markers selected for
COC capture. Currently, CSV and GD2 are particularly promising as markers
based on previously published studies. DISCUSSION/SIGNIFICANCE OF IMPACT:
OS is the most common primary bone tumor and the third leading cause of pediatric
cancer deaths. At diagnosis 80% of patients will present with metastasis, however
only 20% of these cases are clinically detectable. Innovative strategies to identify
patients at risk of metastasis would allow for stratification of intervention therapies.
Currently, tumor recurrence and metastasis are primarily dependent on
diagnostic-imaging modalities such as computerized tomography or positron
emission tomography scans. Unfortunately, these imaging modalities can only
detect tumors of masses of significant size (106 tumor cells). Liquid biopsies may be
a novel alternative to current diagnostic imaging systems to monitor metastatic
incidence and treatment efficacy. The detection of CTCs through routine blood
sampling has the potential to be used clinically for earlier detection, monitoring
the treatment of metastatic cancers and surveying the effect of therapeutic
interventions on metastasis. To date, the majority of the studies on CTCs have
evaluated their presence in carcinomas. Although sarcomas are rare, they
generally have a poor prognosis. This study will address one of the unmet medical
needs in the field of CTC detection; the identification of cell surface OS markers to
improve binding specificity, increase purity, and maintain a high capture efficiency.
This phase of our proposal will evaluate the most abundant and conserved
markers across a panel of OS cell lines. Once a marker or panel of markers is
selected, we will begin to develop a microfluidic device that can be used clinically to
detect CTCs in this disease setting.

2303
Trauma-related acute respiratory distress syndrome (ARDS) in India: Current incidence and management
strategies
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OBJECTIVES/SPECIFIC AIMS: Aim 1: To determine the true incidence of trauma-
related acute respiratory distress syndrome (ARDS) in India. We propose to
perform a prospective observational study to determine the incidence of ARDS in
India. Aim 2: To perform a preliminary assessment of risk factors for ARDS in the
Indian trauma population. We will leverage these findings against the global ARDS
data to provide a foundation for further interventional studies. Aim 3: To evaluate
the current management strategies and patient outcomes from ARDS in trauma
subjects admitted to the Jai Prakash Narayan Apex Trauma Center (JPNATC). These
findings will identify areas in need of practice-based performance improvement in
ARDS therapies in India. METHODS/STUDY POPULATION: This question
prompts an observational study of trauma patients with ARDS in an Indian trauma
population that continues to have substantial in-hospital mortality. The approx-
imate number of ICU-admitted trauma cases for the study period is 1700. Specific
data elements to be collected include patient demographics, comorbidities,
mechanism of injury, Injury Severity Score, risk factors for ARDS, sequential organ
failure and assessment scores, vital signs, laboratory values, and evidence-based
treatments received, including mechanical ventilation and adjunctive therapies.
Outcome data will include discharge location, ICU and hospital length of stay and
OBJECTIVES/SPECIFIC AIMS: One of the driving mechanisms of cancer progression is the reprogramming of metabolic pathways in intermediary metabolism. Cancers increase their energy expenditure by increasing ATP production for utilization in anabolic pathways to increase production of proteins, nucleic acids and lipids. The Warburg effect, where cancer cells predominantly use aerobic glycolysis rather than oxidative phosphorylation to produce ATP, was long thought to be the main initiating pathway in increasing tumor burden. However, compelling new evidence shows that there exists metabolic heterogeneity among and within tumors. Mitochondrial respiration often plays a major role in tumor progression, as many different cancers contain a subpopulation of slow-cycling tumor-initiating cells that are multidrug-resistant and depend on oxidative phosphorylation. These cells represent a target for cancer therapy. In this study, we identified a novel endogenous regulator of mitochondrial respiration, retinoic acid receptor responder 1 (RARRES1). METHODS/STUDY POPULATION: We examined mitochondrial energetics by staining the mitochondria with TMRM and MitoTracker. We then analyzed the apoptotic phenotype of epithelial cells with depletion of RARRES1 with fluorescence-activated cell sorting analysis of annexin V-staining. RESULTS/ANTICIPATED RESULTS: Remarkably, fluorescence-activated cell sorting analysis of annexin V-stained epithelial cells with depletion of RARRES1 were resistant to all studied modes of cell death, implying an effect on a fundamental cell process. By using proteomics, metabolomics, cellular and molecular analyses, our data show that RARRES1 regulates mitochondrial membrane potential and subsequently alters 1-carbon metabolism by modulating the function of the mitochondrial voltage-dependent anion channel. We believe this is the first example of a tumor suppressor protein that functions to directly regulate mitochondrial energetics. Using an extracellular flux analyzer, our data also show that depletion of RARRES1 causes an increase in mitochondrial respiration and ATP production, thus enhancing biosynthetic pathways that drive the pathogenicity and survival of cancers. The metabolic and apoptotic phenotype of RARRES1-depleted cells was reversed by treatment of metformin, a mitochondrial inhibitor. DISCUSSION/SIGNIFICANCE OF IMPACT: These data lay the foundation for metabo-therapy of the many tumor types that exhibit RARRES1 depletion and may have the added benefit of targeting drug-resistant tumor-initiating cells.

Tumor suppressors p53 and ARF control oncogenic potential of triple-negative breast cancer cells by regulating RNA editing enzyme ADAR1

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OBJECTIVES/SPECIFIC AIMS: Triple-negative breast cancer (TNBC) accounts for one-fifth of the breast cancer patient population. The heterogeneous nature of TNBC and lack of options for targeted therapy make its treatment a daunting adventure. The deficiency of tumor suppressors p53 and ARF is one of the known genetic signatures enriched in TNBC. Crucial questions remain about how TNBC is regulated by these genetic alterations. METHODS/STUDY POPULATION: In order to address this issue, we established p53-ARF-defective murine embryonic fibroblast and mammary epithelial cell to study the molecular and phenotypic consequences. Moreover, transgenic mice were generated to investigate the effect of p53/ARF deficiency on mammary tumor development in vivo. RESULTS/ANTICIPATED RESULTS: Increased proliferation and transformation capability were observed in p53/ARF-defective cells, and an aggressive form of mammary tumor was also seen in p53-/-ARF-/- mice. Gene expression profiling and knock-down experiments using shRNAs were conducted to identify inflammatory marker ISG15 and RNA-editing enzyme ADAR1 as potential culprits for the elevated oncogenic potential. Interestingly, we found that the overexpression of ISG15 and ADAR1 is also prevalent in human TNBC cell lines. Reducing ADAR1 expression abrogated the oncogenic potential of human TNBC cell lines, while non-TNBC cells are less susceptible. DISCUSSION/SIGNIFICANCE OF IMPACT: These results indicate critical roles played by the tumor suppressors p53 and ARF in the pathogenesis of TNBC, likely through regulating ADAR1-mediated RNA modifications. Further understanding of this pathway promises to shed light on genetics-driven vulnerabilities of TNBC and inform development of more effective therapeutic strategies.