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TP53 and SF3B1 Mutations in Pituitary Tumours: Unravelling Molecular Drivers of Aggressive Tumour Behaviour

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List of abbreviations

ACTH	adrenocorticotropic hormone
AIP	aryl hydrocarbon receptor interacting protein
ATRX	alpha thalassemia and mental retardation X-linked
CAPTEM	capecitabine temozolomide
CNC	Carney complex
CRH	corticotrophin releasing hormone
CTP/BADx	corticotroph tumour progression after bilateral adrenalectomy
DA	dopamine agonists
ERa	estrogen receptor alpa
ESE	European Society of Endocrinology
FIPA	familial isolated pituitary adenoma
FSH	follicle stimulating hormone
GH	growth hormone
GNAS	guanine nucleotide-binding alpha stimulatory subunit
HPF	high power field
ICA	internal carotid artery
ICI	immune checkpoint inhibitors
LH	luteinizing hormone
MEN1	multiple endocrine neoplasia 1
MEN4	multiple endocrine neoplasia type 4
MGMT	O6-methylguanine DNA methyltransferase
MRI	magnetic resonance imaging
mTOR	mechanistic target of rapamycin
NFPA	non-functioning pituitary adenoma
PitNET	pituitary neuroendocrine tumour
PRL	prolactin
PRRT	peptide receptor radionuclide therapy
SDH	succinate dehydrogenase
SF3B1	Splicing factor 3 subunit 1
SSA	somatostatin analogues
TKI	tyrosine kinase inhibitor
TMZ	temozolomide
TP53	tumour protein p53
TSH	thyroid stimulating hormone
USP8	ubiquitin specific protease 8
USP48	ubiquitin specific protease 48
VEGF	vascular endothelial growth factor
WHO	World Health Organization
X-LAG	X-linked acrogigantism

List of publications

Scientific Publications Summarized in This Thesis

1. Luis Gustavo Perez-Rivas, **Julia Simon**, Adriana Albani, Sicheng Tang, Sigrun Roeber, Guillaume Assié, Timo Deutschbein, Martin Fassnacht, Monica R. Gadelha, Ad R. Hermus, Günter K. Stalla, Maria A. Tichomirowa, Roman Rotermund, Jörg Flitsch, Michael Buchfelder, Isabella Nasi-Kordhishti, Jürgen Honegger, Jun Thorsteinsdottir, Wolfgang Saeger, Jochen Herms, Martin Reincke and Marily Theodoropoulou. **TP53 mutations in functional corticotroph tumours are linked to invasion and worse clinical outcome.** *Acta Neuropathologica Communications.* 2022:10(1), 139. doi:10.1186/s40478-022-01437-1.

2. **Julia Simon**, Luis Gustavo Perez-Rivas, Yining Zhao, Fanny Chasseloup, Helene Lasolle, Christine Cortet, Francoise Descotes, Chiara Villa, Bertrand Baussart, Pia Burman, Dominique Maiter, Vivian von Selzam, Roman Rotermund, Jörg Flitsch, Jun Thorsteinsdottir, Emmanuel Jouanneau, Michael Buchfelder, Philippe Chanson*, Gerald Raverot* and Marily Theodoropoulou***. Prevalence and clinical correlations of SF3B1 variants in lactotroph tumours**. *European Journal of Endocrinology.* 2023:189(3), 372. doi:10.1093/ejendo/lvad114.

Further Publications Resulting During my Time as Doctoral Candidate

1. Adriana Albani*, Luis Gustavo Perez-Rivas*, Sicheng Tang, **Julia Simon**, Kristin Elisabeth Lucia, Paula Colón-Bolea, Jochen Schopohl, Sigrun Roeber, Michael Buchfelder, Roman Rotermund, Jörg Flitsch, Jun Thorsteinsdottir, Jochen Herms, Günter Stalla, Martin Reincke, and Marily Theodoropoulou. **Improved pasireotide response in USP8 mutant corticotroph tumours in vitro**. *Endocrine-Related Cancer*. 2022:*29*(8), 503. doi: 10.1530/ERC-22-0088.

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  Porto, Portugal, 2020

Confirmation of Co-authors

Hereby I declare that all authors contributed to the publications gave written informed consent to use these publications in the frame of my dissertation.

1. Introduction

The pituitary gland is the master gland of the endocrine system controlling diverse functions, such as growth, metabolism and reproduction. It is situated in the *sella turcica* of the sphenoid bone at the base of the skull and is connected to the hypothalamus via the pituitary stalk [1]. Functionally and anatomically the pituitary gland is divided into the posterior lobe (neurohypophysis), which stores and releases oxytocin and vasopressin, and the anterior lobe (adenohypophysis) that produces and secretes the adenohypophyseal hormones including growth hormone (GH), prolactin (PRL), adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH) and the gonadotrophins follicle stimulating hormone (FSH) and luteinizing hormone (LH) [1,2]. Each of these hormones is synthesized in specialized cells: GH in somatotroph, PRL in lactotroph, ACTH in corticotroph, TSH in thyrotroph and the gonadotrophins in gonadotroph cells. Developmentally these anterior pituitary cell types originate from three lineages depending on the transcription factor expressed: PIT1 gives rise to somatotroph, lactotroph and thyrotroph, TPIT to corticotroph and SF1 to gonadotroph cells [1,2].

1.1 Pituitary tumours

Tumours of the anterior pituitary account for 17.2% of all intracranial neoplasms, representing the second most common type after meningioma [3,4]. They are usually benign, but present with symptoms of hormone hypersecretion and/or tumour mass effects that include visual field defects and hypopituitarism [5]. GH-secreting tumours cause acromegaly, lactotroph tumours result in hyperprolactinaemia, corticotroph tumours are responsible for Cushing's disease, while tumours that are not accompanied by hormone hypersecretion are collectively termed as non-functioning pituitary adenomas (NFPA) [6]. The overall prevalence of pituitary tumours is 77-115 cases per

100.000, with lactotroph tumours representing the biggest group (40-66%), followed by NFPA (15-43%), somatotroph (8.5-13%) and corticotroph tumours (2-6%) [7-10].

The 2022 World Health Organization (WHO) classification of endocrine and neuroendocrine tumours classifies pituitary adenomas, or pituitary neuroendocrine tumours (PitNETs), according to their transcription factors (TPIT, PIT1 and SF1)[11]. Accordingly, somatotroph, lactotroph and thyrotroph tumours belong to PIT1-lineage, corticotroph to TPIT-lineage and gonadotroph tumours to SF1-lineage [12]. Lactotroph, somatotroph and corticotroph tumours are further classified in sparsely or densely granulated [12]. The term “metastatic PitNET”, formerly pituitary carcinoma, refers to the presence of metastasis [12].

Pituitary tumours are classified according to size into micro- (<10mm), macro- (≥10mm) and giant adenoma (>40mm). In addition, they can be classified according to patterns of invasion into surrounding structures and proliferation markers using the following classification schemes [13-20].

1.1.1 **Hardy classification**

The modified Hardy-Wilson classification determines invasion into sphenoid bone and suprasellar extension of the tumour. It considers destruction of the sellar and divides it into five groups (0 to IV): grade 0 refers to tumours without sellar enlargement, grade I and II to tumours enclosing within the sellar and grade III or IV to likely invasive tumours that show sellar erosion. Grade I-IV can be further sub classified in five subgroups according to the extrasellar extension patterns: A-C classify increasing amount of symmetrical suprasellar extension and D-E asymmetric parasellar extension [13-15,21,22].

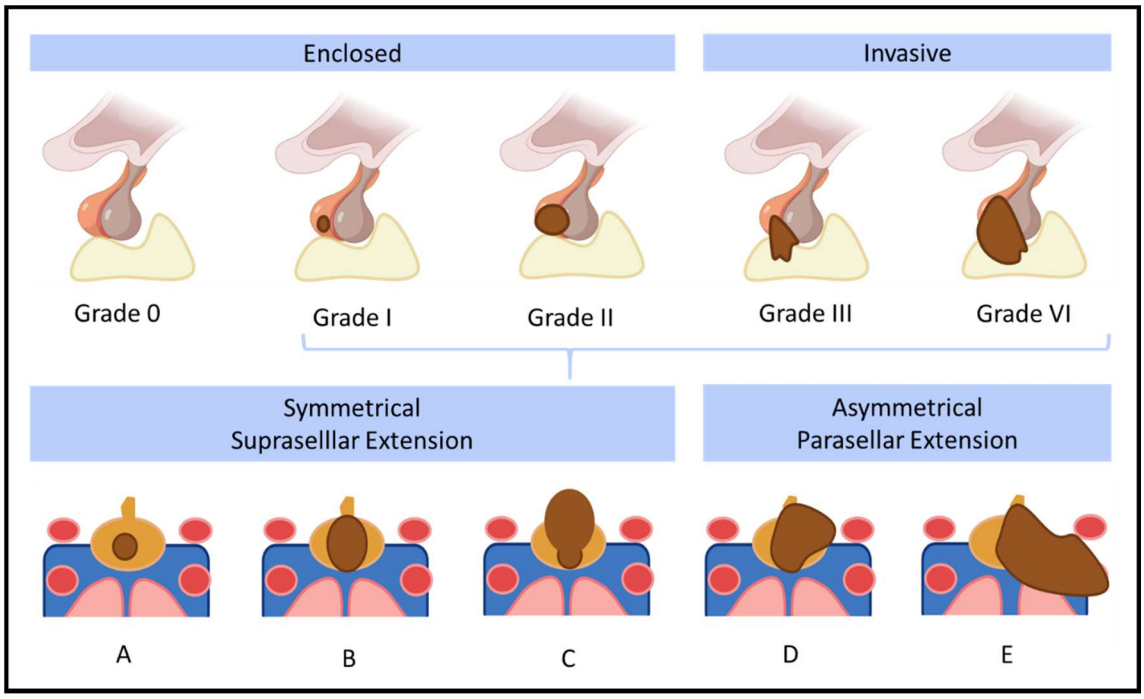

Figure 1. Hardy classification**.** Grade 0-IV gives invasion into spenoid bone: grade 0. Intact, grade I. intact with dilated floor, grade II. intact with expanded fossa, grade III. localized sellar destruction and grade IV. broad sellar destruction. Grade III and IV are invasive into sphenoid bone, whereas grade 0-II are enclosed within anterior pituitary. Grade A-E refers to symmetrical or asymmetrical parasellar extension of the pituitary tumour. Created with BioRender.com

1.1.2 **Knosp classification**

Knosp classification utilizes the information on the tumour's parasellar extension obtained by magnetic resonance imaging (MRI) to categorize pituitary tumours in grade 0 (no extension in the cavernous sinus), 1 (enlarges into cavernous sinus without encasing the internal carotid artery (ICA)), 2 (the tumour encases the ICA without invasion), 3 (partially invades the ICA) and 4 (completely encases and invades the ICA). Knosp grade 3 and 4 pituitary tumours are very likely to be invasive into cavernous sinus [17,22].

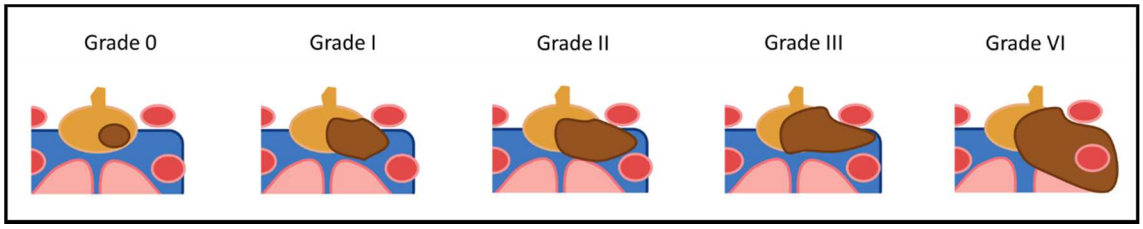

Figure 2. Knosp classification: grade 0. No extension into cavernous sinus, grade I. enlargement into cavernous sinus without encasing internal carotid artery (ICA), grade II. encasing ICA, grade III. partial invasion of ICA, grade IV. complete encasing of ICA and invasion. Created with BioRender.com

Incomplete resection of the pituitary tumour is mainly due to invasion into cavernous sinus [23].

Low Hardy and Knosp grades have been associated with a favourable surgical outcome whereas Hardy/Knosp grades 3 or 4 correlated with lower rates of surgical cure [24].

1.1.3 **Trouillas classification (French five-tiered)**

In contrast to Hardy and Knosp classification, the French five-tiered prognostic classification suggested by Trouillas et al.[18] combines invasion and proliferation of pituitary tumours and aims to predict recurrence of pituitary tumour. In addition to the cavernous sinus invasion, it considers a tumour proliferative when two out of the following three markers are present:

1. Ki-67 index >3%

2. mitotic count higher than 2/10 HPF (high powered field)

3. positive p53-immunostaining [18].

Table 1. Trouillas classification

Grade	Invasion	Proliferation
1a	non-invasive and	non-proliferative
1b	non-invasive and	proliferative
2a	invasive and	non-proliferative
2b	invasive and	proliferative
3	metastatic	

Multiple independent cohorts supported the Trouillas classification's predictive significance for tumour development [25-30].

1.2 Pituitary tumour genetics

Pituitary tumours are mainly sporadic neoplasms with only ~5% occurring in syndromic/familial setting [31]. The genetic landscape of pituitary tumours is diverse and more than half of the genetic background is uncertain [5,32] .

Genetic syndromes presenting with pituitary tumours include multiple endocrine neoplasia (MEN) 1 [33,34], MEN4 (germline mutations in cyclin-dependent kinase inhibitor p27-*CDKN1B*) [35,36], McCune Albright (*GNAS*) [37], Carney complex (CNC; *PRKAR1A*) [38,39], 3Pas (pituitary adenoma, paraganglioma and pheochromocytoma; germline mutations in succinate dehydrogenase subunits-*SDHx* or SDH complex assembly actor 2 protein-*SDHAF2* or MYC-associated factor X-*MAX*) [40-43]. In rare cases, DICER1 syndrome (also known as pleuropulmonary blastoma familial tumour syndrome; germline loss-of-function mutations in *DICER1* gene) may also present with pituitary blastomas (uncommon tumours of the pituitary gland that display undifferentiated Rathke epithelium) or tumours, mainly in infants/paediatric patients [44-48]. Three cases of pituitary tumours were reported

in patients with Lynch syndrome (mutations in genes implicated in DNA mismatch repair, such as, *MSH2, MLH1, MSH6, PMS2* or *EPCAM*) [49].

Pituitary tumours may also occur as nonsyndromic familial isolated pituitary adenomas (FIPA) [31]. About 20% of FIPA is caused by germline mutations in aryl hydrocarbon receptor interacting protein (AIP) gene [50-52]. Patients with germline *AIP* mutations predominantly develop somatotroph pituitary tumours (~80%) already at a young age and these tumours present with accelerated tumour growth, resistance to somatostatin analogues therapy and require more surgeries [53-55]. Variants in other genes such as *CDKN1B* are rarely found in *AIP* mutation negative FIPA patients [56]. Duplication of the *GPR101* gene is the cause of X-linked acrogigantism (X-LAG) and patients with this phenotype present with somatotroph pituitary tumours, mixed somatotroph-lactotroph tumours or pituitary hyperplasia [57,58].

1.2.1 **Sporadic pituitary tumours**

The majority of pituitary tumours are sporadic monoclonal neoplasms. The genetic drivers of sporadic pituitary tumours are in several cases still obscure [5,32]. Somatic variants in genes mutated in tumour predisposing syndrome or isolated cases such as *MEN1*, *CDKN1B* and *AIP* are rare in sporadic pituitary tumours [31,59-61] . At present the genes mutated in >5% of pituitary tumours are *GNAS* and *USP8* [62-64].

GNAS (guanine nucleotide-binding alpha stimulatory subunit) gain-of-function variants (formerly referred to as gsp proto-oncogene) were reported predominantly in ~40% of somatotroph tumours [65,66].

USP8 (ubiquitin specific protease 8) mutational hotspot was discovered by whole exome sequencing in ~40% of corticotroph tumours [67,68]. Except for one heterozygous germline *USP8* variant, mutations in the *USP8* hotspot region are reported to be of somatic origin [69-75]. USP8, a deubiquitinase, is cleaving ubiquitin molecules from target proteins and thus protecting them from lysosomal degradation resulting in subcellular re-localization

[76]. Mutations in the *USP8* hotspot region result in decreased 14-3-3 binding and increased deubiquitinase activity [67]. It is noteworthy that *USP8* mutations were solely identified in corticotroph tumours and not reported in other subsets of pituitary tumours or ectopic ACTH secreting tumours [62-64,67,68,77]. *USP8* variants occur more frequently in female patients with corticotroph tumours and these *USP8* mutant tumours tend to be smaller and less invasive [68,70]. A second mutational hotspot was discovered in another deubiquitinase encoding gene, *USP48*, in 4-28% of *USP8* wild type corticotroph tumours [78-81]. Similarly to *USP8* mutant tumours, *USP48* variants are more prevalent in female patients and *USP48* mutant tumours are smaller compared to wild type tumours, but may be more invasive into the cavernous sinus compared to *USP8* mutant tumours [78,79,81].

Next generation sequencing efforts to identify new recurrent variants in pituitary tumours rekindled the interest on the *TP53* tumour suppressor gene in corticotroph tumours and discovered variants in the *SF3B1* gene in lactotroph tumours [79,82]. As these two genes are focus of my thesis papers, I cover them in more detail below.

TP53

The tumour suppressor gene *TP53*, encoding for tumour protein 53, is commonly altered in human malignancies and these alterations are mainly missense mutations leading to loss of function of p53 [83-85]. Originally, *TP53* variants were considered to be extremely rare events sporadically reported in aggressive cases of corticotroph tumours and carcinomas [86-88]. However, screening efforts with next generation sequencing suggested that *TP53* variants may be more frequent than previously considered in selected tumour cohorts. Whole exome sequencing in cohorts of 18 *USP8* wild-type corticotroph macroadenomas, 27 aggressive corticotroph tumours and 22 aggressive corticotroph tumours and carcinomas revealed somatic *TP53* variants in up to 33% of cases [79,89,90]. Similarly, a case study comprising two patients with pituitary tumours (one metastatic corticotroph tumour and a lactotroph tumour) revealed somatic *TP53* variants in both

tumours that presented with high Ki-67 index, elevated numbers of mitoses and strong immunostaining for p53 [91]. A case report of a *USP8/USP48* wild type metastatic corticotroph tumour reported a somatic *TP53* variant alongside variants in the *NF1*, *PTEN* and *ATRX* genes [92]. *TP53* missense variants were also identified in three aggressive pituitary tumours (one corticotroph and two somatotroph) [93]. Finally, screening a paediatric patient with an aggressive lactotroph tumour revealed a germline *TP53* variant, and this is the first reported case of Li-Fraumeni syndrome first presenting with a pituitary tumour [94].

Although these reports indicate a link between *TP53* variants and aggressive tumour behaviour in pituitary tumours, a significant association could not be established due to small number of cases [79,89,90]. This is primarily due to the rarity of disease that hinders large scale genetic screenings and larger, multicentre studies were needed to establish the prevalence and clinical phenotype of *TP53* variants.

SF3B1

Splicing factor 3 subunit 1 (SF3B1) is part of the U2 dependent major splicing complex and is essential for branch site recognition during splicing processes [95,96]. Change of function mutations in the *SF3B1* gene result in aberrantly spliced transcripts and modified gene expression [95,97,98]. *SF3B1* variants are reported in various human cancers, including cutaneous, mucosal and uveal melanoma, chronic myelomonocytic leukaemia, chronic lymphocytic leukaemia, breast cancer and pancreatic cancer [99-108]. Screening of lactotroph tumours reported a mutational hotspot in the *SF3B1* gene (p.Arg625His) in ~20% of cases [82]. In contrast, other whole exome sequencing studies in other cohorts did not identify *SF3B1* variants [62-64]. Therefore, the prevalence of these variants in lactotroph tumours needed to be established in independent lactotroph tumour cohorts.

1.3 Pituitary tumour treatment

Pituitary surgery is the primary treatment for the majority of pituitary tumour types [109]. Depending on the kind of pituitary tumour, the approved tumour-targeting pharmacological treatment focuses on dopamine agonists (DA) and somatostatin analogues (SSA).

Dopamine agonists, like cabergoline, bromocriptine and quinagolide, constitute the first-line treatment for patients with lactotroph tumours, where they effectively suppress hyperprolactinaemia and cause tumour shrinkage in ~95% of the cases [110-112]. If the tumour growth is not reduced despite high dose therapy of DA or the patient does not tolerate medical therapy due to side effects, pituitary surgery or radiotherapy are second line options for treatment of lactotroph tumours [111,113].

Somatostatin analogues may be used as first line treatment in acromegaly. In addition, the SSA pasireotide is approved for the management of Cushing's disease [114,115].

In Cushing's disease, use of periphery targeting pharmaceuticals such as steroidogenesis synthesis inhibitors (ketoconazole, metyrapone, osilodrostat or mitotane) or glucocorticoid receptor antagonists (mifepristone) is considered, when cortisol levels are persistently elevated despite surgery and/or tumour targeting treatment [115]. In addition, bilateral adrenalectomy may be considered, which is effective but carries the risk of corticotroph tumour progression due to the removal of the glucocorticoid negative feedback (CTP/BADx, previously known as Nelson -or Nelson-Salassa- syndrome) [116,117].

Finally, radiation and stereotactic radiosurgery are considered, independently of pituitary tumour type, for patients that continue to experience clinically relevant tumour growth despite medical intervention [118,119].

1.4 Aggressive pituitary tumours and pituitary carcinomas

Pituitary tumours are mostly benign, nonetheless a small subset develops aggressive behaviour due the course of disease [120,121]. The 2018 guidelines of the European Society of Endocrinology (ESE) defines aggressive pituitary tumours as tumours with "unusually rapid tumour growth rate or tumour growth, as determined with magnetic resonance imaging, despite optimal standard therapy" [113].

Metastatic pituitary tumours constitute ~0.2% of all pituitary tumours and aggressive pituitary tumours are estimated to be 3-4 times more frequent than that [18,120-128]. Corticotroph tumours, which present the smallest group of all pituitary tumour types, are the most common type when considering only aggressive pituitary tumours and pituitary carcinomas, with a prevalence of 40%, followed by lactotroph tumours (~26%) [128-130].

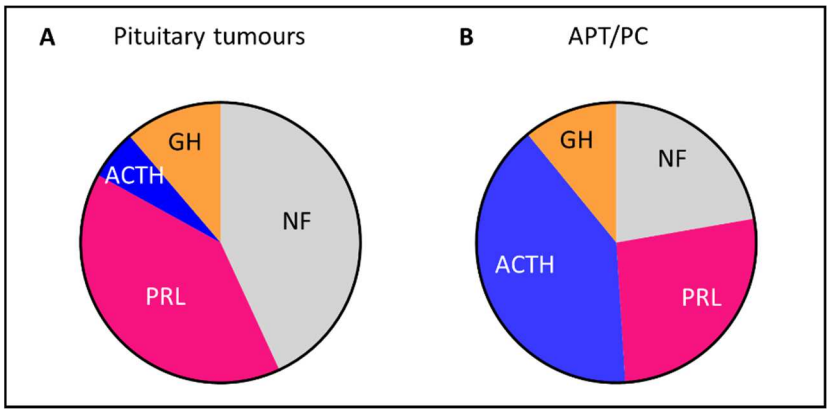

Figure 3. A. Percentages of pituitary tumour type: Non-functioning pituitary tumours (NF) (43%) and lactotroph (PRL) (40%) are the most common pituitary tumour types, followed somatotroph (GH) (11%) and corticotroph (ACTH) (6%) tumours B. Considering only aggressive pituitary tumours (APT) and pituitary carcinomas (PC) corticotroph tumours (40.1%) present the biggest group, followed by lactotroph (26,7%), non-functioning (22,7%) and somatotroph tumours (11%). Adapted from Burman et al. [128].

1.4.1 Management of aggressive pituitary tumours

By definition aggressive pituitary tumours do not respond to standard treatment options including surgery, radiotherapy and pituitary-targeting medical treatment [113]. Thus, after exhaustion of standard therapies, temozolomide (TMZ), an alkylating agent inducing irreversible DNA damage, is the first line medical therapy for aggressive pituitary tumours

alone or in combination with radiotherapy (Stupp protocol) [113,131-133]. Furthermore, some case reports and clinical trials (NCT03930771) investigated the combination of TMZ and capecitabine (CAPTEM) with mixed results [134,135]. TMZ treatment is reported to be effective in ~40-50% of cases and lack of TMZ response is attributed to high expression of the DNA repair enzyme O6-methylguanine DNA methyltransferase (MGMT) [129,136]. Alternative therapeutic approaches include peptide receptor radionuclide therapy (PRRT), which shows partial response or stabilized disease in patients naïve to TMZ treatment, but its efficacy is compromised by previous TMZ treatment [137-141]. Other agents, such as tyrosine kinase inhibitors (lapatinib, erlotinib, sunitinib, apatinib, imatinib), VEGF inhibitor (bevacizumab), mTOR-inhibitor (everolimus), CDK4/6-inhibitor (palbociclib), immune checkpoint inhibitors (ICI) (ipilimumab and nivolumab or pembrolizumab) and cytostatic agents (5-Fluorouracil) were reported to result in partial response or stable disease in some but not all cases of aggressive pituitary tumours [130,138,141-143].

In metastatic pituitary tumours the loco-regional treatment of the metastases is considered, which includes surgical resection of the metastasis, focused radiotherapy and targeted therapies against the metastasis [113,141].

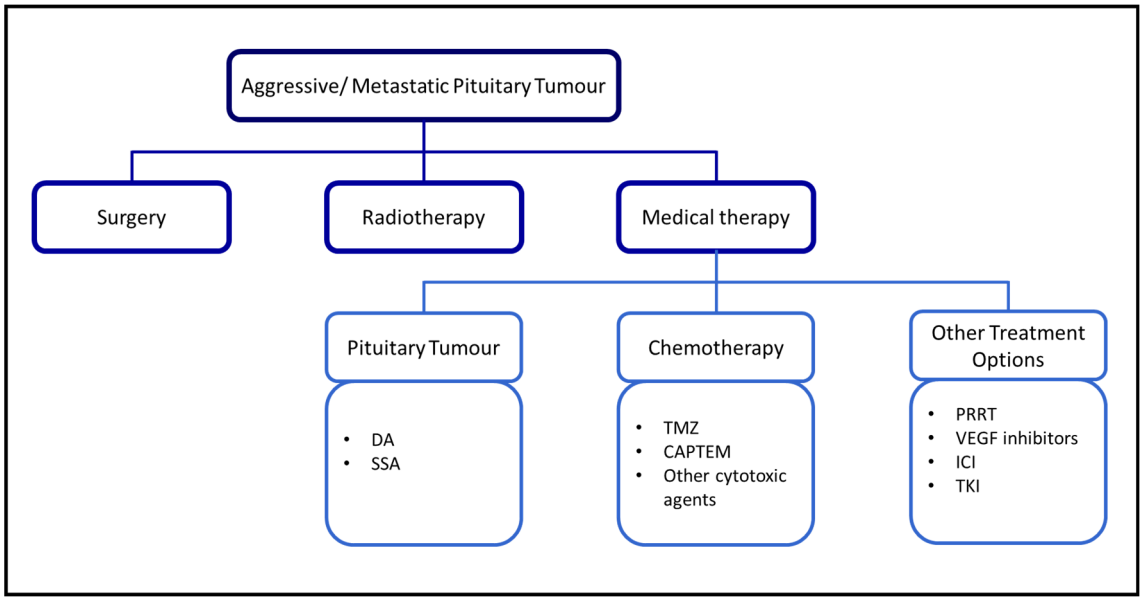

Figure 4. Treatment of aggressive and metastatic pituitary tumours. DA, dopamine agonist; SSA, somatostatine analogue; TMZ, temozolomide; CAPTEM, capecitabine temozolomide; PRRT, peptide receptor radionuclide therapy; ICI, immune checkpoint inhibitors; TKI, tyrosine kinase inhibitors. Adapted from De Sousa and McCormack. [144].

1.4.2 **Potential predictors of aggressive behaviour**

It is still challenging to anticipate which pituitary tumours will develop aggressive behaviour and research in the field has been hindered by the lack of a uniform, universally accepted definition of aggressiveness [128,145,146]. For example, invasion is important to predict surgical outcome, but it is not enough on its own to designate a tumour as aggressive [113,132]. In the WHO classification from 2004, the term "atypical adenoma" is defined by proliferation index Ki-67 (MIB-1) ≥3% and positive immunostaining for p53 protein [147-149]. The term "atypical adenoma" was then removed from the 4th WHO classification of 2017 for endocrine tumours as well as the ESE guidelines for aggressive pituitary tumours (2018) due to its poor prognostic significance [113,149-153].

In the ESE clinical practice guidelines for aggressive pituitary tumours a Ki67-index above 3% indicates that p53 staining and mitotic count should also be determined and considered [113]. A retrospective study in two tertiary centres suggested that proliferative markers as Ki67 index and mitotic count, but not p53 immunostaining, are prognostic factors for aggressive tumour behaviour [30]. In fact, a systematic review concluded that initial Ki67 index ≥ 10% may predict the appearance of metastasis in the future [154]. In addition, grade 2b according to the Trouillas classification, which refers to invasive and proliferative tumours, have a 2.3 to 12-fold and 3.5 to 4.8-fold higher risk for tumour recurrence and progression, respectively, compared to grade 1a [18,29,30,155]. Another study examining Trouillas grade in 43 aggressive pituitary tumours (including 20 metastatic pituitary tumours), emphasized that Trouillas grade 2b or 2b* (Ki67 index ≥10%) tumours have higher risk of malignancy and personalized therapeutical options should be well-considered for patients with grade 2b pituitary tumours [29,145].

Aside the aforementioned potential markers, there are no general predictors of aggressive behaviour due to the variability between pituitary tumour types. In the latest WHO classification released in 2022, pituitary tumour subtypes with a high risk for

aggressive behaviour include sparsely granulated somatotroph tumours, Crooke cell or sparsely granulated corticotroph tumours, lactotroph tumours in men, densely granulated lactotroph tumours and immature PIT1-lineage tumours [12,144]. The likelihood of aggressive behaviour of lactotroph and corticotroph tumours is higher in male individuals [128-130,156]. Switching from non-functioning to functioning in corticotroph tumour is also considered as an indicator of aggressive behaviour [128,130].

Molecular markers associated with aggressive behaviour in lactotroph tumours include loss of heterozygosity in chromosome 11, lower oestrogen receptor alpha (Era) expression and increased VEGF expression, as well as downregulation of the microRNAs (miR-183, miR-340, miR-744 and miR-98), but none was established as predictive marker for aggressiveness [157]. Variants in *RB1, HRAS* and *PIK3CA* in aggressive tumours and carcinomas were identified in isolated case reports, but are rarely reported in whole exome sequencing series (reviewed in [128]). Although no significant correlation between *SF3B1* variants and aggressive pituitary tumour behaviour was proclaimed, they were reported in two cases of aggressive lactotroph tumours, suggesting that they may also play a role in the development of aggressive tumour behaviour [82,158,159].

As mentioned above, there have been sporadic reports of mutations in the mismatch repair genes (*MSH2*, *MSH6*, *MLH1*), but *TP53* and *ATRX* (alpha thalassemia and mental retardation X-linked) variants have been repeatedly documented in aggressive pituitary tumours and carcinomas, mostly in corticotroph and to a lesser extent PIT1-lineage tumours. In addition, variants in *PTEN* or *DAXX* were found to occur concomitantly with *TP53* and/or *ATRX* variants in mutant aggressive corticotroph tumours and corticotroph carcinomas [64,79,90,92,128].

It is noteworthy that in cases of available tumour tissue from multiple surgeries, the variants for the genes examined were found in all specimens indicating early genetic event in pituitary tumorigenesis [89,90,128,160]. This suggests that screening for variants could predict aggressive tumour behaviour already from initial surgical specimens.

1.5 Aims of this dissertation

The objective of this thesis was to identify potential genetic markers predicting aggressive behaviour in pituitary tumours. To assess this, the prevalence of somatic variants in the *TP53* and *SF3B1* genes was examined in corticotroph and lactotroph tumours, respectively, and mutational status was then correlated with clinical and histopathological data in order to establish their correlation with aggressive tumour behaviour. The outcome of these studies was published in two papers, which were peer reviewed and published in international journals.

The first publication reports on the prevalence of *TP53* variants in the relatively large cohort of 86 corticotroph tumours. To evaluate the effect of *TP53* variants on the course of disease, clinical parameters were associated with the mutational status. The first publication sought to ascertain the prevalence of *TP53* variants in corticotroph tumours and their correlation with clinicopathological characteristics and course of disease.

The second publication reports on the prevalence of *SF3B1* variants in a large multicentre cohort of lactotroph tumours from 282 patients. The aim of the second publication was to establish the prevalence of *SF3B1* variants in a large lactotroph tumour cohort and their correlation with clinical features and aggressive tumour behaviour.

2. Summary

Aggressive behaviour of pituitary tumours is defined by an accelerated growth rate or tumour growth despite standard therapy. Although mostly benign, a small percentage of pituitary tumours presents with aggressive behaviour due the course of disease. Why some tumours develop aggressive features remains unclear and no general driver mutations for aggressive behaviour are reported. Whole exome sequencing studies revealed that variants in genes frequently mutated in other cancers, like *TP53* and *SF3B1* may be more frequent than previously thought in certain pituitary tumour types (corticotroph and lactotroph respectively). While for *TP53* there is already evidence of a link to aggressive behaviour especially in corticotroph tumours, for *SF3B1* the association with aggressive behaviour remained unclear.

In this thesis, I investigated the prevalence of both *TP53* and *SF3B1* mutations in large cohorts of corticotroph and lactotroph tumours, respectively, and correlated the outcome of the mutation analysis with clinical parameters.

The first publication examined *TP53* mutations and clinical parameters in a cohort of 86 corticotroph tumours comprising of both *USP8* mutant (n= 25) and wild type (n= 61). Mutations in the *TP53* gene were identified in 15% (9/61) of patients with *USP8* wild type corticotroph tumours. In comparison to wild type tumours, *TP53* mutant tumours had higher Knosp grades and presented more frequently with parasellar invasion. Patients with *TP53* mutant tumours required more clinical procedures including more surgeries, radiotherapy, temozolomide or pasireotide treatment. Moreover, disease specific mortality was increased in the mutant group. All these parameters indicate an aggressive behaviour of *TP53* mutant corticotroph tumours.

The second publication investigated *SF3B1* variants in a previously unpublished multicentre cohort of 282 patients with lactotroph tumours. Two mutations were identified in the *SF3B1* hotspot by Sanger Sequencing: the previously published variant c.1874G>A p.Arg625His and the undescribed in lactotroph tumours variant c.1873C>T

(p.Arg625Cys). In total, *SF3B1* variants were detected in 2.5% of lactotroph tumours, but when considering only metastatic lactotroph tumours, the prevalence rose to 50%. *SF3B1* mutant tumours were more frequently classified with the higher grades in Trouillas Five-Tiered (≥2b) or Knosp classification (grade 3 or 4) and metastasis occurred more frequently in *SF3B1* mutant tumours compared to the wild type group. Similarly, the need for stronger therapeutic interventions as well as the shorter progression free survival indicate a more aggressive course of disease in patients with *SF3B1* mutant lactotroph tumours.

Both publications of this dissertation expand our knowledge on the impact that variants in *TP53* and *SF3B1* may have in pituitary tumours and disease presentation. While other factors leading to aggressive tumour behaviour remain obscure, variants in these two genes are associated with a more aggressive course of disease. Given the severity of disease in patients with aggressive pituitary tumours, screening of corticotroph and lactotroph tumours for *TP53* and *SF3B1* variants, respectively, may facilitate the precise and timely patient management.

3. Zusammenfassung

Aggressives Verhalten von Hypophysentumoren ist definiert durch eine beschleunigte Wachstumsrate oder Tumorwachstum trotz Standardtherapie. Obwohl die meisten gutartig sind, zeigt ein kleiner Prozentsatz der Hypophysentumoren im Verlauf der Erkrankung ein aggressives Verhalten. Warum manche Tumoren aggressive Merkmale entwickeln, ist nach wie vor unklar, da keine allgemeinen Treibermutationen für aggressives Verhalten bekannt sind. Whole-Exome-Sequenzierungsstudien haben gezeigt, dass Varianten in Genen, die bei anderen Krebsarten häufig mutiert sind, wie *TP53* und *SF3B1*, bei bestimmten Hypophysentumoren (kortikotrop bzw. laktotrop) häufiger vorkommen könnten als bisher angenommen. Während es für *TP53* bereits Hinweise auf einen Zusammenhang mit aggressivem Verhalten vor allem bei kortikotropen Tumoren gibt, blieb für *SF3B1* der Zusammenhang mit aggressivem Verhalten unklar.

In dieser Arbeit habe ich die Prävalenz von *TP53*- und *SF3B1*-Mutationen in großen Kohorten kortikotroper bzw. laktotroper Tumore untersucht und die Ergebnisse der Mutationsanalyse mit klinischen Parametern korreliert.

In der ersten Veröffentlichung wurden *TP53*-Mutationen und klinische Parameter in einer Kohorte von 86 kortikotropen Tumoren untersucht, die sowohl *USP8*-Mutanten (n= 25) als auch Wildtypen (n= 61) umfasste. Mutationen im *TP53*-Gen wurden bei 15% (9/61) der *USP8*-Wildtyp kortikotropen Tumoren gefunden. Mutierte Tumoren wiesen höhere Knosp-Klassifizierung auf und zeigten im Vergleich zu Wildtyp-Tumoren häufiger eine paraselläre Invasion. Patienten mit *TP53*-mutierten Tumoren benötigten mehr therapeutische Eingriffe, einschließlich mehr Operationen, Strahlentherapie, Temozolomid- oder Pasireotidtherapie. Außerdem war die krankheitsspezifische Sterblichkeit in der Patientengruppe mit mutierten Tumoren erhöht. All diese Parameter deuten auf ein aggressives Verhalten von *TP53*-mutierten kortikotropen Tumoren hin.

In der zweiten Veröffentlichung wurden *SF3B1*-Mutationen in einer bisher unveröffentlichten, multizentrischen Kohorte von 282 Patienten mit laktotropen Tumoren untersucht. Durch Sanger-Sequenzierung wurden zwei Mutationen im *SF3B1*-Hotspot identifiziert: die bereits veröffentlichte Variante c.1874G>A p.Arg625His und die bei laktotropen Tumoren unbeschriebene Variante c.1873C>T (p.Arg625Cys). Insgesamt wurden *SF3B1*-Mutationen in 2,5 % der laktotropen Tumoren nachgewiesen, aber wenn man nur metastatische laktotrope Tumore berücksichtigt, stieg die Prävalenz auf 50 %. *SF3B1*-mutierte Tumore wurden häufiger mit den höheren Graden der fünfstufigen Trouillas- (≥2b) oder Knosp-Klassifikation (Grad 3 oder 4) eingestuft, und *SF3B1*-mutierte Tumore wiesen häufiger Metastasen auf im Vergleich zur Wildtyp-Gruppe. Auch die Notwendigkeit stärkerer therapeutischer Interventionen sowie das kürzere progressionsfreie Überleben deuten auf einen aggressiveren Krankheitsverlauf bei Patienten mit *SF3B1*-mutierten laktotropen Tumoren hin.

Beide Veröffentlichungen dieser Dissertation erweitern unser Wissen über die Auswirkungen von Varianten in *TP53* und *SF3B1* auf Hypophysentumore und die Krankheitsentstehung. Während andere Faktoren, die zu einem aggressiven Tumorverhalten führen, nach wie vor unklar sind, werden Varianten in diesen beiden Genen mit einem aggressiveren Krankheitsverlauf in Verbindung gebracht. Angesichts der Schwere der Erkrankung bei Patienten mit aggressiven Hypophysentumoren kann ein Screening von kortikotropen und laktotropen Tumoren auf *TP53*- bzw. *SF3B1*-Mutationen die präzise und rechtzeitige Behandlung der Patienten erleichtern.

4. My Contribution

4.1 My contribution to Publication I

For Publication I with the title "*TP53* mutations in functional corticotroph tumours are linked to invasion and worse clinical outcome" published in *Acta Neuropathologica Communications* in 2022, I extracted DNA from corticotroph tumours and amplified the *TP53* coding region for Sanger sequencing. In addition, I contributed to the analyses of the chromatograms and compilation of the clinical datasets.

4.2 My contribution to Publication II

For Publication II with the title "Prevalence and clinical correlations of *SF3B1* variants in lactotroph tumours" published in *European Journal of Endocrinology* in 2023, I collected a relatively large cohort of lactotroph tumours and extracted tumour DNA, amplified the *SF3B1* hotspot region in Exon14 and prepared the samples for Sanger sequencing. I analyzed the chromatograms and variants, compiled the clinical and histopathological data from the patients, and conducted the statistical analyses. I prepared the tables, figures and wrote the original and revised drafts of this manuscript.

5. Publication I

TP53 mutations in functional corticotroph tumours are linked to invasion and worse clinical outcome

Authors: Luis Gustavo Perez-Rivas, **Julia Simon**, Adriana Albani, Sicheng Tang, Sigrun Roeber, Guillaume Assié, Timo Deutschbein, Martin Fassnacht, Monica R. Gadelha, Ad R. Hermus, Günter K. Stalla, Maria A. Tichomirowa, Roman Rotermund, Jörg Flitsch, Michael Buchfelder, Isabella Nasi-Kordhishti, Jürgen Honegger, Jun Thorsteinsdottir, Wolfgang Saeger, Jochen Herms, Martin Reincke and Marily Theodoropoulou

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6. Publication II

Prevalence and clinical correlations of *SF3B1* variants in lactotroph tumours

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