EndoCompass Neuroendocrinology

Pituitary Diseases and Neuroendocrine Neoplasms (NEN)

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1. Introduction

Pituitary disease can manifest across the whole lifespan and includes a variety of pathologies, from congenital hypothalamo-pituitary disorders to pituitary tumours and other lesions in this area. The rarity of many of these conditions not only poses diagnostic and management challenges but it also impacts research efforts to understand their pathogenesis and factors implicated in their prognosis. Comprehensive understanding of the mechanisms of tumour development, regrowth and aggressive behaviour remains deficient, disadvantaging the discovery of more effective therapeutic agents. Congenital hypothalamo-pituitary disorders, with the plethora of their phenotypic manifestations, require further elucidation of their underlying pathophysiology, as does the often-daunting hypothalamic syndrome. Finally, the application of technical and methodological advances (precision medicine, preclinical models, machine learning and artificial intelligence) comprises a promising challenge, in that their expected contribution to the progress of translational research features as a major priority in modern neuroendocrinology.

Neuroendocrine neoplasms (NEN) are malignancies arising from the diffuse endocrine system, which is comprised of neuroendocrine cells dispersed throughout the bronchopulmonary and gastrointestinal tracts. Incidence and particularly prevalence rates of these neoplasms are steeply rising, making gastroenteropancreatic NEN the 2nd most prevalent malignancy in the gut and the 10th most prevalent malignancy overall [1, 2]. NEN diagnosis and treatment are both challenging because of their heterogeneous biological behaviour, with more than 60% being metastatic at diagnosis. A major unmet need is the paucity of curable/effective therapeutic options. The only treatment that offers cure is surgery, possible in few patients with localised disease, while unresectable NENs are treated with a variety of non-curable options (somatostatin analogues, peptide receptor radioligand therapy, targeted agents, or chemotherapy), which eventually fail due to drug resistance, stressing the need for new therapeutic avenues [3]. Another unmet need is the lack of reliable biomarkers to guide management [4], as patients with the same tumour grade/stage behave heterogeneously in terms of time to tumour progression, tumour recurrence or treatment response.

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2. Investigate mechanisms of tumourigenesis and possible new treatment options in adult pituitary disease

i. *Explore genetic and epigenetic factors, including the role of chromatin architecture and regulatory DNA sequences in pituitary and hypothalamic disorders*

[redacted]

a) *Introduction to area and state of the art*

Understanding the interplay of genetic and epigenetic factors is essential to improve diagnosis and treatment in patients with pituitary adenomas and arginine vasopressin deficiency (AVP-D). The majority of pituitary adenomas are sporadic, while ~5 % are familial or isolated that often present at younger age and have poorer prognosis [1,2]. Recurrent somatic variants in *GNAS* and *USP8* exist in ~40% of somatotrophinomas and ~35 % of corticotropinomas respectively [1]. Aggressive tumours show relatively high prevalence of *TP53, ATRX* and *SF3B1* variants [3,4]. Epigenetic modifications in pituitary adenomas are cell lineage-dependent and less explored [5,6]. Similarly, copy number variations and chromosomal gains and losses in pituitary adenomas are more likely histotype-specific [5]. AVP-D is characterized by genomic alterations on genes involved in the vasopressin/aquaporin axis [7.8].

b) *Future research priorities*

There is need for refined diagnostic procedures for the early diagnosis of familial cases and timely prediction of aggressive tumour behaviour and prognosis. Technical advances are expected to allow us to elucidate the mutational landscape of pituitary tumours beyond *GNAS* in somatotropinomas and *USP8* in corticotropinomas. In the long term, we need to intensify the research on the epigenetic mechanisms that underpin the pathophysiology of pituitary adenomas, focusing on lineage-specific patterns of gene demethylation and chromatin accessibility. We also need to decipher the genetic alterations contributing to hypothalamic disorders. Given the complexity and rarity of cases, interdisciplinary collaboration is essential to effectively address these challenges.

c) *Anticipated impact of these priorities*

Advanced research in pituitary and hypothalamic disorders will have a major impact on understanding their pathophysiology and translating to effective clinical management. Early detection of familial cases of pituitary adenomas, hypothalamic disorders and aggressive behaviour of tumours is essential for timely intervention and improved patient outcome. Unraveling the complex genetic and epigenetic mechanisms underlying these disorders is expected to lead to earlier, more accurate diagnosis and personalised treatment strategies that are effective and with fewer side effects. These advancements will facilitate the precision management of patients with pituitary and hypothalamic disorders.

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ii. *Examine the tumor microenvironment in pituitary tumour pathogenesis and clinical utility.*

[redacted]

a) <u>Introduction to area and state of the art</u>

Beyond genetics and epigenetics, the environment within which a tumour exists is defined as tumour microenvironment (TME) and includes immune and stromal non-tumour cells, vascular structures, and non-cellular components (extracellular matrix, ECM) in a cross-talking continuum. The TME opened a new area of research to shed light not only to the pathophysiology of neoplasms but also to the identification of druggable components of the TME [1]. Recently, the TME has been intensely investigated in pituitary tumours particularly the subset which display an aggressive clinical course with rapid progression and resistance to conventional treatments. Some of the druggable cellular or non-cellular components of the TME have been used in solid tumours as well as in aggressive pituitary tumours. Hence, TME components may have a clinical utility not only as druggable targets but also as prognostic biomarkers for resistance to treatment, leading to more efficient management [2, 3].

b) <u>Future research priorities</u>

The future research priorities in studying TME are based on the investigation of: 1) new TME biomarkers that will identify the more aggressive pituitary tumours in the context of proliferation, the pituitary tumours resistant to conventional treatment; 2) genetic, histopathological and molecular imprints, which enable selection of appropriate drugs to be used in each case as monotherapy or as combination therapy, the timing of early management before progression and the most effective sequence of each agent to be used; 3) artificial intelligence (AI) to build an algorithm for individualised treatments, using a combination of omics, epigenetics andTME factors, including the immune component[4, 5] .

The methodology to achieve the above goals includes the establishment of a biobank (solid and liquid biopsies), as well as common European databases with basal information on patients and characteristics of pituitary tumours, including all the biomarkers studied. Thereafter, randomised, multicentric studies will need to establish the factors influencing aggressiveness and resistant to treatment [6].

c) *<u>Anticipated impact of these priorities</u>*

Immunotherapy and anti-angiogenic therapies that target TME components have been attempted in few published cases of aggressive pituitary tumours, with positive outcomes. The rationale of using these therapies is based on the central premise that the TME impacts the pathophysiology of pituitary tumours. On the other hand, the use of common cenrtalised European pituitary tumour biobanks and databases will help to facilitate and accelerate the identification of the TME components, that can be used more efficiently as prognostic and predictive biomarkers or as druggable targets for pituitary tumour management.

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iii. *Investigate (new) treatment options for resistant prolactinomas, non-functioning pituitary adenomas and Cushing's disease particularly in setting of recurrence*

[redacted]

a) <u>Introduction to area and state of the art</u>

1. Prolactinomas

Resistance to dopamine agonists (DA) treatment is uncommon in microprolactinomas and more frequent in macroprolactinomas and invasive tumours. Primary DA resistance occurs in around 20–30% and 10% of the cases treated with bromocriptine or cabergoline, respectively [1]. Secondary DA resistance is rare and is usually associated with aggressive or malignant tumour behaviour [1]. Management approaches in resistant prolactinomas include changing DA, surgery, radiotherapy, temozolomide (in aggressive tumours) or combinations of these; however, these approaches are not always successful [1-4].

2. Non-functioning pituitary adenomas (NFPAs)

NFPAs have a prevalence of 7-41.3/100,000 population and are associated with various morbidities and increased mortality [5-6]. Surgery combined, or not, with radiotherapy are the main means of treatment aiming to safely remove the tumour and stop its regrowth; despite these approaches, regrowth occurs in several patients (the risk of which is currently defined by clinical and pathological parameters), whereas a subset of these tumours demonstrates aggressive behaviour [7-9]. Following tumour regrowth, repeat surgery and/or radiotherapy are the first line approaches and DA have been tried for prevention of NFPA growth [7, 10-11].

3. Cushing's disease (CD)

CD is associated with increased morbidity and mortality [12]. After surgery, the recurrence rate is around 30%. For those cases, radiotherapy and combination drug therapy, targeted for biochemical control (steroidogenesis-inhibitors, glucocorticoid-receptor-blockers) and tumour control (pasireotide, DA) are available. The latter have only limited effectiveness and, in some cases, severely impairing side effects. In therapy-refractory cases, aggressively growing corticotropinomas and ACTH-secreting carcinomas, there have so far only been off-label therapy trials with temozolomide, and individualised therapy attempts with immune-checkpoint-inhibitors, tyrosine-kinase-inhibitors and angiogenesis-inhibitors, all potentially associated with serious side effects [9]. With the discovery of the activating mutation in the *USP8* gene and changes in regulators of the cell cycle (cyclin-dependent kinases) in CD, there are now potential molecular targets available [13-14].

b) <u>Future research priorities</u>

1. Prolactinomas

- Understand molecular mechanisms involved in resistance to DAs.
- Develop new alternative medical treatments and investigate the efficacy and safety of those proposed to date (e.g., estrogen-receptor antagonists, aromatase-inhibitors, pasireotide, lapatinib, everolimus, immune-checkpoint inhibitors).

2. NFPAs

- Understand mechanisms implicated in NFPA regrowth after primary treatment.
- Identify molecular prognostic factors associated with regrowth.
- Identify targets for pharmacotherapy that will prevent regrowth or be used as primary medical treatment, particularly for tumours not requiring prompt surgical intervention.

3. Cushing's disease

- Identify further molecular mechanisms involved in corticotroph tumours.
- Further investigate existing drugs for molecular targets (i.e., seliciclib) and develop new, tumour-targeted and well-tolerable, ideally oral therapy for recurrent CD.

c) <u>Anticipated impact of research</u>

In all of the above types of tumours, the outputs of the proposed research will improve management pathways through expanding treatment options and applying personalised medicine. In the long term, they will have a positive impact on the outcomes and prognosis of the patients.

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iv. *Identify molecular factors associated with invasive or predicting aggressive behavior in pituitary adenomas and develop targeted therapies*

[redacted]

[redacted]

[redacted]

a) Epidemiology, societal impact, research state of the art

The prevalence of invasive and aggressive pituitary tumours varies widely depending on the definition/criteria used and on the type of patients included. Nevertheless, aggressive pituitary tumours are rare; a recent study concluded that around 0.5% of all clinically detected pituitary tumours have an aggressive course [1]. On the other hand, invasive pituitary tumours are frequent (14-40% of surgically treated cases) [2, 3]. Both invasive and aggressive pituitary tumours incur increased morbidity and mortality.

Radiological evidence of tumour extension into the cavernous sinus is not synonymous with invasion *per se*, as this extension can correspond solely to tumour expansion [4, 5]. Moreover, the modified Knosp classification, widely used to define invasiveness, was only intended to predict the probability of intra-operatively observed cavernous sinus invasion and the surgical outcome [6]. Thus, prior to identifying molecular factors associated with invasiveness, clear definitions are needed.

Predicting aggressive behaviour remains challenging. A combined clinicopathological classification has proven its value in predicting progression or recurrence following surgery [7], but remains nevertheless limited in predicting future aggressive behaviour. Recently, a Ki67 index ≥10% has been proposed to predict future malignancy [8], but its value has not been yet proved.

Identification of targeted therapies, including combinations, along with establishing predictors of response to treatment, are most needed, especially following temozolomide failure.

b) Future research priorities

1. Homogenise how “invasiveness” and “expansion” are defined both for clinical and research purposes.
2. Investigate whether 1) “invasiveness” and/or 2) “expansion” are associated with worse prognosis, independent of the fact that invasiveness/expansion leads to incomplete surgical removal of the tumour.
3. From a biological point of view, investigate whether true “invasiveness” has a different molecular and cellular basis compared to simple “expansion”. Dissect these mechanisms to identify a marker usable in clinics, as well as new therapeutic targets. We strongly recommend the use of precision technological approaches (e.g., single-cell transcriptomics, as opposed to bulk transcriptomics), followed by validation at the protein level, and ideally, outcomes further investigated by mechanistic *in vitro* and/or *in vivo* studies. The application of other cutting-edge technologies (such as single-cell multi-omics, spatial transcriptomics etc.) is strongly encouraged.
4. Given the rarity of aggressive pituitary tumours and carcinomas, it is of utmost importance to create pan-EU or at least national databases (with clinical, pathology and molecular data) coupled with biobanks that are as comprehensive as possible (tumour tissue, blood) in order to 1) understand tumour biology, 2) identify therapeutic targets, and 3) identify predictive/prognostic markers. Including all surgical specimens available in the biobank (initial tumour, recurrences, metastases) will be crucial to 1) investigate at which point the tumour phenotype has changed and hence, test whether the identified markers of aggressiveness

could serve as predictive makers, prior to clinical evidence of aggressive behaviour, and 2) study potential predictors of response to different treatments.

5. Similar to the above, a combination of multi-omics studies and wet lab approaches should be applied, ideally within consortia with both strong bioinformatics and wet lab expertise.
6. Test the identified predictive markers of future aggressiveness on large cohorts of both aggressive and benign pituitary tumours with a long follow-up (at least 5 years), in order to assess the prevalence and the specificity of the marker. While waiting for additional markers, one marker already proposed in the literature and simple to test would be an initial Ki67 index ≥10% (see above).
7. Given the rarity of patients requiring therapeutic options beyond temozolomide, pan-EU or at least national multi-centric studies are needed to assess the efficacy and the potential predictors of response to such treatments.

c) Anticipated impact of future research

From a biological perspective, this research is expected to increase the understanding of the tumourigenesis-related processes linked to invasiveness and aggressiveness. From a clinical perspective, this research is expected to inform future guidelines and to directly benefit the patients, leading to personalised approaches, decreased morbidity and mortality, and increased quality of life and survival.

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3. Investigate mechanisms of disease and possible new treatment options in pediatric pituitary and neuroendocrine disease

[redacted]

i. Congenital hypothalamo-pituitary disorders

a. Epidemiology, societal impact, research state of the art

Congenital hypopituitarism (CH) is characterised by single/multiple pituitary hormone deficiencies (MPHD), often associated with midline brain/facial/eye and/or forebrain abnormalities including Septo-Optic Dysplasia (SOD) and holoprosencephaly. It affects 1:3000-1:4000 children with an incidence of SOD in Europe of 1.9-2.5 per 100,000 births. Patients with CH, particularly SOD, can manifest autism, complex behavioural disorders, sleep dysregulation, and hypothalamic dysfunction including inexorable obesity. The impact on their families and wider society can be considerable, with few patients able to take up full time employment, and many likely requiring lifelong supervised care. There is an increased risk of mental health problems including self-harm.

Research suggests that CH arises from abnormal hypothalamo-pituitary development. Variants in over 67 genes have been identified, but account for only 10% of patient phenotypes. These genes encode transcription factors, signalling molecules, eukaryotic translation initiation factors, and components of splicing machinery. Inheritance can be X-linked, autosomal dominant or recessive with variable penetrance [1, 2].

While SOD represents a spectrum of malformations with unpredictable endocrine and neuroimaging phenotypes, CH associated with MPHD is more homogeneous [3]. Stalk and posterior pituitary abnormalities increase the risk of early-onset deficits.

b. Future research priorities

There needs to be a greater understanding of the phenotypic heterogeneity observed, including growth in the absence of GH, unusual patterns of GH secretion, evolution of hormonal deficiencies and pubertal development and obesity in SOD. Clinical features such as impaired olfaction, pain insensitivity and autonomic dysfunction need to be studied in more detail. Improved neuroimaging techniques with better visualisation of the hypothalamus and pituitary stalk may help predict clinical/metabolic outcomes and aid targeted therapy. Long-acting GH and glucocorticoids may improve quality of life, alongside treatments for hypothalamic obesity.

Research should also focus on mental health, neurocognition, behaviour, autism, Attention Deficit Hyperactivity disorder, aggression with self-harm, and sleep disorders.

Additionally, given that the majority of cases remain genetically unexplained, we need to improve our understanding of the aetiology of CH using comprehensive, unbiased modern multi-omic techniques including genomic, epigenomic, transcriptomic, and proteomic approaches with the inclusion of artificial intelligence.

c. Anticipated impact of future research

Better understanding of the underlying pathophysiology and underlying molecular basis will not just improve genetic counselling, but also lead to improved prediction of the clinical trajectory of endocrinopathies and associated hypothalamic dysfunction, with better clinical outcomes.

ii. Acquired hypothalamo-pituitary disorders

a. Epidemiology, societal impact, research state of the art

Acquired hypothalamo-pituitary dysfunction can be caused by (supra) sellar tumours or their treatment, traumatic brain injury, infection, autoimmune processes, granulomatous disease, iron overload or vascular insults. Endocrinopathies and hypothalamic dysfunction can evolve, causing significant morbidity and mortality. The close proximity to the optic chiasm may cause associated visual impairment. The combination of hypothalamo-pituitary dysfunction, visual impairment and neuro-behavioural disturbance can severely impact the quality of life of patients and their families, with significant socioeconomic cost, and increases the risk of mortality [4, 5]. Current research focusses heavily on the genetic aetiology of these tumours to identify novel therapeutic targets.

b. Future research priorities

Targeted therapies may aid in limiting the role of surgery and radiotherapy (e.g. craniopharyngiomas [6], Cushing disease) or in overcoming treatment resistance (e.g. prolactinomas, somatotropinomas). Elucidation of tumourigenic pathways may aid development of newer, less toxic treatments. Additionally, more intensive treatment may be offered for tumours deemed high risk at a molecular level. Neuroimaging needs to improve to achieve more accurate diagnoses and to grade hypothalamic tumoural invasion and the degree of disruption of the surrounding hypothalamic neurocircuitry in order to increase the precision of treatment strategies. New therapies also need to be developed for other rarer causes of acquired hypothalamo-pituitary dysfunction such as hypothalamitis and hypophysitis, and sarcoidosis or granulomatous disease.

c. Anticipated impact of future research

Improved neuroimaging and molecular techniques will improve diagnostic accuracy and risk stratification of acquired hypothalamic-pituitary lesions [7], as well as the development of new treatments for tumours in this region, with fewer off-target effects, reducing the injury incurred by

neurosurgery and radiotherapy on healthy brain tissue. Inclusion of markers of hypothalamo-pituitary dysfunction in clinical trials for novel oncology treatments will clarify the effect of such interventions on long-term quality of life. Overall, this will improve progression-free and event-free survival, reduce toxicity and adverse effects on the hypothalamo-pituitary axis, optimise metabolic and reproductive outcomes, and ultimately improve the quality of life of survivors and their families.

iii. Hypothalamic syndrome

a. Epidemiology, societal impact, research state of the art

The "hypothalamic syndrome" encompasses a range of clinical features: dysregulated appetite and thirst, inexorable morbid obesity, multiple endocrinopathies (including ACTH and AVP deficiencies), thermoregulation issues, fatigue, behavioural, mental health, and sleep difficulties [8-11].

Due to our currently incomplete understanding of the complex neuroendocrine hypothalamic circuitries, many of these problems remain untreatable with resulting morbidity and disability, with a consequent burden on health systems as well as national economies. .

Recently, newer molecular techniques have revealed the components of the hypothalamic circuitry, and the importance of hypothalamic hormones in appetite and weight regulation [8]. Correlation of function with neuroimaging markers has been difficult as conventional MRI cannot precisely define the hypothalamic nuclei.

b. Future research priorities

At present there are no universal biochemical or neuroradiological diagnostic criteria for the hypothalamic syndrome. Development of more precise neuro-endocrine, genomic and epigenomic markers to pinpoint pathogenic changes need to be developed, to better understand and treat the various domains of hypothalamic dysfunction. Similarly, the aetiology of other congenital hypothalamic syndromes such as ROHHAD(NET) needs establishing. Advanced neuroimaging is needed to more precisely define hypothalamic damage and determine how this correlates with the phenotypes observed. Prospective intervention studies are needed for the treatment of hypothalamic obesity and fatigue. The high rate of neuropsychological disturbances, autistic spectrum and mental health disorders associated with hypothalamic syndromes also need to be better understood to develop effective interventions.

c) *Anticipated impact of future research*

The development of successful therapies for hypothalamic obesity will reduce long-term complications such as cardiovascular morbidity and type 2 diabetes. Identifying neuroradiological correlates of hypothalamic dysfunction will allow the development of medical treatments and reduce the risk of complications by improving the precision of neurosurgery and radiotherapy. Understanding the nature of congenital hypothalamic syndromes such as ROHHAD(NET) will aid early diagnosis, genetic counselling, and development of targeted therapies for this life-limiting condition. Overall, achievement of these research priorities will improve the long-term quality of life and reduce long-term mortality for these patients as well as the significant socioeconomic burden on health and social care systems.

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4. Technical and Methodological developments to boost translational research in pituitary and neuroendocrinology

a) Develop precision medicine approaches for pituitary tumours (e.g., identify markers predicting pituitary tumour response to surgery or medical therapies)

[redacted]

a) *Introduction to area and state of the art*

Precision medicine for pituitary tumours involves tailoring diagnosis, treatment, and monitoring strategies to the individual characteristics of each patient and their tumour. This requires identification of biomarkers guiding patient treatment based on individual conditions, as it is being pioneered in acromegaly [1]. Evolution of image guidance and visualisation technologies in pituitary surgery, including intraoperative MRI suites and biologic imaging strategies will be critical in this regard [2,3]. Indeed, endoscopic transsphenoidal approach has improved earlier surgical outcomes [4]; yet pituitary tumour anatomical complexity and heterogenous biology challenge their management, demanding multimodal integrating surgery, radiosurgery, radiation therapy, and medical therapy [5].

b. Future research priorities

Precision pituitary medicine development will require genomic sequencing of each tumour to identify specific mutations, gene expression patterns, and molecular signatures, and to predict their impact on

tumour behaviour. This could facilitate attaining a true clinico-molecular classification and stratification of tumours and to identify molecular targets in specific tumour subtypes. Circulating biomarkers (e.g., hormones, cytokines, metabolite levels) could leverage this approach, jointly informing on tumour progression, invasion, and therapy response, improving patient monitoring and prognosis prediction. Advancing this strategy requires developing algorithms integrating clinical, molecular, and imaging data to stratify patients into risk groups based on tumour aggressiveness, recurrence potential, and treatment responsiveness, tailoring surveillance protocols and treatment interventions according to individual risk profiles. Additionally, targeted therapies will have to be developed, such as small molecule inhibitors, monoclonal antibodies, or peptide receptor ligands, selectively disrupting tumour growth and hormone secretion while minimising off-target effects. This will only be possible through advanced integrative computational models able to predict treatment outcomes, recurrence risk, and long-term prognosis for individual patients, while enabling algorithm refinement by machine learning from patient outcomes and real-world data. Naturally, this necessitates interdisciplinary and international collaborations among researchers, clinicians, patient advocates, and industry partners.

c. Anticipated impact of future research

Integrating these strategies will accelerate discovery and translation and boost precision medicine approaches for pituitary tumours, which can revolutionise their management and offer personalised diagnostic, therapeutic, and prognostic solutions tailored to the unique characteristics of each patient. Implementation of precision pituitary tumour medicine will impact the future of research, healthcare systems and patient care by providing a more accurate diagnosis and prognosis prediction tools to help tailoring treatment. Also, molecular profiling can lead to more effective and personalised therapies, improving patient outcomes and quality of life and helping to overcome treatment resistance by targeting molecularly targeted therapies to each tumour. Overall, the future impact of precision medicine on pituitary tumours promises to be transformative, offering patients more personalised and effective treatment options while advancing our understanding of tumour biology and improving healthcare outcomes.

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b) *Development of preclinical models: organoids, human cell lines and animal models to model hypothalamic and pituitary pathophysiology*

[redacted]

a) *Epidemiology, societal impact, research state of the art*

Establishing *in vitro* or *in vivo* models to study hypothalamus/pituitary function is inherently challenging due to the complexity of this neuroendocrine axis, which involves intricate interactions between diverse cell types and multiple hormones, regulated through dynamic feedback mechanisms. Accurately modeling these interactions in the laboratory requires precise control of differentiation and functional maintenance of specific cell types. Pituitary tumour cell models first established in 1968 (rat GH3, mouse AtT-20; later, rat MMQ cells) are still some of the few cell lines widely used in research despite the limitation of their rodent origin [1]. Numerous attempts to develop human cell lines have failed to faithfully replicate any specific type of pituitary tumour. To circumvent this, labs commonly employ primary cell cultures derived from human pituitary tumours, a more pathophysiologically relevant model compared to immortalised cell lines, although with limited lifespan and high variability [2, 3]. Fortunately, recent advances in cell culture techniques, stem cell and organoid research facilitated the development of human and murine pituitary organoids derived from embryonic stem cells, induced pluripotent stem cells, fetal or adult stem cells [4]. Indeed, various models are currently available for the normal and affected pituitary, including hypopituitarism, craniopharyngioma and anterior pituitary tumours, especially corticotropinomas and prolactinomas, which may serve to evaluate drugs, assess tumour growth and restore pituitary function [5]. Similar approaches have been employed for hypothalamic studies [6], and, although no perfect models can recapitulate pituitary/hypothalamus dysfunction, several recent models can be used depending on the disease [7]. *In vitro* systems lack the capacity to mimic the complex feedback loops and environmental influences (stress, nutrition, circadian rhythms), further limiting hypothalamic-pituitary studies. Nevertheless, 3D chips are being developed to emulate hypothalamus-pituitary crosstalk [8].

b. Future research priorities

Future research should prioritise the development of pituitary and hypothalamic organoid models to better replicate the complex cellular interactions and dynamic feedback mechanisms inherent to these systems. Advances in stem cell technology and bioengineering must be leveraged to create more accurate and functional *in vitro* models. Additionally, developing 3D culture systems and organ-on-a-chip technologies will be crucial to simulate physiological conditions such as stress, nutrition, and circadian rhythms, as well as enabling detailed investigation of disease mechanisms, leading to the discovery of new therapeutic targets and more effective treatments. Efforts should be focused on creating human-derived cell lines to improve translational relevance and facilitate personalised medicine applications for neuroendocrine disorders.

c) Anticipated impact of future research

Development of more accurate and functional models should revolutionise the understanding of pituitary and hypothalamic pathophysiology. These advancements will improve drug screening processes, reduce the dependence on animal models, and facilitate personalised treatment strategies. Ultimately, this research holds the potential to restore pituitary function in patients with related disorders, significantly improving their quality of life and clinical outcomes.

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c) Use of machine learning and artificial intelligence in basic research and clinical management of pituitary diseases

[redacted]

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a) *Epidemiology, societal impact, research state of the art*

Artificial intelligence (AI) and machine learning (ML) have gained momentum in recent years as integrative techniques for diagnosis and intervention in pituitary diseases, enabling more holistic and multidimensional approaches and managing the increasingly large data associated with clinical and basic research [1]. For clinical research, the main fields of ML application are predictive algorithms for tumour aggressiveness and clinical outcomes of surgery and medical treatment [2, 3]. Algorithms may become helpful tools in complex differential diagnoses, but differences in training sets could affect the replicability of results. For basic research, ML is usually applied to pathomic and transcriptomic studies.

b. Future research priorities

Two critical issues in ML studies are appropriate sample size and algorithm/model homogeneity. Many different algorithms have been used in pituitary research to train datasets with a broad range of subjects included. Moreover, predictive algorithms are often designed to replicate clinician-made evaluations (texture, development of pituitary deficiencies), which limits their impact on clinical decisions. Surgical outcomes are usually center-specific. With larger, collaborative datasets, radiomic studies could help unveil other disease-specific characteristics of pituitary neoplasms. Moreover, longitudinal studies with standardised follow-up intervals might help identify patients who require more proactive interventions than those in which “watchful waiting” could be a reasonable approach.

For basic research, pathomic studies could provide insightful results on pituitary neoplasm recurrence and aggressiveness and patient response rate to standard medical regimens. Algorithm analysis of pathological images, ideally integrated with clinical and transcriptomic data, could help identify reliable predictors guiding treatment and follow-up regimens. Moreover, identifying neoplasm-specific biomarkers in peripheral blood could help develop reliable “liquid biopsies,” allowing less invasive diagnosis and surveillance.

Complex ML techniques such as neural networks are often burdened by a lack of explainability, which is a mainstay of transparency in the use of AI algorithms in healthcare. However, ML-assisted clinical decisions will become increasingly prevalent in the upcoming decades. Therefore, the aim should be to combine data from different countries and settings, minimising training-related biases such as the underrepresentation of minorities.

c) *Anticipated impact of future research*

Patient-centered precision medicine is the future of healthcare, where each person will be characterised by an increasingly larger amount of data. Patients with pituitary disorders face numerous challenges during their lifespan, often with little knowledge of what their path will be at diagnosis. Predictive algorithms could help healthcare professionals identify “high” and “low” risk patients, probability of remission, and complications development, empowering people with pituitary disorders to be part of the decision-making process. Moreover, ML techniques could help bridge knowledge gaps in rare pituitary diseases by unveiling new patterns and developing novel therapeutic strategies.

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5. Understanding Neuroendocrine Neoplasm (NEN) biology

i. Predictive and prognostic biomarkers and how to improve preclinical/experimental models in NEN

[redacted]

a) *Epidemiology, societal impact, research state of the art*

Neuroendocrine neoplasms (NENs) are divided into well-differentiated neuroendocrine tumours (NETs) and poorly differentiated neuroendocrine carcinomas (NECs) [1]. The most important prognostic biomarker of NENs is Ki-67, marking cycling cells, a crucial tool for grading [2]. For diagnostic purposes, alterations in TP53 and RB1 are considered biomarkers of NEC [3]. In contrast, MEN1, DAXX, and/or ATRX mutations are typically associated with NETs in some districts [4]. DNA methylation profiling has recently been proposed as a powerful source of diagnostic and prognostic biomarkers [5]. With the introduction of new therapeutic strategies into the clinic, new biomarkers with predictive value are urgently needed.

b. *Future research priorities*

Beyond the distinction between NETs and NECs, the entire NEN category should be intended as a continuous entity, without the well-demarcated boundaries imposed by classification needs. Thus, one of the most crucial research priorities is to address the large grey areas within each neoplasm subgroup, such as the G2 category, which includes tumours with totally different proliferation and clinical behaviour. In this category, the most advanced next-generation techniques should be implemented, including single-cell omics, including epigenomics, and transcriptomics [6]. Furthermore, spatial transcriptomics for investigating the tumour microenvironment represents one of the promising methodologies to further explore this heterogenous tumour context [7].

Historically, improvements in the establishment of experimental models of NET have been modest. Cell lines derived from different NETs were generated, but most cell lines are only partially representative for their NETs of origin. Similar issues relate to the use of NET animal models. NET xenograft models are difficult to establish and generally slow-growing [8]. In recent years, promising 3D spheroid and organoid models of mainly high-grade NET and NECs were established [9, 10], but there is still an unmet need for preclinical models of low-grade NETs. The majority of NET models do not address tumour heterogeneity or incorporate the complex tumour micro-environment. Future research should address these issues, i.e. by 3D techniques and novel technologies such as tumour-on-a chip [11].

c. Anticipated impact of future research

Developments in molecular and preclinical research in the NENs field are expected to foster the progress in understanding the mechanisms underlying neoplasm formation, and, ultimately, to fundamentally improve their diagnostics, therapeutic methods and patient outcomes. Thus, identification of new biomarkers accompanied by design of reliable molecular tests, and advances in anatomical and functional imaging techniques will improve the current methods to diagnose and monitor NENs and to predict their progression. Likewise, advancements in treatment strategies, including new therapeutic targets in the field of biotherapy, molecular targeted therapies, radioisotope therapies, chemotherapy and immunotherapy, as well as combinations of these therapies, should provide more effective treatment options, especially for advanced NENs. Additionally, innovative surgical techniques, including minimally invasive methods will foster improved outcomes. However, as we embrace the era of precision medicine, personalised, individualised treatment approaches based on genetic tests will become available to optimise therapy selection and sequencing, and thereby minimise side effects. Most importantly, research and technological advances will focus on the patient, leading not only to better treatment outcomes and enhanced survival rates, but also to an improvement in the quality of life of patients. In summary, research advances should facilitate a better understanding of NEN pathobiology and leverage the development of improved and early diagnostic tools and more effective treatments, ultimately benefiting NEN patients and healthcare systems alike.

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6. How to Improve Therapy in NEN

[redacted]

a) *Epidemiology, societal impact, research state of the art*

The existing treatment options in advanced NEN are limited, highlighting the unmet need for novel therapeutic approaches. One of the new options includes immune-based treatments which have been at the epicenter of cancer therapy in recent years. Immunotherapy has been evaluated in NENs of different origin and differentiation but with limited efficacy shown only in some high-grade NENs, and particularly as combination therapy [1]. Another option includes cancer vaccines either based on tumour-associated or on tumour-specific antigens. CEA-antigens, and chimeric antigen receptors (CARs) are under development [2]. The molecular profile of patients has also been exploited with limited success in rare individual cases where druggable mutations have been identified [3]. Additionally, the identification of the preferred sequence in the existing therapies represents another major unmet need, and despite some first insights from recent studies, a general approach to be applied in clinical practice is missing [4].

b) *Future research priorities*

Future research priorities in NEN treatment are based on personalised medicine, specifically focusing on the investigation of 1) new biomarkers to identify the disease and appropriate timing of early management before progression; 2) patient-derived organoids for closer to real-life drug efficacy screening; 3) pathological and/or molecular imprints to individualise the sequence and combinations of treatments (omics, immunological, epigenetic and microenvironmental factors). The methodology should include the establishment of biobanks (including solid and liquid biopsies) inside a common European network of expert centres, to integrate information on patients and their respective NENs including disease features and treatment sequence/outcomes. Moreover, this approach will allow to perform randomised, multicentric studies focusing on molecular profiles pre-/post-surgery and pre-/post-therapies, by investigating omics, metabolic alterations, cancer immunology, microenvironment and to map the different neoplasms. Integrating the use of novel models such as organoids, 3D tumour cultures and xenografts into this research network will enable testing of novel drugs and potential cancer vaccines. Finally, artificial intelligence (AI) can help pinpoint the right algorithms for combination and sequential therapies for each patient-group.

c. Anticipated impact of future research

The establishment of a common European biobank and registry network will facilitate the identification of novel prognostic and predictive biomarkers, of right timing and sequencing of existing drugs, as well as of novel efficient individualised approaches, ultimately improving NEN patients' prognosis and quality of life.

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7. Fibrosis in NET (from mesenteric fibrosis to carcinoid heart disease)

[redacted]

a) Introduction to area and state of the art

NET retain the ability to secrete hormonally active substances and consequently to elicit hormonal symptoms in patients. The most common hormonal syndrome, encountered in approximately 20% of NET patients, is the carcinoid syndrome (CS), which is characterised by secretory diarrhoea, flushing and fibrotic complications [1], and reduced quality of life compared to patients without CS [2, 3]. CS is predominantly encountered in patients with a metastatic NET originating from the small intestine, followed by lung, pancreas, ovary, or thymic origin. Although serotonin is considered a critical mediator of the CS, its precise pathophysiology and determinants are incompletely understood [4].

Importantly, fibrotic complications may be observed in patients with NET, usually within the context of the CS, but not exclusively, and include mesenteric fibrosis (MF) and carcinoid heart disease (CHD). MF is a pathognomonic feature of mesenteric metastases of small intestinal NET, characterised by radiating fibrotic strands from a central mesenteric mass. Local fibrosis can induce venous ischemia, ileus, obstruction, and perforation of the bowel and is associated with an impaired overall survival. CHD, encountered in 20-30% of patients with CS, is characterised by right-sides heart valve and endocardial fibrosis, potentially leading to tricuspid valve and pulmonary valve regurgitation and stenosis and ultimately right-sided heart failure with increased mortality and morbidity. In both MF and CHD, there appear to be a crucial role for local and/or systemic NET cells derived serotonin and activation of its 5-HT2b receptor [5]. Besides serotonin, several other pro-fibrotic factors, such as prostaglandins, histamine, bradykinin, tachykinins (substance P, neurokinin A, neuropeptide K) or growth factors (transforming growth factor-β (TGF-β), platelet-derived growth factor, connective tissue growth factor) have been postulated to contribute to the development of NET-associated fibrosis. Moreover, mitochondrial dysfunction reportedly also contributes to the fibrotic process, but the specific mechanisms of mitochondrial involvement in this setting are unclear. The use of 5-HIAA, a serotonin metabolite, for patients at risk of CHD diagnosis and follow-up, is possible only in patients

with serotonin hypersecretion, within a specific diet and laboratory constraints. To this end, there are no specific/sensitive markers to assess the presence, severity, and risk of fibrosis in clinical practice to date.

b) Future research priorities

A better understanding of the pathophysiological mechanisms within the tumour cell and its micro-environment will help to better delineate the therapeutic targets for NET-associated fibrosis. Specifically, for CHD, we proposed to the priorities to be:

- Establish a multinational, multidisciplinary working group to study basic, translational, and clinical aspects of CHD by combining patient databases, imaging records and biobank samples of blood and tissue.
- Identify NET-secreted endocrine factors and local cellular and paracrine mediators of CHD in *ex vivo* and *in vitro* experimental models.
- Improve clinical risk stratification of CHD by combining patient and tumour characteristics, circulating biomarkers and imaging features.
- Develop therapeutic strategies for effective identification and prevention of CHD (progression) in high-risk patients with CS through RCTs.
- Determine the optimal timing of surgical valve replacement for CHD.

For MF, we propose to the priorities to be:

- Develop of an experimental model that successfully recapitulates the tumour micro-environment.
- Identify tumour-/ patient-specific factors within the tumour micro-environment that determine the development of MF.
- Develop a risk assessment tool to identify which patients with MF require preventive surgical resection.
- Discover novel medical targets to prevent progression of MF and test these in multicentric RCTs.

c) Anticipated impact of these priorities

Given the paucity of current knowledge on the pathophysiology of NET-associated fibrosis, these future research endeavors will increase our understanding of the most relevant molecular and cellular contributors. Together with superior risk stratification for the development or progression of fibrosis, they will lead to the first interventional clinical trials aimed specifically at treating/preventing NET-associated fibrotic complications. This will ensure high-grade evidence needed to change clinical practice and improve patient outcomes.

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8. Conclusions

There is no doubt that hypothalamo-pituitary disease remains enigmatic in many of its aspects, ultimately affecting patients' prognosis and outcomes. The many unmet gaps in research described above require attention and action from funders, policy makers, health care systems, clinicians, and academics for the benefit of those living with these conditions in the years to come.

Likewise, despite some recent advances in the field of NEN diagnosis and treatment, there is still limited improvement in the real-life approach to these diseases and the related patient outcomes. International funding should focus in promoting further research to fill the gap existing today between our knowledge and the heterogeneous and still unsolved biological behavior of these rare cancers.