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With the participation of:

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AASNE AARSAND

LABORATORY DIAGNOSIS OF PORPHYRIAS – CHALLENGES AND POTENTIAL SOLUTIONS

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Getting a timely porphyria diagnosis is essential for good patient outcome, but requires high quality of all steps of the total testing process. Firstly, the clinician must consider porphyria as a potential cause of the patient's symptoms and the correct sample materials must be collected, transported, and stored under appropriate conditions. Thereafter, a diagnostic strategy must be selected considering the patient's symptoms and clinical situation, and the relevant biomarkers must be analysed with sufficiently sensitive and specific methods. The analytical results must be interpreted taking the clinical information into account and communicated with interpretative comments and further recommendations to the clinician who must act upon them. For non-specialist laboratories, the quality of porphyria-related services is presently unknown. For specialist laboratories, results from the Ipnet External Quality Assessment Scheme which assesses most aspects of the total testing process show that most participants report the correct diagnoses for patients with the most common porphyrias. However, for more unusual porphyrias or in cases with low or borderline diagnostic marker concentrations, some laboratories do not establish the correct diagnoses. Generally, for porphyria-related analyses there is a lack of harmonization and standardization, and there are variations in diagnostic strategies, analytical performance and reference/decision limits in use that may likely affect patient outcome. Over the last years, the Ipnet External Quality Assessment Scheme and the Ipnet Laboratory Working Group as well as other parties have launched different initiatives addressing these issues, including investigating the sources of variation for urinary ALA and PBG results between Ipnet specialist laboratories, circulating calibrators for erythrocyte protoporphyrin and developing Ipnet best practice diagnostic guidelines, but additional work is required to further improve the quality of porphyria diagnostics.

JASMIN BARMAN-AKSÖZEN

IRON IN THE ERYTHROPOIETIC PROTOPORPHYRIAS

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Iron is essential for life, but it is also a highly reactive substance whose absorption, utilisation and distribution in the body is strictly regulated. In the biosynthesis of haem, iron is a substrate for the final enzymatic reaction in which ferrochelatase (FECH) incorporates ferrous iron into protoporphyrin IX (PPIX) to form haem. However, iron also regulates the translation of the mRNA of delta-aminolevulinate synthase 2 (ALAS2), the first and rate-limiting enzyme of the erythroid haem biosynthesis. This mechanism establishes a link between the availability of iron and the capacity of the haem biosynthetic pathway, ensuring that iron is used efficiently and preventing the accumulation of toxic haem precursors in the event of iron deficiency. Apparently, this balance is disturbed by pathogenic variants in some genes of the haem biosynthetic pathway: a partial deficiency in FECH cause erythropoietic protoporphyria (EPP1, OMIM # 177000) and leads to an accumulation of large amounts of PPIX in the erythrocytes. In most, but not all, patients, EPP1 also causes hypochromic microcytic anaemia and a disturbance in the iron metabolism consistent with iron deficiency, such as low ferritin, low transferrin saturation and low hepcidin blood concentrations, as

well as an increase in soluble transferrin receptor and erythrocyte zinc protoporphyrin (ZnPP). Interestingly, gain-of-function mutations in ALAS2 cause X-linked erythropoietic protoporphyria (XLEPP, OMIM # 300752), which is characterised by an accumulation of PPIX and ZnPP in the erythrocytes and similar disturbances in the iron metabolism as seen in EPP1. Clinically, the two diseases are indistinguishable and are characterised by painful phototoxic burn injuries in tissues exposed to visible light and an increased risk of developing PPIX-mediated liver failure. While in XLEPP the accumulation of PPIX can be well explained by the overly active ALAS2 enzyme, the situation in EPP1 is less clear: In EPP1, the overproduction of PPIX despite an apparent iron deficiency indicates an underlying disturbance in the regulation between iron metabolism and haem biosynthesis.

Recently, several clinical trials and case series have been published in which either iron supplementation was tested as a possible treatment option in EPP1 and XLEPP or in which iron deprivation was used as a therapeutic strategy to lower blood PPIX concentrations and/or to prevent/reverse liver damage. The presentation will discuss the results of these studies in the light of recent findings such as the increase in ALAS2 mRNA levels observed in EPP1 and raise the question of whether the erythropoietic porphyrias should generally be understood as overproduction diseases.

YONATAN EDEL
REEM A. MUSTAFA

IPNET PROJECT FOR THE DEVELOPMENT OF EVIDENCE-BASED GUIDELINES FOR ACUTE PORPHYRIA

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Abstract:

In 2022, IPNET convened an international panel of experts to develop evidence-based guidelines for the management of patients with acute porphyria. The guidelines utilized previously-agreed on definitions of key terms and patients subgroups with porphyria.

The panel prioritized 13 PICO questions to be addressed. In addition to conducting systematic reviews of the published literature, and for questions with scarce evidence the panel utilized the expert evidence approach to inform the recommendations. The panel used the GRADE Evidence to Decision framework to structure the discussion and guide the judgment about different factors that informed developing each of the recommendations. After initial agreement on the recommendations by the panel, a small group (2-3 panelists) led the writing of the recommendations and related implementation remarks. A final draft of the recommendations was then circulated for voting by the whole panel.

In this session, we provide an update about progress to date. We also provide a preview of the methods and examples of recommendations.

VERONICA FIORITO

INHIBITING HEME SYNTHESIS TO MODULATE CELL METABOLISM AND COUNTERACT TUMOR INITIATION AND PROGRESSION

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Metabolic rewiring is a hallmark of malignant cell transformation, facilitating tumor initiation and progression. Heme plays a significant role in regulating cell metabolism, not only as the prosthetic group of proteins and enzymes involved in aerobic respiration but also because pathways controlling heme homeostasis are intricately linked with those governing nutrient utilization.

Mounting experimental and epidemiological evidence suggests that dysregulated heme homeostasis accelerates the development and progression of various cancers. Specifically, heightened heme biosynthesis is a recognized characteristic of cancer, emerging as a crucial in vivo dependency in KRAS-mutated Non-Small Cell Lung Cancer (NSCLC) and Pancreatic Ductal Adenocarcinoma (PDAC).

Through genetic and pharmacological interventions, we demonstrated that reducing ALAS1 activity, the first and rate limiting enzyme in the heme biosynthetic pathway, in cellular and animal models of NSCLC and PDAC led to substantial metabolic alterations. This included constraining the glycolytic phenotype of KRAS-mutated tumor cells and fostering oxidative metabolism. This metabolic transition correlated with impaired tumor initiation, as well as reduced tumor cell proliferation and dissemination.

In conclusion, our findings identify ALAS1 as a crucial factor in regulating the metabolic rewiring that sustains tumor growth and dissemination, thus advocating for its targeting as a therapeutic strategy.

ANTONIO FONTANELLAS

A CLINICALLY RELEVANT MODEL OF ACUTE INTERMITTENT PORPHYRIA (AIP) DEVELOPED IN ADULT NON-HUMAN PRIMATES (NHPS) CAN BE FULLY RESCUED BY SYSTEMIC MESSENGER RNA REPLACEMENT THERAPY

Antonio Fontanellas

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Acute intermittent porphyria (AIP) is a rare metabolic disorder caused by haploinsufficiency of hepatic porphobilinogen deaminase (PBGD), the third enzyme of the heme biosynthesis. Individuals with AIP experience neurovisceral attacks closely associated with hepatic overproduction of potentially neurotoxic heme precursors. We replicated AIP in adult non-human primates (NHPs) through selective knockdown of the hepatic ***PBGD*** gene and evaluated the therapeutic efficacy of human PBGD (hPBGD) mRNA rescue.

Intrahepatic administration of an AAV vector containing shRNA against endogenous PBGD mRNA resulted in sustained PBGD activity inhibition in liver tissue for up to 7 months post-injection. The administration of porphyrinogenic drugs to NHPs induced hepatic heme synthesis, elevated urinary porphyrin precursors, and reproduced acute attack symptoms in patients with AIP, including pain, motor disturbances, and increased brain GABAergic activity. The model also recapitulated functional anomalies associated with AIP, such as reduced brain perfusion and cerebral glucose uptake, disturbances in hepatic TCA cycle, one-carbon metabolism, drug biotransformation, lipidomic profile, and abnormal mitochondrial respiratory chain activity. Additionally, repeated systemic administrations of hPBGD mRNA in this AIP NHP model restored hepatic PBGD levels and activity, providing successful protection against acute attacks, metabolic changes in the liver, and CNS disturbances. This approach demonstrated better efficacy than the current standards of care for AIP.

In conclusion, this novel model significantly expands our understanding of AIP at the molecular, biochemical, and clinical levels, and confirmed the safety and translatability of multiple systemic administration of hPBGD mRNA as a potential etiological AIP treatment.

IQBAL HAMZA

INTER-ORGAN HEME TRANSPORT AND SIGNALING

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Heme is a vital but cytotoxic cofactor that must be transported in a highly controlled manner through membranes via specific intra- and inter-cellular pathways. However, the genes and pathways responsible for heme trafficking remain poorly understood. Caenorhabditis elegans, a microscopic translucent animal is unique amongst free-living animals as it cannot synthesize heme but acquires heme for sustenance. Thus, C. elegans is an ideal animal model to identify heme trafficking pathways as it permits organismal heme homeostasis to be directly manipulated by controlling environmental heme. Heme is imported apically into the intestine by HRG-1-related permeases and exported basolaterally by MRP-5/ABCC5 to extra-intestinal tissues. Loss of mrp-5 causes embryonic lethality that can be suppressed by heme supplementation raising the possibility that MRP-5-independent heme export pathways must exist. Here we show, by performing a forward genetic screen in mrp-5 null mutants, that loss of the vesicular cargo sorting Adaptor Protein complexes (AP-3) fully rescues mrp-5 lethality and restores heme homeostasis. Remarkably, intestinal heme accumulation due to mrp-5-deficiency causes a concomitant deficit in the lysosomal heme importer HRG-1 abundance and localization. Loss of both MRP-5 and AP-3 subunits resurrects HRG-1 levels and localization, thus underscoring the crucial role of HRG-1 in dictating mrp-5 mutant phenotypes. In the absence of MRP-5, heme is exported by SLC49A3 homolog, a previously uncharacterized transporter. Live-cell imaging reveals vesicular coalescence that facilitates heme transfer between the importers and exporters at the interface of lysosomal-related organelle. These results define a mechanistic model for metazoan heme trafficking and identifies SLC49A3 as a promising candidate for heme export in mammals.A modern approach to the determination of drug safety in acute porphyria.

RICHARD J. HIFT

A MODERN APPROACH TO THE DETERMINATION OF DRUG SAFETY IN ACUTE PORPHYRIA

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There is a significant association between drug exposure and the risk of an acute porphyria attack. It is therefore important that health professionals and patients are able to distinguish between safe (non-porphyrogenic) and dangerous (porphyrogenic) drugs. Clinical evidence is unreliable due to individual variability in drug response, its respective nature, and the poor quality of many reports. Experimental models using animals and cell cultures have failed to provide are sufficiently accurate predictions to guide prescribing. Predicting drug safety based on metabolic disposition has proven highly effective. Porphyrogenicity is associated with ALAS1 up-regulation in response to hepatocyte heme depletion. This is a characteristic consequence of the direct up-regulation of CYP expression or mechanism-based irreversible CYP inactivation. Drugs which are not associated with this metabolic disposition appear to be safe.

A detailed understanding of a drug's metabolism can therefore reliably predict its porphyrogenicity even in the absence of actual clinical or laboratory-based experience. The Norwegian Porphyria Center (NAPOS) database, based on this predictive model, has become a standard resource for patients and health professionals. It is currently being updated and modernized with the assistance of an IPNET working group. Individual variability in drug response remains a challenge. Factors such as the individual's metabolome and multiple exposures to inducing factors may contribute to this variability. Observations suggest that patients with recent acute attacks or biochemically active porphyria are more susceptible to drug reactions. Thus, prescribing recommendations are more stringent for those with active porphyria, while a lenient approach is permissible for others.

LOUISA G. KLUIJVER

PREDICTING AND IMPROVING OSTEOPOROSIS IN ERYTHROPOIETIC PROTOPORPHYRIA; A LONGITUDINAL COHORT STUDY

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Background: Erythropoietic protoporphyria (EPP) is a rare inherited metabolic disease, causing lifelong painful phototoxic reactions, minimal sunlight exposure, and vitamin D deficiency. Previous studies reported a high osteoporosis prevalence in EPP patients. The objective is to identify those at risk for osteoporosis and assess treatment effects on bone mineral density (BMD) in EPP.

Methods: In this longitudinal ambispective single centre cohort study data from patient files and two-time questionnaire from adult patients diagnosed with EPP who underwent at least one dual-energy X-ray absorptiometry scan (DEXA-scan) at the Erasmus Medical Centre, Rotterdam, the Netherlands between 2012 and 2023 was used.

Results: 82.7% (n=115) of patients exhibit a Z-score below 0 standard deviation (SD). Osteopenia was found in 39.5% (n=55), while osteoporosis affected 15.3% (n=21) of patients. There were 50 osteoporosis-related fractures in 34.2% of patients. Aging (OR 1.08; CI: 1.03-1.12), persistent vitamin D deficiency (OR 1.11; 95% CI: 1.00-1.23) and a low body-mass index (BMI) (OR 0.91; 95% CI: 0.82-0.99) increased the odds of osteopenia/osteoporosis. Patients with a vitamin D deficiency (OR 5.51; 95% CI: 1.69-17.92) and no cholecalciferol at baseline (OR 0.22; 95% CI: 0.04-1.34) had the highest odds of increasing in BMD.

Conclusions: Vitamin D status plays a crucial role in both preventing osteoporosis and improving BMD. This population is a natural model for lack of sunlight exposure and vitamin D deficiency, underlining the importance of lifelong adequate vitamin D levels for bone health in both the EPP and general population.

ERIC LAI

NON-CANONICAL ROLE OF ALAS1 AS AN INHIBITOR OF RNA INTERFERENCE

Seungjae Lee, Sangmi Lee, Robert Desnick, Makiko Yasuda, Eric C. Lai

RNA interference (RNAi) is a fundamental strategy for gene silencing via small interfering RNAs (siRNAs) that program Argonaute (Ago) effectors, and is related to endogenous silencing via microRNAs (miRNAs). Although most efforts focus on factors that are required for miRNA/RNAi function, biological systems are typically subject to repression. Using molecular genetic approaches, we uncover ALAS1, the first enzyme in the heme biosynthesis pathway, as a repressor of miRNA accumulation in cells and in mice. This was non-intuitive, since heme is well-known to play a positive role as a cofactor for the nuclear miRNA processing machinery. Our mechanistic analyses indicate that, under heme-replete conditions, loss of ALAS1 does not affect RNase III-mediated steps of miRNA biogenesis (i.e. Drosha or Dicer activity). Instead, we find that ALAS1, but not other core heme biosynthesis factors, limits the assembly and activity of the Argonaute effector complex. Notably, ALAS1 is the target of one the few current FDA-approved siRNA drugs, namely Givosiran. Accordingly, our data may have broader implications for RNAi therapies. We provide evidence that loss of ALAS1 enhances siRNA-mediated knockdown, suggesting potential for ALAS1 depletion as an RNAi adjuvant.

MATTIAS LISSING

LONG-TERM COMPLICATIONS IN ACUTE PORPHYRIAS

Mattias Lissing

Life expectancy in patients with acute porphyria (AP) has improved significantly over time, mainly due to increased knowledge, preventive measures and new treatment options. The care for patients with AP is hence increasingly focused on long-terms complications that often arise in asymptomatic patients years after the typical age of active symptomatic porphyria disease. Long term complications in acute porphyria are diverse, often multi-factorial and involve several organ systems. They include chronic neurological symptoms, high blood pressure, porphyria associated kidney disease and hepatopathy with an increased risk of primary liver cancer.

Chronic neurological symptoms in patients with AP such as pain, fatigue, nausea and psychiatric symptoms involve both the central, peripheral and autonomous nervous systems. Symptoms are more common in patients with severe recurrent AP disease. The neurological symptoms associated with AP have a significant impact on quality of life and timely assessment of main underlying cause of symptoms as well as both preventive and symptomatic treatment are of high importance. High blood pressure (HBP) and porphyria associated kidney disease (PAKD) are common conditions in patients with a history of active AP. Although HBP and PAKD are causally related, both are independently related to AP and since both conditions are mainly asymptomatic at early stages and easy to assess, regular controls are recommended.

The liver is considered the main site of porphyrin precursor accumulation and it is not surprising that elevated liver enzymes are reported in patients with AP, mainly related to acute attacks. No study has however found a clear association between AP and liver fibrosis or cirrhosis and histology studies are scarce and provide few specific findings. A strong association between acute intermittent porphyria (AIP) and primary liver cancer (PLC), mainly hepatocellular carcinoma, is well established although the association is less clear for the rarer AP forms variegate porphyria (VP) and hereditary coproporphyria (HCP). AP-associated PLC is related to lifetime biochemical activity and age, but individual risk assessments are difficult and currently the best option to reduce mortality related to PLC in AP is the implementation of annual or biannual surveillance with ultrasound in patients aged 50 or higher.

OSCAR MILLET

INNOVATIVE THERAPIES IN CONGENITAL ERYTHROPOIETIC PORPHYRIA (CEP)

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Congenital erythropoietic porphyria, also known as Günther's disease, results from a deficient activity in the fourth enzyme of the heme pathway, the uroporphyrinogen III synthase (UROIIIS). Ciclopirox (CPX) is an off-label drug, topically prescribed as an antifungal. A few years ago, we demonstrated that it also acts as a pharmacological chaperone in CEP, correcting the specific activity in deleterious mutations in UROIIIS. We also produced an oral formulation that is devoid of gastrointestinal problems, a common feature in the oral administration of ciclopirox. Here, we will briefly discuss the advances of the development of this drug for the treatment of CEP, currently in phase I/II as an oral formulation.

ANNA E. MINDER
CHANTAL PELTENBURG

AFAMELANOTIDE FOR VARIEGATE PORPHYRIA RELATED SKIN SYMPTOMS

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Background

Variegate porphyria (VP) is a rare autosomal-dominant acute porphyria that can also manifest with cutaneous symptoms on light-exposed skin. Whilst there are treatments available for acute manifestations, there are no approved medicinal products for the prevention of phototoxicity and related dermatological symptoms. We evaluated the efficacy and safety of afamelanotide to reduce disease severity and related symptoms and improve quality of life (QoL).

Methods

This proof-of-concept, phase IIa, open-label trial was conducted in Switzerland and the Netherlands. All 6 patients received a subcutaneous implant containing 16mg of afamelanotide every 28 days (a total of 6 implants). Disease severity was measured by the clinical global impression of change (CGIC), patient global impression of change (PGIC), skin condition score (from the VP-QoL), investigator global assessment using an 11-point Likert-type visual analogue scale (VAS IGA). New lesions were counted by the physicians. The duration of sun exposure (patient daily diary) and adverse events (AEs) were recorded over a 196-day period. QoL was assessed via the VP-derived QoL in Epidermolysis Bullosa (VP-QoLEB) questionnaire, VP QoL and the WPAI:GH. Adverse Events were recorded throughout the study.

Results

Disease severity was markedly reduced, with 'much improved' median CGIC and PGIC scores of +2.5 by the end of treatment (EOT) on day 168. There was a median VAS

IGA score change of 3.0-points, with patients exhibiting reduced 'mild fragility' with 'no fresh wounds/blisters', and 'near to no fragility' of the skin of the face, hands and forearms following treatment. The daily hours of sunlight exposure increased until Day 112 whilst the number of new lesions reduced from a median of 10.0 at baseline to a median of 2.5 lesions by the EOT. The VP-QoL showed a reduction in the median total fragility by 12 points and an improved skin condition median score by 19.5 points by EOT. The VP-QoLEB improved following afamelanotide by a median of 12.0-points and the WPAI:GH median improvement on regular daily activities by 3.5 points by EOT. Related AEs were mild to moderate and largely in keeping with the existing safety profile of afamelanotide; one SAE occurred during the study but was assessed to be unrelated to the study drug.

Conclusions

Afamelanotide was well-tolerated, demonstrated a favourable safety profile and was associated with an improvement in disease severity and QoL in patients with VP.

BINDU D. PAUL

ROLE OF THE BILIRUBIN/BILIVERDIN REDUCTASE AXIS IN NEUROPROTECTION

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Bilirubin is one of the most frequently measured metabolites in the blood, yet its exact functions in vivo are still obscure. Biliverdin reductase A (BVRA) is the main biosynthetic enzyme for bilirubin and a component of heme catabolism responsible for converting biliverdin to bilirubin. While the antioxidant and anti-inflammatory roles of bilirubin in vitro and in peripheral tissues have been well studied, the functions of bilirubin in the brain have been less explored. We show that bilirubin, being lipophilic, protects the lipid rich compartments of cells and prevents lipid peroxidation and plays a complementary role to the protective effects of the major water soluble antioxidant, glutathione (GSH), which predominantly protects the hydrophilic compartments. The brain is lipid-rich and metabolically highly active and is especially susceptible to lipid peroxidation. We have shown that mice lacking BVRA display elevated lipid peroxidation, mitochondrial dysfunction and compromised ability to neutralize free radicals and are highly susceptible to neuronal damage. Furthermore, we have shown that bilirubin directly scavenges superoxide radicals (O2·−) generated during mitochondrial respiration and mediates neuroprotection. Additionally, we show that BVRA, mediates synaptic signaling through the focal adhesion kinase (FAK). Thus, studying the actions of BVRA and bilirubin will yield deeper insights into a novel and hitherto underappreciated neuroprotective pathway in the brain, which can be harnessed to develop therapeutics for neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and stroke.

PETRO E.PETRIDES

TOWARDS A REAL WORLD EVIDENCE (RWE) IN THE TREATMENT OF ACUTE PORPHYRIA WITH GIVOSIRAN

Petro E.Petrides

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'Randomized clinical trial' (RCT) evidence (ENVISION trial with strict inclusion and exclusion criteria) is the Gold standard which has lead to the regulatory approval of Givosiran and the incorporation of this novel treatment into practice. 'Real-world evidence' (RWE) which refers to the safety and efficacy of Givosiran in daily clinical practice (in unselected patients with different comedications, comorbidities or organ impairments) is necessary to supplement the experiences obtained by RCTs. RWE with Givosiran has until now only been reported by the French group with 25 patients (Poli et al, Mol Gen Metab 2022) and several individual case presentations (e.g. Graff et al, Front Genetics 2022). Following up our initial observation of a severe acute necrotizing pancreatitis in a Givosiran treated patient (Petrides et al, Ann Hematol 2021) and its possible origin (drug induced, homocysteine induced, idiopathic ?) we are still intrigued by this complication. Lipase elevation and pancreatitis has been observed in RCT (Kuter et al, J Hepatol 2023) as well as RWE (Poli see above). I will discuss a 46 year old male who became symptomatic at the age of 40 years. Two years later we diagnosed his AIP (PBG-Deaminase 10,9 nmol/l/s (13,3-24,7); Genotyping: c973C>T in Exon15). Abdominal ultrasound revealed two gallstones. After several sporadic attacks treated with hemin he was offered Givosiran; after the first injection his situation did not improve but got worse (abdominal pain, nausea, loss of power) as seen by others (Ma et al, 2022). At the same time lipase (> 3 x UN), creatinine and homocysteine rose despite vitamin B6 supplementation. The next two injections were reduced by 50% but his state did not change so that therapy was discontinued. I will discuss gallbladder and pancreas interactions and a potential interference of Givosiran in this situation to explain some of symptoms.

We have recently analyzed RWE with Momelotinib, a novel JAK2 inhibitor in myelofibrosis (a rare hematological disorder) in 60 patients treated in 16 centers in Germany (Jilg S. et al. MoReLife – Momelotinib in Real-Life…. Ann Hematol, in press) and confirmed its role in daily clinical practice. In a similar way a multicentric, multinational prospective observational study should be initiated for Givosiran (GivRealLife-Analysis) which addresses efficacy, safety, dosing, adherence and drug survival of this important therapy.

PATRICIA RENARD

A ROLE FOR HEME SYNTHESIS AND SUCCINATE IN THE REGULATION OF PLURIPOTENT STATES TRANSITIONS

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Embryonic stem cells (ESCs) have been stabilized in vitro under two main distinct developmental states : a naïve pre-implantation stage and a primed post-implantation stage. Mitochondria undergo deep morphological and metabolic modifications during the naïve-to-primed transition: the naïve stem cells have few roundly-shape mitochondria with poorly developed cristae but they use both glycolysis and oxidative metabolisms. Surprisingly, although the mitochondria of primed cells are more elongated with more developed cristae, no oxygen consumption is detected in those cells.

Beside the oxidative phosphorylation, we have shown that another partly mitochondria-localized metabolic pathway, the heme synthesis pathway, is also important for the transition from naïve to primed state. Indeed, the exit of the naïve state is impaired upon blockade of the heme biosynthesis pathway linked to the incapacity to activate MAPK- and TGFβ-dependent signaling pathways.

In addition, the heme synthesis inhibition promotes the acquisition of 2 cell-like cells features in a heme-independent manner. This phenomenon is caused by a mitochondrial succinate accumulation as the inhibition of heme synthesis provokes the accumulation of the substrates of this pathway, including succinate. We have shown that in these conditions, succinate accumulates not only in mitochondria, but also in the other cell compartments and even leaks out of the cell. The extracellular succinate acts as a paracrine/autocrine signal, able to trigger the 2 cell-like reprogramming through the activation of its plasma membrane G-protein coupled receptor, SUCNR1.

Overall, this study unveils a new mechanism underlying the maintenance of pluripotency under the control of heme synthesis.

ELIANE SARDH

USE OF GIVOSIRAN IN ACUTE HEPATIC PORPHYRIAS: EXPERIENCE FROM SWEDEN AND FRANCE

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Background:

Givosiran (Givlaari®) is an RNA interference therapeutic that targets hepatic ALAS1 mRNA, and approved for the treatment of adults with recurrent acute hepatic porphyria (AHP) defined as 4 or more acute attacks per year. The clinical trials that led to approval effectively demonstrated that givosiran prevents recurrent acute porphyria attacks based on a fixed dose schedule of 2.5 mg/kg every 4 weeks and that clinical efficacy increases with continued dosing up to 36 months. However, there are several questions on how to use givosiran in clinical practice.

Methods:

We retrospectively analysed the long-term follow-up of 47 recurrent AHP patients treated with givosiran in two expert centres in France and in Sweden.

Results:

22 Swedish and 25 French recurrent AHP patients were included in this retrospective observational study. 9 Swedish patients and 7 French patients were included during the Phase 1/2 and 3 studies of givosiran. The median age at onset of disease was 30 years for the French patients (15-42) and 32 years for the Swedish patients (19-44). The median time of recurrent disease before treatment start was 2,7 years for the Swedish patients (0,2 – 22) and 15.7 years for the French patients (2 – 34). All Swedish patients were dosed monthly for the first six months and after that at dosing intervals between

every six- eight week depending on patients' availability. Eighteen of the 22 Swedish patients have discontinued treatment with givosiran. Two patients discontinued due to severe adverse events (anaphylaxis and severe LFT increase), one patient due to severe nausea, 15 patients when being clinically and biochemically stable for at least 12 months. When analyzing time to remission, two treatment groups could be identified; the delayed group that required about 4 years to reach remission and the immediate group that reached remission after about 2 years on treatment. Further analysis showed that the delayed group were those who had had the most severe disease. The sustained efficacy of givosiran in most French patients enabled to adapt the frequency of administration in accordance with monthly ALA level measurements. In 42% of patients, givosiran was administered only when heme precursor levels were increasing. Two patients discontinued, one due to a severe adverse event of acute pancreatitis and the other because of a desire to become pregnant. In both the Swedish and French patient group, the most common adverse effects were ALT elevations, fatigue, nausea and hyperhomocystinuria.

Conclusion:

Givosiran significantly reduced attack rates in both cohorts. These results show that the dosing schedule for givosiran can be individualized based on the patient's clinical and biochemical response and that treatment can be stopped when the patient has been in clinical and biochemical remission for >12 months. The time to reach remission depends on the severity and duration of the disease before starting givosiran.

MIGUEL SOARES

UNVEILING AND TRANSLATING THE EVOLUTIONARY CONSERVED ROLE OF HEME IN MALARIA

Miguel Soares, PhD

Instituto Gulbenkian de Ciência

Malaria is an ancestral disease caused by parasites from the *Plasmodium* genus. Beyond its profound impact on human evolution and social development, malaria remains a major medical and socio-economic hindrance in geographical areas where disease transmission persists.

Plasmodium parasites invade, develop and replicate in the red blood cells of their infected hosts. To mitigate the oxidative stress imposed by the redox activity of the iron contained in the prosthetic heme groups of hemoglobin, the parasite converts heme into redox-inert hemozoin crystals. This peculiar strategy contrasts with the canonical heme detoxifying pathway of virtually all other living organisms, where heme is cleaved into biliverdin and bilirubin, via two independent reactions catalyzed by heme oxygenase 1 and biliverdin reductase A, respectively. The reason why *Plasmodium spp.* parasites evolved to avoid this evolutionary conserved pathway is not understood.

Here I will present experimental data supporting the idea that the canonical heme detoxifying is highly deleterious to blood stages of *Plasmodium spp.* Moreover, I will present how this evolutionary vulnerability can be exploited therapeutically to target blood stages of *Plasmodium* and limit malaria severity and mortality.

PENELOPE E. STEIN

BASIC NEEDS IN COUNTRIES WHERE THERE IS A LACK OF SPECIALIST PORPHYRIA SERVICES

Penelope E Stein

The diagnosis and treatment of porphyria is a significant challenge in developing countries, in common with other rare diseases. While there is increasing awareness of the burden of rare diseases globally, patient outcomes are often poor. Poverty and poor access to healthcare are major obstacles, as well as difficulty obtaining a timely and accurate diagnosis, lack of experienced healthcare providers and lack of access to high-cost treatments, all of which are compounded by limited resources. Moreover in many low income countries, the priority of rare diseases is simply eclipsed by the need to focus on basic healthcare issues such as maintaining nutrition and preventing communicable diseases.

The Acute Porphyria International Support Group is a new working group of the International Porphyria Network. Its aim is to offer support to healthcare professionals looking after patients with suspected acute porphyria in countries where specialist care is not available. This may include arranging for biological samples to be couriered to a porphyria laboratory in Europe or the North America for diagnostic testing without charge, management advice and better access to treatments. A summary of our experience will be presented.

EMANUELA TOLOSANO

INHIBITING HEME SYNTHESIS TO MODULATE CELL METABOLISM AND COUNTERACT TUMOR INITIATION AND PROGRESSION

Emanuela Tolosano

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Metabolic rewiring is a hallmark of malignant cell transformation, facilitating tumor initiation and progression. Heme plays a significant role in regulating cell metabolism, not only as the prosthetic group of proteins and enzymes involved in aerobic respiration but also because pathways controlling heme homeostasis are intricately linked with those governing nutrient utilization.

Mounting experimental and epidemiological evidence suggests that dysregulated heme homeostasis accelerates the development and progression of various cancers. Specifically, heightened heme biosynthesis is a recognized characteristic of cancer, emerging as a crucial in vivo dependency in KRAS-mutated Non-Small Cell Lung Cancer (NSCLC) and Pancreatic Ductal Adenocarcinoma (PDAC).

Through genetic and pharmacological interventions, we demonstrated that inhibiting ALAS1, the first and rate limiting enzyme in the heme biosynthetic pathway, in cellular and animal models of NSCLC and PDAC led to substantial metabolic alterations. This included constraining the glycolytic phenotype of KRAS-mutated tumor cells and fostering oxidative metabolism. This metabolic transition correlated with impaired tumor initiation, as well as reduced tumor cell proliferation and dissemination.

In conclusion, our findings identify ALAS1 as a crucial factor in regulating the metabolic rewiring that sustains tumor growth and dissemination, thus advocating for its targeting as a therapeutic strategy.

IÑAKI F. TROCONIZ

COMPUTATIONAL APPROACHES FOR MAPPING HEME BIOLOGY

Iñaki F. Troconiz

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The heme biology represents a complex system that comprises several elements that interact each order through enzyme governed synthesis and degradation processes that are tightly regulated by feedback mechanisms. The role of the heme system is of extraordinary importance with regard key physiological mechanisms, and therefore its dysregulation is associated to severe disease conditions.

Understanding systems as the hemo represents a difficult challenge due the highly dynamic and variable environment. In that particular context, the building of computational multiscale models which merge treatment and disease features mechanistically through the integration of data from multiple sources represents an appealing approach to increase disease understanding and to accelerate discovery and development. This integrative perspective takes particular relevance in the case of certain alterations of the heme pathway resulting in rare diseases as the acute intermittent porphyria where the development of new therapeutics is hampered by the limited knowledge of the disease pathophysiology and lack of eligible patients for clinical trials.

Several models developed in the pre-clinical arena for different therapeutic alternatives (eg., mRNA based) will be presented. Those models are capable to unravel several mechanisms in the heme pathway, such as mRNA translation, increased PBGD activity in liver, and haem precursor metabolism) as well as the regulation in ALA synthesis from data limited mostly to amount of different hemo precursors measured in urine in 24 h. intervals.

Those above mentioned processes will be quantitatively characterized by the corresponding model parameters, that are used to simulate time course of the key components of the heme pathway in different in-silico scenarios showing the translational impact of the quantitative approach.

BRUCE WANG

NATURAL HISTORY OF ACUTE HEPATIC PORPHYRIAS IN US PATIENTS

Bruce Wang
(San Francisco, US)

Background: The longitudinal study (LS) of the porphyrias is a NIH funded natural history study of the porphyrias in the United States. The goal is to assess the natural history, biochemical changes, therapeutic effects, and potential association with other medical conditions. To date more than 400 patients with acute hepatic porphyrias (AHP) have been enrolled, with some patients followed for more than 10 years. We have previously described the baseline characteristics of the first 108 AHP patients. Here we provide an update to describe the baseline characteristics of more than 400 AHP patients. Methods: From 2009 to 2023, 404 patients of AHP were enrolled in the LS, including AIP (330 cases), VP (49 cases), HCP (24 cases), and ADP (1 case). Clinical data including demographic characteristics (gender, race, ethnicity, family history), signs and symptoms, genetic testing, biochemical parameters (ALA, PBG, urine/serum creatinine, AST, ALT), as well as treatment history involving Hematin and Givosiran was entered into a centralized database. Selected features were compared to normal population values using various statistical analysis tools, including the Chi-Square test, T-test, and Pearson correlation. These analyses were performed within the SPSS Statistics 27.0.1 environment. Results: Among 404 patients in the study, 85% (345) were white, 80% (322) were females, and, mostly were diagnosed within ages of 33.1±14.58. Patients were categorized based on their clinical presentation and frequency of attacks into 3 groups: recurrent (4 or more attacks annually), sporadic, and asymptomatic. Asymptomatic patients were further separated into those with abnormal ALA/PBG and those with normal ALA/PBG. We looked for clinical correlation between these groups with biochemical factors, DNA mutation, and diversity and severity of symptoms. The most common symptoms associated with acute attacks were abdominal pain, nausea, weakness, heartburn, and high blood pressure, and their incidences were significantly increased in porphyria compared to normal population as control. AHP diagnosis remains delayed, with a mean of 9 years after the onset

of symptoms, though this is improved from our earlier report. Forty one percent (167) of patients have received intravenous Hematin, the majority of whom had recurrent acute attacks. Such a delayed diagnosis would significantly increase the occurrence of chronic medical conditions including chronic kidney disease with different stages (mean estimated GFR=72.00) over the period before the diagnosis of porphyria. Conclusion: AHP remain undiagnosed for many years following the onset of symptoms. This delayed diagnosis can impact the quality of life and lead to the development of chronic medical conditions over time. A comprehensive understanding of the porphyria population in the United States can assist healthcare providers in achieving earlier diagnoses and implementing appropriate management strategies.

JESSICA ZUCMAN-ROSSI

MOLECULAR MECHANISMS IN HEPATOCARCINOGENESIS

Prof. Jessica Zucman-Rossi

Centre de Recherche des Cordeliers, Université Paris Cité

Hepatocellular carcinomas develop after a multistep process of tumorigenesis from normal hepatocyte with a crucial point at which malignant transformation and then cancer progression occur. In this process, activation of oncogenic pathways are key alterations that can be promoted by environmental exposure or endogenous conditions in the body such as hormonal or metabolic dysregulations. Moreover, malignant transformation of the cells is modulated by the constitutional genetic background of the individuals, local cell injury, such as inflammation, and the immune response. Another level of complexity is the nature of cell at the origin of the cancer development that will impact the phenotype of the resulted cancers. We have a specific focus on the liver because it is an organ particularly exposed to toxics, that can regenerate and in which different types of tumors can develop defined by their histological features. Specific types of malignancies are developed at different ages with progressive a progressive increase incidence starting with rare hepatoblastoma in children, then fibrolamellar carcinoma in young adults and hepatocellular carcinoma that are the most frequent after 60 years old. Also, the differential role of gender (with female developing more benign tumors and male more cancers) and the metabolic syndrome that contribute to both benign and malignant tumorigenesis is primordial to take into account. The specific role of HMBS mutation in hepatocarcinogenesis within the general context of HCC molecular mechanisms.

Biosketch

Jessica Zucman-Rossi, MD, PhD was trained in internal medicine and oncology. Her research area mainly investigates the **genetics and biology of cancers and benign tumors**, in adults and children. Her team has a major interest in understanding the **relations between exposure to toxic agents, diet habits, genetic predisposition, chronic diseases, including viral infections**, and their cooperation in the mechanisms of carcinogenesis. They discovered genetic predispositions to benign and malignant liver tumors, including HNF1A in adenoma, WNT3A/WNT9A, and AAV2 insertions in hepatocellular carcinoma. They also performed **seminal discoveries** in identifying **tumor molecular classifications and their translation into the clinical care of patients**. Her team is strongly interested in deciphering the cell plasticity and heterogeneity of liver cancers and finding new therapeutic targets in these diseases.

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