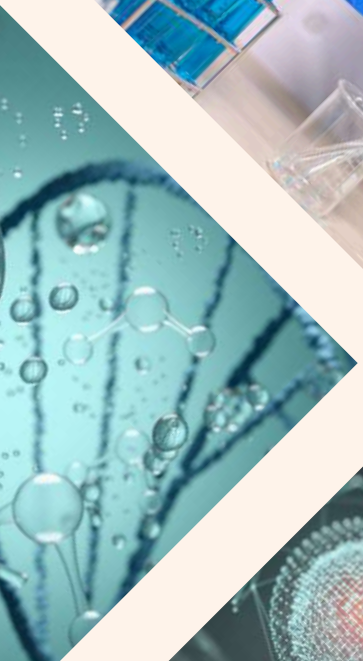
EPTRI
European Paediatric Translational Research Infrastructure

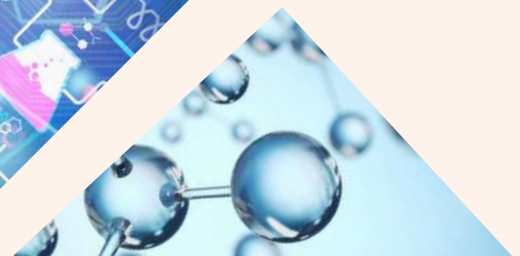
# EPTRI SCIENTIFIC MEETING

## BOOK OF ABSTRACTS

## BOLOGNA, ITALY

## 13-15 MARCH, 2025





*“EPTRI Paediatric Medicines Discovery TRP”*

# SPEAKER SECTION

## SPEAKER: FEDERICA D’AMICO

### Alterations of the microbiota in lactating Göttingen Minipig sows treated with metformin.

Federica D'Amico[1] Silvia Bencivenni[1], Augusta Zannoni[1], Domenico Ventrella[1], Alberto Elmi[2], Maria Laura Bacci[1], Mikael Niku[3], Patrizia Brigidi[1], Monica Forni[1], Silvia Turroni[1]

1 – University of Bologna; 2 – University of Pisa; 3 – University of Helsinki

Background: Early gut microbiota dysbiosis can affect child’s health and has been linked to the onset and progression of several diseases later in life (e.g., asthma, allergies, obesity, type 2 diabetes). Breast milk is recognized as a major driver of the structure and dynamics of the gut microbial community from the earliest moments of infant life. In addition to nutritious and prebiotic compounds, milk is associated with a specific microbiota that is shaped by several maternal factors, including gut microorganisms and medications (e.g., metformin, an antidiabetic drug). However, the impact of the latter on the milk microbiota is still largely unknown. In this context, the Göttingen Minipig has been proposed as the most appropriate animal model to study the safety of drug use during pregnancy and lactation due to its physiological and metabolic similarities to humans, milk volume and ethical factors. Furthermore, this animal model seems promising for microbiota studies due to its genetic stability and microbiological control, and has been used to study gut microbiota modifications in other contexts. In this study, we investigated the gut and milk microbiota composition of Göttingen Minipig sows receiving prolonged metformin administration, and followed the dynamics of the gut microbiota of the corresponding piglets.

Methods: One week after farrowing, four sows received oral metformin (500 mg/day) for 2 weeks, followed by 850 mg/day during the third week. Stool and milk samples were collected from each sow 5-6 days after farrowing and at the end of each weekly metformin treatment. In addition, rectal swabs were collected from piglets before treatment and weekly during the metformin administration. Characterization of all microbial ecosystems was performed by Illumina 16S rRNA amplicon sequencing.

Results: The milk microbiota of Göttingen Minipig sows after metformin administration showed an increase of biodiversity that correlated with higher drug doses. Increased levels of Prevotella and Ruminococcus, and reduced relative abundance of Fusobacterium and Porphyromonas were observed after the last week of treatment. As expected, the gut microbiota of sows was strongly affected by metformin use, with a significant reduction in health-associated Oscillospiraceae members over time. Analysis of piglet gut microbial communities is ongoing.

Preliminary conclusions: In addition to demonstrating the relevance of the Göttingen Minipig as a valid model for studying the impact of medications on maternal microbiota, our results suggest that both milk and gut microbiota of sows may be affected by metformin intake and thus have an impact on piglet gut microbial development in a critical timeframe.

### SPEAKER: SULEMAN KHAN ZADRAM

### Innovative Targeting of GD2-positive Childhood Neuroblastoma tumors with Photooncolytic phage nanovector platforms.

Suleman Khan Zadran[1], Giovanni Perini[1]

1 – University of Bologna, Department of Pharmacy and Biotechnology

Background: Disialoganglioside-GD2 is a key molecular target for neuroblastoma (NB) immunotherapy which is based on the employment of GD2-targeting antibodies. However, about 50% of treated patients can experience tumor relapse due to limited immune-mediated cytotoxicity and poor antibody penetration into tumors.

Aim: To address this problem, we developed a penetrating photooncolytic phage nanovector platform that selectively targets GD2-expressing NB cells.

Results: The phage bioconjugates, functionalized with photosensitizers, result in specific oncolysis of GD2-positive NB cells upon light irradiation, without

affecting GD2-negative ones. Notably, the bioconjugates penetrate deep into GD2-positive tumor spheroids to induce breakdown and cell death. Additionally, to overcome resistance from GD2 loss, often linked to poor prognosis, we introduced a CRISPRa strategy to reactivate GD2 expression in GD2-negative cells.

Conclusion: The approach offers a minimally invasive and highly effective strategy to target NB and addresses critical aspects of anti-GD2 resistance.

## POSTER SECTION

### Advances in pediatric nephrology: AI-based repurposing studies to identify new promising vasopressin V2 receptor ligands.

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Summary of purpose: Abnormal vasopressin functioning is associated with different disorders including diabetes insipidus (DI), the inappropriate secretion of antidiuretic hormone (SIADH) that are characterized by hypo- and hypernatremia in children and require timely recognition and treatment.

Autosomal recessive polycystic kidney disease (ARPKD) is also associated with altered vasopressin V2 receptor (V2R) responses. ARPKD is a rare genetic disorder highly significant in pediatric nephrology. Hepatorenal fibrocystic syndrome and renal cysts are observed in ARPKD pediatric patients. Importantly, clinical manifestations may occur in utero or at birth. ARPKD shares many

similarities with Autosomal dominant polycystic kidney disease (ADPKD) including increased cyclic adenosine monophosphate (cAMP). The vasopressin V2 receptor (V2R) antagonist, tolvaptan, has been recently approved for the treatment of progressive disease in adult ADPKD patients. Conversely, there is no current cure for ARPKD. Activation of V2R not only regulates renal water reabsorption but is also involved in controlling cell proliferation, cancer, and the enlargement of cysts in PKD patients. Based on these findings, the major aim of this study is to identify new molecules modulating V2R-dependent signaling. To this purpose, an inverse screening of a large collection of known drugs was performed to identify novel V2R ligands that might modulate different receptor-mediated effects.

Methodology: The inverse screening campaign was run by using PLATO, a homemade target fishing platform. Structure-based studies were also carried out. Renal collecting duct MCD4 cells, stably expressing human V2R and aquaporin-2 (AQP2), were used as an experimental model to test the effects of drugs. Confocal analysis and Western Blotting studies were carried out to show the expression and the localization of the V2R. Fluorescence Resonance Energy Transfer (FRET), and calcein fluorescence quenching (CFQ) were applied to evaluate changes in intracellular cAMP and DDAVP-induced water flux.

Results: The initial screening yielded 185 candidate V2R modulators, having predicted IC50 values against V2R better than 0.1 μM. Among these, five molecules with predicted IC50 towards V2R below 50 nM were prioritized for further analysis. Confocal analysis and Western Blotting studies confirmed the expression of V2R in MCD4 cells, used as an experimental in vitro model.

Therefore, FRET studies were carried out to test whether these compounds affect the DDAVP-induced cAMP responses. Interestingly, we found that one of these, known to be a selective antagonist of the purinergic receptor P2Y12-R, potentiates the effect of DDAVP by increasing the intracellular level of cAMP. More importantly, another drug, coded as F2544, at 1 nM concentration significantly reduced the DDAVP-dependent cAMP production. Functional CFQ studies revealed that this drug reduced the DDAVP-induced water reabsorption, with effects comparable to tolvaptan, which is a well-established V2R antagonist used as a positive control. In this respect, an in-depth computational investigation showed a nice overlap of the molecular interaction fields generated from the binding sites of V2R and F2544. Finally, molecular docking simulations returned a promising posing and scoring of F2544 in the V2R binding site.

Conclusions: Our studies identified for the first time new V2R ligands by applying an AI-based approach. Combining in-depth computational investigations and functional studies, F2544 was prioritized for being repurposed for treating diseases associated with abnormal V2R signaling, such as ARPKD in pediatric nephrology.