Personalized Management of Pheochromocytoma and Paraganglioma

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Background and Relevance

- Pheochromocytomas (PCCs) and paragangliomas (PGLs) (together referred to as PPGLs) are endocrine tumors originating from neural crest–derived cells:
  - adrenal medulla
  - sympathetic (mostly below the diaphragm) paraganglia
  - parasympathetic (anterior thoracic and head and neck) paraganglia
- According to the latest WHO classification, all PPGLs are considered to have metastatic potential, replacing the previous term “malignant”.

Background and Relevance

- Current evaluation of the metastatic potential of a PPGL is based on a multifactorial risk assessment according to:
  - tumor size (≥5 cm)
  - extra-adrenal location
  - the presence of a SDHB mutation
  - a dopaminergic phenotype (eg, plasma methoxytyramine more than 3-fold above the ULN)
  - high Ki-67 index

Background and Relevance

- IHC staining of tumor tissue for SDHB:
  - a valuable method for identifying patients likely to have SDHB mutations
- The method has reasonably high sensitivity but a lower specificity of around 84%.
- The ***combination of SDHB IHC and metabolite profiling*** with machine learning algorithms considerably improves the accuracy of both methods for identifying ***functional SDHB mutations***.

Background and Relevance

- These methods for screening for SDHx mutations are reasonable for quickly identifying patients for high metastatic risk.

- However, accurate genetic testing remains indispensable.

Background and Relevance

- the *Pheochromocytoma of the Adrenal Gland Score* (**PASS**) and *Grading of Adrenal Pheochromocytoma and Paraganglioma* (**GAPP**) score are the only globally used ***risk-stratification systems*** based on histological features (the GAPP score also includes PGLs and additionally involves the catecholamine phenotype).
- A PASS score $\geq$ 4 indicates potential malignant behavior:
  - Sensitivity: close to 100%
  - Specificity: 75%.

Background and Relevance

- Metastatic behavior cannot be reliably predicted with a PASS score ≥ 4, but rule-out of malignant behavior with a PASS score < 4 or a GAPP score of < 3 seems to be fairly reliable.
- It also seems reasonable to include all aforementioned risk factors together with the PASS/GAPP score for a more accurate risk assessment.
- However, until this has been proven in larger patient cohorts, regular follow-up of all patients remains mandatory

Background and Relevance

- When currently known germline mutations are taken into account:
  - 30% to 35% of patients with PPGLs are affected by germline mutations
  - a further 35% to 40% show somatic driver mutations
- In combination, ***mutations*** in more than 20 PPGL driver genes have been identified in around ***70% of all patients with PPGLs***, and these are divided into 3 main molecular clusters:
  - pseudohypoxia cluster 1 (1A and 1B)
  - kinase-signaling cluster 2
  - Wnt signaling cluster 3.

Background and Relevance

- These clusters also translate into clinical, biochemical, and imaging signatures which may guide follow-up and therapy, facilitating a cluster-specific (personalized) patient management plan.

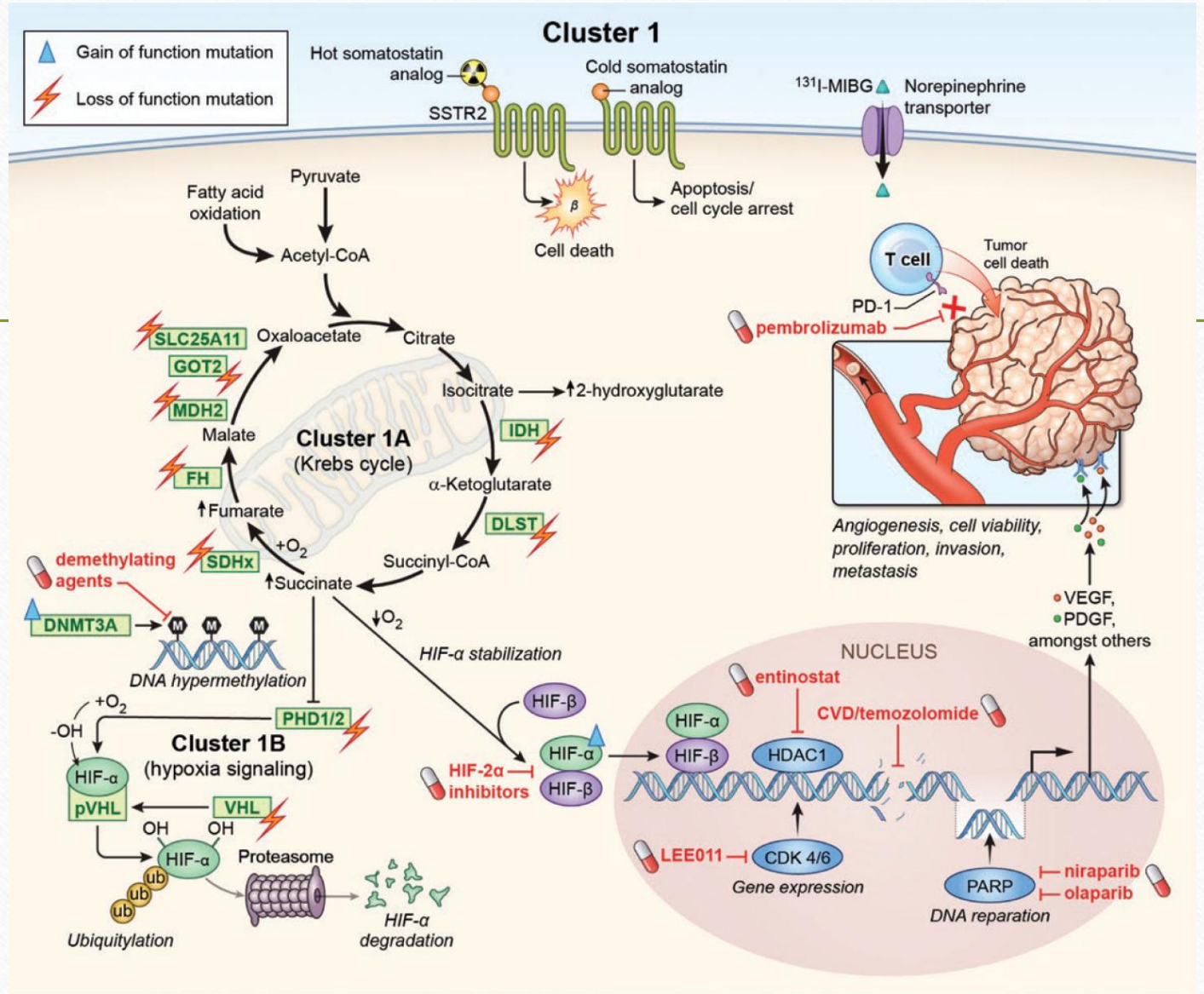
- *Genetic testing* is recommended for *every patient* since confirmation of the cluster affiliation has been shown to have a *positive impact on PPGL management and outcomes*.

- this review focuses on specific *PPGL* clusters.

Molecular Cluster 1
Pathophysiology and Signaling Pathways

- Cluster 1 is termed the pseudohypoxic cluster since the tumors of this cluster are characterized by activation of pathways that mimic hypoxia signaling.

- Cluster 1 is divided into 2 subclusters:
  - 1A: gene mutation in the Krebs cycle, almost 100% are germline mutations
  - 1B: gene mutation in the hypoxia-signaling pathway, about 25% are germline mutations


Cluster 1
Gain of function mutation
Loss of function mutation
Hot somatostatin analog
Cold somatostatin analog
SSTR2
Cell death
Apoptosis/ cell cycle arrest
131I-MIBG
Norepinephrine transporter
Pyruvate
Fatty acid oxidation
Acetyl-CoA
SLC25A11
Oxaloacetate
Citrate
GOT2
MDH2
Malate
Isocitrate
2-hydroxyglutarate
IDH
Cluster 1A
(Krebs cycle)
α-Ketoglutarate
FH
Fumarate
DLST
+O2
demethylating agents
SDHx
Succinyl-CoA
Succinate
DNMT3A
DNA hypermethylation
↓O2
HIF-α stabilization
+O2
-OH
PHD1/2
Cluster 1B
(hypoxia signaling)
HIF-α
pVHL
VHL
OH
OH
HIF-α
Proteasome
ub
Ubiquitylation
HIF-α degradation
HIF-β
HIF-α
HIF-β
HIF-2α inhibitors
T cell
Tumor cell death
PD-1
pembrolizumab
Angiogenesis, cell viability, proliferation, invasion, metastasis
VEGF,
PDGF,
amongst others
NUCLEUS
entinostat
CVD/temozolomide
HIF-α
HIF-β
HDAC1
LEE011
CDK 4/6
Gene expression
PARP
niraparib
olaparib
DNA reparation

Molecular Cluster 1
Pathophysiology and Signaling Pathways

- These mutations lead to stabilization of HIF-2α and thus, among other actions, promote angiogenesis, tumor progression, migration, invasion, and metastasis.

- Taken together, these studies provide a rationale for ***targeting HIF-2α and DNA methylation in cluster 1 PPGLs***.

Molecular Cluster 2
Pathophysiology and Signaling Pathways

- Cluster 2 comprises mutations in the following genes associated with tyrosine kinase signaling: *RET*, *NF1*, *HRAS*, *TMEM127*, *MAX*, *FGFR1*, and rare cases with *Met*, *MERTK*, *BRAF*, and *NGFR*.
- These mutations lead to activation of tyrosine kinase-associated signaling pathways, such as PI3K/AKT, RAS/RAF/ERK, and mTORC1, and finally as a common intersection point with cluster 1 to increased synthesis of HIF-α and, among other changes, to enhanced cell growth, cell survival, and tumor formation.
- Most patients with these tumors show a better clinical outcome compared to patients with cluster 1–related tumors.

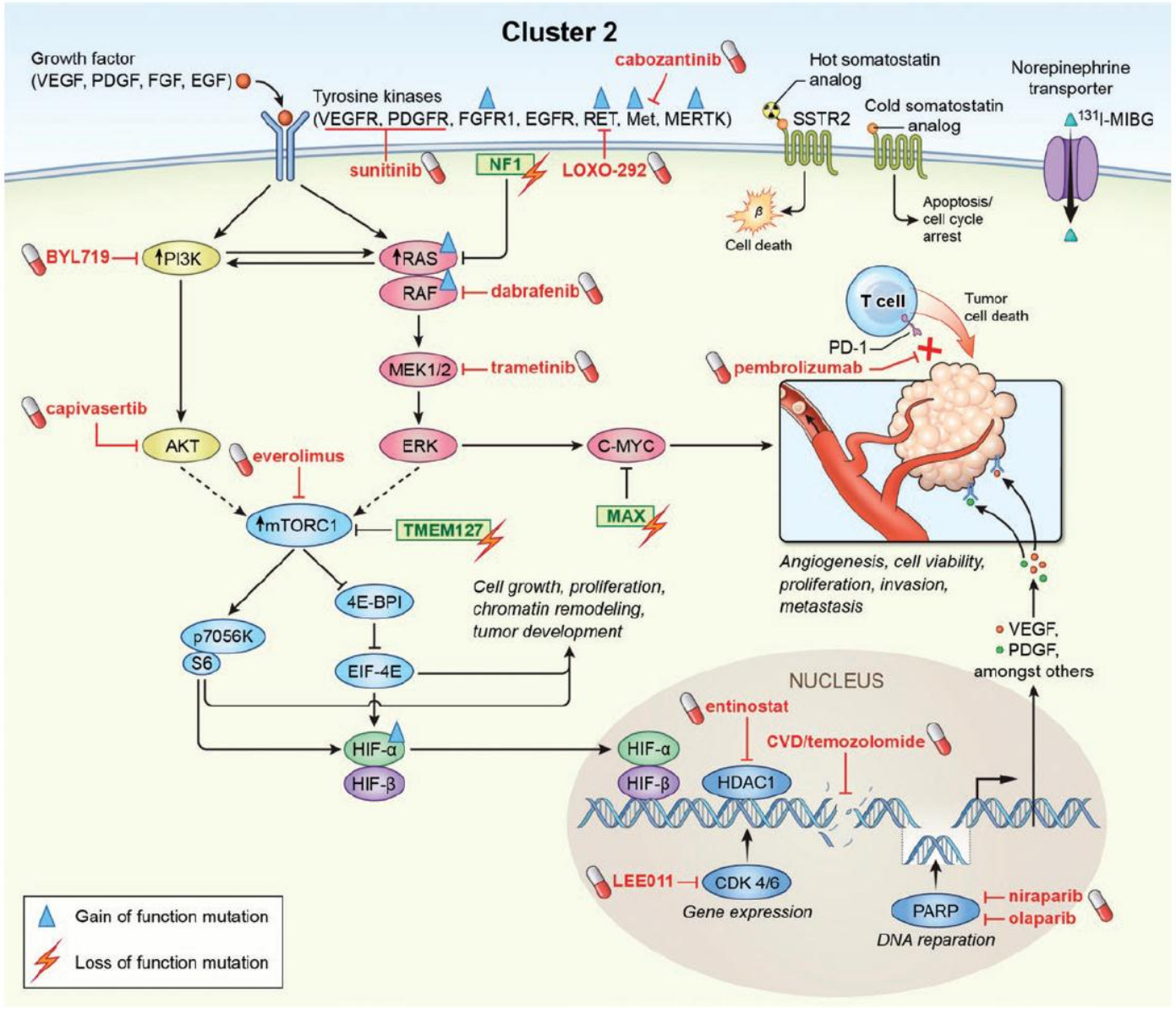


Figure 5. Gene mutations leading to an activation of kinase signaling pathways (cluster 2) and derived molecular targets for a personalized therapy. Mutations in *RET, NF1, HRAS, TMEM127, MAX, FGFR1, Met, MERTK, BRAF* and *NGFR* activate phosphatidylinositol-3-kinase (PI3K)/AKT, mammalian target of rapamycin (mTORC1)/p70S6 kinase (p70S6K), and RAS/RAF/ERK signaling pathways. Highlighted in red are potential drugs that address the molecular changes in cluster 2 PPGLs and are in preclinical and clinical evaluation. Molecular-targeted signaling pathway inhibitors (alone and in combination) might be specifically effective in these tumors. Moreover, similar to cluster 1, cluster 2 PPGLs provide the somatostatin receptor (possibly lower expression compared to cluster 1) and the norepinephrine transporter (possibly higher expression compared to cluster 1) as potential targets for the treatment of these tumors. Immune checkpoint inhibitors and drugs addressing DNA repair and synthesis mechanisms are furthermore under evaluation.

Molecular Cluster 3
Pathophysiology and Signaling Pathways

- Cluster 3 comprises the *MAML3* fusion gene and somatic mutations in *CSDE1* associated with overactivation of Wnt- and s-catenin signaling leading, among others, to angiogenesis, proliferation, survival, invasion, metastasis, and deregulation of metabolism.

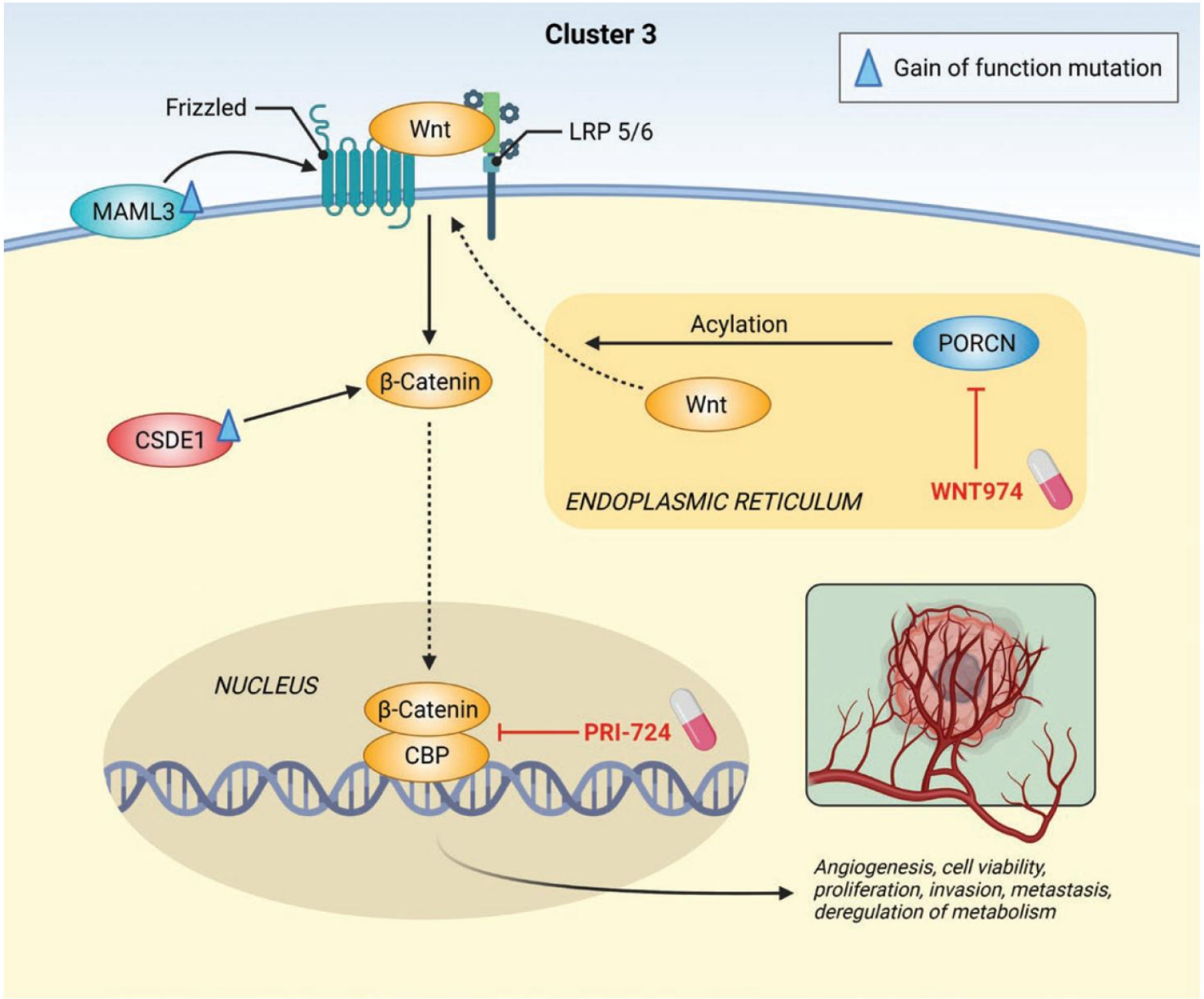


Figure 6. Gene mutations leading to an activation of Wnt signaling (cluster 3) and derived molecular targets for a personalized therapy. Mutations in *MAML3* and *CSDE1* activate Wnt/ß-catenin signaling. Highlighted in red are potential drugs that address the molecular changes in cluster 3 PPGLs and are in preclinical evaluation.

Molecular Cluster 1
Penetrance, Epidemiology, and Metastatic Risk

- Patients who belong to ***cluster 1*** group, especially those with *SDHB* mutations:
  - often ***present at a young age*** (<20 years of age, some presenting at 5 years of age or less)
  - are predisposed to ***multiple and recurrent tumors with metastatic spread***.
  - *SDHA/B/C/D* mutations: inherited in an AD fashion

Molecular Cluster 1
Penetrance, Epidemiology, and Metastatic Risk

Table 1. Penetrance of cluster 1–related PPGLs

Penetrance	*SDHB*	*SDHA*	*SDHC*	*SDHD*	*VHL*
50 years	21%				
60 years	42% and 22%, respectively			43%	
80 years	25-65%				
Lifetime estimate	22%	1.7%	8.3%		15-20%

Molecular Cluster 1
Penetrance, Epidemiology, and Metastatic Risk

- patients with metastatic PPGL:
  - 50-60%: cluster 1 mutation
  - 2.3-4%: cluster 2 mutation

- In a systematic literature review:
  - **24.3%** of patients with ***cluster 1*** tumors showed metastases specially in SDHB mutation carriers
  - ***4.1%*** of patients with ***cluster 2*** tumors showed metastases
  - ***11.4%*** of patients with ***cluster 3*** tumors showed metastases

Molecular Cluster 1
Penetrance, Epidemiology, and Metastatic Risk

Table 2. Metastatic risk and location of cluster 1–related PPGLs

Mutation	Metastatic risk	Location
SDHB	35-75%	Sympathetic/parasympathetic PGLs, less commonly PCCs
SDHA	30-66%	Sympathetic/parasympathetic PGLs, very rarely PCCs
SDHC	low	Sympathetic/parasympathetic PGLs, less commonly PCCs
SDHD	15-29%	Sympathetic/parasympathetic (often head and neck) PGLs and PCCs
HIF2A/EPAS1	>30%	Sympathetic/rarely parasympathetic PGLs and PCCs
VHL	5-8%	PCCs, less commonly sympathetic PGLs, and rarely parasympathetic PGLs
SDHAF2	not known	Parasympathetic (head and neck) PGLs

Molecular Cluster 1
Penetrance, Epidemiology, and Metastatic Risk

- *SDHx*-mutated tumors are mostly located at extra-adrenal locations, while *VHL*, *FH*, and *HIF2A/EPAS1*-related tumors are located at both intra- and extra-adrenal sites.

Molecular Cluster 1
Penetrance, Epidemiology, and Metastatic Risk

- Although cluster 1 is associated with the highest metastatic risk, patients with tumors of this cluster group only showed a trend to shorter overall survival in a multivariate analysis.

- Interestingly, although 70% of children with *SDHB*-related tumors developed metastases at a median age of 16, the estimated 5-, 10-, and 20-year overall survival rate was relatively favorable (100%, 97%, and 78%, respectively).

- Recent studies consistently report that apart from the absence of metastases, both younger age (< 40 years in 1 study) and smaller size of the primary tumor (< 5 cm) at first diagnosis is associated with a better prognosis and survival.

Molecular Cluster 2
Penetrance, Epidemiology, and Metastatic Risk

Table 8. Penetrance/prevalence, metastatic risk, location of cluster 2–related PCCs

	RET	NF1	TMEM127	MAX
Penetrance	Around 50% (50%-80% multiple)	Around 7%-8% (12% multiple)	Penetrance unknown, prevalence around 2% (single tumors)	Penetrance unknown, prevalence around 1% (67% multiple)
Metastatic risk	<5% (3.5%)	Around 2%-12%	Mostly benign	Around 10%
Location		adrenal		

Molecular Cluster 2
Penetrance, Epidemiology, and Metastatic Risk

- Metastatic risk of cluster 2–related PCCs is low and *RET*, *NF1*, *TMEM127* and *MAX* mutations are almost exclusively associated with PCCs.
- In one study, only 2.3 % of all metastatic cases belonged to cluster 2.
- Nevertheless, metastatic spread remains possible for any tumor of the cluster 2 group.

Molecular Cluster 3
Penetrance, Epidemiology, and Metastatic Risk

- For cluster 3–related tumors, only somatic mutations have been identified to date.
- Wnt-altered PPGLs with *MAML3* fusion genes were all associated with metastatic disease and showed poor aggressive-disease-free survival
- They have an aggressive phenotype with high risk of multiplicity, recurrence, and metastases.
- Wnt signaling–related tumors seem to be mainly located within the adrenals (PCCs).

Molecular Cluster 3
Penetrance, Epidemiology, and Metastatic Risk

- Cluster 3-related tumors are identified by somatic mutations and are associated with a high risk of recurrence, multiplicity, and metastases.
- Wnt signaling–related tumors seem to be mainly located within the adrenals (PCCs).

Clinical Presentations

- Testing for PPGL is usually based on one of several reasons:
  - a known germline mutation
  - a previous history of a PPGL
  - an incidentally discovered adrenal or extra-adrenal mass compatible with a PPGL
  - clinical signs and symptoms

- Many clinical signs and symptoms are relatively nonspecific, such as headache or hypertension (in an increasingly obese population). Nevertheless, some signs and symptoms are more prominent in screened patients with than without PPGL.

Clinical Presentations

- A clinical feature score (−1 to +7 points) for signs and symptoms to triage patients according to their likelihood of PPGLs has very recently been published (applies to *all* clusters):
  - 1 point for each specific sign: pallor, hyperhidrosis, tremor (max. 3 points)
  - 1 point for each specific symptom: palpitations, nausea (max. 2 points)
  - 1 point for a BMI < 25
  - 1 point for a HR > 85 beats per minute
  - while for obesity (BMI > 30 , 1 point is subtracted
- A high clinical feature score ≥ 3 points indicates a 5.8-fold higher likelihood of having a PPGL compared with a lower score

Clinical Presentations

- Patients with *cluster 1* PPGLs have *lower basic symptom scores* and *less often suffer from tremor, anxiety/ panic, and pallor* (related to catecholamine excess) compared with patients with cluster 2 PCC.

- Some reports suggest that patients with cluster 1-related PPGLs *may present more often with sustained hypertension* due to the continuous release of norepinephrine into the circulation

- *Cluster 2*–related PPGLs are more likely to be associated with *higher basic symptom scores* . Their signs and symptoms are mainly of an *episodic nature* with *tremor, anxiety/panic and pallor*, and *older age at first diagnosis* associated with *paroxysmal excessive catecholamine secretion* (triggered by stimuli) due to a *high catecholamine content*, but *low rates of constant secretion* and a well-developed regulatory control, in contrast to cluster 1 tumors.

Molecular Cluster 1
Clinical Presentations

- Spells —less likely in cluster 1 PPGLs—may be triggered by certain medications, food, beverages (containing tyramine such as red wine and beer), surgery, anesthesia, endoscopy, severe stress, or elevated intraabdominal pressure (palpation, defecation, pregnancy).
- Medications that have the potential to induce a catecholamine crisis include: glucocorticoids, metoclopramide, droperidol, MAO inhibitors, TCAs, opiates, naloxone, glucagon, certain antibiotics (linezolid), drugs for obesity management (phentermine, sibutramine), and chemotherapy.

Clinical Presentations
Molecular Cluster 1

- Nevertheless, some patients may be asymptomatic, especially those with small (<2 cm) tumors where there is low catecholamine production or more generally in cases where tumors produce and metabolize but do not secrete appreciable amounts of catecholamines.
- **SDHx mutated** and ***other cluster 1-related PPGLs*** have ***lower catecholamine contents than other tumors***; in some cases, particularly for PGL in the head and neck, the tumors may be nonfunctional (no catecholamine production, also known as "silent").
- For these cases, identification based on catecholamine-related signs and symptoms or biochemical testing is not possible.
- Measurements of chromogranin A, a biomarker of neuroendocrine tumors, may be useful in some of these cases.

Clinical Presentations

- Catecholamine-related signs and symptoms of ***patients with metastatic PPGLs (mostly related to cluster 1)*** are mainly secondary to secretion of ***norepinephrine***; in contrast, the signs and symptoms of ***other PPGLs (but particularly those associated with cluster 2*** mutations) can reflect additional secretion of ***epinephrine***.
- Despite these differences, signs and symptoms per se cannot be used to reliably distinguish metastatic from nonmetastatic patients.

Clinical Presentations
Molecular Cluster 2

- For *RET*-related PCCs the predominant stimulation of beta-adrenoceptors by epinephrine is presumably responsible for the presentation of episodic tachycardia/palpitations and paroxysmal hypertension rather than sustained hypertension.
- However, only around 50% of patients with *RET*-related PCCs present with signs and symptoms, which may reflect negligible or low rates of catecholamine secretion as well as discovery as part of screening programs.
- Similarly, patients with NF1-related PCCs can often be asymptomatic and normotensive

Biochemistry
Molecular Cluster 1

- In contrast to cluster 2, ***most cluster 1*** PPGLs present with a ***noradrenergic phenotype***, as assessed by ***elevated*** plasma concentrations of ***normetanephrine*** and ***no or relatively small increases in metanephrine***.

- These tumors may also be associated ***with or without elevations of plasma dopamine***

Biochemistry
Molecular Cluster 1

- PPGLs of the ***cluster 1*** group are characterized by ***lower tumoral catecholamine contents***, but ***higher rates of catecholamine secretion per mass of tumor tissue***, compared with cluster 2 adrenergic tumors.
- This is potentially of clinical relevance since the higher rates of catecholamine secretion per mass of tumor tissue may reflect a more continuous pattern of secretion in noradrenergic than adrenergic tumors.
- **SDHB-related PPGLs**, in particular, present with ***lowest tumoral catecholamine contents*** and, outside of screening programs, ***large tumor size at diagnosis***

Biochemistry
Molecular Cluster 1

- Possibly, large tumor size at diagnosis might reflect in part the low tumoral contents of catecholamines and often dopaminergic biochemical features that might be expected to result in an asymptomatic clinical presentation.
- ***Increases of plasma free normetanephrine and/or 3-methoxytyramine*** with ***no or minimal increases of metanephrine*** (LC-MS/MS) **point uniquely and accurately** to the diagnosis of a ***cluster 1 PPGL***.
- The higher risk of metastasis in noradrenergic than adrenergic PPGL (29.1% nonadrenergic vs 10.4% adrenergic) most likely simply reflects the association of the former with cluster 1 mutations and the latter with cluster 2 mutations.
- In contrast, a dopaminergic phenotype appears to be an independent risk factor of metastatic disease

Biochemistry
Molecular Cluster 1

- In general, for the diagnosis of PPGLs, plasma free normetanephrine, metanephrine, and 3-methoxytyramine are superior to the measurement of the urinary metabolites.
- For the plasma measurements, more than a 2-fold increase above ULN provides a high suspicion of a PPGL
- however, this is possible only with accurate measurement methods (ideally, LC-MS/ MS) and appropriately applied pre-analytics (such as blood sampling after remaining in a supine position for at least 20 minutes)

Biochemistry
Molecular Cluster 2

- With the exception of *MAX*-related PCCs (mixed or noradrenergic phenotype), ***cluster 2***–related tumors have an ***adrenergic phenotype*** (assessed by ***elevated plasma or urinary metanephrine***).
- This adrenergic signature ***indicates a more mature phenotype***.
- Adrenergic tumors are characterized by high catecholamine content and high rates of production of metanephrines, but overall low rates of catecholamine secretion and well-developed secretory control.

Biochemistry
Molecular Cluster 3

- Highest chromogranin A overexpression among all clusters.
- Catecholamine phenotype unknown.

Imaging

- CT imaging (native plus contrast-enhanced phase): high screening sensitivity (about 100%) but low specificity (about 50%) for PCCs (native phase >10 HU). (PCCs are associated with cluster 1B and cluster 2).
- MRI imaging: higher sensitivity for head and neck and sympathetic PGLs (mostly cluster 1A-related) compared with CT.
- MRI overall preferable for children and long-term follow-up of children and adults.
- CT superior to MRI for lung metastases, MRI superior to CT for liver metastases.
- combining both modalities may be considered in the follow-up of cluster 1 mutation carriers or in patients with a history of cluster 1-related PPGL.

Imaging
Molecular Cluster 1

- The highest sensitivity and specificity for detection of cluster 1-related metastatic and multifocal PPGLs is provided by functional (ie, molecular) imaging.
- Functional imaging is recommended for:
  - staging of metastatic/ multifocal disease
  - presurgery staging of a PCC ≥5 cm or any PGL
  - after surgery in patients with oligo-metastatic/ multifocal disease
  - initial screening and optional in follow-up of adult *SDHx* mutation carriers.

Imaging

Table 3. Most sensitive functional imaging modalities for cluster 1A/1B

Functional imaging	*SDHx*-related (cluster 1A)	*VHL*-related (cluster 1B)	*EPAS1*(*HIF2A*)/*PHD1/2/FH*-related (cluster 1B)
First choice	[^{68}Ga]-DOTA-SSA PET/CT	[^{18}F]FDOPA PET/CT	[^{18}F]FDOPA PET/CT
Second choice	[^{18}F] FDG PET/CT ([^{18}F]DOPA PET/CT for head and neck PGLs)	[^{68}Ga]-DOTA-SSA PET/CT	[^{18}F]FDG PET/CT

Molecular Cluster 2
Imaging

- High screening sensitivity for cluster 2–related PCCs: abdominal CT imaging (native phase >10 HU plus contrastenhanced phase) or contrast-enhanced abdominal MRI.
- Since cluster 2–related tumors are usually located intra adrenally, anatomic abdominal imaging with CT or MRI is usually sufficient for tumor localization.
- MRI overall preferable for children and long-term follow-up of children and adults.
- CT superior to MRI for lung metastases, MRI superior to CT for liver metastases.
- For PCCs ≥ 5 cm: Additional presurgery contrast-enhanced thoracic CT or functional imaging to exclude metastases.

Molecular Cluster 2
Imaging

- For inconclusive results on anatomic imaging or staging of metastatic/multifocal disease:
  - the most sensitive functional imaging method for cluster 2–related PCCs: [18F]FDOPA PET/CT
  - the second most sensitive one: [68Ga]- DOTA-SSA PET/CT

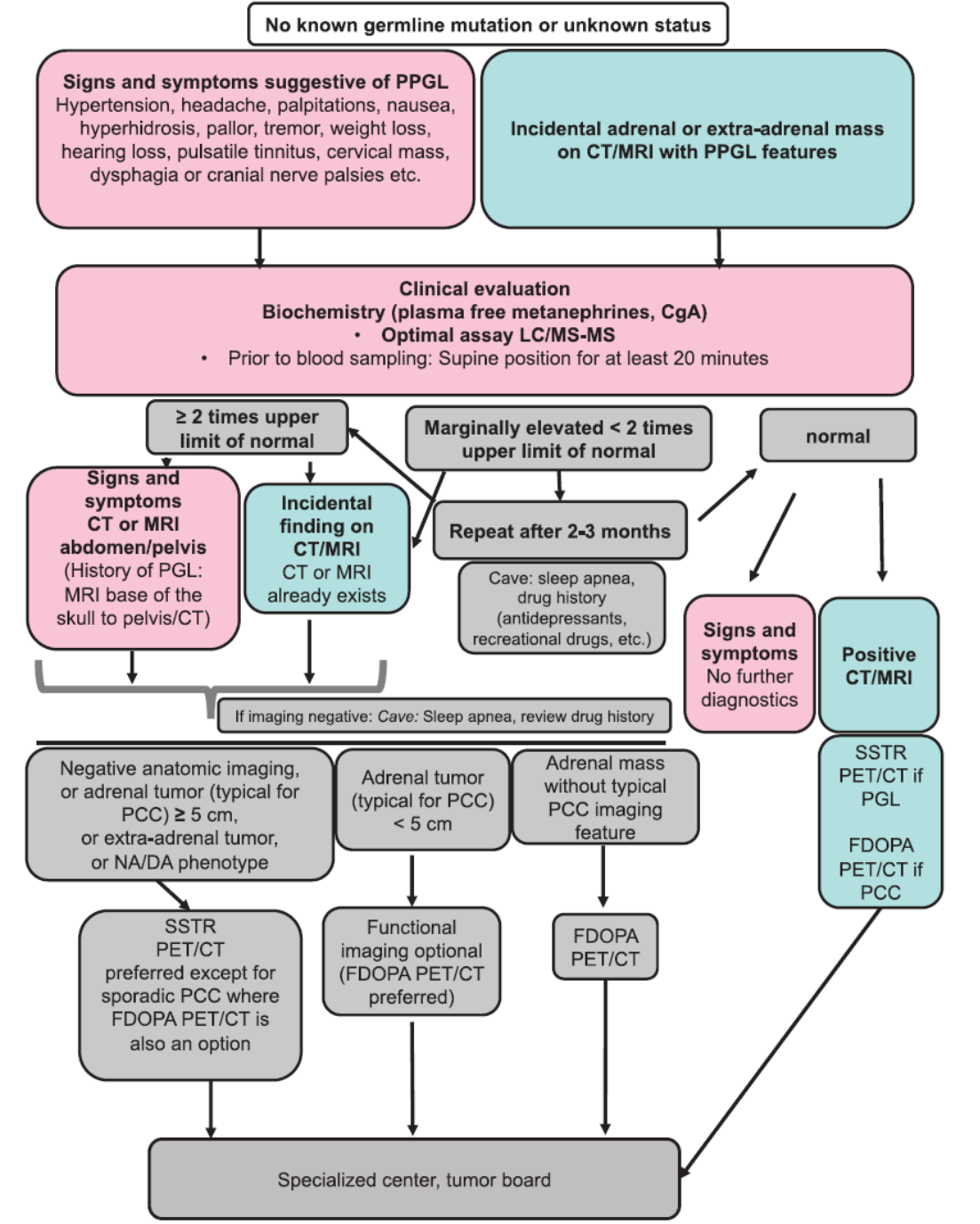
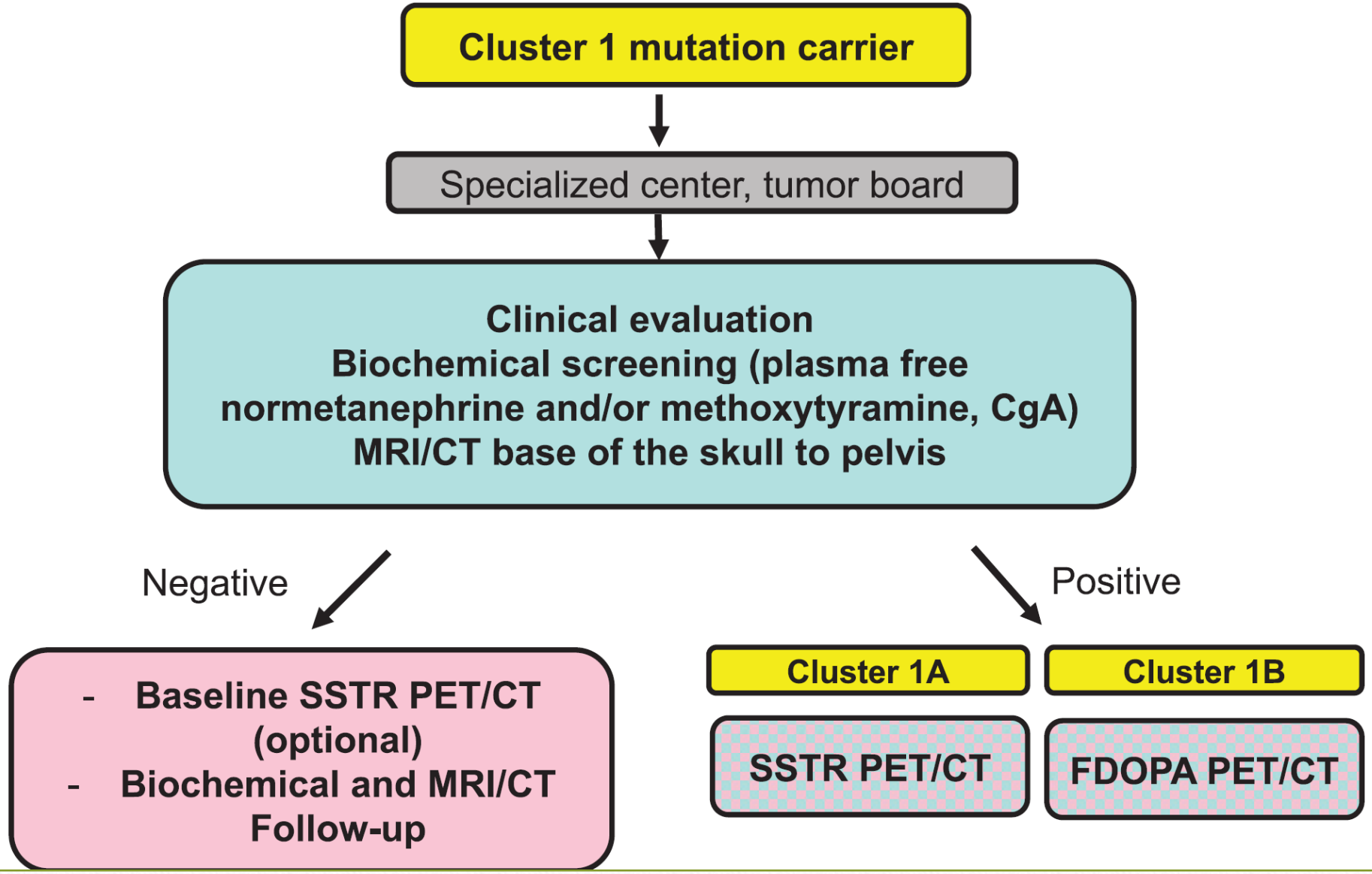
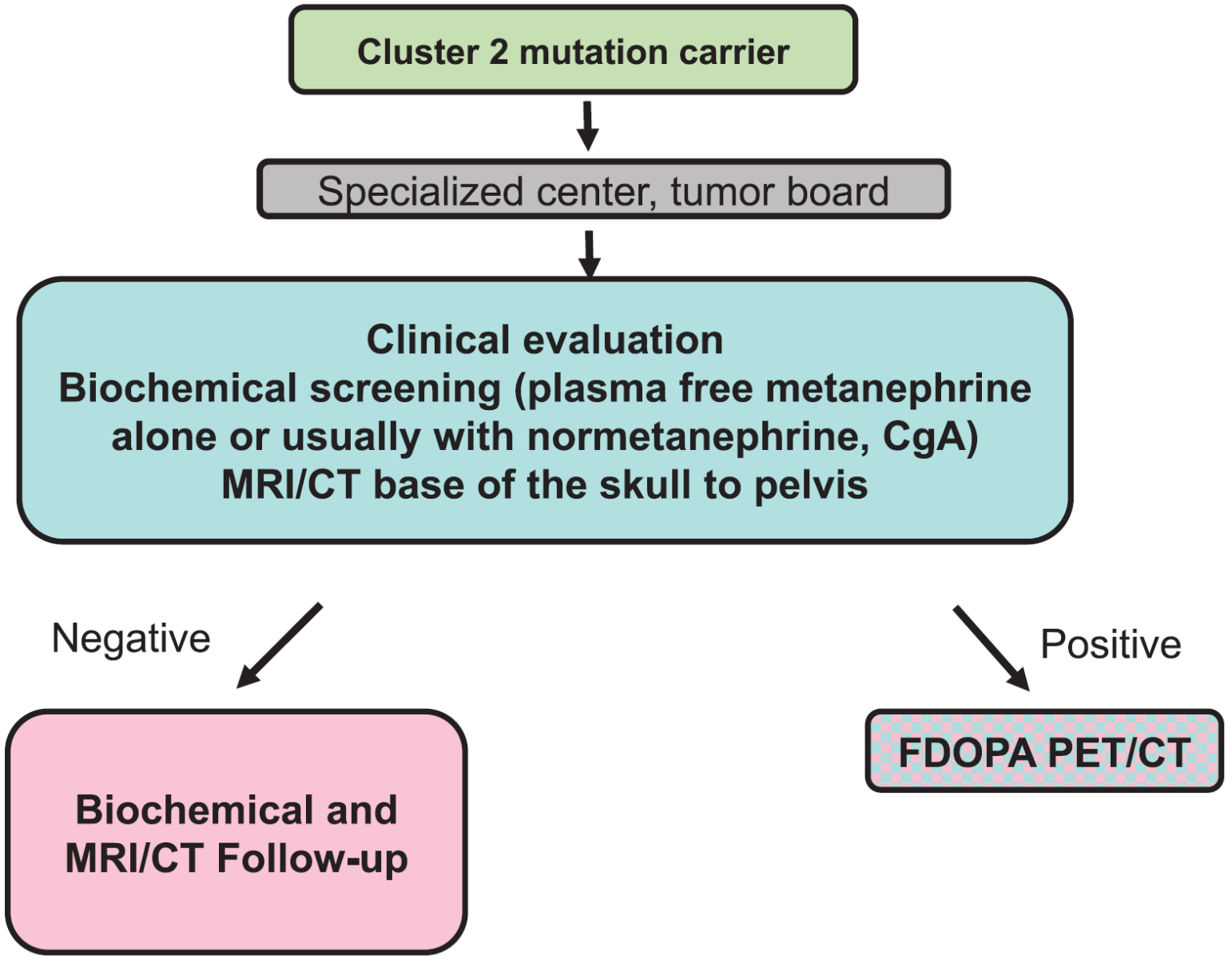


Figure 2. General diagnostic flow-chart.


Cluster 1 mutation carrier
Specialized center, tumor board
Clinical evaluation
Biochemical screening (plasma free normetanephrine and/or methoxytyramine, CgA)
MRI/CT base of the skull to pelvis
Negative
Positive
- Baseline SSTR PET/CT (optional)
- Biochemical and MRI/CT Follow-up
Cluster 1A
Cluster 1B
SSTR PET/CT
FDOPA PET/CT


Cluster 2 mutation carrier
Specialized center, tumor board
Clinical evaluation
Biochemical screening (plasma free metanephrine alone or usually with normetanephrine, CgA)
MRI/CT base of the skull to pelvis
Negative
Positive
Biochemical and MRI/CT Follow-up
FDOPA PET/CT

Molecular Cluster 3
Imaging

- For anatomic imaging, the same applies as for the other clusters; however, the most sensitive functional imaging modality is unknown.

Follow Up

- In general, every patient with any of the following criteria should undergo lifelong follow-up:
  - germline mutation
  - history of PGL
  - age <20 years at initial diagnosis
  - tumor size ≥ 5 cm
  - multiple or recurrent PPGLs
  - noradrenergic/dopaminergic phenotype

Follow Up
Molecular Cluster 1

Table 4. Follow-up of asymptomatic *SDHx* mutation carriers

Follow-up of *asymptomatic SDHx* mutation carriers	Adults	Children
Initial screening	Clinical examination (including bp), biochemical testing, MRI (base of the skull to pelvis), [^{68}Ga]-DOTA-SSA PET/CT	Clinical examination (including bp), biochemical testing, MRI (base of the skull to pelvis) (initiation: at the age of 6-10 and 10-15 years for *SDHB* and *SDHA/C/D* mutation carriers, respectively)
Follow-up	Every 12 months clinical examination (including bp) & biochemical testing (plasma > urine), every 24-36 months MRI (base of the skull to pelvis) (no consensus on alternating MRI and PET/CT)	Every 12 months clinical examination (including bp), every 24 months biochemical testing, every 24-36 months MRI (base of the skull to pelvis)

Abbreviations: bp, blood pressure; MRI, magnetic resonance imaging; PET/CT, positron emission tomography/computed tomography.

Follow Up
Molecular Cluster 1

- The authors of the current review suggest performing an MRI (base of the skull to pelvis), alternating with a low-dose chest CT plus MRI (base of the skull, neck, abdomen, pelvis) in order to reach a higher sensitivity for lung metastases.
- Genetic testing in children should only be performed if tumor screening is considered, and tumor screening should only be performed following the discovery of a mutation.
- The 2- to 3-year imaging intervals were chosen since *SDHB*-related tumors can be found as early as 2 years after initial negative screening.

Follow Up
Molecular Cluster 1

Table 5. Follow-up of cluster 1A/1B mutation carriers *with a history* of a PPGL

Follow-up of cluster 1 mutation carriers *with a history* of a PPGL	History of metastatic PPGL, history of sympathetic PGL, *SDHA/B, FH HIF2A/EPAS1*-related PPGLs	History of head and neck PGL, *SDHC/D/AF2, VHL*
Biochemistry	6-12 months (for HIF2A/EPAS1 including hematocrit)	12 months
Imaging (MRI base of the skull to pelvis, possibly alternating with low-dose chest CT plus MRI base of the skull, neck, abdomen, pelvis)	12-24 months (initially 12, then 12-24 months)	24-36 months (24 months for *SDHD*) *VHL* mutations: risk of renal cell cancer, consider abdominal MRI every 12 months; optic fundus examination every 12 months; CNS tumors, CNS MRI every 24-36 months.

Abbreviations: CNS, central nervous system; CT, computed tomography; MRI, magnetic resonance imaging; PGL, paraganglioma; PPGL, pheochromocytoma/paraganglioma.

Follow Up
Molecular Cluster 2

Table 9. Follow-up of *asymptomatic* cluster 2-mutation carriers

Follow-up of *asymptomatic* mutation carriers	*RET*	*NF1*
Clinical and biochemical evaluation	Initial screening by the age of 11-16 years depending on the specific mutation, then every 12 months (higher penetrance) Risk of primary hyperparathyroidism and medullary thyroid carcinoma (every 12 months calcitonin, calcium, PTH if applicable)	Initial screening by the age of 10-14 years, then every 36 months (lower penetrance)

Follow Up
Molecular Cluster 2

Table 10. Follow-up of cluster 2 mutation carriers *with a history* of a PCC

Follow-up of cluster 2 mutation carriers *with a history* of a PCC	*RET* (high/moderate risk for PCC), *NF1*, *TMEM127*, *MAX*	*RET*(low risk for PCC)
Clinical and biochemical evaluation	12 months	12 months
Imaging (abdominal/pelvic MRI)	At least every 5 years	optional

Follow Up
Molecular Cluster 2

- For each patient with first diagnosis of a cluster 2-related PCC ≥5 cm, a chest CT would be reasonable to rule out metastatic disease.

- however, this is unnecessary in the routine follow-up of these mutation carriers due to their low metastatic risk and almost exclusively adrenal location (PCCs) of cluster 2–related disease.

Follow Up
Molecular Cluster 3

- Optimal follow-up is unknown for these rare tumors.
- However, according to a reported high risk of recurrence, multiplicity, and metastases, follow-up should be performed by analogy with cluster 1A-related PPGLs.

Treatment

- Whenever possible, curative surgery should be the therapy of choice.
- Minimally invasive adrenalectomy is the preferred surgical standard.
- In contrast to ***cluster 2*** PPGLs, ***adrenal-sparing surgery*** should *not* be favored over ***total adrenalectomy*** in most ***cluster 1*** tumors, since these tumors have a high risk of recurrence and metastatic spread, particularly *SDHB*-mutant tumors.
- Although cortical sparing surgery is associated with development of recurrent disease in about 13% of patients with germline mutations in *RET* (cluster 2 PPGLs) or *VHL*, this is not associated with decreased survival and can be considered for less aggressive PPGLs.
- Surgery of a primary tumor in a patient with metastatic disease may be considered if there is a mass effect or reason to decrease high catecholamine levels to alleviate their organ-related damage/dysfunction.

Treatment

- Recent studies suggest that surgical removal of a primary tumor might be associated with improved overall survival, although this remains controversial.
- removing a very large primary PPGL in the presence of numerous, small metastatic lesions may improve uptake of various PPGL-specific radiopharmaceuticals in certain patients (if radionuclide therapy is planned).
- There are several anecdotal reports that suggest a potential beneficial effect of presumably curative surgery of the primary tumor and the metastases in oligometastatic disease. However, much more evidence is required for any firm recommendations.
- At least 7-14 days before surgery, other medical procedures (such as endoscopy), or systemic therapies, alpha-adrenoceptor blockade should be initiated in biochemically positive cases, unless this is purely elevated dopamine/3-methoxytyramine. To reach a seated blood pressure target $<130/80$ and HR 60 to 70 in a seated and 70 to 80 for an upright position.
- Beta-adrenoceptor blockers should be administered 2 to 3 days after alpha-adrenoceptor blockade is initiated.

Treatment

- There are no generally approved systemic treatment options for metastatic PPGLs, apart from high-specific activity (HSA) [131I]-MIBG in the United States
- there are practiced standards of therapy for metastatic PPGLs including:
  - chemotherapy (cyclophosphamide, vincristine, and dacarbazine [CVD] or temozolomide monotherapy)
  - Radionuclide therapy ([131I]-MIBG, [177Lu]-DOTATATE)
  - tyrosine kinase inhibitors (TKIs) (sunitinib, cabozantinib)
  - Immunotherapy
- To date, CVD chemotherapy is one of the most established therapies in aggressive and rapidly progressive PPGLs, and it has shown to be ***particularly effective*** in patients with ***cluster 1 SDHB mutations***. Its :
  - Disease control rate: 48% to 83%
  - Progression-free survival (PFS): 20 to 40 months

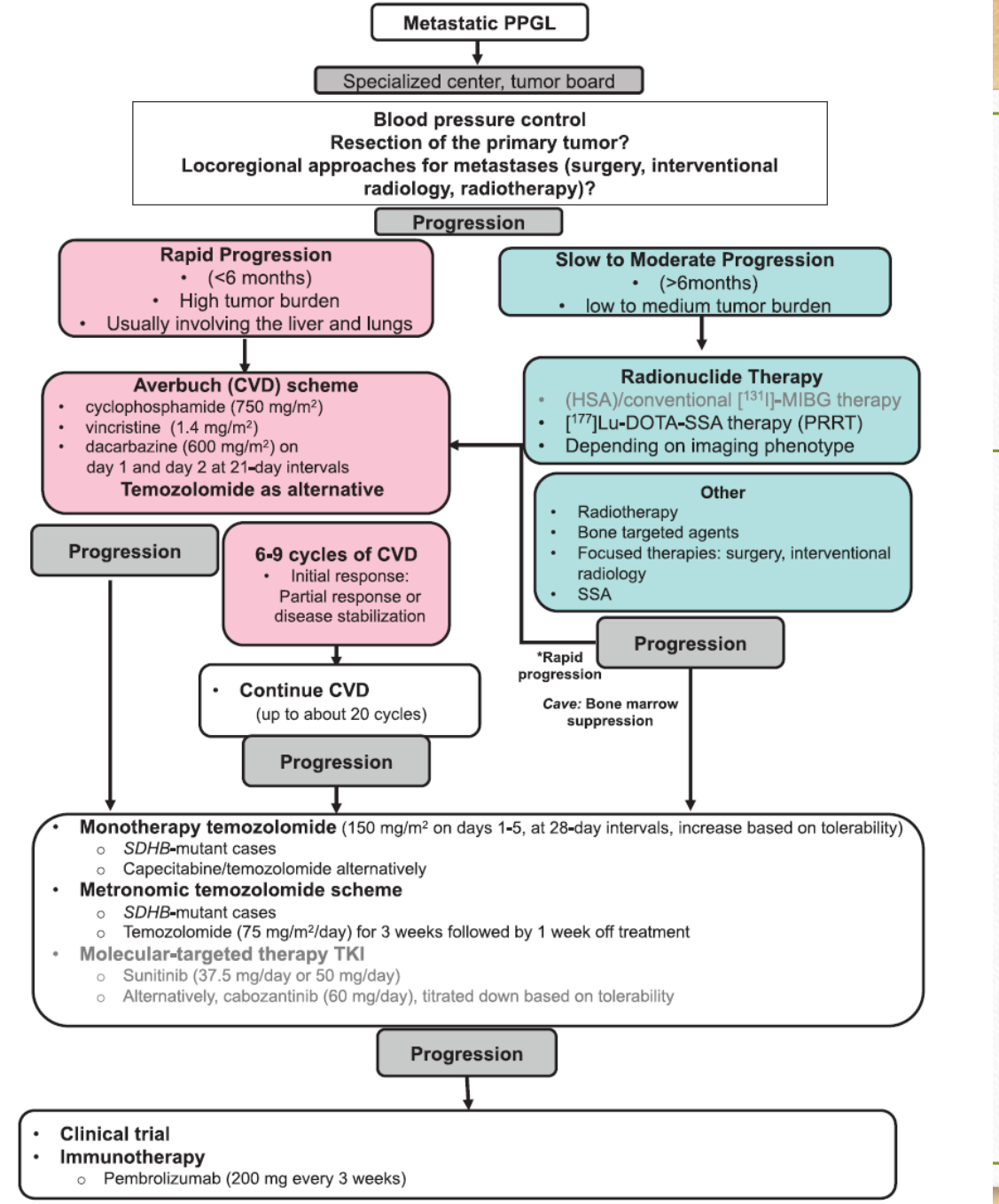
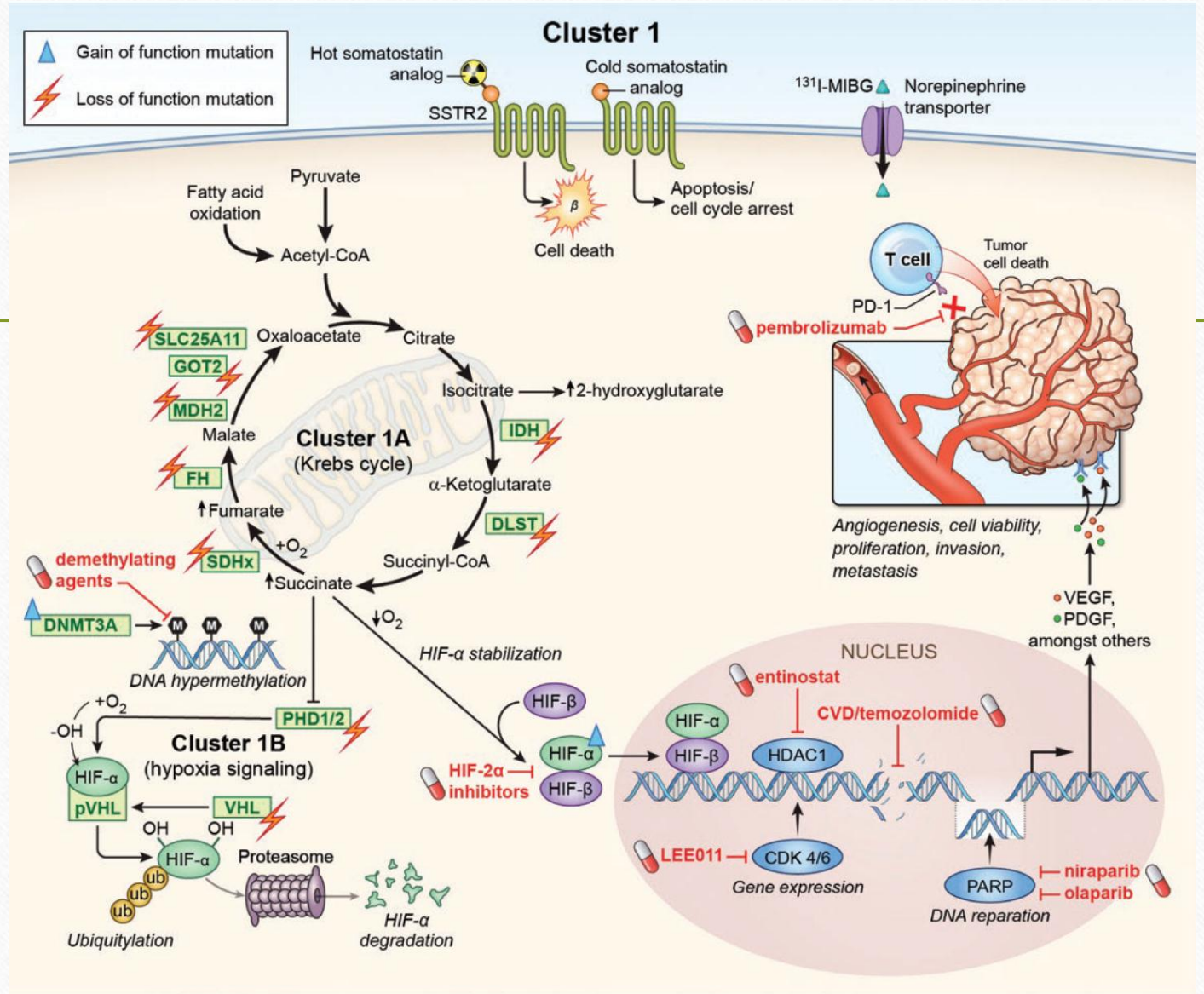


Figure 4. Flow-chart for systemic therapy of metastatic disease (1, 4, 22); black letters: potentially interesting therapy for cluster 1; gray letters: potentially interesting therapy for cluster 2. Abbreviations: SSA, somatostatin analogues; TKI, tyrosine kinase inhibitor.


Cluster 1
Gain of function mutation
Loss of function mutation
Hot somatostatin analog
Cold somatostatin analog
SSTR2
Cell death
Apoptosis/ cell cycle arrest
131I-MIBG
Norepinephrine transporter
Pyruvate
Fatty acid oxidation
Acetyl-CoA
SLC25A11
Oxaloacetate
Citrate
GOT2
MDH2
Malate
Isocitrate
2-hydroxyglutarate
IDH
Cluster 1A
(Krebs cycle)
α-Ketoglutarate
FH
Fumarate
DLST
+O2
demethylating agents
SDHx
Succinyl-CoA
Succinate
DNMT3A
DNA hypermethylation
↓O2
HIF-α stabilization
+O2
-OH
PHD1/2
Cluster 1B
(hypoxia signaling)
HIF-α
pVHL
VHL
OH
OH
HIF-α
Proteasome
ub
Ubiquitylation
HIF-α degradation
HIF-β
HIF-α
HIF-β
HIF-2α inhibitors
T cell
Tumor cell death
PD-1
pembrolizumab
Angiogenesis, cell viability, proliferation, invasion, metastasis
VEGF,
PDGF,
amongst others
NUCLEUS
entinostat
CVD/temozolomide
HIF-α
HIF-β
HDAC1
LEE011
CDK 4/6
Gene expression
PARP
niraparib
olaparib
DNA reparation

Treatment
Molecular Cluster 1

- However, state-of-the-art of therapy is based mostly on retrospective data, with only few prospective and no RCTs.
- As a future perspective, the combination of temozolomide with PARP inhibitors may enhance efficacy in cluster 1 tumors.
- A clinical phase 2 trial investigating temozolomide in combination with the PARP inhibitor olaparib is currently recruiting (NCT04394858)

Treatment
Molecular Cluster 1

- Alpha-particle emitting radionuclides may have advantages over conventional beta-particle emitters, and PRRT with an alpha-emitter (225Ac DOTATATE) has shown promising preliminary results in gastro-enteropancreatic neuroendocrine tumor patients who are stable or refractory to 177[Lu]-DOTATATE PRRT.

- In general, the need of therapy always has to be carefully balanced against the danger of severe bone marrow suppression, especially if radionuclide therapy is followed by chemotherapy or vice-versa.

Treatment
Molecular Cluster 1

- As an alternative, tyrosine kinase inhibitor therapy may be considered subsequent to radionuclide therapy (instead of chemotherapy)

- this will even become more relevant if the FIRST-MAPP study as the first randomized placebo-controlled clinical trial in the field of PPGL is able to confirm efficacy of sunitinib in these patients.

Treatment
Molecular Cluster 1

- The mTORC1 inhibitor everolimus (a signaling pathway inhibitor) has already been approved for the antiproliferative treatment of midgut and pancreatic NETs.

- There is evidence from our own translational studies in human PPGL primary cultures that these molecular-targeted drugs (alone but primarily in combination) are potentially effective in kinase signaling cluster 2–related disease, but they might be less effective in cluster 1–related disease.

Treatment
Molecular Cluster 1

- There are also 2 small, recent prospective phase 2 clinical studies suggesting that immunotherapy might be an option in selected cases with no other remaining therapeutic options.

- This requires further investigation but highlights the need for germline testing of each patient and/or somatic testing of each tumor in order to define such correlations between cluster affiliation (mutation) and therapeutic response.

Treatment
Molecular Cluster 1

- For midgut and pancreatic NETs, with a Ki-67 < 10%, "cold" somatostatin analogs (biotherapy)– octreotide LAR and lanreotide autogel—have been approved for anti-proliferative therapy in Europe and the United States.

- PPGLs with strong SSTR2 expression, particularly cluster 1 *SDHx*-related PGLs, which also show best responses to PRRT, might be treated by analogy. However, data from prospective studies are lacking and are urgently needed.

Treatment

- Additionally, antiresorptive therapies, such as bisphosphonates and denosumab, are regularly administered in the case of larger (usually numerous) bone metastases, by analogy with other neuroendocrine tumor bone metastases.
- Conventional external beam radiation therapy (cEBRT) or stereotactic radiosurgery are also well-established approaches for the therapy of single (often more-rapidly growing) bone and other metastases in oligo-metastatic disease in order to attenuate tumor growth or alleviate mass effects, and for symptomatic relief.
- Locoregional therapy approaches, including radiofrequency ablation or cryoablation, may also be reasonable approaches for single metastases such as liver metastases in oligo-metastatic disease.
- The use of cEBRT in a surgical tumor bed is currently unproven.

Treatment
Molecular Cluster 2

- Therapy is not yet cluster-specific; however, systemic therapy is only infrequently necessary in cluster 2–related disease since only around 2% to 4% of metastatic PPGLs bear cluster 2 mutations.

- Nevertheless, we suggest potential individual cluster 2–specific current and future systemic therapy approaches, such as: [131I]MIBG therapy; kinase signaling pathway–related TKIs (sunitinib, cabozantinib, LOXO-292, lenvatinib, axitinib among others); and other specific targeted signaling pathway inhibitors alone and in combination (PI3K/AKT/mTORC1 inhibitors and RAF/ MEK/ERK inhibitors)

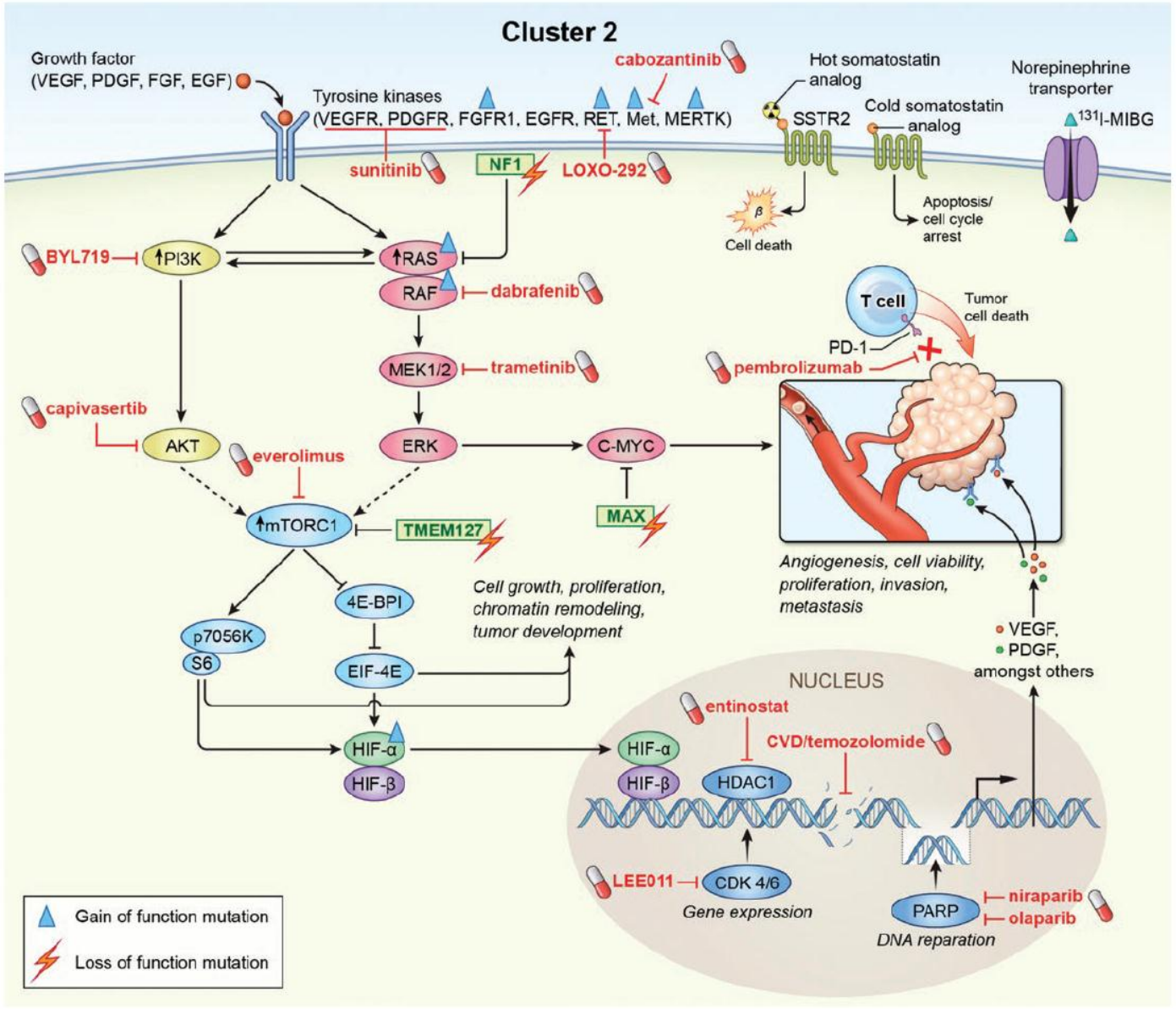


Figure 5. Gene mutations leading to an activation of kinase signaling pathways (cluster 2) and derived molecular targets for a personalized therapy. Mutations in *RET, NF1, HRAS, TMEM127, MAX, FGFR1, Met, MERTK, BRAF* and *NGFR* activate phosphatidylinositol-3-kinase (PI3K)/AKT, mammalian target of rapamycin (mTORC1)/p70S6 kinase (p70S6K), and RAS/RAF/ERK signaling pathways. Highlighted in red are potential drugs that address the molecular changes in cluster 2 PPGLs and are in preclinical and clinical evaluation. Molecular-targeted signaling pathway inhibitors (alone and in combination) might be specifically effective in these tumors. Moreover, similar to cluster 1, cluster 2 PPGLs provide the somatostatin receptor (possibly lower expression compared to cluster 1) and the norepinephrine transporter (possibly higher expression compared to cluster 1) as potential targets for the treatment of these tumors. Immune checkpoint inhibitors and drugs addressing DNA repair and synthesis mechanisms are furthermore under evaluation.

Treatment
Molecular Cluster 3

- Regarding molecular-targeted therapies, targeting Wnt signaling seems reasonable for cluster 3–related tumors.

- In different NET cell lines (also overexpressing chromogranin A), the research group of Nolting and Auernhammer et al showed good efficacy of the PORCN inhibitor WNT974, which inhibits Wnt signaling, and of the s-catenin inhibitor PRI-724.

Table 11. Individualized management plan depending on the cluster affiliation

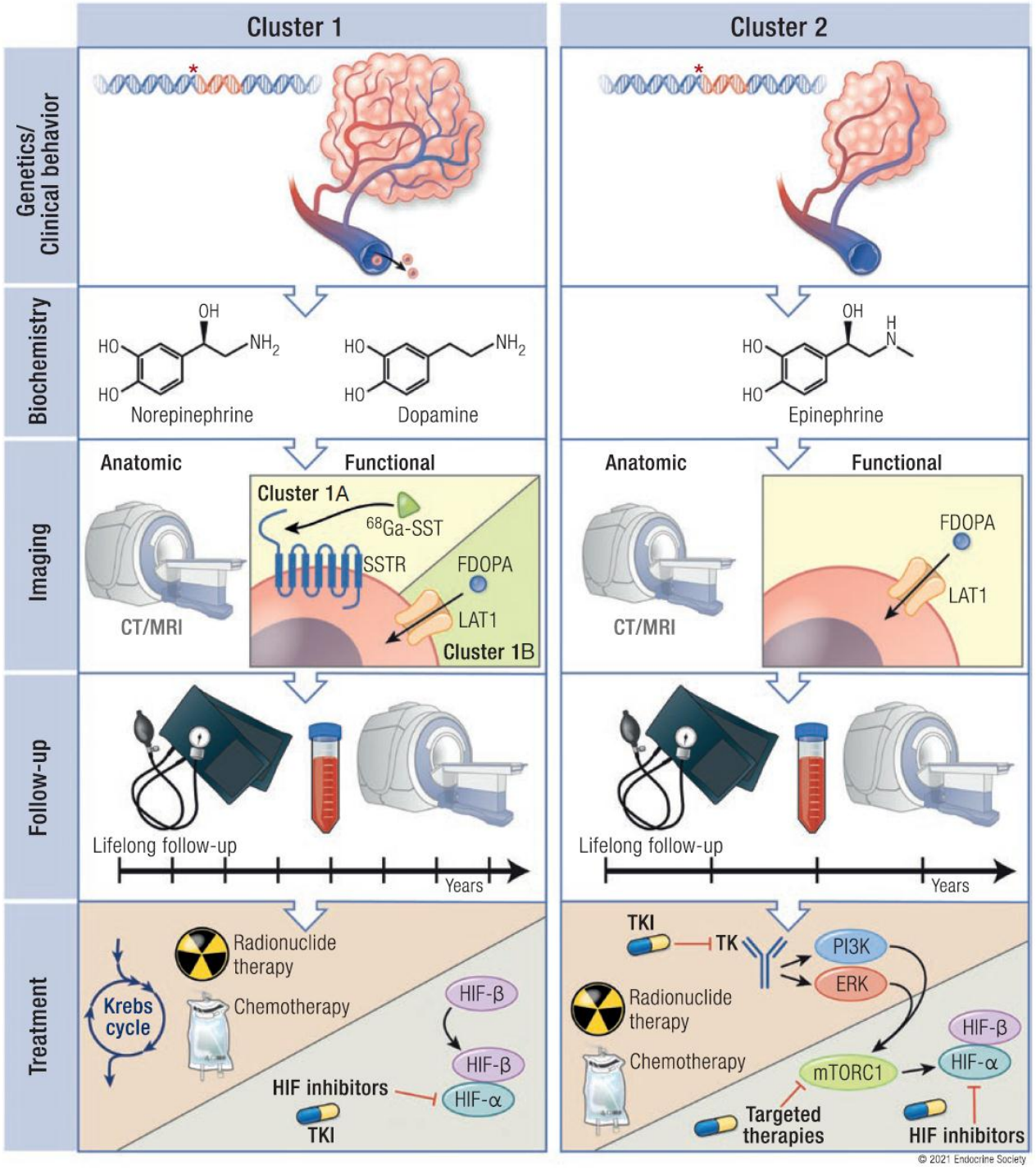
Cluster	Cluster 1A (Krebs cycle-related): *SDHx* (*SDHA, B, C, D, F2*), *FH*, *MDH2* (10%-15% of PPGL)	Cluster 1B (*VHL/EPAS1*-related): *VHL*, *EPAS1(HIF2A)*, (15%-20% of PPGL)	Cluster 2 (kinase signaling–related): *RET, NF1, MAX, TMEM127, HRAS* (50%-60% of PCC/PGL)	Cluster 3 (Wnt signaling–related): *CSDE1, MAML3* (5%-10% of PCC/PGL)
Percentage of germline mutations	Almost 100% germline	25% germline (0%*EPAS1(HIF2A)*)	20% germline	0% germline
Signaling pathways	Pseudohypoxia, Krebs cycle-related, HIF-2α stabilization	Pseudohypoxia, *VHL/EPAS1*-related, HIF-2α stabilization	Kinase signaling: PI3K/AKT, RAS/RAF/ERK, mTORC1/p70S6K	Wnt signaling
Biochemistry	Noradrenergic/dopaminergic (low catecholamine content, constant release)	Noradrenergic (low catecholamine content, constant release)	Adrenergic with additional elevation of normetanephrine (high catecholamine content, better secretory control, episodic secretion)	Unknown, highest CgA overexpression
Symptoms	More likely constant hypertension and tachycardia/-arrhythmia	More likely constant hypertension and tachycardia/-arrhythmia	More likely episodic "spells", higher sign/symptom scores, more likely tremor, anxiety/panic, pallor	Unknown
Imaging	[^{68}Ga]-DOTA-SSA PET/CT (except for *FH*)	[^{18}F]FDOPA PET/CT (also for *FH*)	[^{18}F]FDOPA PET/CT	Unknown
Tumor location	Mostly extra-adrenal	Adrenal, extra-adrenal	Adrenal	Adrenal
Metastatic risk	High-intermediate	Intermediate-low	Low	High-intermediate
Age of presentation	Early (20-30 years old, earliest 5 years old)	Early, some during childhood	Late (40-50 years old), some can present early (earliest 10 years old)	Unknown
Therapy	Surgery, systemic: CVD, temozolomide, SSTR2-based radionuclide therapy (PRRT), (HSA) [^{131}I]-MIBG, TKIs	Surgery, systemic: CVD, temozolomide, SSTR2-based radionuclide therapy (PRRT), (HSA) [^{131}I]-MIBG, TKIs	Surgery, systemic in rare cases: (HSA) [^{131}I]-MIBG, TKIs, SSTR2-based radionuclide therapy (PRRT), CVD, temozolomide	Surgery, systemic: CVD, temozolomide, SSTR2-based radionuclide therapy (PRRT), (HSA) [^{131}I]-MIBG, TKIs

Black letters: potentially specifically interesting for cluster 1; gray letters: potentially specifically interesting for cluster 2

Abbreviations: CgA, chromogranin A; CVD, cyclophosphamide/vincristine/dacarbazine; HSA, high-specific activity; MIBG, meta-iodobenzylguanidine; PCC, pheochromocytoma; PET/CT, positron emission tomography/computed tomography; PGL, paraganglioma; PPGL, pheochromocytoma/paraganglioma; PRRT, peptide receptor radionuclide therapy; SDHA/x, succinate dehydrogenase subunit A/x; SSTR, somatostatin receptor; TKI, tyrosine kinase inhibitor; VHL, von Hippel–Lindau.

Table 12. Individualized follow-up of patients *with a history* of a PPGL depending on the underlying mutation status and disease characteristics

Follow-up (*history* of a PPGL)	*High risk*	*Intermediate risk*	*Low risk*
	History of metastatic PPGL, history of sympathetic PGL, *SDHA, SDHB,* and *FH* (*FH* limited data), *HIF2A/EPAS1*	History of head and neck PGL, history of high-risk PCC (noradrenergic, ≥5 cm, recurrent, multiple) *SDHAF2, SDHC, SDHD, VHL, NF1, MAX, TMEM127, RET* with high/moderate risk for PCC	History of low-risk PCC (adrenergic, <5 cm), *RET* with low risk for PCC
Clinical, biochemistry	6-12 months (for *HIF2A*/EPAS1including hematocrit)	12 months (6 months for high-risk PCC)	12 months
Imaging (MRI base of the skull to pelvis/ MRI base of the skull, neck, abdomen, pelvis plus low-dose contrast-enhanced chest CT, alternating, for cluster 1; MRI abdomen/pelvis for cluster 2)	12-24 months (with history of disease initially 12, then 12-24 months) 6-12 months for history of very large primary PPGLs or those with large necrosis, high Ki67, and vascular and lymphatic invasion	24-36 months for *SDHAF2, SDHC, SDHD* (24 months for *SDHD*),*VHL* At least every 5 years for *NF1, MAX, TMEM127, RET* (only abdominal/pelvic MRI)	Optional
Special cases	For *HIF2A/EPAS1:* optic fundus examination every 12 months; PCC ≥5 cm: preoperative staging with additional contrast-enhanced chest CT or functional imaging History of nonfunctioning PPGL: Alternating, MRI (base of the skull to pelvis)/MRI base of the skull/ neck/abdomen/pelvis plus low-dose contrast-enhanced chest CT every 24 months History of metastatic PPGL/ sympathetic PGL: functional imaging 3-6 months postsurgery, afterwards, alternating, yearly MRI (base of the skull to pelvis)/MRI base of the skull/neck/ abdomen/pelvis plus low-dose chest CT, possibly functional imaging every 24-36 months	*VHL* mutations: risk of renal cell cancer, consider abdominal MRI every 12 months; optic fundus examination every 12 months; CNS tumors, CNS MRI every 24-36 months; *RET* mutations: risk of primary hyperparathyroidism and medullary thyroid carcinoma (every 12 months calcitonin, calcium, PTH if applicable)	
Postsurgery	Clinical and biochemical follow-up 3-6 weeks after surgery (after recovery)		


Cluster 1
Cluster 2
Genetics/ Clinical behavior
Biochemistry
Imaging
Follow-up
Treatment
OH
HO
HO
NH2
Norepinephrine
Dopamine
H
N
Epinephrine
Anatomic
Functional
CT/MRI
Cluster 1A
68Ga-SST
SSTR
FDOPA
LAT1
Cluster 1B
Lifelong follow-up
Years
Radionuclide therapy
Chemotherapy
Krebs cycle
HIF-β
HIF-α
HIF inhibitors
TKI
TK
PI3K
ERK
mTORC1
Targeted therapies
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