## Q3 2033 The soluble guanylate cyclase stimulator IWP-121 inhibits renal inflammation and fibrosis in human renal proximal tubular cells and in the Dahl Salt-Sensitive rat model

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Objective: To determine the effects of the soluble guanylate cyclase (sGC) stimulator, IWP-121, on renal inflammation and fibrosis in human renal proximal tubular cells (RPTCs) and in the Dahl-ss rat model of hypertension, heart failure and kidney dysfunction.

Results: IWP-121 stimulated cGMP formation and phosphorylation of vasodilator-stimulated phosphoprotein (VASP) in human RPTCs, confirming the existence of sGC-cGMP signaling in these cells. Treatment of RPTCs with TGFβ for 24 h induced elongated cell morphology changes and loss of cell-cell contact. IWP-121 at 1 and 10 μM almost completely reversed these phenotypic changes. Exposure of RPTCs to TGFβ for 48 h resulted in up to 70% apoptosis, an effect that was significantly inhibited by IWP-121. Monocyte chemoattractant protein-1 (MCP-1) is up-regulated in renal diseases including diabetic nephropathy. MCP-1 levels in RPTCs were enhanced 4- to 5-fold after treatment with TNFα and returned to basal level when cells were treated with IWP-121 or anti-TNFα antibody. Consistent with the effects observed in RPTCs, IWP-121 statistically reduced pro-inflammatory (TGFβ, TNFα , MCP-1, IL-6) and pro-fibrotic (collagens type1α1 and type 3α1, α-SMA ) gene expression in kidneys from Dahl-ss rats. In addition, IWP-121 reduced serum inflammatory markers (MCP-1, IL-6, OPN-1, and TIMP-1). Finally, we showed that TGFβ activated phosphorylation of SMAD3 in human RPTCs; this effect was also blocked by IWP-121.

Conclusion: IWP-121 demonstrated anti-inflammatory and anti-fibrotic activities in *in vitro* and *in vivo* models. The anti-fibrotic mechanism appears to be through inhibition of TGFβ-mediated SMAD3 signaling. These data suggest that sGC stimulation may represent a mechanism for the treatment of inflammatory and fibrotic diseases.

## Q3 2034 Podoplanin discriminates distinct stromal cell populations and a novel progenitor subset in the liver

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Podoplanin/gp38$^+$ stromal cells present in lymphoid organs play a central role in the formation and reorganization of the extracellular matrix and in the functional regulation of immune responses. gp38$^+$ cells are present during embryogenesis and in human livers of primary biliary cirrhosis. Since little is known about their function, we studied gp38$^+$ cells during chronic liver inflammation in models of biliary and parenchymal liver fibrosis and steatohepatitis. gp38$^+$ cells were analyzed using flow cytometry and confocal microscopy and the expression of their steady state and inflammation-associated genes were evaluated from healthy and inflamed livers. gp38$^+$ cells significantly expanded in all three models of liver injury, and returned to baseline levels during regression of inflammation. Based on CD133 and gp38 expression numerous subsets could be identified that were negative for CD133 (gp38$^{hi}$CD133$^-$, gp38$^{low}$CD133$^-$ and gp38$^-$CD133$^-$). Moreover, among the CD133$^+$ cells, previously identified as progenitor population in injured liver, two subpopulations could be distinguished based on their gp38 expression (gp38$^-$CD133$^+$, CD133$^+$gp38$^+$). Importantly, the distribution of the identified subsets in inflammation illustrated injury-specific changes. Moreover, the gp38$^+$CD133$^+$ cells exhibited liver progenitor cell characteristics similar to the gp38$^-$CD133$^+$ population, thus representing a novel subset within the classical progenitor cell niche. Additionally, these cells expressed distinct sets of inflammatory genes during liver injury.

Conclusion: Our study illuminates a novel classification of the stromal/progenitor cell compartment in the liver and pinpoints a hitherto unrecognized injury-related alteration in progenitor subset composition in chronic liver inflammation and fibrosis.

## Q3 2035 Th2-driven Innate Immune Responses in the Development of Lung Fibrosis induced by Multi-walled Carbon Nanotubes

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Aberrant tissue responses to persistent deposition of foreign bodies lead to organ fibrosis through orchestrated, yet poorly understood, mechanisms. We used multi-walled carbon nanotubes (MWCNT) to develop a mouse model of lung fibrosis, which features a prominent acute phase inflammation followed by chronic interstitial fibrosis. Genome-wide microarray analyses of the lungs exposed to MWCNT identified a range of differentially expressed genes that potentially function in the regulation of the acute-to-chronic transition, through pathways of immune and inflammatory regulation, response to stress and extracellular stimuli, and cell migration and adhesion. In particular, T helper 2 (Th2)-driven innate immune responses were significantly enriched. We then demonstrated that MWCNT induced the expression of Th2 cytokines interleukin (IL)-4 and IL-13, and a panel of signature downstream genes including Il4i1, Chia, and Ccl11/Eotaxin. Induction of Th2 cytokines took place in CD4+ T lymphocytes indicating the activation of Th2 cells. Furthermore, induction involved the activation of STAT6 via phosphorylation of STAT6 and up-regulation of GATA-3 that controls the transcription of Th2 target genes. Our study uncovers the activation of Th2-driven immune/inflammatory responses during pulmonary pathologic fibrosis development induced by MWCNT.

This work was funded to QM by CDC/NIOSH.

## Q3 2036 Single cell analysis reveals dynamic transitions within distinct subpopulations of Fibro-Adipogenic Progenitors (FAPs) implicated in skeletal muscle regeneration or fibrosis

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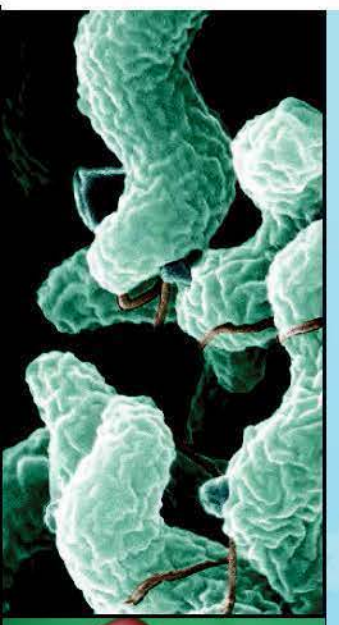
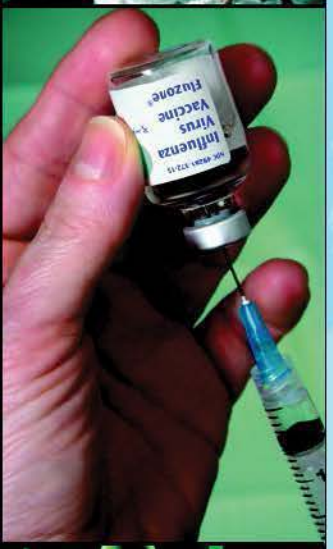
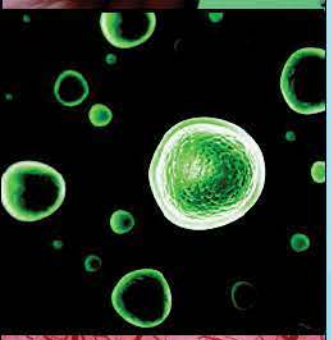
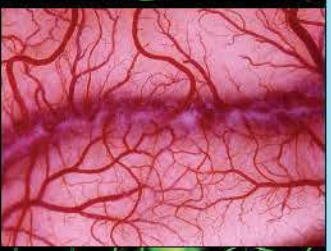
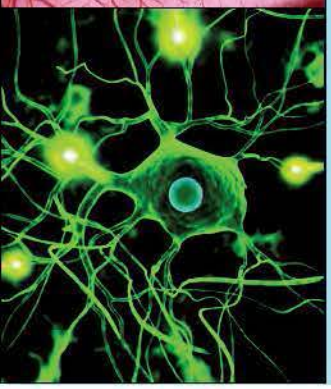
One common feature in chronic muscular degenerative disorders is the ability of diseased muscles to either counter the disease progression by compensatory regeneration or undergo fibrosis. Understanding the biological and molecular basis of the stages of disease progression can influence patient response to treatments. Recently identified mesenchymal Fibro-Adipogenic Progenitors (FAPs) located in skeletal muscle interstitium are contributors to both compensatory regeneration and fibro-adipogenic degeneration of muscles in Duchenne Muscular Dystrophy (DMD). FAPs promote muscle regeneration by releasing pro-myogenic paracrine factors. In pathological circumstances such as DMD, FAPs are the major drivers of fibrotic scarring and intramuscular fatty infiltration. This disease stage-dependent activity indicates an intrinsic heterogeneity of FAPs. Our laboratory demonstrated that HDAC inhibitors (HDACi), which promote regeneration in DMD muscles, target FAPs. To address the relationship between FAPs heterogeneity and different activity in healthy or dystrophic muscles, we used Fluidigm platform to profile FAPs gene expression at single cell level. We have compared FAPs profiles from skeletal muscles of young and old wild type, either unperturbed or induced to regenerate by notexin injury, and from dystrophic mdx mice. Our data revealed that FAPs consist of distinct subpopulations with specific gene co-expression patterns and biological activities. Based on our data we re-designed flow cytometry protocol in order to isolate individual FAPs subpopulations for their further functional studies. Other than elucidating the pathogenesis of DMD and other muscular disorders, FAPs signature can provide novel biomarkers of disease progression and response to therapeutic interventions, such as the recent clinical trial with the HDACi Givinostat.









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