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REVIEW
ENDOCRINE SIDE-EFFECTS OF ANTI-CANCER DRUGS

mAbs and pituitary dysfunction: clinical evidence and pathogenic hypotheses

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Abstract

mAbs are established targeted therapies for several diseases, including hematological and solid malignancies. These agents have shown a favorable toxicity profile, but, despite their high selectivity, new typical side-effects have emerged. In cancer patients, pituitary dysfunction may be mainly due to brain metastases or primary tumors and to related surgery and radiotherapy. Anticancer agents may induce hypopituitarism in patients cured for childhood cancers. These agents infrequently affect pituitary function in adult cancer patients. Notably, hypophysitis, a previously very rare disease, has emerged as a distinctive side-effect of ipilimumab and tremelimumab, two mAbs inhibiting the cytotoxic T-lymphocyte antigen-4 receptor, being occasionally seen with nivolumab, another immune checkpoint inhibitor. Enhanced antitumor immunity is the suggested mechanism of action of these drugs and autoimmunity the presumptive mechanism of their toxicity. Recently, ipilimumab has been licensed for the treatment of patients affected by metastatic melanoma. With the expanding use of these drugs, hypophysitis will be progressively encountered by oncologists and endocrinologists in clinical practice. The optimal management of this potentially life-threatening adverse event needs a rapid and timely diagnostic and therapeutic intervention. Hypopituitarism caused by these agents is rarely reversible, requiring prolonged or lifelong substitutive hormonal treatment. Further studies are needed to clarify several clinical and pathogenic aspects of this new form of secondary pituitary dysfunction.

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Introduction

In the last years, the increasing understanding of molecular mechanisms of diseases has allowed remarkable improvements in the diagnosis and treatment of several conditions, including cancer. Many of the disease-specific or pathogenic molecules are revealed as detectable and even 'druggable targets' by highly selective proteins, such as mAbs (1, 2).

The availability of these synthetic proteins has lead to the creation of numerous new diagnostic applications and clear improvements in those already existing (3). Immunofluorescence, immunohistochemistry, or epitope-specific immunoblotting are only a few examples of the current techniques based on mAbs.

Moreover, a number of mAbs are currently used for the pathogenic treatment of rheumatologic diseases and other conditions sustained by unwanted immune/inflammatory response (e.g. arthritis, inflammatory bowel diseases, acute rejection of kidney transplants, etc.) (4, 5, 6).

MAbs, either as single drugs or combined with cytotoxics, have improved clinical outcomes in patients affected by various hematological and solid malignancies

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(7, 8). These agents exert their anticancer activity by different mechanisms of action, including the alteration of signal transduction in the downstream intracellular pathways and the neutralization of soluble ligands (7). Moreover, mAbs may activate the antibody-dependent or the complement-mediated cytotoxicity and may target the immunosuppressive crosstalk between immune system and cancer cells, inducing a tumor-specific immune response (7). MAbs can even deliver cytotoxic drug or radionuclide in the proximity of the target cells (9).

Toxicity profiles described with mAbs considerably differ from those seen with cytotoxic agents and are usually mild to moderate, although life-threatening adverse events may occur (7). Unusual toxicities have been associated with a specific class of mAbs, becoming characteristic of that class of drug. Skin toxicities may be induced by anti-epidermal growth factor receptor mAbs, approved for the treatment of advanced colorectal cancer and head and neck cancer (8, 10). Hypertension, thromboembolic events, hemorrhage, proteinuria, wound leakage, fistulas, and bowel perforation are associated with bevacizumab, an anti-vascular endothelial growth factor mAb, approved for the treatment of patients affected by advanced colorectal, breast, lung, kidney, and ovarian cancers and glioblastoma (8, 11, 12). Symptomatic and asymptomatic cardiotoxicity may be induced by trastuzumab, an anti-HER2 mAb, approved for patients with HER2-positive breast and advanced gastric cancer (8, 13). Pneumonia, tuberculosis, demyelination, drug-induced lupus, hepatotoxicity, or lymphomas are seen amongst patients treated with the anti-tumor necrosis factor α (TNF-α) mAbs (14). Recently, hypophysitis has been recognized as an adverse event triggered by some mAbs acting as immune checkpoint inhibitors (15, 16).

Herein, the epidemiological and clinical data on pituitary dysfunction induced by mAbs are reviewed, focusing on the suggested pathogenic mechanisms sustaining hypophysitis induced by anti-cytotoxic T-lymphocyte antigen-4 (CTLA4) mAbs and by other immunoregulatory anticancer mAbs.

Drug-induced pituitary dysfunction

Various anticancer treatments may induce selective deficit of pituitary hormones (17). Usually, these endocrine side-effects manifest and progress subclinically (17, 18). In cancer patients, irradiation of brain primary tumors or metastases may often cause various degrees of pituitary damage (18). Pituitary dysfunction may also occur even when the radiation field does not apparently involve the pituitary or hypothalamic area (18). Anticancer agents may induce hypopituitarism in patients cured for childhood cancers (17). These agents infrequently affect pituitary function in adult cancer patients. Single cases of hypopituitarism have been attributed to interferons (with or without ribavidin) in patients affected by hepatitis C (19, 20, 21, 22, 23), but not in cancer patients. Conversely, pituitary dysfunction is a relatively common side-effect induced by new mAbs that inhibit specific immune checkpoints (24). Pituitary disorders induced by these agents have been reported as 'hypopituitarism' or 'hypophysitis' with different degrees of toxicity (Table 1). The mAbs subclass of immune checkpoint inhibitors includes mAbs targeting different receptors, including the CTLA4 and the programed death 1 (PD1) or the PD1 ligand 1 (L1) (PD1-L1) (25).

mAbs inducing pituitary dysfunction

Anti-CTLA4-mAbs

CTLA4 is a key immune checkpoint molecule that downregulates T-cell activation and proliferation (26). CTLA4 contributes to control autoimmunity and in the presence of cancer it limits the expansion of tumor-specific effector T-cells, favoring cancer immune tolerance (26). CTLA4 blockade with an anti-CTLA4 mAb (anti-CTLA4-mAb) results in the removal of the negative immune-modulatory effect of CTLA4. This action elicits therapeutic benefit (27), but it may also remove CTLA4-mediated protection from auto-immunity, and is responsible for a new spectrum of autoimmune–inflammatory side-effects, classified as immune-related adverse events (IRAEs) (28). Two mAbs inhibiting CTLA4 activity have been evaluated for clinical use: ipilimumab and tremelimumab. In two phase III trials, ipilimumab, a fully human anti-CTLA4 IgG1-mAb, improved for the first time the overall survival in patients with metastatic melanoma (29, 30). Based on this exciting finding, in March 2011, ipilimumab was approved by the FDA as first- and second-line monotherapy for unresectable or metastatic melanoma, followed by the EMA approval of the drug for previously treated advanced melanoma (8). Tremelimumab, another anti-CTLA4-mAb, did not show similar efficacy (31). The toxicity profile of anti-CTLA4-mAbs appears characteristic compared with other anticancer agents. IRAEs frequently involved the gastrointestinal tract, liver, skin, and anterior pituitary. Rarer IRAEs may involve thyroid, adrenals, central and peripheral nervous system (i.e. aseptic meningitis, Guillan-Barré syndrome, myastenia gravis, optic neuropathy), eyes (episcleritis or uveitis), lung, pancreas, heart, kidney, and bone marrow (red blood cells aplasia) (27). These toxicities are occasionally (if any) encountered with other immunomodulating agents (i.e. interferons) or with cytotoxic drugs. The CTLA4-mAbs delivered dose seems to influence the frequency and severity of IRAEs (27). IRAEs induced by anti-CTLA4-mAbs were mild to moderate in most patients, and the recovery was obtained by medical treatments as indicated in protocol guidelines (32). However, more severe grade of IRAEs, particularly severe or

Table 1 The incidence of pituitary dysfunction in studies on mAbs inhibiting immune checkpoints.

References	Type of trial, treatment schedule	Clinical setting	Number of patients	Incidence of pituitary dysfunction	Other endocrine adverse events
Ipilimumab					
(36)	Phase I trial 3 mg/kg every 3 weeks, or 3 mg/kg→dose reduced to 1 mg/kg every 3 weeks+ vaccination with modified HLA-A*0201-restricted peptides from gp100 MAA	Pretreated stage IV melanoma	56	G3–4 hypophysitis: one pt (1.8%)	Not reported
(37)	Phase I–II trial 3 mg/kg+peptide vaccinations or intra-patient dose escalation→peptides (HLA-A*0201 status)	Pretreated stage IV melanoma	139	G3–4 hypophysitis: 13 pts (9%)	G1–2 hypothyroidism: three pts (2%)
(38)	Phase I trial 3 mg/kg→1 mg/kg or all doses at 3 mg/kg q 3 weeks	mRCC	61	G3–4 hypopituitarism: two pts (3.3%)	G3–4 primary adrenal insufficiency: one pt (1.6%)
(15)	Retrospective study 3 mg/kg every 3 weeks	Pretreated stage IV melanoma and RCC	113 50	G3–4 hypophysitis: 8/163 pts (4.9%), 6/113 melanoma pts (5%), 2/50 RCC pts (4%)	Not reported
(39)	Phase I–II trial 3→9 mg/kg (intra-patient dose escalation)	Pretreated stage IV melanoma	46	G3–4 hypophysitis: eight pts (17%); (5 mg/kg: one pt), (9 mg/kg: seven pts)	G3–4 hypothyroidism: one pt (2.2%)
(77)	Phase II trial 3 mg/kg every 3 weeks×four for a maximum two courses	Metastatic pancreas ADC	27	G2–3 hypopituitarism: one pt (3.7%)	Not reported
(78)	Phase I trial 3 mg/kg→monthly 1 mg/kg×3 months (dose level 1), → escalation to 3 mg/kg monthly×4 months (dose level 2)	Relapsed/refractory B-cell NHL	18	G1–2 hypophysitis: one pt (6%)	Not reported
(29)	Phase III trial 3 mg/kg every 3 weeks for four doses with or without gp100 vs gp100 alone	Pretreated unresectable stage III/IV melanoma	676	Hypophysitis: G3, two pts (1.5%) in ipilimumab arm; G3, two pts (0.5%) in combination arm; 0 in gp100 arm; Hypopituitarism: G3–4, two pts (1.6%); G1/2, one pt in ipilimumab arm; G3, two pts (0.8%); G1–2, one pt in the combination arm	G1–2 hypothyroidism: two pts (1.5%) in ipilimumab arm; G3, one pt (0.3%); G1/2, five pts (1.6%) in the combined arm; G1/2, two pts (1.5%) in gp100 arm Adrenal insufficiency: G1–2, two pts (1.5%) in ipilimumab arm; G3, two pts (0.5%); G1–2 one pt (0.3) in the combination arm
(79)	Phase II trial 0.3, 3, 10 mg/kg q 3 weeks for four cycles (induction)→q 3 months (maintenance)	Pretreated unresectable stage III/IV melanoma	217	Endocrine-IRAEs were globally reported: Grade 3–4: 0.3 mg/kg: 0 pts; 3 mg/kg: two pts (1%); 10 mg/kg: one pt (0.5%) In the 3 mg/kg group, hypopituitarism lead to treatment withdrawal	
(43)	Phase I trial 10 mg/kg q 3 weeks×4→q 3 months+ bevacizumab 7.5 mg/kg (Cohort 1) or 15 mg/kg (cohort 2) q 3 weeks	Unresectable stage III/IV melanoma (naïve/not)	21	Hypophysitis (grade not specified): three pts (14%)	Thyroiditis (grade not specified): four pts (19%)

Table 1 *Continued*

References	Type of trial, treatment schedule	Clinical setting	Number of patients	Incidence of pituitary dysfunction	Other endocrine adverse events
(80)	Phase II trial 10 mg/kg q 3 weeks for four cycles (induction)→ q 3 months (maintenance)	Pretreated, unresectable stage III/IV melanoma	155	Endocrine-IRAEs were globally reported: G3: two pts (1.3%); G4: 0 pts	
(50)	Phase II trial compassionate use 10 mg/kg q 3 weeks for four doses→every 12 weeks in case of CB	Refractory melanoma	53	G2–3 hypophysitis with adrenal insufficiency: two pts (4%)	G2 hypothyroidism: one pt (2%)
(41)	Phase I trial 1–10 mg/kg escalating dose+PSA–Tricom vaccine	Chemotherapy refractory/naïve mHRPC	30	Panhypophysitis: four pts (13.3%) (5 mg/kg: G2, two pts; 10 mg/kg: G3, one pt, G2, one pt)	Hypothyroidism: four pts (13.3%) (5 mg/kg: G2, two pts; 10 mg/kg: G2, two pts); Adrenal insufficiency: three pts (10%) (5 mg/kg: G3, one pt; 10 mg/kg: G3, one pt; G2, one pt)
(46)	Phase IIb trial Paclitaxel 175 mg/m^2+ carboplatin (AUC 6)± placebo/ipilimumab	Stage III/IV NSCLC	204	G3 hypophysitis/hypopituitarism: one pt (0.5%) in the concurrent ipilimumab arm	Not reported
(81)	Phase II trial 10 mg/kg i.v. q 3 weeks up to four doses (induction)→10 mg/kg q 12 weeks (maintenance)	Pretreated, unresectable stage III/IV melanoma	EAP; 830	Endocrinopathies: 4%	
(42)	Phase I trial 0.3, 1, 3, 5 mg/kg escalating dose+ GVAX	CT-naïve mCRPC	28	Hypophysitis: G2, one pt (3.6%); G3, two pts (7.1%) at 3.0 mg/kg dose level; G2, two pts (7.1%); G3, two pts (7.1%) at the 5.0 mg/kg dose level	Not reported
(40)	Phase II trial 3 or 10 mg/kg every 6–8 weeks for 12 months→maintenance ipilimumab at 10 mg/kg every 3 months. In HLAA*0201+ pts, ipilimumab infusions were accompanied by three separate subcutaneous vaccine (tyrosinase, gp100, MART-1)	Resected stage IIIc/IV melanoma	75	Hypophysitis: 11 (15%) pts	Hypothyroidism (G1): one (1.3%) pt
Tremelimumab					
(51)	Phase I trial 0.01–15 mg/kg every 90 days	Metastatic malignancies	39	G2 hypopituitarism: one pt (2.5%) (15 mg/kg)	G1 hypothyroidism: one pt (2.5%) G1 hyperthyroidism: one pt (2.5%) (15 mg/kg)
(82)	Phase II trial 15 mg/kg i.v. every 90 days	Refractory or relapsed melanoma	251	G3–4 hypophysitis: one pt (0.4%)	Thyroid disorders: eight pts (3.2%)
(31)	Phase III trial 15 mg/kg i.v. every 90 days (arm A) vs CT (arm B)	Naïve unresectable stage IIIc/IV melanoma	325 (arm A)	'Hypothalamic or pituitary gland disorders': six pts (2%) No case in arm B	'Primary thyroid disorders': 17 (5%) vs one (2%) pt in arm B Primary adrenal insufficiency: four pts (1%) vs no case in arm B

Table 1 *Continued*

References	Type of trial, treatment schedule	Clinical setting	Number of patients	Incidence of pituitary dysfunction	Other endocrine adverse events
Anti-PD1 mAbs					
(67)	Phase I trial Nivolumab 0.1–10 mg/kg every 2 weeks (escalating dose)	Various advanced solid malignancies	296	Hypophysitis: one pt (<1%)	Thyroiditis: <1%. Adrenal insufficiency: 0 TSH increase: nine pts (3%) (0.1 mg/kg: two pts G1–2; 1 mg/kg: two pts G1–2; 3 mg/kg: two pts G1–2; 10 mg/kg: three pts G1–2+1 pt G3–4) Hypothyroidism: seven pts (2%) (0.3 mg/kg: one pt G1–2; 1 mg/kg: two pts G1–2; 3 mg/kg: one pt G1–2; 10 mg/kg: two pts G1–2+one pt G3–4) Hyperthyroidism: three pts (<1%) (0.1 mg/kg: one pt G1–2; 3 mg/kg: one pt G1–2; 10 mg/kg: one pt G1–2+one pt G3–4)

AEs, adverse events; CT, cytotoxic chemotherapy; DLT, dose limiting toxicity; EAP, expanded access program; ED-SCLC, extensive disease-small-cell lung cancer; G, toxicity grade according to CTC-NCI criteria; G1, mild; G2, moderate; G3, severe; G4, life threatening; GVAX, granulocyte–macrophage colony-stimulating factor gene-transfected tumor cell vaccine; HRPC, hormone resistant prostate cancer; IRAEs, immune-related adverse events; NSCLC, nonsmall cell lung cancer; Pretr, pretreated; PSA, prostate specific antigen; Pt(s), patient(s).

life-threatening diarrhea/colitis/diverticulitis, hepatitis, and hypophysitis and even drug-related deaths (2.2%) were reported (33, 34). In the majority of cases, the onset of IRAEs seems to depend on the organ damaged by the drug (33, 35). Skin IRAEs are typically reported after an average of 3–4 weeks, those affecting the gastrointestinal tract and liver occur after 6 or 7 weeks, while endocrine adverse events tend to occur after 9 weeks (32, 35). The difference in IRAEs time of onset remains unexplained (33). Skin and endocrine disorders are usually more persistent. Gastrointestinal symptoms resolve more rapidly, although were most frequently associated with severe complications (bowel perforation/obstruction or peritonitis) if not readily recognized (33). Similarly hypophysitis, if not promptly diagnosed and properly treated, may be life threatening due to secondary adrenal insufficiency.

Anti-CTLA4-mAbs and pituitary dysfunction

Anti-CTLA4-mAbs have been reported to induce pituitary dysfunction (hypophysitis, anti-CTLA4-H, and/or hypopituitarism) with a variable incidence (0–17%) of 4.5% in the largest trial (29). The reasons explaining the wide range of incidence are still unknown. The onset of the pituitary damage secondary to anti-CTLA4-mAbs is independent from the tumor types, having been reported in patients with various solid and hematologic malignancies (kidney, prostate, pancreatic, lung cancer, melanoma, lymphoma). However, as ipilimumab and tremelimumab have been mostly studied in patients with kidney and prostate cancer and melanoma, the majority of data comes from these settings. Notably, not in all trials, a surveillance for endocrine toxicity (i.e. adrenocorticotropin, cortisol, thyroid-stimulating hormone, and free thyroxine serum levels) was required by protocol, and therefore some cases may have been missed. The dosage of the drugs seems to influence the rate of anti-CTLA4-H. In initial small studies on patients affected by metastatic melanoma with lower doses of the drug (1–3 mg/kg), a lower incidence of hypophysitis (1.8%) was reported (36). However, in a larger phase II study including 139 advanced/metastatic cutaneous melanoma patients receiving 1–3 mg/kg with or without a peptide vaccine, severe to life-threatening hypophysitis was recorded in 9% of patients (37). Two out of 61 patients (3.3%) with metastatic renal cell cancer who received ipilimumab (1–3 mg/kg) experienced hypophysitis (38). In a trial evaluating ipilimumab (3–9 mg/kg intra-patient dose escalation) in patients affected by cutaneous melanoma who had previously received other treatments, eight out of 46 (17%) patients had hypophysitis (one patient at the dose of 5 mg/kg and seven patients at the dose of 9 mg/kg) (39). In the first published trial evaluating toxicity and tolerability of ipilimumab in patients with radically resected stage IIIc/IV melanoma (adjuvant setting) with doses of 3 and 10 mg/kg (at the extended dosing interval of 6–8 weeks), 11 out of 75 (15%) patients had G1–3 hypophysitis. In this trial, patients who resulted HLAA*0201-positive received three separate subcutaneous vaccines (tyrosinase, gp100, MART1) in addition to ipilimumab. Approximately 60% were pretreated with other drugs active against melanoma. The authors did not observe a significant difference in toxicity

when comparing the two ipilimumab dosing regimens. It is unclear what the influence of the administration of vaccines is in triggering hypophysitis. In this report, six (8%) patients required replacement glucocorticoids due to hypophysitis for longer than 2 years (40). In the phase III trial leading to the FDA approval of ipilimumab (676 pretreated patients with unresectable stage III/IV melanoma receiving the drug as a single agent or combined with the peptide vaccine gp100 or gp100 alone), 'hypophysitis' was reported in two (1.5%) patients in ipilimumab only and in two (0.5%) patients in the combination arm. Furthermore, drug-related 'hypopituitarism' was reported in six (1.2%) patients (two in the ipilimumab arm and two in the combination arm respectively) (29).

In a phase I trial on patients with metastatic castration-resistant prostate cancer (mCRPC), ipilimumab (1–10 mg/kg) was administered in combination with an anti-prostate-specific antigen (PSA) vaccine (41). Hypophysitis occurred in four out of 30 (13.3%) patients on the higher doses (5 mg/kg, two patients; 10 mg/kg, two patient) (41). In another mCRPC study of ipilimumab (0.3–5 mg/kg) combined with a prostate cancer cell vaccine, moderate to severe hypophysitis was diagnosed in all three patients at the dose of 3 mg/kg, in two of 16 (13%) patients of the 3 mg/kg expansion cohort, and in two of three patients at the higher dose (5 mg/kg) (42). Whether the higher incidence of hypophysitis seen in these trials might be attributed to the higher drug dose, to the concomitant administration of vaccine or both, remains to be elucidated. Preliminary results from the only trial evaluating ipilimumab (10 mg/kg) administered in combination with bevacizumab reported high rates of hypophysitis (14%) (43).

Hypophysitis was never diagnosed when ipilimumab, even at a dose of 10 mg/kg, was combined with chemotherapy in ant studies (30, 44, 45) except one (46). Similarly, hypophysitis was not reported in patients affected by advanced cutaneous melanoma with brain radiotherapy-pretreated metastases (47). The presumptive explanation of these data might reside in the immune cell depletion induced by cytotoxic chemotherapy and radiotherapy. Hypophysitis was not reported among patients with metastatic melanoma who were involved in two of three expanded access programs of ipilimumab as a single agent (3 mg/kg) (48, 49). In the other trial, the incidence of hypophysitis was 4% (50).

Tremelimumab (15 mg/kg) has also been reported to induce hypophysitis, apparently at a lower frequency (0.4–5% of patients). In the first phase I study of tremelimumab, one out of 39 (5%) patients with solid malignancies developed panhypopituitarism due to hypophysitis (51). In the largest phase III trial, 655 untreated patients affected by unresectable stage IIIc or IV melanoma randomly received tremelimumab (15 mg/kg once every 90 days) or chemotherapy (temozolomide or dacarbazine). In the tremelimumab arm, endocrine toxicities occurred in 7.4% of patients (31). 'Hypothalamic or pituitary gland disorders' were reported in 2% of patients, while primary thyroid disorders and primary adrenal insufficiency was diagnosed in 5 and 1% of patients respectively (31). However, data on pituitary dysfunction induced by tremelimumab are quantitatively more limited when compared with ipilimumab. This is the consequence of the negative results on tumor control seen in the tremelimumab pivotal phase III trial that have greatly lessened the clinical development of the drug.

Clinical features of anti-CTLA4-H

Anti-CTLA4-H seems to occur mainly in males. This is the opposite of classic lymphocytic hypophysitis (LyH) (52, 53). Presenting symptoms are not specific, including headache, fatigue, weakness, confusion, hallucinations, memory loss, labile moods, insomnia, anorexia, temperature intolerance, and chills (15, 28, 38). Sometimes, erectile dysfunction and loss of libido were also present (54). Visual impairment, including diplopia may occur, but less frequently compared with LyH (52, 54). In patients receiving ipilimumab at standard doses, symptoms occurred at a median time of 11 weeks after initiation of therapy (before the fourth dose), suggesting a possible cumulative effect (54). However, the onset of hypophysitis has been reported as early as 4 weeks from the first infusion (55). When pituitary hormone levels were evaluated at the onset of symptoms, variable degrees of hypopituitarism were detected (56). In patients with anti-CTLA4-H, the pituitary–thyroid and pituitary–adrenal axes are almost invariably damaged (54, 56). Also, hypogonadotropic hypogonadism is present in most male patients (83–87%) (54, 56). However, as patients who received anti-CTLA4-mAbs were affected by an advanced stage disease, hormonal abnormalities may also result from sickness-induced hypogonadism and/or sick euthyroid syndrome. In a short case series, three out of five patients showed low serum IGF1 levels (56). Prolactin levels may be elevated or low in ~25% of patients (15, 39). Sporadically, the posterior pituitary was affected with diabetes insipidus (55) or SIADH (57).

Anti-CTLA4-H presents radiological features similar to classic LyH. At magnetic resonance imaging (MRI), a pituitary swelling appears in most cases (up to 60–100% of baseline size), sometimes with thickening of the stalk (15). The height of the gland in the sagittal view increases from 3.4–6 to 7.7–11.8 mm (15, 54, 57). Contrast enhancement of the pituitary may or may not be homogeneous (58). However, MRI changes appear to be of lesser magnitude compared with those seen in classic LyH (54) and in some cases, the gland may show normal radiologic features (54, 58).

As in patients with classic LyH (51, 52), high-dose glucocorticoids are used to treat anti-CTLA4-H. The treatment is then slowly tapered according to the

improvement of the clinical and laboratory picture (32). In almost all patients, acute symptoms disappear a few days after the drug withdrawal and the start of glucocorticoids and hormone replacement therapy (15). The efficacy of glucocorticoids generally parallels with rapid pituitary shrinkage on MRI, but enlargement of the gland may decrease more gradually in a variable period of time (4–12 weeks), despite a more rapid reduction of symptoms (54, 56). Importantly, pituitary hormone deficits may be prolonged or even lifelong, despite the prompt initiation of glucocorticoid therapy. Indeed, hypopituitarism is the only potentially irreversible IRAE induced by anti-CTLA4-mAbs (32, 33). Early discontinuation of glucocorticoid therapy may predispose to relapsing hypophysitis. Recovery of pituitary–thyroid function has been reported in 37–50% of patients (32, 33, 54, 56), whereas pituitary–gonadal axis function recovered in 57% of men (15). Very few patients were able to discontinue glucocorticoid replacement due to persistent secondary adrenal insufficiency (28, 54). Unfortunately, at the onset of anti-CTLA4-H or during its course, it is still difficult to predict which patients will develop persistent hypopituitarism.

Similarly, the protective role of glucocorticoids in reducing the incidence/severity of anti-CTLA4-H remains to be investigated. Remarkably, the antitumor effects of CTLA4 blockade seemed not to be influenced by the treatment of anti-CTLA4-H with high-dose glucocorticoids and replacement therapy (27). Patients who experienced anti-CTLA4-H may be safely retreated, based on individual case evaluations (27).

Other mAbs inhibiting immune checkpoints

PD1 is a key immune-checkpoint receptor expressed by activated T cells (59). It is part of the CD28/CTLA4 family and contributes to the maintenance of T-cell functional silence against their cognate antigens (59). PD1 is similar to CTLA4 in structure, but exerts a distinct biologic function, primarily in peripheral tissues (59, 60). PD1 has two known ligands, PD1-L1 (B7-H1) and PD1-L2 (B7-DC), PD1-L1 being the primary PD1 ligand (59, 60, 61). In contrast to CTLA4 ligands, PD1-L1 is selectively upregulated and expressed on many solid tumors (60, 61, 62) and on cells within the tumor microenvironment in response to inflammation (59, 62, 63). In this context, PD1-L1 inhibits cytokine production and the cytolytic activity of PD1+, tumor-infiltrating CD4+, and CD8+ T cells (61, 64, 65). Both PD1 and PD1-L1 play a pivotal role in the ability of tumor cells to evade the host's immune system (59, 60, 61, 62, 63, 64, 65). Human mAbs targeting PD1 or PD1-L1 induce various pharmacological interference, including increase in immune function and antitumor immunity *in vitro* (62, 64, 66). Similarly, in preclinical models, blockade of the PD1 pathway using mAbs directed against both PD1 and PD1-L1 stimulates cytotoxic activity and inhibits tumor growth (59, 60).

Nivolumab (formerly BMS-936558, MDX-1106) is a fully human IgG4 PD1 mAb. It was evaluated in a phase I trial on 296 patients affected by various advanced types of cancer at the escalating doses of 0.1–10.0 mg/kg every 2 weeks. Cumulative response rates ranged between 18 and 28%. Severe drug-related adverse events were reported in 14% of patients, with three deaths due to pulmonary toxicity. Hypophysitis was reported in one (<1%) case (67).

No cases of pituitary dysfunction were reported in initial trials evaluating lambrolizumab, another humanized IgG4 mAb that acts against PD1 (68) or other mAbs targeting PD1-L1 (i.e. BMS-936559 and MPDL3280A) (69, 70).

Discussion

In cancer patients, pituitary dysfunction may occur due to a variety of causes. Hypopituitarism may be a consequence of brain radiotherapy and surgery as well as of metastases that infiltrate or compress the gland or hypothalamus or affect their connections. Anticancer drugs may also induce selective deficit of different pituitary hormones (17). However, all these toxicities are on the whole infrequent (17). Until the advent of anti-CTLA4-mAbs, hypophysitis was never induced by anticancer drugs. It was an exceptional adverse event reported in patients who were treated with interferon for viral hepatitis (49, 50, 51, 52, 53). Furthermore, hypophysitis is *per se* a very rare disease. Up to now, <1000 cases have been reported, although the incidence of this condition may be underestimated (52, 53). Unexpectedly, during the clinical development of anti-CTLA4-mAbs, hypophysitis has emerged as a distinctive side-effect and probably as a new form of secondary hypophysitis. The wide range of anti-CTLA4-H incidence (0–17%) may in part depend on the different drug dosage, being more frequent when higher doses are used. However, it should be emphasized that pituitary hormones monitoring was not required by all trials' protocols. Therefore, transient or mild damage to the pituitary might have been underdiagnosed. It is unclear whether previous anticancer immune therapies may facilitate the onset of pituitary toxicity seen in these patients. Notably, a higher rate of hypophysitis has been reported when ipilimumab was associated with bevacizumab or certain vaccine types, but not with cytotoxic chemotherapy or radiotherapy to melanoma brain metastases (which actually seems to have a protective effect). Recently, hypophysitis is very rare (<1%) or absent in initial trials evaluating other immune checkpoint inhibitors (anti-PD1 and anti-PD-L1 respectively) (67, 68, 69, 70).

No case of hypopituitarism/hypophysitis was attributable to mAbs targeting immune mediators (i.e. TNF-α or interleukins), used in patients affected by rheumatologic diseases or other conditions sustained by

autoimmunity. This might be explained by the inherent mechanism of action of these mAbs and the therapeutic objective which is aimed at reducing rather than enhancing the immune activation triggering the disease. Infliximab, for example, by targeting TNF-α in inflammatory bowel disease, blocks one of the pathogenic effectors of the disease. On the contrary, anti-CTLA4-mAbs, by unleashing one of the molecules involved both in cancer immune escape and autoregulatory activity of the immune response, induce not only tumor response but also IRAEs. The different incidence of hypophysitis between anti-CTLA4- and anti-PD1/anti-PD1-L1 mAbs may be related to the different role of the molecule targeted by these agents in the context of the negative modulation of immune response. Indeed, CTLA4 is induced in T cells at the time of their initial response to antigen to modulate cell-mediated immune response once triggered. The PD1/PD1-L1 pathway is not involved in initial T-cell activation, but it starts later to regulate inflammatory responses in tissues and tumor microenvironment sustained by effector T-cells, limiting tissue damage related to immune activation (25). Therefore, the mAbs-mediated inhibition of PD1/PD1-L1 pathway presumably acts on the modulatory phase of the process more than in the triggering phase of the autoregulatory mechanism.

The key question remains why anti-CTLA4-mAbs trigger the onset of hypophysitis. Unfortunately, almost all aspects of this condition are unclear. Firstly, the exact incidence is unknown. More importantly, the pathogenic mechanisms of anti-CTLA4-mAbs have been only hypothesized (54, 56). Since the presenting clinical features, including MRI and hormone profile, resemble primary LyH and the causative drugs unleash autoimmunity, the suggested pathogenic mechanism is likely to be autoimmunity. However, as in LyH, neither antigen(s) nor the role of pituitary immune microenvironment favoring the onset of this condition has been unveiled. Furthermore, to date no case CTLA4-H has been proven by pathology. The onset of anti-CTLA4-H has been suggested to result from the activity of anti-pituitary antibodies (15). Although the presence of pituitary antibodies in patients on anti-CTLA4-mAbs has been detected, pituitary-triggering antigen(s) and pathogenic antibodies in anti-CTLA4-H remain to be evaluated. Furthermore, potential factors influencing the onset of anti-CTLA4-H, including age, gender, genetic predisposition (specific polymorphisms of *CTLA4* and other genes involved in known autoimmune diseases) have not been elucidated. Overall, the available data show that this condition is more prevalent in males, contrary to most other autoimmune diseases, including autoimmune hypophysitis. However, such male preponderance needs to be confirmed in larger trials. When the potential genetic correlations between *CTLA4* haplotypes and responses in patients receiving ipilimumab were evaluated, no correlation emerged (71, 72). Until now, studies evaluating genetic markers as potential predictors of specific toxicities are still lacking.

It has also been reported that the onset of IRAEs is associated with better clinical outcomes of underlying malignancy (73). However, whether the onset of hypophysitis in patients under anti-CTLA4-mAbs (or a specific grade of this toxicity) may predict better survival and/or response remains to be estimated in further trials with appropriate analytical approach (73).

As in primary LyH, patients on anti-CTLA4-mAbs with symptoms suggesting hypophysitis should promptly undergo pituitary MRI and pituitary function assessment. The possibility of pituitary metastasis should be considered in the presence of a sellar mass in this clinical scenario. If metastasis is high in the differential diagnosis, a biopsy should be considered (74). If anti-CTLA4-H is suspected, the drug should be held. Methylprednisolone (1–2 mg/kg per day i.v.) should be given for a few days, followed by prednisone (1–2 mg/kg per day orally), gradually tapered over 4 weeks (24, 32). An alternative high-dose steroid regimen may be 4 mg dexamethasone every 6 h for 7 days, gradually tapered to 0.5 mg/day and then shifting to a replacement dose of hydrocortisone (24, 75). Notably, as MRI findings may be normal, the treatment should be started as soon as possible once hypopituitarism is documented (76). However, several aspects regarding the treatment with glucocorticoids in anti-CTLA4-H are still unclear. Indeed, it is not known whether high-dose corticosteroids are necessary or if lower doses may suffice. Also, the timing of the tapering should be evaluated as, sometimes, hypophysitis relapse follows the reduction of glucocorticoids to replacement dose. Finally, the potential of the precautionary use of steroids (before starting anti-CTLA4-mAbs) in reducing the onset and/or long-term sequelae of hypophysitis/hypopituitarism, especially in preventing prolonged substitutive treatment, remains to be evaluated.

Many of the current unexplained issues regarding anti-CTLA4-H might be clarified by findings that could emerge from a pathology evaluation on pituitary biopsies of patients presenting with this adverse event. However, to the best of our knowledge, no patient with anti-CTLA4-H has undergone a pituitary biopsy. Indeed, to perform a pituitary biopsy in cancer patients with a suspected anti-CTLA4-H may be unethical, as it is not essential for the diagnosis nor for the treatment of this condition (unless pituitary metastasis is in the differential diagnosis).

Alternatively, evaluations could be performed on biopsy material obtained postmortem. However, postmortem material might not guarantee the quality of pathology assessment. Anti-CTLA4-H remains highly interesting as a unique model of research. Indeed, it allows to monitor the variation of the known pituitary antigens and antibodies in a homogeneous cohort of patients (a specific disease and a known pituitary-damaging drug), testing their role as diagnostic and predictive tools. Obviously, this context appears also as a

fertile ground to research new pituitary antigens. Pituitary antigens and antibodies could be monitored in these patients at baseline, before each cycle of treatment, and during follow-up. Such a study could help to define a series of important clinical, laboratory, and radiological features aimed at better defining the real incidence, the diagnosis, and the pathogenesis of anti-CTLA4-H. The gained information could be helpful in evaluating the potential existence of a subclinical anti-CTLA4-H, the likely impact of this syndrome on the quality of life, and the possible existence of a subgroup of patients with factors that could predispose to develop anti-CTLA4-H. This might not only reduce the rate of life-threatening complications, such as secondary adrenal insufficiency or other pituitary deficits, but also allow an optimized use of these drugs from a pharmaco-economy point of view.

Conclusion

Pituitary dysfunction induced by mAbs is limited to anti-CTLA4 agents and is occasionally seen with a PD1 inhibitor. With the expanding use of ipilimumab, hypophysitis, a previously very rare disease, will be progressively encountered by oncologists and endocrinologists in clinical practice. The key approach to obtain the optimal management of this potentially life-threatening adverse event is a rapid and timely diagnostic and therapeutic intervention. This goal could be obtained with a high level of communication between clinical staff and patients (33). Continued efforts should be made to optimize the therapeutic use of mAbs, as their efficacy may be relatively modest in absolute magnitude compared with their high cost (2). To this aim, discovery of new predictive biomarkers of both activity and toxicity remains a priority.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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