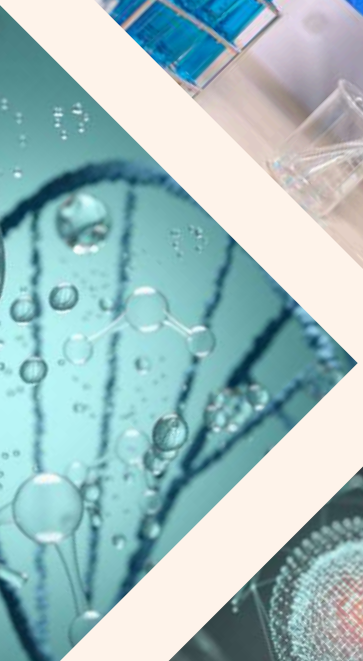
EPTRI
European Paediatric Translational Research Infrastructure

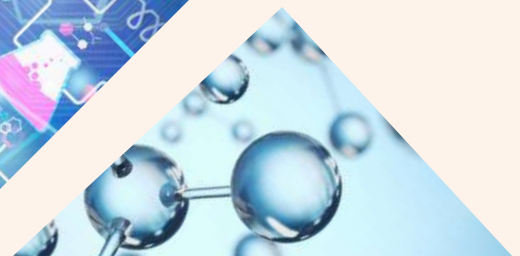
EPTRI SCIENTIFIC MEETING

BOOK OF ABSTRACTS

BOLOGNA, ITALY

13-15 MARCH, 2025





“EPTRI Advances Therapy Medicinal Products TRP”

SPEAKER SECTION

SPEAKER: GIOVANNI MIGLIACCIO

Advancing paediatric care through ATMPs: challenges, opportunities, and the role of EPTRI

Giovanni Migliaccio[1,2]

1 – European Paediatric Translational Research Infrastructure (EPTRI), Leuven, Belgium; 2 – Consorzio per le Valutazioni Biologiche e Farmacologiche (CVBF), Pavia, Italy

Advanced Therapy Medicinal Products (ATMPs) are an emerging class of medicinal products harnessing gene, cell, and tissue-based therapies to treat severe and life-threatening conditions. These therapies provide personalized, regenerative solutions tailored to a patient’s genetic background, disease progression, and immune response. ATMPs are especially crucial for pediatric patients, as many rare monogenic disorders manifest during fetal or neonatal stages, where conventional treatments are either lacking or purely palliative.

Currently, 18 ATMPs have been licensed, with 10 specifically including or dedicated to pediatric patients.

Despite their potential, ATMPs face substantial challenges in development and accessibility. In paediatric medicine, the small patient population limits financial incentives, often resulting in market failures and reduced investment in research and commercialization. Additionally, the complex regulatory approval processes and the high development costs further hinder their broader adoption in clinical settings.

To overcome these barriers, initiatives such as the Pediatric Advanced Medicines Biotech proposal and the European Pediatric Translational Research Infrastructure (EPTRI) are working to enhance accessibility, foster collaborative research, and establish sustainable pricing and marketing strategies. Notably, the EPTRI Thematic Research Platform on ATMPs plays a critical role in driving innovation and ensuring these life-changing therapies reach the paediatric patients who need them most. Addressing financial, regulatory, and logistical barriers is essential for the successful integration of ATMPs into clinical practice, ultimately improving outcomes for children with rare and life-threatening diseases.

POSTER SECTION

Luspatercept, a novel therapeutic approach in patients affected from Beta Thalassaemia Major.

Bledi Kreka[1], Manika Kreka[1], Alma Idrizi[1]

1 – UHCT Mother Terese, Albania Shahini University of Medicine, Tirana, Albania

It is always a challenge deeping insight in haemoglobinopathies concerns.

Haemoglobinopathies (hemoglobin disorders) caused by genetic mutations are autosomal recessive disorders and the most frequent genetic inherited diseases seen worldwide, specifically and above all among Mediterranean countries. Thalassaemia syndromes (Beta Thalassaemia Major/ Intermedia and Sickle Cell Disease) have been the first diagnosed diseases since in intrauterine life using recombinant DNA techniques. So, a better understanding of their pathophysiology has given a spectacular improvement and a considerable impact into these conditions management.

It is estimated annual births of more than 330 000 affected infants who are going to suffer from these disorders. The distribution of these diseases or disorders is historically linked to current or previously malaria endemic regions, however nowadays immigration has led to a worldwide distribution, making them a global health problem.

Till now we still cannot have a permanent resolve of these diseases, unless a successful bone marrow transplantation or Stem Cell Transplantation.

Crucial therapies like regular blood transfusions and an optimal compliance of iron chelation therapy seem to have a great importance for a successful treatment of these individuals promising a longer life expectancy.

During the last decade, new treatment approaches and novel therapies have been proposed, some of which have the potential to change the natural history of these disorders. Indeed, the first erythroid maturation agent, luspatercept and gene therapy have been approved for beta thalassaemia adult patients. A green light is given for use in children and this makes us confident and optimistic in offering a qualitative life to our children affected.

At EHA 2024, the investigators reported safety data from the first part of the two-part study, using Luspatercept, specifically regarding patients with transfusion dependent β-thalassemia who were younger than 18 but no younger than 12 years of age. These data were from 12 patients who had received at least four transfusions in the 24 weeks pre-enrollment. This novel treatment hopes to reduce anemia, to minimize chronic red blood cell transfusions, and mitigate secondary iron overload.

Conclusion: The advances of the understanding of hemoglobinopathies and the specificity in each type of them seem to help scientists to pave the way to develop several novel treatments and novel therapies.

It is now clear that efficacy and safety of many therapies in children and adults is still under study. In the future and upcoming years, the therapeutic approach to these disorders is going to become more

complex and will change the natural history and the real burden of these disorders which really impact individuals and societies.

Venetoclax and Azacitidine in Pediatric High-risk Myeloproliferative Neoplasms: the AIEOP Experience.

Gennaro Pagano[1], Francesco Baccelli[1], Davide Leardini[1], Edoardo Muratore[1], Gianluca Bossu'[1], Tamara Belotti[1], Francesca Gottardi[1], Francesco Venturelli[1], Francesca Vendemini[2], Alessandro Di Gangi[3], Marco Becilli[4], Manuela Tumino[5], Giuliana Beneduce[6], Francesco Saglio[7], Arcangelo Prete[1], Franco Locatelli[4], Riccardo Masetti[1]

1 – Pediatric Hematology and Oncology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy; 2 – Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy; 3 – University of Pisa, Italy; 4 – IRCCS Bambino Gesù Pediatric Hospital, Rome, Italy; 5 – University of Padua, Italy; 6 – AORN Santobono-Pausilipon, Naples, Italy; 7 – Regina Margherita Pediatric Hospital, Turin, Italy;

Background: Refractory and/or relapsed acute myeloid leukemia (AML r/r), advanced myelodysplastic syndromes (MDS-EB), and therapy-related myelodysplastic syndromes/acute myeloid leukemia (t-MDS/AML) represent a significant therapeutic challenge for pediatric hemato-oncologists, as these diseases are often resistant to conventional cytotoxic therapies and have a high relapse rate.

Affected patients have often undergone intensive pretreatment and experienced considerable toxicity, making it crucial to adopt less toxic therapies to minimize further adverse effects.

Aim: In this retrospective, multicenter study, 31 pediatric patients (median age of 10 years, range 2-20) with high-risk myeloid malignancies were treated with a combination of venetoclax and azacitidine (ven-aza) (median number of cycles: 2, range 1-7) at centers affiliated with the Italian Association of Pediatric Hematology and Oncology (AIEOP). The patients were diagnosed with AML r/r (n=18), MDS-EB (n=6), and t-MDS/AML (n=7).

Results: The results showed a complete remission (CR) rate of 48.4%, with an overall response rate (ORR), defined as the sum of CR and PR, of 71%. A total of 58.1% of patients successfully proceeded to hematopoietic stem cell transplantation (HSCT). With a median follow-up of 216 days (32 – 1004) from the start of ven-aza, the one-year event-free survival (EFS) was 53.5% (95% CI: 35.8%-79.9%), significantly higher in patients who underwent HSCT ($p < 0.0001$). Ven-aza demonstrated excellent efficacy in the subgroup of patients with AML r/r harboring KMT2A rearrangement and in patients with MDS-EB with UBTF-TD.

Grade ≥3 adverse events included neutropenia (12 patients), febrile neutropenia (8 patients), fungal infections (2 patients), and hypertransaminasemia associated with diarrhea (1 patient). No treatment-related deaths were reported.

Conclusion: Our study demonstrates that ven-aza represents a safe and effective bridging strategy to HSCT in pediatric and young adult patients with high-risk myeloid malignancies.

Perinatal Cell-Derived Spheroids as a Dual Therapeutic Strategy for Type 1 Diabetes Mellitus.

Francesca Paris[1], Giulio Maltoni[2], Pasquale Marrazzo[3], Aretha Rambaldi[1], Andrea Scozzarella[2], Giuliana Simonazzi[4], Laura Bonsi[5], Stefano Ratti[1], Lucia Manzoli[6], Francesco Alviano[6], Marcello Lanari[2]

1 – Cellular Signalling Laboratory, Anatomy Center, Department of Biomedical Sciences (DIBINEM), University of Bologna, 40126 Bologna, Italy; 2 – Pediatric Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, 40138 Bologna, Italy; 3 – Department of Biomolecular Sciences (DISB), University of Urbino, Urbino, Italy; 4 – Obstetrics Unit, Department of Obstetrics and Gynecology, IRCCS Azienda Ospedaliero-Universitaria Sant'Orsola, 40138 Bologna, Italy; 5 – University of Bologna, 40126 Bologna, Italy; 6 – Cellular Signalling Laboratory, Anatomy Center, Department of Biomedical Sciences (DIBINEM), University of Bologna, 40126 Bologna, Italy

Background: Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disorder characterized by the selective destruction of pancreatic beta cells, leading to absolute insulin deficiency. Current therapies rely on exogenous insulin administration, which does not address the underlying immune dysregulation nor restore endogenous insulin production. Advanced cellular strategies leveraging the regenerative and immunomodulatory properties of stem cells may provide a more comprehensive therapeutic approach.

Aim: This study investigates the potential of perinatal cell-derived spheroids as a dual therapeutic strategy for T1DM, integrating both insulin secretion and immune modulation. We first characterized the immunomodulatory capacity of spheroids composed of Wharton's jelly mesenchymal stem cells (WJ-MSCs) combined with amniotic epithelial cells (AECs). These spheroids were co-cultured with activated peripheral blood mononuclear cells (PBMCs) from healthy donors to assess their effect on pro-inflammatory and regulatory immune populations. Subsequently, we induced endocrine differentiation in AECs to generate insulin-producing cells, confirming the expression of pancreatic markers via immunofluorescence. Differentiated AECs were then integrated with undifferentiated WJ-MSCs to form structured spheroids with both functional insulin secretion and immunomodulatory properties.

Results: Our findings indicate that undifferentiated perinatal spheroids exert significant immunomodulatory effects, attenuating pro-inflammatory responses while promoting anti-inflammatory pathways. Spheroids containing insulin-producing AECs formed cohesive three-dimensional structures and exhibited functional potential for insulin secretion. Further investigations will evaluate the therapeutic efficacy of these spheroids in preclinical models, with a focus on their dual role in immune regulation and metabolic restoration.

Conclusion: These results support the potential of perinatal cell-derived spheroids as a novel cell-based therapy for T1DM, addressing both beta-cell replacement and immune-mediated disease mechanisms. This strategy may pave the way for innovative treatments that surpass conventional insulin therapy, offering a more integrated and durable therapeutic approach for pediatric and adult T1DM patients.

Pioneering paediatric DMD therapies: precision gene editing in iPSC and preclinical mouse models.

Francesca Colli[1], Christian Santangeli[1], Diego Berionni[2], Milena Bellin[2], Francesco Chemello[1]

1 – University of Bologna; 2 – University of Padova

Duchenne muscular dystrophy (DMD) is a severe paediatric neuromuscular disorder caused by mutations in the dystrophin gene, leading to progressive muscle degeneration and early mortality. Despite recent advances, effective treatments remain elusive, highlighting the need for robust translational research models that faithfully represent the paediatric disease environment and inform therapeutic development.

Patient-derived induced pluripotent stem cells (iPSCs) serve as pivotal in vitro platforms to recapitulate the core features of DMD pathology while providing a patient-centric perspective crucial for paediatric research. In our laboratory, we combine these iPSC models with next-generation gene editing tools - specifically base and prime editing - to accurately correct pathogenic mutations responsible for DMD.

To further optimise and assess these approaches, we employ preclinical mouse models of DMD, thus establishing a comprehensive translational pipeline. By integrating the specificity of iPSC-based systems with the physiological complexity of in vivo studies, we can rigorously evaluate safety, efficacy, and delivery strategies, as well as potential off-target effects, prior to clinical translation.

Our research program aligns with the goals of the European Paediatric Translational Research Infrastructure by expediting the development of transformative therapies for children affected by DMD and other rare diseases. By harnessing cutting-edge iPSC technology, established mouse models, and precise gene editing, we aim to accelerate the path from bench to bedside, ultimately improving outcomes for paediatric patients and advancing the field of neuromuscular research.

Off-the-shelf non-viral CARCIK cells derived from Cord Blood: A GMP-compliant approach for the treatment of hematologic malignancies.

Ilaria Pisani[1,2], Giusi Melita[1,2], Simone Naso[1,2], Michele Quaroni[1,2], Patricia Borges de Souza[3], Elisa Gotti[4], Carolina Cuofano[4], Giada Matera[2,5], Daniela Belotti[2,5], Benedetta Cabiati[2,5], Stefania Galimberti[6], Angela Maria Savino[7], Jolanda Sarno[7], Massimiliano Mazza[3], Anna Gaimari[3], Fabio Nicolini[3], Benedetta Rambaldi[8], G Ottaviano[2], G Lucchini[2], G. Dastoli[1,2], A Biondi[1,2], Alessandro Rambaldi[8,9], J Golay[4], M Introna[4], G Gaipa[1,2,5], Sarah Tettamanti[1,2]

1 – Fondazione Tettamanti; 2 – IRCCS San Gerardo, Monza; 3 – Advanced Cellular Therapies and Rare Tumors Unit, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori"; 4 – Center of Cellular Therapy "G. Lanzani", Division of Hematology, ASST Papa Giovanni XXIII, Bergamo; 5 – Laboratorio di Terapia Cellulare e Genica Stefano Verri; 6 – Bicocca Bioinformatics Biostatistics and Bioimaging B4 Center, School of Medicine and Surgery, University of Milano-Bicocca; 7 – School of Medicine and Surgery, University of Milano-Bicocca; 8 – Department of Oncology and Hematology, University of Milan; 9 – Department of Oncology-Hematology, ASST Papa Giovanni XXIII

Chimeric antigen receptor (CAR) T-cell therapy has transformed the treatment of hematologic malignancies. However, its widespread adoption is limited by the challenges associated with autologous manufacturing, including high costs, lengthy production times, and variability in T-cell quality. To overcome these limitations, over the past decade, we have developed a non-viral gene transfer platform utilizing the Sleeping Beauty transposon system and electroporation to engineer Cytokine-Induced Killer (CIK) Cells (PCT/EP2015/075980). With an AIFA-approved Good Manufacturing Practice (GMP) Cell Factory at our institution, we have been conducting academic early-phase clinical trials since 2017 to evaluate the CARCIK-19 product for the treatment of Acute Lymphoblastic Leukemia (NCT03389035, NCT05252403). These trials demonstrated that up to 85% of treated patients achieved complete remission, with a favorable safety profile. These groundbreaking results marked the first successful application of CARCIK therapy worldwide (PMID 32870895, Lussana ASH2022, Rambaldi ASH2022)). Furthermore, an ongoing phase I/II study is currently assessing the safety and efficacy of partially matched donor-derived CARCIK-CD19 cells for the treatment of B-cell non-Hodgkin lymphoma and B-cell chronic lymphocytic leukemia outside the transplant setting (NCT05869279).

To further advance our non-viral platform toward an efficient and cost-effective off-the-shelf CAR T-cell therapy, we explored the feasibility of generating allogeneic CAR-CIK cells derived from cord blood (CB). Cord blood has been widely used in hematopoietic stem cell transplantation due to its advantages, including reduced graft-versus-host disease (GvHD) risk and superior proliferative capacity.

In this study, we optimized a GMP-compliant method for generating CAR-expressing CIK (CARCIK) cells from cryopreserved CB units. CIK cells, which exhibit both T-cell receptor (TCR)-mediated and natural killer (NK)-like activity, provide an effective mechanism for targeting malignant cells while minimizing the risk of inducing GvHD (PMID: 17606446, 16953207, Gaipa ASH2018). The integration of the Sleeping Beauty transposon system enabled efficient CAR transgene delivery into CB-derived CIK cells, leading to robust expansion and stable CAR expression.

We conducted a comparative analysis of the metabolic and functional properties of CB-derived CARCIK cells versus their peripheral blood (PB)-derived counterparts. CB-derived CARCIK cells demonstrated reduced glycolysis, enhanced mitochondrial respiration, and a higher proportion of naïve and memory-like T cells, suggesting superior persistence and longevity. In vivo efficacy was evaluated using a DAUDI xenograft model, where CB-derived CARCIK-CD19 cells exhibited potent antitumor activity, effectively controlling disease progression and prolonging survival.

These findings support the integration of CB-derived CARCIK cells into future clinical applications, offering a readily available, off-the-shelf cellular therapy with a favorable safety profile. The unique immunobiology of CB T cells, combined with the scalability of CIK cell expansion and the efficiency of the Sleeping Beauty transposon system, provides a viable solution to the current limitations of autologous CAR T-cell therapy. Given the urgent need for accessible and cost-effective immunotherapies, CB-derived CARCIK cells represent a promising alternative for the treatment of hematologic malignancies. Ongoing and planned clinical trials will further define their therapeutic potential, paving the way for broader clinical implementation and improved patient outcomes.

Accessing the intrathecal space in a porcine paediatric model: description of

Domenico Ventrella[1], Carlotta Lambertini[1], Alberto Elmi[1,2], Nicolina Cristina Sorrentino[3,4], Luca Laghi[5], Alessandro Fraldi[3,6], Monica Forni[7], Noemi Romagnoli[1], Maria Laura Bacci[1]

1 – Department of Veterinary Medical Sciences, University of Bologna; 2 – Department of Veterinary Sciences, University of Pisa; 3 – Department of Clinical Medicine and Surgery, University of Napoli; 4 – Advance histopathology facility, TIGEM - Telethon Institute of Genetics and Medicine; 5 – Department of Agricultural and Food Sciences, University of Bologna; 6 – CEINGE - Biotecnologie Avanzate Franco Salvatore; 7 – Department of Medical and Surgical Sciences, University of Bologna

The piglet has emerged as one of the most complete and accurate models for neonatal and paediatric translational medicine due to genetical, anatomical and physiological similarities with humans. The porcine brain resembles humans in terms of weight, volume, cortical surface area, myelination, composition and electrical activity, and its development extends from prenatal to early postnatal life. Moreover, due to their longer lifespan, pigs are a great model to study inherited/genetic conditions. All of the above have contributed to the interest toward piglets for gene therapy for neurological disorders. In such scenario, it became pivotal to validate reproducible techniques to access the intrathecal space allowing for cerebrospinal (CFS) sampling and local drug administration. This abstract describes two techniques developed for Cisterna magna (CM) puncture and lumbar spinal catheter placements, as well as some applications.

The CM study, divided into a cadaver phase and an in vivo one, was conducted on 2-30 days-old piglets: after hand palpation of the occipital protuberance, a line identifying the median spinal plane was marked; a second one was then traced between the cranial margins of the atlas wings. A 22G×75mm spinal needle was introduced along the median line to a depth of 4mm, 5mm posteriorly to the intersection of the two lines; the tip of the needle was directed cranioventrally using the cranial margin of the wings as an external landmark. The technique allowed the safe CSF collection in all animals [1].

The second study, once again divided into a cadaver and an in vivo phase, identified the L2-L3 intervertebral space as the best one for percutaneous insertion of spinal catheter, reaching the CM, in 30 days-old piglets. Again, the newly developed technique allowed for safe placement of the device in all subject. The proposed technique requires less skilled operators when compared to the other existing method, which involves surgical approach, and potentially leads to fewer complications [2].

The above-mentioned methodologies were then used in a trial aimed at creating a comprehensive map of Central Nervous System (CNS) transduction by eight recombinant adeno-associated virus (rAAV) serotypes upon cerebrospinal fluid administration in neonatal piglets. rAAV9 showed the highest transduction efficiency and the widest distribution capability, robustly transducing both glia and neurons, including the motor neurons of the spinal cord [3]. Alongside, pre-injection CSF samples from 5, 30 and 50 days-old subjects were used for a metabolomic quantitative profiling of their composition, that highlighted several differences between ages, potentially related to the tightening of the blood-brain barrier [4].

According to the author's experience, focussing on the set up and standardization of the methodologies to be used to deliver drugs intrathecally and to collect uncontaminated CSF samples,

is a mandatory preliminary step allowing for smoother, more refined trials, in full compliance with the 3Rs principle. Moreover, the in-depth quantification of the CSF composition represents a critical tool to better understand the physiology of such an important lab animal, especially prior to establishing and phenotyping disease models.

1. Romagnoli, N. et al. Access to Cerebrospinal Fluid in Piglets via the Cisterna Magna: Optimization and Description of the Technique. Lab. Anim. 2014, 48, 345–348.
2. Lambertini, C. et al. Transdermal Spinal Catheter Placement in Piglets: Description and Validation of the Technique. J. Neurosci. Methods 2015, 255, 17–21.
3. Sorrentino, N.C. et al. A Comprehensive Map of CNS Transduction by Eight Recombinant Adeno-Associated Virus Serotypes Upon Cerebrospinal Fluid Administration in Pigs. Mol. Ther. J. Am. Soc. Gene Ther. 2016, 24, 276–286.
4. Ventrella, D. et al. Age-Related 1H NMR Characterization of Cerebrospinal Fluid in Newborn and Young Healthy Piglets. PloS One 2016, 11, e0157623.