Endocrine-related adverse events following ipilimumab in patients with advanced melanoma: a comprehensive retrospective review from a single institution

Mabel Ryder[1,2,†], Margaret Callahan[4], Michael A Postow[2], Jedd Wolchok[2,3] and James A Fagin[1,2]

[1]Human Oncology and Pathogenesis Program, [2]Department of Medicine and [3]Ludwig Institute for Cancer Research, Memorial Sloan-Kettering Cancer Center, New York, New York, USA
[4]Weill Cornell Medical College, New York, New York, USA
[†]M Ryder is now at Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905, USA

Correspondence should be addressed to M Ryder
Email
ryder.mabel@mayo.edu

Abstract

Novel immune checkpoint blockade with ipilimumab, an antibody blocking the cytotoxic T-lymphocyte antigen 4 (CTLA4), is revolutionizing cancer therapy. However, ipilimumab induces symptomatic, sometimes severe, endocrine immune-related adverse events (irAEs) that are inconsistently recognized and reported. The objective of this review was to comprehensively characterize the incidence, presentation, and management of endocrinopathies following ipilimumab therapy in a single center that is highly specialized in immune checkpoint blockade. We carried out a retrospective analysis of endocrine irAEs in melanoma patients receiving ipilimumab therapy in clinical trials between 2007 and 2013. A total of 256 patients were included in this analysis. We reviewed pituitary-, thyroid-, and adrenal-related hormone test results, as well as radiographic studies and the clinical histories of patients, to identify and characterize cases of hypophysitis, hypothyroidism, thyroiditis, and adrenal dysfunction. Following ipilimumab therapy, the overall incidence of hypophysitis was 8% and that of hypothyroidism/thyroiditis 6%. Primary adrenal dysfunction was rare. Therapy with a combination of ipilimumab and nivolumab, an anti-programmed cell death 1 (PDCD1, also called PD1) receptor antibody, was associated with a 22% incidence of either thyroiditis or hypothyroidism and a 9% incidence of hypophysitis. Symptomatic relief, in particular, for hypophysitis, was achieved in all patients with hormone replacement, although endogenous hormone secretion rarely recovered. In summary, we observed that CTLA4 blockade alone, and in particular in combination with PD1 blockade, is associated with an increased risk of symptomatic, sometimes severe, hypophysitis as well as thyroid dysfunction. Prompt initiation with hormone replacement reverses symptoms. Evaluation and reporting of endocrine irAEs in clinical trials should be done using standardized diagnostic criteria and terminology.

Key Words
- ipilimumab
- nivolumab
- immune-related adverse effects
- hypophysitis
- thyroiditis

Endocrine-Related Cancer (2014) **21**, 371–381

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0499

Published by Bioscientifica Ltd.

Introduction

The recruitment of the immune system to treat metastatic melanoma has been a central focus in the field, given the apparent immunogenicity of melanoma cells. A new generation of immunotherapies targeting negative regulatory receptors on T cells has emerged. Ipilimumab, the prototypic MAB of this class, blocks the cytotoxic T-lymphocyte antigen 4 (CTLA4) on activated T cells (Lipson & Drake 2011). The engagement of CTLA4 by its ligands in the B7 co-stimulatory family suppresses T-cell proliferation, helping restore tissue homeostasis (Egen *et al*. 2002, Pentcheva-Hoang *et al*. 2004). Nivolumab, a second antibody-derived immune regulator, blocks the activation of the anti-programmed cell death 1 (PDCD1, also called PD1) receptor (Topalian *et al*. 2012). The efficacy of targeting immune checkpoints in patients with metastatic melanoma was established in a milestone phase III clinical trial in which ipilimumab, for the first time, improved the overall survival of patients, several of whom exhibited either complete or partial responses or stable disease that was durable for years (Hodi *et al*. 2010). This trial led to the FDA approval of ipilimumab for patients with metastatic melanoma in 2011. Clinical trials are now examining the cooperation between combined CTLA4 and PD1 blockade in patients with advanced melanoma.

Although these immunological checkpoint-blocking antibodies are revolutionizing cancer therapeutics, a unique class of mechanism-based toxicities, termed immune-related adverse events (irAEs), has emerged. Among these, endocrine irAEs are perhaps the least recognized but among the most highly symptomatic irAEs to occur following ipilimumab therapy (Blansfield *et al*. 2005, Dillard *et al*. 2010). Immune-related hypophysitis, the most common endocrine irAE in this analysis, is a particularly challenging diagnosis because before anti-CTLA4 therapy, this was a rare disease confined primarily to *postpartum* women (Caturegli *et al*. 2005). Although the US FDA approval of ipilimumab stipulates that thyroid-stimulating hormone (TSH) levels be evaluated before administration of each dose of ipilimumab as a screen for thyroid dysfunction, there is no requirement for screening pituitary–adrenal biochemistries. This, combined with the lack of common terminology and/or diagnostic criteria used to establish the diagnosis of hypophysitis, most probably explains the highly inconsistent reporting of hypophysitis in published clinical trials, with incidences ranging from 0 to 17% (Corsello *et al*. 2013). In a study carried out by Hodi *et al*. for example, the incidence of hypophysitis was estimated to be 1.5%. This is probably an underestimate, as cases of hypopituitarism, suppressed corticotroph secretion, and adrenal insufficiency, which are probably direct consequences of hypophysitis, were each classified separately (Hodi *et al*. 2010). The identification and reporting of endocrine irAEs are particularly difficult in melanoma clinical trials because empiric therapy for suspected irAEs is often initiated empirically in the absence of confirmatory diagnostic testing due to the high mortality of these patients with stage 4 disease.

The purpose of this review was to present, for the first time, a comprehensive retrospective analysis of the clinical presentation of endocrine irAEs from a single institution in patients with metastatic melanoma treated with ipilimumab in clinical trials. Despite the limitations in assessing endocrine irAEs in these terminally ill patients, we established clearly defined criteria for endocrine irAEs, enabling a more systematic characterization of endocrine irAEs. We demonstrated particular susceptibility of the thyroid and pituitary to combination anti-CTLA4 and anti-PD1 therapy. Despite the often dramatic clinical presentations of endocrine irAEs, in particular, hypophysitis, symptoms are rapidly ameliorated with exogenous hormone replacement. Unlike other irAEs, this often permits patients to continue receiving these immunotherapies from which they may derive significant clinical benefits without the necessity of prolonged therapeutic immune suppression.

Subjects and methods

Ipilimumab-induced endocrine irAEs were retrospectively identified from Bristol-Myers Squibb-sponsored clinical trials ($n=13$) in patients with advanced and/or metastatic melanoma at the Memorial Sloan-Kettering Cancer Center (MSKCC) between 2007 and 2013. The IRB-approved data were gathered by reviewing the electronic medical records and databases of all IRB-consented and -enrolled patients. Five trials encompassing ~65 patients were excluded from this retrospective analysis because laboratory studies were either not performed or performed at a sponsor-designated central facility or the study remained blinded. The included trials and their descriptions are summarized in Table 1. Standard quantitative enzymatic or radioimmunometric assays were performed according to the manufacturers' instructions for the indicated hormone tests.

Induction with ipilimumab was performed with i.v. infusions of 0.3, 3, or 10 mg/kg ipilimumab every 4 weeks for a total of four doses, followed by maintenance

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0499

Published by Bioscientifica Ltd.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0499

Published by Bioscientifica Ltd.

Table 1 Clinical trials involving ipilimumab in patients with metastatic melanoma at MSKCC

NCT protocol ID	Notes	Sample size, gender	Hypophysitis	Primary thyroid dysfunction	Other dysfunction
NCT00495066 (expanded access trial)	Metastatic melanoma, pretreated Ipilimumab 3 or 10 mg/kg 3 mg/kg, $n=74$ (54%) 10 mg/kg, $n=63$ (46%)	$n=137$ Male $n=91$ (66%) Female $n=46$	$n=9$ (6%)[a]	Primary hypothyroidism, $n=2$ (1%, 1:1 male: female) Thyroiditis, $n=1$ (<1%)	Low testosterone, $n=2$ Primary adrenal insufficiency, $n=1$ (male)
NCT00920907	Metastatic melanoma, pretreated, Ipilimumab 10 mg/kg	$n=16$ Male $n=10$ (60%) Female $n=6$	$n=2$ (13%)	Primary hypothyroidism, $n=2$ (12%, 1:1 male: female) Thyroiditis, $n=1$ (6%)	Not assessed
NCT00289640 NCT00162123 NCT00289627 excluded	Metastatic melanoma, pretreated Ipilimumab 10 mg/kg	$n=21$ Male $n=15$ (71%) Female $n=6$	Not assessed	Primary hypothyroidism, $n=3$ of 16 (19%, 2:1 male:female) Other abnormal TFTs, $n=3$ (19%) (all were transiently abnormal)	Low testosterone, $n=1$
NCT01245556	Metastatic melanoma, pretreated Ipilimumab 3 mg/kg + RAF inhibitor (BMS-908662)	$n=3$ Male $n=3$ (100%)	Not assessed	Thyroiditis, $n=1$ (male)[b]	None
NCT00796991	Metastatic melanoma, first line therapy Arm A= carboplatin + paclitaxel + ipilimumab 10 mg/kg Arm B= dacarbazine + ipilimumab 10 mg/kg Arm C= ipilimumab 10 mg/kg	$n=15$ Male $n=6$ (40%) Female $n=9$	Not assessed	Primary hypothyroidism, $n=1$ (7%, female)	Not assessed
NCT01323517	Unresectable melanoma of the limb Limb infusion with dactinomycin + melphalan followed by ipilimumab 10 mg/kg	$n=13$ Male $n=6$ (46%) Female $n=7$	$n=4$ (30%)	Thyroiditis, $n=1$ (8%)[c] Other abnormal TFTs, $n=6$	Low testosterone, $n=2$ Suspicious, $n=1$ (female)[d] Hypercalcemia, $n=1$ (female)
NCT01024231	Metastatic melanoma, pretreated Ipilimumab 3 mg/kg + nivolumab	$n=45$ Male $n=27$ (60%) Female $n=18$	$n=4$ (9%)[e]	Primary hypothyroidism, $n=6$ (13%, 2:4 male:female) Thyroiditis, $n=4$ (9%) Other abnormal TFTs, persistent and/or transient, $n=8$ (18%)	Low testosterone, $n=4$ Autonomous cortisol secretion, $n=1$ (female) Hypercalcemia, $n=1$ (female)
NCT00324155	Advanced/metastatic melanoma, first line Dacarbazine + ipilimumab 10 mg/kg vs placebo	$n=7$ Male $n=5$ Female $n=2$	Not assessed	Primary hypothyroidism, $n=1$ (14%, female)	Not assessed
Total		$n=256$ Male =162(63%) Female =94	$n=19$ of 211 (9%)	Primary hypothyroidism, $n=15$ of 256 (6%) Subacute thyroiditis, $n=8$ of 256 (3%)	Low testosterone, $n=9$ Adrenal dysfunction, $n=3$ Hypercalcemia, $n=2$

[a]$n=44$ patients had at least 1 or more ACTH and/or cortisol level checks.
[b]This patient later developed hypophysitis on ipilimumab + nivolumab study.
[c]Patient later developed hypophysitis.
[d]Suspicious for ectopic ACTH secretion versus early onset adrenal insufficiency.
[e]1 suspicious case with clinical symptoms.

infusions every 3 months for 2 years in selected trials. Screening thyroid function tests (TFTs), consisting of TSH, thyroxine (FT_4 or T_4), and/or triiodothyronine (T_3), were completed in all the patients in the expanded access trial at baseline and during follow-up ($n=173$ patients; Table 1). In the remaining trials, at least 40% of the patients had one or more TFTs done, either at baseline and/or during follow-up. Blood tests for cortisol, adrenocorticotropin (ACTH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and either estradiol or testosterone were performed according to clinical indications and/or investigator preference.

Cases of hypophysitis (Tables 1 and 2) were identified by the presence of one or more of the following: i) secondary adrenal insufficiency, defined by the presence of acute onset of symptoms of adrenal insufficiency associated with biochemically proven low or suppressed serum cortisol levels with inappropriately low ACTH levels in the absence of exogenous steroid treatment; dynamic low- or high-dose ACTH stimulation testing was not performed; ii) suspected or suspicious for secondary hypothyroidism, defined by a low FT_4 level with either a normal or suppressed TSH level that did not normalize on subsequent testing; or iii) in one patient, the presence of symptoms highly suggestive of secondary adrenal insufficiency with incomplete laboratory tests and an magnetic resonance imaging (MRI) study consistent with hypophysitis. Cases identified as suspicious for central hypothyroidism, which can be difficult to distinguish biochemically from sick euthyroid syndrome, were based on the laboratory tests consistent with central hypothyroidism in the context of co-existing central adrenal insufficiency and/or radiographic evidence of hypophysitis. In the majority of patients, we could not screen for secondary hypogonadism because routine LH and FSH levels were not evaluated. On study, none of the patients were found to have growth hormone or insulin-like growth factor levels evaluated and only rare patients had prolactin levels, usually as part of the workup of suspected hypophysitis.

Immune-related primary hypothyroidism (Table 1) was defined by the presence of a TSH level ≥ 10 mIU/l alone with or without a low FT_4 or T_3 level. Immune-related thyroiditis (Table 1) was defined by the presence of a suppressed TSH level with an elevated FT_4 and/or T_3 level. Other thyroid dysfunction, excluding that related to hypophysitis, hypothyroidism, and thyroiditis, was defined by the presence of a low or elevated TSH level with a normal FT_4 and/or T_3 level.

Primary adrenal insufficiency, autonomous adrenal function, ectopic ACTH secretion, and hypercalcemia were rare immune-related events and are individually described in the Results section and in Table 1.

Results

Hypophysitis

In four trials of 211 patients, we identified 19 cases of hypophysitis (Tables 1, 2 and 3) with an overall incidence of 8%. The presenting symptoms of hypophysitis included headaches, nausea, emesis, extreme fatigue, diarrhea, arthralgias, and/or mental status changes. One relatively asymptomatic patient was incidentally detected after abnormal TFTs, performed as part of the protocol design, prompting an evaluation of other pituitary hormones.

Table 3 provides a summary of the clinical characteristics of patients with hypophysitis. The male:female ratio was 11:8, with an overall incidence of 8 and 10% respectively. The median time to onset of symptoms following CTLA4 blockade was 4 months. Only two cases occurred at later time points: 8 and 19 months after initiation of ipilimumab therapy. Symptomatic secondary adrenal insufficiency was present in 16 (84%) patients (Tables 2 and 3). This is probably an underrepresentation as three of the 19 patients did not have adequate corticotropin function evaluations or their axis could not be evaluated due to the presence of exogenous steroids (cases 6, 7, and 9; Table 2). Of these three patients, two (cases 6 and 9; Table 2) were found to have abnormalities of the pituitary on MRI. The third patient, without an MRI, had a very low FT_4 level with an inappropriately normal TSH level (case 7). Three patients (16%) had loss of ACTH secretion alone with preservation of other axes. Case 5 (Table 2) developed irreversible secondary adrenal insufficiency, mild central hypogonadism, and concomitant subclinical primary hypothyroidism with an elevated TSH level, a normal FT_4 level, and rising titers of thyroid peroxidase (TPO) and thyroglobulin (Tg) autoantibodies.

In total, 11 of the 19 patients with hypophysitis had TFTs (Tables 2 and 3) that were either consistent with or suspicious for central hypothyroidism, characterized by a low FT_4 or T_3 level and either a normal or low TSH level. Discrimination between the presence of secondary hypothyroidism, sick euthyroid syndrome, and the effects of exogenous high-dose steroids was difficult in this population of patients. The presence of a significantly low FT_4 level with a normal or low TSH level in patients

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0499

Published by Bioscientifica Ltd.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0499

Published by Bioscientifica Ltd.

Table 2 Cases of hypophysitis following ipilimumab therapy in patients with metastatic melanoma

Case	Age, gender	mg/kg (no. of doses)	Symptoms	Hormone axes affected	Onset of hypophysitis	Biochemistries	Imaging, MRI brain/sella	Recovery of pituitary function	Received high-dose steroids	Status	Non-dermatological irAEs	Protocol no.
1	54, m	10 (3)	Mild fatigue and abnormal screening of TSH	Panhypopituitarism	4 weeks after the third dose	Cortisol[a], am 0.25, ACTH[b] 0.22, TSH[c] 0.03, FT_4[d] 12.87, T_3[e] 2.42, LH[f] 0.4, FSH[g] 2.7, testosterone[h] <3.47, and prolactin[i] 0.53	Enlarged sella	Yes, with normal ACTH stimulation test	Yes	Dead	None	NCT00495066
2	71, m	3 (4)	Fatigue, increased sleep, weight loss, and night sweats/chills	ACTH and TSH	2 months after the fourth dose	Cortisol 0.275, ACTH <1.1, TSH 0.35, FT_4 5.79, LH 9.1, FSH 12.6, and testosterone 74.29	Not done	No	Yes	NED[j]	None	NCT00495066
3	69, m	10 (16)	Nausea, emesis, and dehydration	ACTH	3 months after the fourth dose	Cortisol 0.58, ACTH <1.1, TSH 1.68, FT_4 11.84, T_3 3.77, LH 9.8, FSH 22.5, testosterone 133, and prolactin 0.83	Partial empty sella no abnormal enhancement	No	Yes	NED	None	NCT00495066
4	69, m	3 (4)	Severe headache, poor oral intake, and hypotension	ACTH, LH/FSH, and most probably TSH	2 weeks after the the fourth dose	Cortisol, am 0.6, ACTH <1.1, TSH 0.5, FT_4 11.58, T_3 0.88, LH undetectable, and testosterone <3.47	Ill-defined nodular enhancement of sella, resolved after 2 months	No	Yes	Dead	None	NCT00495066
5	61, m	3 (3)	Headaches	ACTH and LH/FSH. (thyroiditis)	3 weeks after the third dose	TSH 0.04, FT_4 14.93, T_3 2.05 (screening TFTs) TSH <0.02, T_3 1.62, cortisol 0.35, ACTH <1.1, FSH 1.8, testosterone <3.47, TSH <0.02, FT_4 25.35, T_3 0.88, and thyroid peroxidase antibody (TPO Ab)[k] <10	Enlarged sella	Partial recovery of gonadotrophs but not ACTH-persistent thyroiditis	Yes	Dead	None	NCT00495066
6	75, f	3 (2)	Fevers, sweats, and weakness	Possibly TSH and could not assess ACTH	4 weeks after the second dose	Prolactin 3.17, TSH 0.15, FT_4 20.59, estradiol <10[l], LH 11, and FSH 60	Interval increase the in size of sella	Yes; patient weaned off steroids for autoimmune hepatitis, not put on replacement hormones	Yes for hepatitis	Dead	Hepatitis	NCT00495066
7	38, f	3 (8)	Asymptomatic	Possibly TSH, and could not assess ACTH or LH/FSH	15 months after induction	TSH 0.26, FT_4 0.77, and FT_3 0.12. No other laboratory test results available	Not done	Not assessed	No	NED	None	NCT00495066
8	78, m	3 (8)	Flu-like syndrome, nausea, poor appetite, and weakness	Panhypopituitarism	4 weeks after the third dose	Cortisol, am 0.925, ACTH 2.2, TSH 5.88, FT_4 10.16, and testosterone <3.47	Not done	Partial – hypogonadism resolved	Yes	Dead	None	NCT00495066
9	71, m	10 (3)	Fatigue, poor appetite, and weakness	Not adequately assessed	2 weeks after the second dose	ACTH 2.64, no cortisol, TSH 0.7, no FT_4	Thickening and prominence of sella and infundibulum	Not assessed	Yes	Dead	None	NCT00495066

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0499

Published by Bioscientifica Ltd.

Table 2 *Continued*

Case	Age, gender	mg/kg (no. of doses)	Symptoms	Hormone axes affected	Onset of hypophysitis	Biochemistries	Imaging, MRI brain/sella	Recovery of pituitary function	Received high-dose steroids	Status	Non-dermato-logical irAEs	Protocol no.
X[m]	48, m	3 (4)	Fatigue and headache	Possibly TSH and ACTH	Before ipilimu-mab therapy (received anti-PD1 therapy before ipilimumab therapy)	ACTH, am <1.1, no cortisol TSH 4.93, and FT_4 11.06	Normal sella	Not assessed	No	Dead	None	NCT00495066
10	58, f	10 (4)	Headache, blurred vision, nausea, and emesis	Probable ACTH and TSH	1 day after the third dose	Cortisol, am 1.58 (48 h after prednisone therapy), no ACTH, TSH 0.12, FT_4 14.80, T_3 1.77 TSH 0.05, FT_4 13.12, TPO Ab <10, thyroglobulin antibody (Tg Ab)[n] <20 LH 25.8, FSH 53.9, and prolactin 0.31	Homogenous enlargement	No symptoms recurred after prednisone dose tapered	Yes	Dead	None	NCT00920907
11	74, m	10 (8)	Fatigue and weakness	ACTH	2–3 weeks after the fifth dose	Cortisol 0.25, ACTH <1.1, TSH 1.51, FT_4 16.34, LH 2.16, FSH 7.17, and testosterone 159	Normal	No	Yes	Unknown	None	NCT00920907
12	69, f	10 (7)	Severe fatigue and poor appetite	ACTH LH and FSH not assessed	3 months after the first dose	Cortisol, am <0.05, ACTH 2.2, TSH 3.60, FT_4 11.58, T_3 2.76, and prolactin 1.34 Repeat TFTs: TSH 2.73, and FT_4 15.7	Not done	No	Yes	NED	Colitis	NCT01323517
13	71, f	10 (6)	Arthralgias, fatigue,weight loss, and dehydration	ACTH LH and FSH not assessed	8 months after the first dose	Cortisol 0.275, ACTH 1.54, and TSH 2.93	Normal sella	No	Yes	Alive	Hyper-calcemia	NCT01323517
14	77, f	10 (3)	Unknown	TSH and possibly ACTH	2 months after the first dose	Cortisol, am 0.875 (had been on prior steroid therapy), ACTH evalu-ation not done, TSH 0.01, and FT_4 7.72 Repeat TFTs off thyroid hormone: TSH 3.72, FT_4 7.72, and T_3 1.03	Not done	No	Yes	Alive	Colitis	NCT01323517
15	78, m	10 (4)	Headaches and fatigue	Panhypopituit-arism	3 months after the first dose	Cortisol, am 0.25, ACTH 2.86, TSH 0.09, FT_4 9.01, and testosterone <6.94	Patchy enhance-ment of sella with thicken-ing of stalk	No	Yes	NED	None	NCT01323517
16	47, f	3 (4)	Fevers, chills, and fatigue, arthralgias	ACTH and LH/FSH not assessed	3.5 months after the first dose	Cortisol, am <0.125, ACTH <1.1, and TSH 4.80	Not done	No	Yes	NED	1) Thyroiditis 2) Pneumonitis	NCT01024231
17	73, f	3 (4)	Headache, visual changes, fatigue,nausea and emesis, diarrhea, altered mental status	ACTH	3.5 months after the first dose	Cortisol, pm 0.6, ACTH <1.1, TSH 1.53, FT_4 14.03, FSH 18.9, and LH 5.2	Enlargement of sella with heterogeneo-us enhance-ment and focal nodular area of hypointensity	Not assessed	Yes	NED	Pancreatitis	NCT01024231

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0499

Published by Bioscientifica Ltd.

Table 2 *Continued*

Case	Age, gender	mg/kg (no. of doses)	Symptoms	Hormone axes affected	Onset of hypophysitis	Biochemistries	Imaging, MRI brain/sella	Recovery of pituitary function	Received high-dose steroids	Status	Non-dermato-logical irAEs	Protocol no.
18	52, f	3 (5)	Fatigue	ACTH and TSH, LH/FSH not assessed	5 months after 1st dose	Cortisol, am 0.675, ACTH, am 1.1, TSH 2.59, and FT_4 15.06 Repeat TFTs: TSH 1.88 and FT_4 9.78	Not done	Not assessed	Yes	SD[o]	1) Hypo-thyroidism 2) Hepatitis 3) Colitis	NCT01024231
19	74, m	3 (7)	Severe fatigue, arthralgias, and mental status changes	ACTH and TSH and preserved LH/FSH	19 months after the first ipilimumab infusion 6 months after re-induction with ipilimumab 3 months after the first nivolumab infusion	Cortisol, am <0.05, ACTH <1.1, TSH 3.02, and FT_4 11.12 Repeat TFTs: TSH 0.97 and FT_4 11.19	Normal	Not assessed	Yes	SD	1) Thyroiditis 2) Myocarditis	NCT01024231

[a]Cortisol: reference ranges am = 1.25–6.25 nmol/l; pm = 0.75–3 nmol/l.
[b]Adrenocorticotropin (ACTH): reference range 1.32–10.56 pmol/l.
[c]Thyroid-stimulating hormone (TSH): reference range 0.55–4.78 mIU/l.
[d]Free T_4: reference range 11.58–23.17 pmol/l.
[e]Total T_3: reference range 0.924–2.772 nmol/l.
[f]Luteinizing hormone (LH): reference range 2–9 IU/l.
[g]Follicle-stimulating hormone (FSH): reference range 1–18 IU/l.
[h]Total testosterone: reference range 62.80–263.03 pmol/l.
[i]Prolactin: reference range 0.087–0.78 nmol/l.
[j]No evidence of disease.
[k]Thyroid peroxidase antibody (TPO Ab): reference range 0–35 IU/l.
[l]Estradiol: reference range for postmenopausal females, not detectable to 14 pg/ml.
[m]Patient received nivolumab therapy before ipilimumab therapy.
[n]Thyroglobulin antibody (Tg Ab): reference range <20 IU/l.
[o]Stable disease.

Table 3 Clinical characteristics of patients with hypophysitis following CTLA4 blockade

Hypophysitis	*n*=19 (%)
Gender	
Males, *n*=134 total	11 of 134 (8)
Females, *n*=77 total	8 of 77 (10)
Dose of ipilimumab	
3 mg/kg (*n*=119)	10 (8)
10 mg/kg (*n*=92)	9 (10)
Median time to onset of hypophysitis after the first dose of ipilimumab	4 months
Hormone axes involved (confirmed and/or suspected)	
Corticotrophs (could not be assessed in three patients)	16
Thyrotrophs (definite and/or possible; could not be assessed in one patient)	11
Gonadotrophs (could not be assessed in six patients)	5
Definite preservation of corticotroph secretion	0
MRI brain/sella completed	12
Normal/near normal	3 (25)
Abnormal	9 (75)
Received high-dose steroids at diagnosis	18 (95)
Recovery of hormone function	
Not assessed	5
No recovery of axis/axes	9
Recovery of ACTH secretion	3
Recovery of TSH secretion	0
Recovery of LH and FSH secretion	2
Developed additional non-dermatological irAEs	8
Overall status at last follow-up	
Alive	11
Dead	7
Unknown, lost to follow-up	1

with definite secondary adrenal insufficiency and/or radiographic abnormalities of the pituitary on MRI supports the presence of secondary hypothyroidism. The normalization of the TFTs (Table 2), in the absence of thyroid hormone replacement, may support either a transient immunological effect on thyrotrophs or the transient effects of steroids and/or acute illness on TFTs.

Structural imaging of the pituitary was performed with MRI or computed tomography (CT) scan in 12 of the 19 cases. Of the 12 scans, nine (75%) were clearly abnormal, showing either diffuse enlargement and/or abnormal enhancement of the pituitary. In one patient, the acute onset of symptomatic hypophysitis was accompanied by an ill-defined nodular enhancement of the pituitary on MRI that resolved on a follow-up scan. Three patients had normal imaging of the pituitary, including one patient whose scan was initially normal, but on follow-up, the patient was observed to have a severely contracted size of the pituitary. Two other patients had an empty sella or a slight thickening of the pituitary.

The majority of patients who presented with acute, symptomatic hypophysitis received a course of high-dose steroids followed by replacement with physiological hydrocortisone in those with secondary adrenal insufficiency. Treatment with high-dose or physiological steroid replacement and/or thyroid hormone reversed symptoms in all patients. Only three patients had documented biochemical recovery of ACTH secretion, while two had partial recovery of gonadotropic function only and remained with ACTH and TSH deficiencies. No patient in any of the trials had evidence of partial and/or complete central diabetes insipidus. In one patient, pituitary enlargement resolved on interval imaging and after therapy with high-dose steroids.

Of particular interest were the clinical presentations of hypophysitis in the ipilimumab+nivolumab trial. Three of the four confirmed cases of hypophysitis were preceded either by thyroiditis and/or by overt hypothyroidism. A fifth case was highly suspicious for hypophysitis based on the clinical presentation with severe arthralgias and fatigue. The male:female ratio was 1:3, with a 4 and 17% incidence respectively. One of the three patients had an abnormal pituitary on MRI and three had secondary adrenal insufficiency. One patient had secondary hypothyroidism, but the adrenal axis could not be interpreted due to prior steroid use. Overall, 18 of the 45 patients enrolled, including the four patients with hypophysitis, had non-endocrine irAEs, consisting of colitis, pancreatitis, hepatitis, myocarditis, and pneumonitis.

Immune-related hypothyroidism and thyroid dysfunction

We identified 15 cases (6%) of hypothyroidism (Table 1). Patients with preexisting hypothyroidism were excluded from this analysis. The male:female ratio of hypothyroidism was 6:9, which equated to an overall incidence of 4 and 10% respectively. Of the 15 cases, eight (53%) received ipilimumab 3 mg/kg and seven received 10 mg/kg (47%). The onset of hypothyroidism was variable, occurring within the first 5 months and up to 3 years after induction therapy. One patient with a TSH level of 18 reverted to normal in the absence of thyroid hormone replacement. Thyroid autoantibody levels were not measured in most patients. Fatigue was the most common presenting symptom, if present, and improved with thyroid hormone replacement. It is worth noting that six (40%) of the 15 cases of hypothyroidism occurred with combination ipilimumab+nivolumab therapy

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0499

Published by Bioscientifica Ltd.

with an overall incidence of 13% in this trial with a male:female ratio of 2:4.

Six patients developed thyroiditis, in three of whom it occurred on the ipilimumab+nivolumab therapy. In one patient with thyroiditis, thyroid autoantibody levels were not elevated. All patients were asymptomatic and identified by screening TFTs. At least two patients progressed to hypothyroidism. Of the six patients in the ipilimumab+nivolumab trial with either thyroiditis or hypothyroidism, three subsequently developed hypophysitis (cases 23, 25, and 26; Table 2) with symptomatic secondary adrenal insufficiency requiring hydrocortisone replacement therapy.

The prevalence of other abnormal thyroid tests was best characterized in the 137 patients enrolled in the ipilimumab expanded access program as TSH and FT_4 levels were evaluated at baseline and during follow-up in all patients. Of the 137 patients, 20 (15%) had mild abnormalities in TSH or FT_4 at baseline. On study, six (4%) patients were found to have developed subclinical hypothyroidism, defined as a TSH level between 5 and 10 mcU/ml with a normal FT_4 level.

Ipilimumab-associated low testosterone levels

Few measurements of testosterone, estradiol, LH, and/or FSH were done on protocol. Overall, we identified nine patients with low total testosterone levels, defined by a testosterone level below the normal reference range, in the absence of hypophysitis. Sex hormone-binding globulin levels were not measured. The majority of testosterone levels were either transiently low and/or measurements were not repeated. In the expanded access trial, 20 patients had testosterone levels evaluated. In two of these, the levels were low, which persisted in only one patient. In the combination ipilimumab+nivolumab trial, low total testosterone levels were observed in four of 45 patients. Most had concurrent non-endocrine irAEs and were receiving high-dose steroids, suggesting that the etiology of the low testosterone on immunotherapies is probably complex and/or multifactorial.

Ipilimumab-associated adrenal dysfunction

We identified two cases of primary adrenal dysfunction (Table 1), though this may represent an underestimation, as routine adrenal laboratory tests were not done and/or some patients received exogenous steroids for irAEs. One patient developed symptomatic primary adrenal insufficiency on ipilimumab therapy defined by a morning cortisol level $<5\ \mu g/dl$ with elevated ACTH levels. A cosyntropin stimulation test confirmed the diagnosis. This patient had bilateral adrenal nodules that were consistent with metastases and the most likely etiology was adrenal insufficiency. One patient had suspicious autonomous, ACTH-independent cortisol secretion defined by morning cortisol levels between 10 and 17 μg/dl with suppressed ACTH levels (<5 pg/ml) and in the absence of exogenous steroids. It was not clear from the medical record whether this patient had any clinical features suggestive of Cushing's syndrome (CS), and no definitive workup for CS was completed at the time of this review.

Miscellaneous endocrinopathies

One female patient developed asymptomatic and high ACTH levels on the dactinomycin+melphalan limb infusion followed by ipilimumab trial. Laboratory tests were normal immediately before ipilimumab therapy. On study, the ACTH levels were found to have increased to 70 and 180 pg/ml (reference range 6–48 pg/ml) with serum cortisol levels of 15 μg/dl. A 250 μg cosyntropin stimulation test and a 24-h urine cortisol test were normal. There was no clinical evidence of adrenal insufficiency or CS. An MRI of the brain was consistent with a meningioma, and a CT scan of the chest and abdomen revealed normal adrenal glands and a 1 cm stable lung nodule that was non-avid on fluorodeoxyglucose positron emission tomography. The etiology and clinical significance of the acute and sustained rise in ACTH levels remain unclear, but may represent the early development of either ectopic ACTH CS or primary adrenal insufficiency.

Two female patients were incidentally diagnosed with hypercalcemia, defined as a serum calcium level >11 mg/dl. Both patients had a low but detectable parathyroid hormone (PTH) level. One patient presented with concurrent secondary adrenal insufficiency due to hypophysitis. She received one infusion of bisphosphonate therapy that was followed by normal calcium levels on long-term follow-up. A second asymptomatic patient developed incidental acute hypercalcemia with calcium levels >12 mg/dl and a PTH level of 20 pg/ml. In the absence of any calcium-lowering agent therapy, and while continuing on ipilimumab+nivolumab therapy, the calcium level normalized spontaneously.

Discussion

The role of CTLA4 in broad, immune-mediated homeostasis was established in murine studies in which deletion of *Ctla4* resulted in widespread T-cell infiltration followed by

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0499

Published by Bioscientifica Ltd.

multiorgan autoimmune destruction and death. In humans, polymorphisms in the *CTLA4* gene confer increased susceptibility to a variety of autoimmune diseases, including Hashimoto's thyroiditis (HT), type 1 diabetes mellitus, and Addison's disease (Ueda *et al.* 2003, Blomhoff *et al.* 2004). Thus, blocking CTLA4, while promoting anti-tumor immunity, results in an array of immune-mediated toxicities.

In our retrospective review of melanoma patients treated with ipilimumab at the MSKCC, we identified hypophysitis as the most common endocrine irAE followed by hypothyroidism. The higher overall incidence of hypophysitis in the studies (8%) reviewed compared with that in most of the published trials probably reflects our well-defined clinical, biochemical, and radiographic criteria for identifying cases, which is highly inconsistent and ambiguous in published clinical trials (Hodi *et al.* 2010). We demonstrated that the clinical presentation of hypophysitis is often non-specific and may overlap with cancer-related constitutional symptoms. Even with careful review of the data from our trials, it is clear that the incidence of either occult or subclinical hypophysitis is probably underrepresented because of inadequate anterior pituitary hormone evaluations and the presence of exogenous steroids that may mask the symptoms and biochemistries of hypophysitis and that at least 25% of patients with hypophysitis in this analysis had a normal pituitary MRI. Measurements of pituitary autoantigens, which are presently not clinically available, may improve our ability to diagnose hypophysitis (Tzou *et al.* 2008), but require further investigation and validation. In the interim, our data provide a strong rationale for monitoring ACTH and cortisol levels in patients receiving ipilimumab and/or nivolumab therapy, similar to the routine TFTs being done to screen for hypothyroidism/thyroiditis, which is less frequent and symptomatic compared with hypophysitis.

Symptoms of hypophysitis, which are often associated with significant morbidity, were rapidly reversed with the initiation of steroid therapy. Nearly all patients included in this analysis received high doses of steroids that were tapered over 4 weeks, yet only a minority of patients recovered endogenous hormone secretion. Although high-dose steroids are standard of care for acute hypophysitis, our analysis suggests that physiological steroid replacement may be sufficient for most patients with hypophysitis and that higher-dose steroid regimens could be reserved for patients with persistent inflammatory symptoms, such as headaches and vision changes. A few patients do recover either complete and/or partial anterior pituitary function, making it reasonable in due course to carefully assess for the recovery of endogenous pituitary hormone secretion.

There is a clear association between CTLA4 and sporadic, autoimmune thyroid disease in humans and mice (Tomer *et al.* 2001, Ueda *et al.* 2003). Yet, compared with all irAEs, hypothyroidism is less frequent. The incidence of hypothyroidism in our analysis was 6%, but it ranged from 1 to 14% within individual trials. The presence or absence of thyroid autoantibodies, which are clearly established in HT and linked to *CTLA4* polymorphisms, has not been well characterized following CTLA4 blockade. Routine measurements of these autoantibodies, in addition to TFTs, will better clarify whether hypothyroidism/thyroiditis more closely resembles sporadic HT or a form of subacute thyroiditis.

It is worth noting that the combination anti-CTLA4+ anti-PD1 therapy resulted in a high incidence of hypothyroidism/thyroiditis, which, in turn, was followed by the development of hypophysitis. In two large phase I clinical trials in patients treated with anti-PD1, there was only a 0–1% incidence of hypophysitis and a 3% incidence of hypothyroidism (31;32). Our data, in a smaller number of patients, are the first to suggest that disinhibition of CTLA4 and PD1 may have additive effects on the induction of hypothyroidism and/or thyroiditis, which, in turn, is associated with an increased susceptibility to hypophysitis. Larger studies are required to better clarify these observations.

In a recent comprehensive review of published trials with anti-CTLA4 therapy, it has been suggested that hypophysitis is selective for males (Corsello *et al.* 2013). On the contrary, we demonstrated that this is more probably related to the increased proportion of males in melanoma clinical trials than a gender predilection. A dose dependency of anti-CTLA4 therapy and irAEs has also been proposed (Corsello *et al.* 2013). Our data are conflicting. The overall incidence of hypophysitis was nearly equal between 3 and 10 mg/kg ipilimumab. Yet, in the expanded access trial, the incidence of hypophysitis was 8 and 4% following therapy with 3 and 10 mg/kg ipilimumab respectively. In smaller trials with 10 mg/kg ipilimumab therapy in previously untreated patients, the incidence of hypophysitis was as high as 30%. It is possible that both dosage and previous exposure to anti-neoplastic therapies may differentially affect susceptibility to hypophysitis.

Primary adrenal disorders were a rare event in keeping with published studies (Corsello *et al.* 2013). The incidence of other endocrine irAEs, specifically hypercalcemia and hypogonadism, was less frequently tested. When present, they were most often only transiently abnormal.

Our analysis, though comprehensive and performed at a single center that is highly specialized in the use of immune checkpoint inhibitors, has limitations. Both the retrospective nature of the analysis and the limited

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0499

Published by Bioscientifica Ltd.

comprehensive endocrine evaluations in many patients represent challenges in accurately diagnosing endocrine irAEs. Despite these limitations, our data demonstrate that CTLA4 blockade produces a myriad of irAEs and, among these, hypophysitis is the most common endocrine irAE that is associated with a high morbidity and has the potential for increased mortality. In patients presenting with acute fatigue, weight loss, diarrhea, nausea, emesis, and/or arthralgias, a workup for endocrinopathies, in particular, hypophysitis, should be done. Prompt therapy ameliorates symptoms of endocrinopathies and permits continued therapy with these immunotherapies that show promising and durable clinical responses in patients with metastatic melanoma, many in patients who had progressed on multiple conventional therapies. To better characterize the incidence of endocrine irAEs in future trials, we recommend that the grading of endocrinopathies according to the Common Terminology Criteria for Adverse Events be amended to more specifically reflect the clinical and biochemical diagnosis of endocrine irAEs. The latter should be based on case-driven comprehensive endocrine hormone and thyroid autoantibody evaluations, radiographic imaging, and, when feasible, dynamic, ACTH-stimulated cortisol evaluations. This will enable a better understanding of the spectrum of endocrine irAEs arising from these therapies that will be critical to their continued success. This is particularly relevant as immunological checkpoint blockade with anti-CTLA4 and/or anti-PD1, in combination with molecular targeted drugs, chemotherapies, and/or radiation, is rapidly emerging as a viable cancer therapeutic in a broader range of malignancies.

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

Funding
Dr J Wolchok received consulting fees from Bristol-Myers Squibb, Ziopharm Oncology, Polynoma, and Merck and grant support from Bristol-Myers Squibb, GlaxoSmithKline, and MedImmune on behalf of his institution. Dr M A Postow received grant support from Bristol-Myers Squibb on behalf of his institution and served as an unpaid consultant for and received reimbursement for travel expenses from Bristol-Myers Squibb.

Author contribution statement
Dr M Ryder wrote the manuscript, performed the chart reviews, and analyzed the data. Dr M Callahan performed the chart reviews, obtained patient data, and reviewed and helped revise the manuscript. Dr M A Postow and Dr J Wolchok obtained patient data and technical information and helped revise the manuscript. Dr J A Fagin was involved in the writing and revision of the manuscript.

Acknowledgements
The authors thank Valerie Russell and Charlotte Ariyan for providing access to IRB-approved patient databases for this study.

References

Blansfield JA, Beck KE, Tran K, Yang JC, Hughes MS, Kammula US, Royal RE, Topalian SL, Haworth LR, Levy C *et al.* 2005 Cytotoxic T-lymphocyte-associated antigen-4 blockage can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. *Journal of Immunotherapy* **28** 593–598. (doi:10.1097/01.cji.0000178913.41256.06)

Blomhoff A, Lie BA, Myhre AG, Kemp EH, Weetman AP, Akselsen HE, Huseby ES & Undlien DE 2004 Polymorphisms in the cytotoxic T lymphocyte antigen-4 gene region confer susceptibility to Addison's disease. *Journal of Clinical Endocrinology and Metabolism* **89** 3474–3476. (doi:10.1210/jc.2003-031854)

Caturegli P, Newschaffer C, Olivi A, Pomper MG, Burger PC & Rose NR 2005 Autoimmune hypophysitis. *Endocrine Reviews* **26** 599–614. (doi:10.1210/er.2004-0011)

Corsello SM, Barnabei A, Marchetti P, De Vecchis L, Salvatori R & Torino F 2013 Endocrine side effects induced by immune checkpoint inhibitors. *Journal of Clinical Endocrinology and Metabolism* **98** 1361–1375. (doi:10.1210/jc.2012-4075)

Dillard T, Yedinak C, Alumkal J & Fleseriu M 2010 Anti-CTLA-4 antibody therapy associated autoimmune hypophysitis: serious immune related adverse events across a spectrum of cancer subtypes. *Pituitary* **13** 29–38. (doi:10.1007/s11102-009-0193-z)

Egen JG, Kuhns MS & Allison JP 2002 CTLA-4: new insights into its biological function and use in tumor immunotherapy. *Nature Immunology* **3** 611–618. (doi:10.1038/ni0702-611)

Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC *et al.* 2010 Improved survival with ipilimumab in patients with metastatic melanoma. *New England Journal of Medicine* **363** 711–723. (doi:10.1056/NEJMoa1003466)

Lipson EJ & Drake CG 2011 Ipilimumab: an anti-CTLA-4 antibody for metastatic melanoma. *Clinical Cancer Research* **17** 6958–6962. (doi:10.1158/1078-0432.CCR-11-1595)

Pentcheva-Hoang T, Egen JG, Wojnoonski K & Allison JP 2004 B7-1 and B7-2 selectively recruit CTLA-4 and CD28 to the immunological synapse. *Immunity* **21** 401–413. (doi:10.1016/j.immuni.2004.06.017)

Tomer Y, Greenberg DA, Barbesino G, Concepcion E & Davies TF 2001 CTLA-4 and not CD28 is a susceptibility gene for thyroid autoantibody production. *Journal of Clinical Endocrinology and Metabolism* **86** 1687–1693. (doi:10.1210/jcem.86.4.7372)

Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB *et al.* 2012 Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *New England Journal of Medicine* **366** 2443–2454. (doi:10.1056/NEJMoa1200690)

Tzou SC, Lupi I, Landek M, Gutenberg A, Tzou YM, Kimura H, Pinna G, Rose NR & Caturegli P 2008 Autoimmune hypophysitis of SJL mice: clinical insights from a new animal model. *Endocrinology* **149** 3461–3469. (doi:10.1210/en.2007-1692)

Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, Hunter KM, Smith AN, Di Genova G *et al.* 2003 Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* **423** 506–511. (doi:10.1038/nature01621)

Received in final form 8 January 2014
Accepted 30 January 2014

Published by Bioscientifica Ltd.