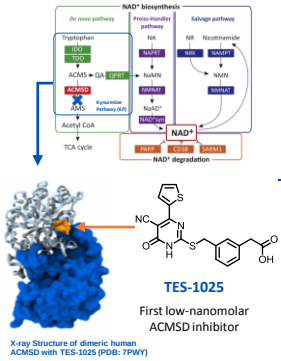
# ACMSD Inhibition Decreases Mortality in Sepsis by Promoting an Anti-inflammatory Phenotype

Roberto Pellicciari[1], Francesca De Franco[1], Nicola Giacchè[1], Janet Robertson[1], Paride Liscio[1], Hernando Gomez[2], Marco Gargaro[3], Francesca Fallarino[3], and John Kellum[2]

*[1]Tes Pharma s.r.l. Corciano, Perugia, Italy. [2] University of Pittsburgh Medical Center, Pennsylvania, USA. [3] Department of Medicine and Surgery, Perugia, Italy.*

## Introduction

α-Amino-β-carboxymuconic-ε-semialdehyde Decarboxylase (ACMSD) is an enzyme of Kynurenine Pathway, mainly expressed in liver and kidney and which represents a branch point in the de novo $NAD^+$ biosynthesis pathway that directs the conversion of tryptophan to $NAD^+$.
Tes Pharma has discovered and characterised the first nanomolar and selective ACMSD inhibitor: **TES-1025** (*Pellicciari R, et al. J Med Chem. 2018, 61, 745-759*), demonstrating that ACMSD inhibition increases intracellular $NAD^+$ levels and has protective effects in preclinical models of metabolic diseases of both the liver (NASH) and kidneys, Acute Kidney Injury (AKI) (*Katsyuba E, et al. Nature, 2018, 563, 354-359*).
Our previous results highlight that ACMSD inhibition by our preclinical candidate **TES-1025** is a novel therapeutic approach for kidney diseases without treatments, such as, Acute Kidney Injury (AKI) and Chronic Kidney Disease (CKD) (*Kellum JA, et al. Nat Rev Nephrol. 2019, 15(2), 65-66; Manrique-Caballero EL, et al. Antioxid Redox Signal. 2021, 35,1449-1466*), liver metabolic disease such as NASH, and Acute-on-Chronic Liver Failure (ACLF), or combined liver-kidney diseases, such as, the Hepato-Renal Syndrome (HRS).



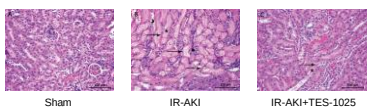




**TES-1025**
First low-nanomolar ACMSD inhibitor

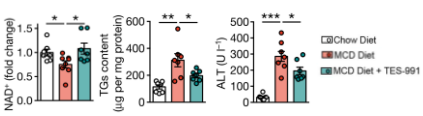
X-ray Structure of dimeric human ACMSD with TES-1025 (PDB: 7PWY)

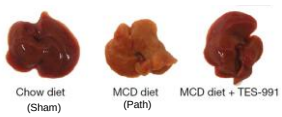
### In-vivo Efficacy in Ischemia/Reperfusion Acute Kidney Injury (IR-AKI) Mouse Model


Treatment with TES-1025 (15 mg/kg/day) demonstrated protective effects in IR-AKI preclinical models, in terms of renal function and kidney histology

### In-vivo Efficacy in Methionine-Choline Deficient (MCD) diet NASH mouse model




ACMSD inhibition, by TES-991 (bioisostere of TES-1025, at 15 mg/kg/day) protects the liver from NASH, increasing $NAD^+$ and reducing triglycerides (TGs) and ALT biomarkers

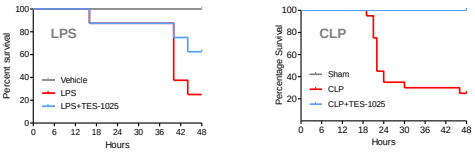
**Here, we disclose novel therapeutic applications for TES-1025, revealing unique physiological functions of ACMSD in the modulation of systemic inflammatory response, decreasing mortality in sepsis preclinical models by promoting an anti-inflammatory phenotype.**

## Methods

**Lipopolysaccharide (LPS) and cecal ligation and puncture (CLP)-induced models of acute inflammation/sepsis in adult C57BL/6 mice**. In the first study mice were exposed to LPS at 15mg/kg or vehicle (volume 10ml/kg) then sacrificed at 48h. In the second study mice were exposed to CLP or sham surgery in a model of abdominal sepsis. In both studies, animals were randomised to treatment with TES-1025 or vehicle, with survival at the 48h timepoint as the primary outcome.
**Effect of ACMSD inhibition on macrophage polarisation**. Resident liver macrophages (Kupffer cells) in culture were exposed to LPS for 24 hours, and then treated with TES-1025 or DMSO. Changes in macrophage phenotype, were determined by measuring M1 and M2 markers, such as iNOS, TNFα, IL-6 and Arginase-1 (Arg-1), Mannose receptor-2 (Mrc-2), IL-10, respectively.
**Effect of ACMSD inhibition on inflammatory marker genes in HK2 cells**. $2*10^5$ HK2 cells were stimulated with TGFβ (10ng/mL) and TES-1025 (1,10, 50 and 100µM) for 16 hours. $1.5*10^5$ HK-2 cells were stimulated with LPS (30µg/mL) and TES-1025 (10, 50µM) for 24 hours. Gene expression of fibrotic and inflammatory genes has been performed by RT-PCR.

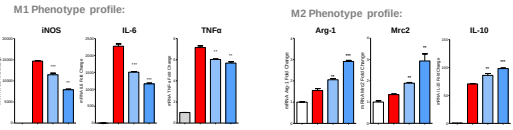
## Results

### TES-1025 Increases Survival Against Both LPS- and CLP-induced Models of Acute Systemic Inflammation



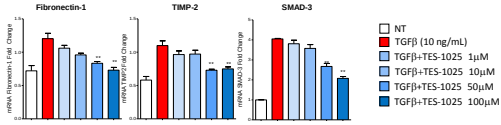

TES-1025 (50mg/kg) promotes survival in both LPS- and CLP-treated mice compared to controls.

### TES-1025 Promotes Polarisation of Macrophages Towards the Anti-inflammatory M2 Phenotype

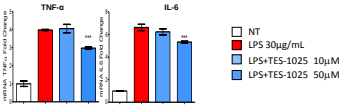



M1/M2 gene marker expression changes in Kupffer cells induced by TES-1025 against LPS-insult.
One way Anova **p<0.01; ***p<0.001 vs CLP.

### TES-1025 Reduces the Expression of Both Pro-fibrotic and Inflammatory Genes in Renal Tubular Cells




TES-1025 mediated suppression of TGF-β1 / SMAD-3 signalling drives reverses TGF-β1-induced changes fibrotic gene marker expression in HK-2 cells. One way Anova **p<0.01 vs TGF-β1.




TES-1025 reduces inflammatory marker gene expression in HK-2 cells.
One way Anova **p<0.001 vs LPS

## Conclusions

We have previously demonstrated that ACMSD inhibitors are efficacious in kidney and liver diseases associated with $NAD^+$ depletion.
Here we show that ACMSD inhibition protects from death in both LPS- and CLP-induced models of severe inflammation which may be driven by an anti-inflammatory effect promoted by a shift, in the functional M1/M2 phenotype of liver macrophages during the response to infection/inflammation.
Overall these results expand the therapeutic opportunities for ACMSD Inhibitors, unravelling peculiar physiological functions of ACMSD in the modulation of de novo NAD+ biosynthesis pathway, on the road the discovery of further therapeutic applications of **TES-1025** for the treatment of systemic inflammatory diseases of kidney and liver.