ESE Research Roadmap/EndoCompass chapter by cancer working group: Endocrine causes of cancer and Endocrine consequences of cancer

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Introduction

There are unambiguous links between endocrine agents and various cancers. The two most common cancers in Europe are the hormone-dependent cancers, namely estrogen receptor (ER) positive breast cancer, which comprises 75% of all breast cancer cases and prostate cancer, the most common cancer in men. These two major cancers are mediated by endogenous endocrine pathways, but their initiation and progression can be influenced by a myriad of other variables. The association between physiologic and environmental endocrine agents and cancer initiation is complex and varied, but what is also apparent, are endocrine-related health issues that are a direct consequence of cancer (or the treatments used for cancer patients). As such, malignant tumor growth is both a consequence of endocrines, as well as the cause of endocrine disorders and diseases. These complex, but critical issue will be discussed, with a focus on the impact of these issues on society and the requirements needed to address these clinical problems. Specific recommendations for future research will be provided to tackle these health-related problems.

Subtopic 1. Endocrine-related cancers.

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Section 1.1 Estrogen Receptor positive breast cancer

1.1.1 Background information and the clinical problem. Breast cancer accounts for 30% of all newly diagnosed cancers in women in Europe and Estrogen Receptor (ER) positive breast cancers represent 3 out of every 4 cases (Stearns and Davidson, 2014; Allison et al, 2020). In these hormone-dependent breast cancers, the developmental ER transcriptional pathway re-emerges later in life to initiate tumor formation and disease progression. It is known that other endocrine pathways can impinge on ER activity, in particular pregnancy-associated hormones although the link with cancer risk has been a complex and controversial topic for many years. The use of synthetic progesterone-based compounds called progestins in various female physiological contexts, such as the contraceptive pill and hormone-replacement therapy (HRT) have been transformative. Progestins mimic the endogenous hormone progesterone and activate an intracellular receptor called the Progesterone Receptor. Historical epidemiological data from HRT trials suggested that the inclusion of a progestin in HRT could increase a women's risk of breast cancer by two to three times (Chlebowski et al, 2003; Chlebowski et al, 2010). The immediate impact was substantial, with HRT usage declining by 40-70% in European countries (Antoine et al, 2016), as a result of the purported link between progestins and breast cancer risk, meaning that many women dealt with the debilitating menopausal symptoms, to avoid the breast cancer risk associated with HRT use. Pre-clinical work supported these findings and suggested that progestins could be pro-tumorigenic and could promote breast cancer. The 18-year follow-up on the HRT trials (Manson et al, 2017) has suggested that the initial conclusions were incorrect and that overall survival is not affected by the use of progestins and that the perceived risk of getting breast cancer and the implied increased mortality, was not accurate. In addition, recent pre-clinical data has suggested that any progestin-mediated cell growth from is a consequence of promiscuous compounds that activate Androgen Receptor (AR) and that pure PR ligands pose no risk (Shamseddin et al, 2021). Epidemiological data from France has supported this concept for years: namely that pure PR agonists are protective, whereas mixed ligands that can also activate other nuclear receptors like AR, can be associated with increased breast cancer risk (Fournier et al, 2005; Fournier et al, 2008). The perception of PR and its agonist ligands as cancer promoting agents is not accurate and paradigms in the field need to be re-evaluated, particularly in light of the fact that PR

agonists have been shown to be effective cancer treatments, but have fallen out of favour because of the inaccurate perception around the risks associated with these ligands.

1.1.2 Recommendations for future research. A better understanding of the role of these ligands is required. Critically, future work needs to make the distinction that progestins should not be grouped as one class of molecules and instead, progestins should be separated based on those that are pure PR agonists and those that have cross-reactive activity on other nuclear receptors. The reclassification of progestins that are specific to PR and those that can activate other hormonal receptors (such as AR) needs to be considered as separate entities and their utility should be re-evaluated and exploited for physiological and pathological use where appropriate. Based on these issues, the following recommendations are being proposed:

1. A need to re-evaluate different classes of progestins, to assess pure PR ligands as a separate entity, when compared to 'progestins' that can also regulate other hormone receptors, particularly AR and Glucocorticoid Receptor (GR). These agents need to be reclassified and studied (biologically and clinically) as distinct entities. Re-education of the public around the genuine breast cancer risks, when using these different agents in HRT formulation.
2. Investment into fundamental research to assess the contradictions observed with hormone receptor ligands for the treatment of ER+ breast cancer. A consortium-based approach to identify the most physiologically-relevant models for studying hormonal cross-talk in ER+ breast cancer. The use of these agreed models to directly assess PR agonists vs antagonists as treatments, as well as AR agonists vs antagonists as treatments in ER+ breast cancer. An evaluation of the clinical context that is most appropriate for these specific endocrine ligands. A specific emphasis needs to be placed on the potential clinical utility of these agents in metastatic disease, which represents the unmet clinical need.
3. Investment into new approaches for treating ER+ breast cancer, that does not involve additional approaches to block the ER itself (i.e. SERMs, SERDs, PROTACs) or downstream effectors of the ER pathway (i.e. CDK4/6 inhibitors). This would involve a better understanding of cross-talk between ER and other nuclear receptors (NRs), so that existing ligands for other NRs can be effectively exploited to indirectly modulate ER activity. This work would also involve the identification of upstream regulators of ER that can be therapeutically targeted. These would likely include ER-associated pioneer factors and epigenetic proteins that contribute to *cis*-regulatory elements that can ultimately be used by ER for transcriptional activity. Therapeutic interventions that target proteins functionally upstream of ER will likely bypass known treatment resistance mechanisms.

Section 1.2 Prostate cancer:

1.2.1 Background information and the clinical problem. Prostate cancer represents the tumour type with the highest incidence among the european male population (Globocan 2022). On a early stage, most of these tumours (>95%) are localized (prostate-confined) and androgen receptor (AR) positive adenocarcinomas, arising from the luminal-epithelial prostatic cells. Given the AR-driven nature of this disease, androgen deprivation therapy (ADT), consisting on the systemic inhibition of the androgen production, represents the main pharmacological approach for treating prostate cancer. ADT is especially effective when combined with radical therapeutic approaches such as surgery and/or radiotherapy (Messing et al., 2006; Denham et al., 2019; Kishan et al., 2022). Unfortunately, approximately 20% of the patients undergoing ADT eventually develop resistance, progressing to a phenotype known as castration-resistant prostate cancer (CRPC). Despite the development of therapeutic strategies in recent years, such as AR signaling inhibitors (ARSI) like the androgen precursor synthesis inhibitor "abiraterone" and the second-generation AR inhibitors "enzalutamide," "apalutamide," and "darolutamide," CRPC remains a lethal disease, due to its progression to these drugs. Notably, a substantial body of data from published clinical trials demonstrates that an

intensified inhibition of the AR-driven oncogenic program through ADT+novel ARSI yields clinical benefits compared to ADT alone. This is observed across patients in both castration-sensitive (e.g., LATITUDE, STAMPEDE, ENZAMET, ARCHES, TITAN, EMBARK) and castration-resistant (e.g., AFFIRM, PREVAIL, PROSPER, SPARTAN, ARAMIS) settings (Westaby et al., 2021). Molecular data obtained from these and various other clinical trials, along with findings from experimental approaches, highlight that the main resistance mechanisms to ADT and ARSI involve the restoration of AR signaling through gain-of-function AR gene amplification, AR gain-of-function mutations, upregulation of constitutively active AR splice variants, increased intratumoral androgen biosynthesis, or the bypass of AR by switching to other receptors (e.g., Glucocorticoid Receptor) to drive transcription of a subset of AR-regulated genes (Watson et al., 2015). However, there is also a subset of tumours which get transdifferenciated into an AR-negative or AR-independent status, a phenotype also called neuroendocrine prostate cancer, which is associated with a pronouncedly poor prognosis. While AR-negative neuroendocrine prostate cancer plays a crucial role in ARSI resistance, since this subset does not express the ARSI target, there remains a lack of consensus regarding its prevalence. For instance, a study of 150 CRPC patients reported over 96% of patients exhibiting adenocarcinoma histology, with only 2.9% showing adenocarcinoma with neuroendocrine differentiation (Robinson et al., 2015). Conversely, a study by Small et al. revealed a different landscape, with RNA-sequencing analyses of biopsies from CRPC patients resistant to abiraterone or enzalutamide showing only 33% displaying adenocarcinoma phenotype, while 12% had neuroendocrine features and 27% exhibited an intermediate type distinct from either neuroendocrine or adenocarcinoma (Small et al., 2015). Considering the challenge in targeting the aggressive phenotypes that emerge in response to ARSI, it has been suggested that reducing treatment selective pressure may offer a potential therapeutic approach. Strategies such as intermittent versus continuous ADT for castration sensitive prostate cancer patients have been proposed, with reported inconclusive results (Hershman et al., 2016; Aggarwal et al., 2024). Therefore, despite the demonstrated clinical benefit of hormonal therapy for prostate cancer patients, there is still a need for optimization to address the associated progressive therapy-resistant phenomena.

1.2.2 Recommendations for future research. AR continues to be the primary therapeutic target for prostate cancer. However, a deeper understanding of the resistant mechanisms driving persistent AR signaling is essential to identify novel therapeutic approaches aimed at preventing or overcoming ARSI resistance. Additionally, further research efforts are warranted to elucidate the biology of transdifferentiated neuroendocrine prostate cancer, with the aim of uncovering effective therapeutic targets for this phenotype. Addressing these two objectives is hampered by the considerable challenge posed by intratumour and intra/interpatient heterogeneity. Therefore, additional research, including single-cell analyses across various settings, and taking into accound clinical and patient-related factors (e.g., metabolic status), is imperative. In light of these considerations, the following recommendations are proposed:

1. Investment in innovative strategies to disrupt persistent AR signaling and treat advanced prostate cancer. Delving deeper into our understanding of AR splicing, particularly the biology of AR-V7 (recognized as the most clinically relevant AR splicing variant since it lacks the ligand binding domain targeted by AR inhibitors), emerges as a critical research focus. Therefore, comprehensive research efforts are warranted to identify novel approaches that prevent the emergence or suppress constitutively active AR splicing variants. For example, inhibitors of the AR and AR-V7 common N-terminal domain (e.g., EPI-7170) and PROteolysis TArgeting Chimeras (PROTACs) have been recently developed and their clinical efficiency is being evaluated in ongoing clinical trials. Additionally, exploration of other promising areas of interest should prioritize the discovery of novel regulators of upstream AR transcriptional activity, such as pioneer factors and AR/AR-V7 co-regulators. These approaches hold promise

for influencing AR activity regardless of its copy number, protein levels, and/or mutation status.

2. Considering clinical factors, particularly metabolic status, is crucial for clinical decision-making in prostate cancer management. Prostate cancer predominantly affects elderly males (>60 years old), often coinciding with comorbidities like metabolic conditions (e.g., diabetes) and concomitant medication use, which can impact prostate cancer microenvironment and treatment outcomes. Given the pronounced regulatory endocrine component of this tumour type, understanding how the metabolic status of the patients influences prostate cancer biology is paramount and should be considered for clinical decisions. Furthermore, while some studies suggest that metabolic-related drugs may exert antitumour effects on prostate cancer, further fundamental investigation and clinical data are necessary to fully understand the underlying molecular mechanisms and elucidate their clinical efficacy. Bridging this gap between fundamental research and clinical translation is essential for integrating metabolic interventions into prostate cancer treatment strategies effectively.
3. Identifying novel targets for translating into the development of therapeutic strategies to effectively address CRPC remains a critical unmet clinical need. Despite recent advancements, such as the approval of PARP inhibitors for tumours with DNA repair deficiencies and the promise of AKT inhibitors for PTEN loss tumours, substantial research is still required to identify better predictive biomarkers for selecting sensitive populations and guiding clinical decision-making. Moreover, it is imperative to explore alternative oncogenic pathways beyond AR in prostate cancer. Certain tumours, like neuroendocrine prostate cancer, circumvent AR signaling, emphasizing the necessity for innovative, long-lasting approaches and the discovery of predictive biomarkers to benefit prostate cancer patients.
4. Overcoming the immunosuppressive microenvironment in prostate cancer remains a significant challenge. While immunotherapy has revolutionized cancer treatment in certain tumour types, its efficacy in prostate cancer is hampered by factors such as poor expression of immune checkpoint proteins and the presence of a strongly immunosuppressive tumour microenvironment. Although sipuleucel-T has been approved for prostate cancer treatment, its clinical utility is limited according to the IMPACT clinical trial. Current perspectives in this field focus on two main strategies: 1) identifying global and effective immune targets for prostate cancer treatment, and 2) Turning immunesuppressive into inflamed prostate cancer microenvorenment.

Subgroup 2: Acute and Late endocrine effects of childhood cancer or its treatment, including check point inhibitors

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Late endocrine complications after treatment for childhood and young adulthood cancer

Improved survival for childhood cancer has resulted in a new population of childhood cancer survivors, currently 500 000 in Europe, with a survival rate of ~81%, (Bhotta et al 2022). Childhood cancer survivors (CCS) are at increased risk of treatment-related late complications, with a cumulative burden of 4.7 severe complications per survivor at age 50, including endocrine complications, cognitive dysfunction and cardiovascular disease (Suh E, Stratton KL, Lancet Oncology, 2020). Endocrine sequele are among the most common late complications and up to 50% experience at least one endocrine disorder among children and young adults treated for childhood cancer (Mostoufi-Moab S et al. 2016). The risk of long-term complications depends on the tumour type and location, age at diagnosis, oncological treatment, and genetic polymorphism (Brignardello et al. Eur J Endocrinol 2013, Sanch PC et al. 2017 Endocrinol Diab Nutr). The highest risk is seen in CCS of CNS-tumours, leukemias, bone tumours and Hodgkins disease. The most common endocrine complications are hypothalamic-pituitary insufficiency, hypothyroidism, thyroid cancer, gonadal dysfunction, low bone mineral density and

metabolic syndrome (Casano-Sancho P et al. 2022 Cancers). New directions in research include novel risk-prediction strategies, genetic predisposition , the development of improved early detection strategies (< 5 years after diagnosis) and new interventions to prevent the long term consequences of endocrine complications such as short stature, obesity, cardiovascular disease and psychological impairment. The lack of randomised controlled trials for surveillance and treatment of endocrine complications in CCS is challenging as the treatment protocols are changing over time and comparison between different treatment eras is complicated. The aim for future research should include to identify the survivors at high-risk for late toxicity through the use of a panel of biomarkers. This will develop precision medicine, which will enhance survivors outcomes and minimize unnecessary treatment. Childhood cancer is a rare disease, including many different diagnoses and treatments, which calls for action to gather European researchers to build an infrastructure with large cohorts of CCS for research.

Hypothalamic-pituitary disorders after cancer treatment

Hypthalamic-pituitary (HP) disorders are frequently reported in childhood cancer survivors (CCS) (Clement et al. 2016 JCO, van Iersel 2022 Endocrine Reviews) and are associated with impaired growth, pubertal development and reproductive function. Cranial radiotherapy is the primary cause of HP dysfunction as a late complication (van Iersel et al, Endocrine Reviews 2022; van Santen 2020, Chemaitilly 2019 Laughton et al. 2008. JCO). Tumour location and surgery involving the HP region result in early onset of HP dysfunction. Radiation-induced HP dysfunction may appear months to many years after radiotherapy (Laughton et al. 2008. JCO, van Iersel 2022), with GH deficiency in most cases being the first and ACTH deficiency being the last pituitary hormone to be deficient. Diabetes insipidus is not a late effect of cancer treatment but may occur during cancer treatment due to the tumor or neurosurgery. If DI newly occurs in the late effects clinic it may be a sign of tumour recurrence warrenting additional investigations.

Exposure to cranial radiotherapy is less common today in non CNS survivors and prophylactic CNS radiotherapy in ALL survivors is no longer a routine treatment (Link et al. JCEM). Further, therapy with protons instead of photons may affect the prevalence of HP dysfunction by reducing the scatter to normal tissue (Eaton BR 2016 Neuro-oncol). Few studies have reported chemotherapy as a cause of HP dysfunction, but this was not confirmed in larger cohorts (Bakker B 2004 BMT; van Iersel 2022). However, targeted chemotherapy such as imatinib, tyrosine kinase inhibitor (TKI) and immune system modulators may cause pituitary dysfunction (Corsello SM 2013 JCEM), for which surveillance is advocated . Hypothalamic dysfunction, resulting in pituitary dysfunction plus obesity, sleep disturbance , temperature dysregulation and behanioural problems, may be severe in children after treatment for suprasellar tumors and may aggravate in time . Known risk factors are tumor location and extent of neurosurgery. The effect of radiotherapy on the hypothalamus is greatly unknown. Hypothalamic obesity will be discussed in the ENDoCompass chapter on hypothalamic-pituitary disorders in more detail.

Future research priorities:

- To study the prevalence of hypothalamic dysfunction following radiotherapy (fotons or protons) during childhood

- To study the need for prolonged follow up; when to stop screening for HP dysfunction?

- To study the effect of targeted therapies during childhood on HP dysfunction.

- To study the consequences of HP dysfunction in CCS on metabolic health, bone health, psychosocial development, sexual performance and fatigue.

- To perform collaborative (European) studies collecting CCS

Thyroid disorders after cancer treatment

Thyroid disorders, such as hypothyroidism, hyperthyroidism, thyroid nodules and thyroid cancer, are frequent after treatment for childhood cancer (Mostofi-Moab S 2016 JCO, Clement et al, Cancer Treatment Reviews 2018, doi: 10.1007/s00259-022-05762-4). Primary hypothyroidism is one the most frequent reported complications after radiotherapy to the neck, especially in Hodgkin lymphoma with a prevalence of up to 50% (Sklar et al. 2000 JCEM). MIBG treatment for childhood neuroblastoma may result in hypothyroidism, thyroid nodules and thyroid cancer. Chemotherapy, such as busulphan and cyclophosphamide, has been associated with hypothyroidism (Bakker B 2004 BMT). Further, TKI has been shown to cause hypothyroidism (Lodish MB 2013 JCEM). Hyperthyroidism can present after neck or craniospinal radiotherapy (Mostofi-Moab S 2016. JCO). Thyroid cancer that occurs as secondary cancer after childhood cancer treatment may require a different approach than primary thyroid cancer due to previous exposure to toxic treatment, differences in genetic background or individual experiences of the patient (van Santen and Verburg , Eur J Endocrinol. 2020). For screening of secondary thyroid cancer , a shared decision model was proposed.

Future research priorities:

- To study the mechanism of TKI-induced hypothyroidism.

- To study the longterm TKI-induced complications

- To study the effect of shared decision making on surveillance for secondary thyroid cancer

- To develop a IGHG recommendation for surveillance of thyroid dysfunction in CCS

- To update the IGHG recommendation for surveillance of thyroid cancer in CCS

Safety of growth hormone therapy

Concerns have been raised regarding the influence of hormone replacement on cell growth and metabolism in CCS, however current consensus guidelines have stated to consider GH treatment to be safe in GH deficient disease free CCS (Boguszewski 2022 Europ J Endocrinol). Published clinical guidelines on hormone replacement are not specific to CCS as they need to take into consideration risks of tumor recurrence and secondary malignancy (Sklar CA 2018 JCEM).,).

So far, no association has been found between GH therapy in GH deficient CCS and tumor recurrence or secondary neoplasia (endocrine socjety 4, 25, Savendahl et al, Tentourier et al, Sklar CA 2018 JCEM, Scottish guideline network SIGN 2013, van Iersel L 2022 Endocrine reviews). Consensus was found to start GH after 3 months in children with benign disease such as cranoipharyngioma, and after one year for treatment after malignant disease. Researchgaps include when to start GH therapy in GH deficient children after completion of cancer treatment, which dose of GH to aim for, the effect of long-acting GH on growth and tumor recurrence in CCS and GH therapy in children who receive maintenance treatment such as with TKI. The benefit of GH therapy in adult CCS on body composition, bone health, metabolic syndrome and quality of liferemains to be studied.

Future research priorities:

- Timing of starting GH treatment after or during in children with chronic low grade disease) treatment for childhood cancer

- The effect of GH treatment on final height, body composition and tumor outcome in GH deficient children direclty after completion of cancer treatment,

- The effect of long-acting GH on growth and tumor recurrence in CCS

- A randomized controlled trial on the effect of GH therapy on body composition, bone health, metabolic syndrome and quality of life in adult CCS.

Bone health

Low bone mineral density (BMD) is reported in up to 36 % of CCS (van Atteveld JE, Lancet Diabetes Endocrinol. 2023) with highest risk among ALL, CNS tumors and stem cell transplanted survivors. (Hudson 2013 JAMA). The standardised incidence ratio of any first fracture has been shown to be 3·53 (95% CI 3·06-4·06) for males and 5·35 (4·46-6·52) for females. Impact of cancer diagnosis on the bone (ALL), glucocorticoid treatment, chemotherapy (methotrexate), cranial radiotherapy, nutrional status vit D deficiency, lifestyle and endocrine conditions such as GH deficiency, hypogonadism are suggested mechanism for low BMD (Mostoufi-Moab 2012 JEM, Cohen 2012 Ped Blood Cancer, Atteveld Lancet 2023). New cancer treatment protocols, including TKIs, may result in deficits in BMD, but more research is needed to improve the understanding of the negative effects of these agents on the bone health.

Future research priorities:

- to study the effect of TKIs effect on the bone health

- to study the effects of treatment with GH, thyroid hormone and sex steroids on bone health in CCS?

- to study the indications and effect of bifosphonates on bone health outcomes in CCS

- to study ways to prevent toxicity of chemotherapy on bone development

Obesity and Metabolic syndrome

Overweight, obesity and the metabolic syndrome is prevalent in CCS. In a recent analysis of the Dutch CCS cohort, an overweight prevalence of 46.3% was found in men and 44.3% in women (obesity 11.2% and 15.9%, morbid obesity 2.4% and 5.4%). Especially CCS have a significantly higher risk for obesity and metabolic syndrome compared to siblings and general population which is associated with pituitary dysfunction (Mostofi-Moab 2016 JCEM, Smith 2014 Cancer, Agular M 2015 JAMA, van Schaik, JCO, Pluimakers EJE 2023). Survivors with hypothalamic involvement of their tumor are at highest risk for morbid obesity due to hypothalamic dysfunction (Lustig 2003, JCEMm Muller et al Nature) (See chapter X on hypothalamic-pituitary disorders). In addition, ALL survivors treated with prophylactic cranial radiotherapy are at high risk for obesity (Zhang 2014 Pediatrics). Survivors treated with total body irradiation (TBI) have an increased risk for Diabetes mellitus and insulin resistance, with an even higher risk among those treated with cranial radiotherapy in addition to TBI (Friedman 2017 Biol Blood marrow transplant). Suggested explanation is decreased lean mass and different body fat distribution in CCS treated with TBI.

Future research priorities:

- The effect of surveillance for and timely referral of obesity in the Late effects clinic on cardiovascular and psychosocial later outcomes.

- The effect of standardised controlled interventions with physical activity and life style factors on obesity in CCS.

- Management of acquired hypothalamic obesity (described in the chapter on hypothalamic-pituitary disease)

Overall anticipated impact on future research

An increased knowledge on endocrine late complications after childhood cancer, including the development of precision medicine, will allow the clinicians to better identify the survivors at high-risk for late toxicity. This will enhance survivors outcomes and minimize unnecessary treatment. We will know more about targeted therapies during childhood and the effect on endocrine dysfunction, including the need for prolonged follow up.

Oncofertility: effect of oncological treatment on spermatogenesis and children's health

[redacted]

CCS are at increased risk for gonadal insufficiency with impaired fertility after treatment with radiotherapy and after exposure to alkylating agents, which, for both modalities, is dose-dependent (Skinner 2017 Lancet Oncology; van Dorp JCO 2016). Testicular radiation doses greater than 4 Gy, ovarian radiation doses greater than 5 Gy, hypothalamic pituitary RT greater than 22 to 30 Gy in girls, and TBI in both boys and girls are known risks for premature gonadal failure and infertility.

Epidemiology, societal impact, research state of the art of gonadal toxicity in men

Thanks to the availability of highly efficient cancer treatments, the number of young men who recovers from cancer is steadily increasing, exceeding 95% for testicular cancer and 80% for childhood cancers and many types of lymphoma.

The most frequent cancers in post-pubertal subjects are testis cancer (TC) and hematological malignancies (HM). While fertility preservation based on sperm cryopreservation is highly recommended, in the majority of cases, spermatogenesis recovers after a few months/years from the end of treatment, implying that natural conception after genotoxic treatment is a viable alternative to the use of cryopreserved spermatozoa. Current literature data is relatively scarce concerning the effect of these therapies on sperm DNA integrity and epigenetic changes and data mainly refers to up to 2 years from the end of therapies. Recently, high sperm DNA fragmentation persisting even after 3 years from the end of therapies was reported both in testis cancer and in HM patients (Farnetani 2023, Farnetani 2024). These studies questioned the safety of natural conception after the canonic 24 months of waiting period, underlying the need for personalized pre-conception counselling. However, it is still unclear if these genetic changes in spermatozoa of cancer survivors are related to therapy or the malignant disease per se (Al-Jebari et al 2019).

Epidemiological studies comparing the frequency of malformations in children born to cancer survivors versus cancer free fathers are scarce. The largest study to date reports a 17% significant increase in major malformations, but the cohort is extremely heterogeneous in terms of type of cancers, treatments and the time for conception after the oncological therapies (Stahl et al 2011). Apart from the malformation rate, no other health conditions have been studied so far. At this regard, it is relevant to note that epigenetic changes in spermatozoa (Chan et al 2023) may lead to diseases, which are not always detectable at birth and they may even lead to a transgenerational effect. Finally, de novo mutation rate after oncological treatments was addressed only by a single, small study leaving open the question about the risk estimate for monogenic and complex diseases (Maher et al 2019).

Future research priorities:

To identify genetic and clinical markers indicative of a higher susceptibility to chemotherapy- and radiotherapy induced gonadal toxicity (males and females)

There is an urgent need to better understanding the genotoxic effects of cancer disease as such and also that of oncological therapies on sperm DNA and their consequences and persistency over time. Objectives: 1) analysis of large cohorts of subjects prior to and after being treated with different regimens of chemo/radiotherapies in order to define: i) the type and entity of genotoxic effect (sperm DNA fragmentation and DNA methylation) and ii) its persistency over time in function of the cancer disease as well as treatment type and dosage. Such parameters should be evaluated prior and after treatment for at least 5 years follow-up (or over 5 years if pathological values are detected).

-the development of a reliable technology able to detect the de novo mutation rate in sperm suspension prior and after therapy

-very large epidemiological studies on birth defects in children born to cancer survivors able to provide risk estimate for specific cancer types/treatment groups rather than providing a general estimate for all cancers.

-large prospective epidemiological studies focusing not only on malformation rate but also on other health outcomes of children

Anticipated impact of future research

A deeper knowledge on the genotoxic effect of oncological treatments on spermatozoa and the development of diagnostic tests would allow an informed decision making on the timing of conception and on the choice between natural versus the use of cryopreserved spermatozoa in an ART context. Large epidemiological (especially prospective studies) on children taking into consideration specific treatment groups would allow a more precise risk estimate and similarly to the in vitro studies would allow a personalized pe-conception counselling.

Immune check point inhibitors

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Immune checkpoints (ICs) are molecules that modulate the duration and amplitude of physiological immune function, playing important roles in maintaining immune homeostasis. Immune checkpoint inhibitors (ICIs) are antibodies that target certain immune checkpoints (ICs), such as programmed death 1 (PD-1), its ligand (PD-L1), or cytotoxic T-lymphocyte antigen-4 (CTLA-4) (Chang LS et al 2019). ICIs have emerged as a powerful new tool for various types of cancers, namely, lymphoma, melanoma, lung cancer, renal cell carcinoma, urothelial carcinoma, etc. The indications of ICIs are currently increasing. As these ICs are crucial for immunological self-tolerance, such therapies can trigger autoimmune adverse effects, affecting a number of organs. Endocrinopathies are among the most common complications, including thyroid dysfunction, hypophysitis, and more rarely diabetes mellitus (DM) and primary adrenal insufficiency (Chang LS et al 2019; Husebye ES et al 2022). The time of onset of endocrinopathies presents a great range (weeks to months after the initial dose). Furthermore, the severity of endocrinopathies presents a great range and grading systems are used in the clinical practice. Combination of ICIs appears to increase the risk of endocrine complications. It has been hypothesized that autoimmune complications after ICIs may be a positive predictor of immunotherapy response (Paschou SA et al 2021; Husebye ES et al 2022). ICIs therapy can be continued in most cases, high-dose glucocorticoids are rarely required for management, while endocrine deficiency usually persists necessitating lifelong replacement, except for cases with transient thyroiditis (Husebye ES et al 2022).

Recommendations for future research.

ICIs have significant endocrine side-effects that physicians treating these patients need to know about, since they are relatively frequent, and their management differs from other immune-related adverse events in three key ways: (i) ICIs therapy can be continued in most cases, (ii) high-dose glucocorticoids are rarely required for management, and (iii) endocrine deficiency usually persists, necessitating lifelong replacement, except for cases with transient thyroiditis. Based on these issues, the following recommendations are being proposed:

1. Investment into international big data registries. There is a surprising lack of reports on endocrinopathies related to ICIs therapy in large patient databases which include data on adequately diagnosed ICIs endocrinopathies in patients followed up over time.

2. As the range of ICIs is expanding, there is a need to explore not only side-effects but also the outcome of oncologic and endocrine treatments. It has been hypothesized that autoimmune complications after ICIs may be a positive predictor of immunotherapy response.

3. The grading systems should be evaluated and standardized. ICIs therapy can be continued in most cases and high-dose glucocorticoids are rarely required for management.

4. Exact dosing of hormones replacement and dose adaptation through lifetime should be studied. Endocrine deficiency usually persists, necessitating lifelong replacement, except for cases with transient thyroiditis.

Anticipated Impact of Future Research.

International efforts through big data registries and collaborative studies will help to improve screening, early diagnosis, grading systems and individualized management -both short term and long term- of endocrinopathies related to ICIs therapy. These efforts will ultimately reduce burden and costs for patients, their families, national health systems and the society.

Familial Endocrine Cancer Syndromes

[redacted]

Epidemiology, societal impact, research state-of-the-art

A. Epidemiology

Familial endocrine cancer syndromes are a heterogeneous group of rare hereditary endocrine disorders, usually transmitted in an autosomal dominant pattern. In each of these syndromes, the germline genetic alteration predisposes carriers to a specific set of endocrine and non-endocrine tumors and conditions. These conditions vary in expressivity and penetrance at the individual level: the mutation does not necessarily lead to manifestations or to the full pattern of manifestations that define the syndrome.

The most common of these familial endocrine cancer syndromes are

- Multiple Endocrine Neoplasia Type 1 (MEN1, OMIM#131100, ORPHA#625), caused by germline inactivating variants in the *MEN1* tumour suppressor gene. MEN1 is inherited as an autosomal dominant trait. MEN1 is characterized by 3 major compounds, parathyroid tumours, duodenopancreatic neuroendocrine tumours and, pituitary neuroendocrine tumours, and minor associations such as lung neuroendocrine tumor, thymic neuroendocrine tumor, adrenal tumor. Non-endocrine: cutaneous lesions, breast cancer. Estimated prevalence is 1/20.000-40.000. The

overall penetrance is 76-99% by age 50, and 99% by age 88 (including pHPT >95%, dpNET 80%, and PitNET in 50-80%) (Brandi doi: 10.1210/endrev/bnaa031).

- Multiple endocrine neoplasia type 2 (MEN 2A, OMIM#171400, ORPHA#247698; MEN 2B, OMIM#162300, ORPHA#247709), due to mutations of the *RET* proto-oncogene, leading to medullary thyroid carcinoma in about 100% of cases, pheochromocytoma in about 50% and parathyroid tumours in 10-20% (the latter only in MEN 2A, whereas MEN 2B, the most aggressive and rare form usually due to M918TRET mutations may also be associated with additional endocrine and non-endocrine features). MEN2 is inherited as an autosomal dominant trait. The estimated prevalence is 1/40.000 (Mathiesen, doi: 10.1016/j.semcancer.2021.03.035).
- Von Hippel Lindau Syndrome (VHL, OMIM#193300, ORPHA#892), due to mutations in the VHL tumour suppressor gene, resulting in an inconsistent association with retinal, brain and medullary hemangioblastoma, pancreatic neuroendocrine tumours, renal cell carcinoma and/or pheochromocytoma. VHL is inherited as an autosomal dominant trait. Its prevalence is estimated to be 1/53.000. The penetrance is > 80% for at least one disease after the age of 60 (Jafri, doi: 10.1530/EJE-11-0497; Mougel, doi 10.1136/jmg-2023-109550).
- Pheochromocytoma Paraganglioma Syndrome (OMIM#115310, 168000, 601650, 605373, 614165; ORPHA#29072), mainly due to mutations in genes encoding succinyl dehydrogenase subunits (*SDHx*), leading to an increased risk of developing adrenal (also called pheochromocytoma) or extra-adrenal (autonomic nervous system) paragangliomas. Some rare cases of pituitary tumors have been described in the 3 PA syndrome. Pheochromocytoma are found in 0.1 to 0.6% of patients with hypertension. The penetrance is variable, depending on the gene (Crona, doi 10.1210/er.2017-00062; Buffet, doi 10.1016/j.beem.2020.101416).

B. Economic and health care burden, wider societal impact, impact on the environment as a whole

Access to comprehensive genetic testing is important, for both diagnosis and management. For example, in MEN2, predictive genetic testing is recommended for first-degree relatives of mutation carriers, often already in early childhood. This allows identification of persons not at-risk for syndrome-related tumors as well as timely initiating screening in identified mutation-carriers, and in case of MEN2, determining the optimal age of thyroidectomy (Wells, doi:10.1089/thy.2014.0335). However, in some countries, the diagnosis of a genetic disorder may have an impact on the ability to obtain health insurance, life insurance and the ability to obtain a mortgage, which may lead to reluctance to undergo genetic testing.

Patients with these familial endocrine cancer syndromes can experience a long delay in diagnosis, leading to added psychosocial burden as well as increased morbidity and mortality both in patients and family members. As blanket population screening is undesirable (apart from, debatably, MEN2B) given the rarity of these disorders, there is a need **to identify easy to use and highly specific clues for generalists to aid early diagnosis of these disorders**.

Patients with familial endocrine cancer syndromes are advised to follow a lifelong, disorder-specific, screening program aimed at early detection of manifestations to allow timely intervention to prevent subsequent morbidity and mortality. And while this has improved outcomes, concerns have been raised, for example in MEN1, about the burden of these screening programs in terms of radiation exposure, financial aspects, toxicity, psychological burden and the increased detection of small indolent tumours that may or may not become clinically relevant (Newey, doi: 10.1210/jendso/bvac001). Thus there is a need for **biomarkers that may allow a more personalized and risk-based approach.**

In patients with familial endocrine cancer syndromes, morbidity and mortality are caused by the tumours that arise as part of the syndrome but also by the sequelae of (surgical) treatment, such as hypoparathyroidism and endocrine/exocrine pancreatic insufficiency in MEN1, hypothyroidism in MEN2, adrenal insufficiency in MEN2 and familial PPGL syndrome and dialysis in VHL. There is a need for **targeted, non-invasive or minimally invasive early interventions and more optimal, risk-based timing of interventions**.

C. Research state of the art

For these familial endocrine cancer syndromes, the last two to three decades after the identification of their respective genes (such as *VHL* in 1993 (Latif, doi: 10.1126/science.8493574), *RET* in 1993 (Donnis-Keller, doi: 10.1093/hmg/2.7.851) or *MEN1* in 1997 (Chandrasekharappa doi: 10.1126/science.276.5311.404.; Lemmens, doi: 10.1093/hmg/6.7.1177)) have tremendously increased the knowledge of 1) the function of these genes and their molecular pathways, 2) the epidemiology of the syndromes, their phenotype and the natural history of the tumour manifestations, thanks to national multicenter collaborative cohort studies and registries 3) phenotype-genotype correlations with clear implications for management, including the discovery of new drugs that target the genetic alteration, the pathway or the receptors (such as anti-RET tyrosine kinase inhibitors in MEN2, belzutifan in VHL, and theranostic advances in PPGL) (Hadoux, doi: 10.1056/NEJMoa2309719; Jonasch, belzutifan, doi: 10.1056/NEJMoa2103425; taieb, doi: 10.1530/ERC-19-0165).

Future Research priorities

Axis 1: To decipher the natural history of diseases to optimize treatment through large international epidemiological registries coupled with biobanking with the aim of:

1. **Define the modifying factors of tumorigenesis, i.e.** look at the genomic and epigenomic background beyond the gene of interest, in particular trying to define some protective or exacerbating genetic or epigenetic factors. This would require whole genome and omics approaches, ideally comparing for example patients from the same family but with different disease profiles or specifically evaluating patients with the more aggressive forms of endocrine cancer syndromes. Although epigenetic/environmental triggers may be involved in accelerating tumorigenesis in endocrine cancer syndromes, no specific large-scale studies have been conducted to identify them.
2. **Improve early diagnosis of rare diseases for early care and to reduce delays in diagnosis:** Some rare diseases may start with non-endocrine or non-specific signs. Research should focus on identifying these early, specific, signs in order to improve the education of non-endocrine specialists to help them recognize the early signs of each disease. For example, MEN2B, the most aggressive and rarest form of MEN2, can lead to medullary thyroid cancer early in life: however, clinical signs of this cancer will be detectable at a stage when the disease is already aggressive; some authors have shown that severe gastrointestinal symptoms should be a warning signal that allows early detection of MEN2B (Brauckhoff, doi: 10.1016/j.surg.2008.08.028; Castinetti, doi: 10.1016/S2213-8587(18)30336-X)
3. **Optimise genetic screening programmes, based on large studies with high levels of evidence to** improve the cost-effectiveness of genetic testing in patients with suspected hereditary disease. This point must include the future neonatal genomic screening programs.
4. **Optimise screening protocols and develop shared decision-making tools** for the detection and management of the disease manifestations. For example, in MEN1, this would help to define the optimal strategy for mild hypercalcemia, or for small non secreting neuroendocrine tumors.
5. **Identify novel approaches for individual risk prediction** to enable personalized screening, monitoring and timing of intervention. These may include blood-based biomarkers, functional imaging, dynamic prediction models based on clinical data or AI-based models. For example, there is a variable penetrance among patients with SDHx mutations: however, all patients are followed

the same way as there is no way to determine on which patients are at a low or high risk of developing PPGL (Benn, doi: 10.1530/ERC-15-0268).

6. **Optimise and personalise treatment using phenotype-genotype correlations.** Although thousands of mutations have been identified worldwide in all of these endocrine cancer syndromes, genotype-phenotype correlations have not yet been identified in all of them, because of the lack of large enough registries, or the lack of harmonization of epidemiological, genetic and clinical data to draw firm conclusions about a type of mutation or set of mutations in a given syndrome. For example, while VHL missense mutations appear to confer an increased risk of pheochromocytoma compared with truncating variants or gene deletions, the follow-up of pheochromocytoma is the same for every patient with VHL, regardless of the mutation, but may differ between the guidelines of different societies (Mougel, doi 10.1136/jmg-2023-109550).
7. **Characterise the psychosocial consequences of lifelong surveillance and survivorship care**. Endocrine cancer syndromes are lifelong conditions that require regular follow-up even after successful treatment of the tumour. This has psychosocial consequences for patients and families, and appropriate support cannot be provided by physicians alone (van Leuwaarde, doi: 10.1159/000508374 ; Rodrigues, doi: 10.1089/thy.2016.0148; Henderson, doi: 10.1111/cen.14823). Patients' advocacy groups are a cornerstone in the management of these patients. Psychosocial research is needed to determine the impact on patients and carers/families' quality of life to improve the long-term consequences of these endocrine cancer syndromes. Furthermore, issues regarding counseling about advanced family planning deserve attention.

<u>Axis 2: To develop advanced preclinical models that recapitulate tumorigenesis in the human context:</u>

1. **To identify new specific therapeutic and theranostic targets, focusing on the gene or specific signaling pathways, to improve outcomes in patients with unresectable and/or metastatic tumours.** The recent clinical results of RET-targeting tyrosine kinase inhibitors in patients with RET+ medullary thyroid cancer, or of Belzutifan, an inhibitor of the Hif2a pathway (HIF is involved in the VHL oxygen-sensing pathway), show that there is room for further therapeutic research focusing on the direct effects of mutations on the targets (as opposed to non-specific treatments aimed at destroying metastases) (Hadoux, doi: 10.1056/NEJMoa2309719; Jonasch, belzutifan, doi: 10.1056/NEJMoa2103425; taieb, doi: 10.1530/ERC-19-0165).
2. **To identify new preventative and therapeutic strategies to allow early targeted interventions** before tumours reach an advanced stage. In these familial endocrine cancer syndromes where we are now screening patients prospectively and identifying associated tumours early, there is a unique window of opportunity for cancer interception that, if exploited, could improve prognosis immensely.
3. **To elucidate tissue-restricted tumour development and progression.**
4. **To enable high-throughput pharmacological screening.**
5. **To test combined specific and non-specific treatments** that could dramatically change the prognosis of endocrine cancer syndromes.

Anticipated Impact of Future Research

Coordinating national and international efforts through registries and collaborative preclinical studies will help to improve the early diagnosis, the management and monitoring of these rare diseases, through the prism of an individualized approach. This will also reduce the burden of this group of syndromes, in terms of cost to patients by avoiding long-term treatments or sequelae, and to society.

References

Stearns V and Davidson NE. Chapter 45: Adjuvant Chemo Endocrine Therapy. In: Harris JR, Lippman ME, Morrow M, Osborne CK, eds. *Diseases of the Breast*. 5th ed. Philadelphia: Wolters Kluwer Health; 2014.

Allison KH, Hammond MEH, Dowsett M, et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update. *J Clin Oncol*. 2020;38(12):1346-1366. doi:10.1200/JCO.19.02309.

Chlebowski RT, Hendrix SL, Langer RD, Stefanick ML, Gass M, Lane D, Rodabough RJ, Gilligan MA, Cyr MG, Thomson CA, Khandekar J, Petrovitch H, McTiernan A; WHI Investigators. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial.

JAMA. 2003 Jun 25;289(24):3243-53

Chlebowski RT, Anderson GL, Gass M, Lane DS, Aragaki AK, Kuller LH, Manson JE, Stefanick ML, Ockene J, Sarto GE, Johnson KC, Wactawski-Wende J, Ravdin PM, Schenken R, Hendrix SL, Rajkovic A, Rohan TE, Yasmeen S, Prentice RL; WHI Investigators. Estrogen plus progestin and breast cancer incidence and mortality in postmenopausal women. JAMA. 2010 Oct 20;304(15):1684-92.

Antoine C, Ameye L, Paesmans M, de Azambuja E, Rozenberg S. Menopausal hormone therapy use in relation to breast cancer incidence in 11 European countries.

Maturitas. 2016 Feb;84:81-8.

Manson JE, Aragaki AK, Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Chlebowski RT, Howard BV, Thomson CA, Margolis KL, Lewis CE, Stefanick ML, Jackson RD, Johnson KC, Martin LW, Shumaker SA, Espeland MA, Wactawski-Wende J; WHI Investigators. Menopausal Hormone Therapy and Long-term All-Cause and Cause-Specific Mortality: The Women's Health Initiative Randomized Trials.

JAMA. 2017 Sep 12;318(10):927-938

Shamseddin M, De Martino F, Constantin C, Scabia V, Lancelot AS, Laszlo C, Ayyannan A, Battista L, Raffoul W, Gailloud-Matthieu MC, Bucher P, Fiche M, Ambrosini G, Sflomos G, Brisken C. Contraceptive progestins with androgenic properties stimulate breast epithelial cell proliferation. EMBO Mol Med. 2021 Jul 7;13(7):e14314. doi: 10.15252/emmm.202114314.

Fournier A, Berrino F, Clavel-Chapelon F. Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N cohort study. Breast Cancer Res Treat. 2008 Jan;107(1):103-11. doi: 10.1007/s10549-007-9523-x.

Fournier A, Berrino F, Riboli E, Avenel V, Clavel-Chapelon F. Breast cancer risk in relation to different types of hormone replacement therapy in the E3N-EPIC cohort. Int J Cancer. 2005 Apr 10;114(3):448-54.

Chang LS, Barroso-Sousa R, Tolaney SM, Hodi FS, Kaiser UB, Min L. Endocrine toxicity of cancer immunotherapy targeting immune checkpoints. Endocr Rev 2019;40:17-65.

Husebye ES, Castinetti F, Criseno S, Curigliano G, Decallonne B, Fleseriu M, Higham CE, Lupi I, Paschou SA, Toth M, van der Kooij M, Dekkers OM. Endocrine-related adverse conditions in patients receiving immune checkpoint inhibition: an ESE clinical practice guideline. Eur J Endocrinol 2022;187(6):G1-G21.

Paschou SA, Stefanaki K, Psaltopoulou T, Liontos M, Koutsoukos K, Zagouri F, Lambrinoudaki I, Dimopoulos MA. How we treat endocrine complications of immune checkpoint inhibitors. ESMO Open 2021;6(1):100011.

Bassett, J H, S a Forbes, a a Pannett, S E Lloyd, P T Christie, C Wooding, B Harding, G M Besser, C R Edwards, J P Monson, J Sampson, J a Wass, M H Wheeler, and R V Thakker. 1998. 'Characterization of mutations in patients with multiple endocrine neoplasia type 1.', American journal of human genetics, 62: 232-44.

Brandi, M. L., S. K. Agarwal, N. D. Perrier, K. E. Lines, G. D. Valk, and R. V. Thakker. 2021. 'Multiple Endocrine Neoplasia Type 1: Latest Insights', Endocr Rev, 42: 133-70.

Chandrasekharappa, S. C., SC Guru, P Manickam, SE Olufemi, FS Collins, MR Emmert-Buck, LV Debelenko, Z Zhuang, IA Lubensky, LA Liotta, JS Crabtree, Y Wang, BA Roe, J Weisemann, MS Boguski, SK Agarwal, MB Kester, YS Kim, C Heppner, Q Dong, AM Spiegel, AL Burns, and SJ Marx. 1997. 'Positional Cloning of the Gene for Multiple Endocrine Neoplasia-Type 1 ', Science, 276: 404-07.

de Laat, J. M., R. B. van der Luijt, C. R. Pieterman, M. P. Oostveen, A. R. Hermus, O. M. Dekkers, W. W. de Herder, A. N. van der Horst-Schrivers, M. L. Drent, P. H. Bisschop, B. Havekes, M. R. Vriens, and G. D. Valk. 2016. 'MEN1 redefined, a clinical comparison of mutation-positive and mutation-negative patients', BMC Med, 14: 182.

Lemmens, I, WJ Van de Ven, K Kas, CX Zhang, S Giraud, V Wautot, N Buisson, K De Witte, J Salandre, G Lenoir, M Pugeat, A Calender, F Parente, D Quincey, P Gaudray, MJ De Wit, CJ Lips, JW Höppener, S Khodaei, AL Grant, G Weber, S Kytölä, BT Teh, F Farnebo, and RV Thakker. 1997. 'Identification of the multiple endocrine neoplasia type 1 (MEN1) gene', Human molecular genetics, 6: 1177-83.

Romanet, P., A. Mohamed, S. Giraud, M. F. Odou, M. O. North, M. Pertuit, E. Pasmant, L. Coppin, C. Guien, A. Calender, F. Borson-Chazot, C. Beroud, P. Goudet, and A. Barlier. 2019. 'UMD-MEN1 Database: An Overview of the 370 MEN1 Variants Present in 1676 Patients From the French Population', J Clin Endocrinol Metab, 104: 753-64.