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

Mabel Ryder1,2,†, Margaret Callahan4, Michael A Postow2, Jedd Wolchok2,3 and James A Fagin1,2

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

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 (PD1D1, 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
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 radioimmunoassay methods 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
### Table 1 Clinical trials involving ipilimumab in patients with metastatic melanoma at MSKCC

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

<sup>a</sup> n = 44 patients had at least 1 or more ACTH and/or cortisol level checks.

<sup>b</sup>This patient later developed hypophysitis on ipilimumab + nivolumab study.

<sup>c</sup>Patient later developed hypophysitis.

<sup>d</sup>Suspicious for ectopic ACTH secretion versus early onset adrenal insufficiency.

<sup>e</sup>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 (FT₄ or T₄), and/or triiodothyronine (T₃), 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, adrenocorticotropic hormone (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 FT₄ 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 FT₄ or T₃ level. Immune-related thyroiditis (Table 1) was defined by the presence of a suppressed TSH level with an elevated FT₄ and/or T₃ 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 FT₄ and/or T₃ 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 FT₄ 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 FT₄ 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 FT₄ or T₃ 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 FT₄ level with a normal or low TSH level in patients...
Table 2  Cases of hypophysitis following ipilimumab therapy in patients with metastatic melanoma

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, gender</th>
<th>mg/kg (no. of doses)</th>
<th>Symptoms</th>
<th>Hormone axes affected</th>
<th>Onset of hypophysitis</th>
<th>Biochemistries</th>
<th>Imaging, MRI brain/sella</th>
<th>Recovery of pituitary function</th>
<th>Received high-dose steroids</th>
<th>Status</th>
<th>Non-dermatological irAEs</th>
<th>Protocol no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54, m</td>
<td>10 (3)</td>
<td>Mild fatigue and abnormal screening of TSH</td>
<td>Panhypopituitarism</td>
<td>4 weeks after the third dose</td>
<td>Cortisol&lt;sub&gt;a&lt;/sub&gt;, am 0.25, ACTH&lt;sub&gt;b&lt;/sub&gt; 0.22, TSH&lt;sub&gt;c&lt;/sub&gt; 0.03, FT&lt;sub&gt;4&lt;/sub&gt; 12.87, FT&lt;sub&gt;T&lt;/sub&gt; 2.42, LH 0.4, FSHP 2.7, testosterone&lt;sub&gt;d&lt;/sub&gt; &lt; 3.47, and prolactin&lt;sub&gt;e&lt;/sub&gt; 0.53</td>
<td>Enlarged sella</td>
<td>Yes, with normal ACTH stimulation test</td>
<td>Yes</td>
<td>Dead</td>
<td>None</td>
<td>NCT00495066</td>
</tr>
<tr>
<td>2</td>
<td>71, m</td>
<td>3 (4)</td>
<td>Fatigue, increased sleep, weight loss, and night sweats/chills</td>
<td>ACTH and TSH</td>
<td>2 months after the fourth dose</td>
<td>Cortisol 0.275, ACTH &lt; 1.1, TSH 0.35, FT&lt;sub&gt;4&lt;/sub&gt; 5.79, LH 9.1, FSH 12.6, and testosterone 74.29</td>
<td>Not done</td>
<td>No</td>
<td>Yes</td>
<td>NED</td>
<td>None</td>
<td>NCT00495066</td>
</tr>
<tr>
<td>3</td>
<td>69, m</td>
<td>10 (16)</td>
<td>Nausea, emesis, and dehydration</td>
<td>ACTH</td>
<td>3 months after the fourth dose</td>
<td>Cortisol 0.58, ACTH &lt; 1.1, TSH 1.68, FT&lt;sub&gt;4&lt;/sub&gt; 11.84, T&lt;sub&gt;3&lt;/sub&gt; 3.77, LH 9.8, FSH 22.5, and testosterone 133, and prolactin 0.83</td>
<td>Partial empty sella no abnormal enhancement</td>
<td>No</td>
<td>Yes</td>
<td>NED</td>
<td>None</td>
<td>NCT00495066</td>
</tr>
<tr>
<td>4</td>
<td>69, m</td>
<td>3 (4)</td>
<td>Severe headache, poor oral intake, and hypotension</td>
<td>ACTH, LH, FSH, and most probably TSH</td>
<td>2 weeks after the fourth dose</td>
<td>Cortisol 0.56, ACTH &lt; 1.1, TSH 0.5, FT&lt;sub&gt;4&lt;/sub&gt; 11.58, T&lt;sub&gt;3&lt;/sub&gt; 0.88, LH undetectable, and testosterone &lt; 3.47 TSH 0.04, FT&lt;sub&gt;4&lt;/sub&gt; 14.93, T&lt;sub&gt;3&lt;/sub&gt; 2.05 (screening TFTs) TSH 0.02, T&lt;sub&gt;3&lt;/sub&gt; 1.62, cortisol 0.35, ACTH &lt; 1.1, FSH 1.8, testosterone &lt; 3.47, TSH &lt; 0.5, FT&lt;sub&gt;4&lt;/sub&gt; 25.35, T&lt;sub&gt;3&lt;/sub&gt; 0.88, and thyroid peroxidase antibody (TPO Ab&lt;sup&gt;k&lt;/sup&gt;) &lt; 10</td>
<td>Ill-defined nodular enhancement of sella, resolved after 2 months</td>
<td>No</td>
<td>Yes</td>
<td>Dead</td>
<td>None</td>
<td>NCT00495066</td>
</tr>
<tr>
<td>5</td>
<td>61, m</td>
<td>3 (3)</td>
<td>Headaches</td>
<td>ACTH and LHFSH, (thyroiditis)</td>
<td>3 weeks after the third dose</td>
<td>Partial recovery of gonadotrophs but not ACTH-persistent thyroiditis</td>
<td>Partial empty sella no abnormal enhancement</td>
<td>No</td>
<td>Yes</td>
<td>Dead</td>
<td>None</td>
<td>NCT00495066</td>
</tr>
<tr>
<td>6</td>
<td>75, f</td>
<td>3 (2)</td>
<td>Fevers, sweats, and weakness</td>
<td>Possibly TSH and could not assess ACTH</td>
<td>4 weeks after the second dose</td>
<td>Prolactin 3.17, TSH 0.15, FT&lt;sub&gt;4&lt;/sub&gt; 20.59, estradiol &lt; 10, LH 11, and FSH 60</td>
<td>Interval increase in size of sella</td>
<td>Yes</td>
<td>Patient weaned off steroids for autoimmune hepatitis, not put on replacement hormones</td>
<td>Yes for hepatitis</td>
<td>Dead</td>
<td>Hepatitis</td>
</tr>
<tr>
<td>7</td>
<td>38, f</td>
<td>3 (8)</td>
<td>Asymptomatic</td>
<td>Possibly TSH, and could not assess ACTH or LHFSH</td>
<td>15 months after induction</td>
<td>TSH 0.26, FT&lt;sub&gt;4&lt;/sub&gt; 0.77, and FT&lt;sub&gt;T&lt;/sub&gt; 0.12. No other laboratory test results available</td>
<td>Not done</td>
<td>No</td>
<td>NED</td>
<td>None</td>
<td>NCT00495066</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>78, m</td>
<td>3 (8)</td>
<td>Flu-like syndrome, nausea, poor appetite, and weakness</td>
<td>Panhypopituitarism</td>
<td>4 weeks after the third dose</td>
<td>Cortisol, am 0.925, ACTH 2.2, TSH 5.88, FT&lt;sub&gt;4&lt;/sub&gt; 10.16, and testosterone &lt; 3.47</td>
<td>Not done</td>
<td>Partial – hypogonadism resolved</td>
<td>Yes</td>
<td>Dead</td>
<td>None</td>
<td>NCT00495066</td>
</tr>
<tr>
<td>9</td>
<td>71, m</td>
<td>10 (3)</td>
<td>Fatigue, poor appetite, and weakness</td>
<td>Not adequately assessed</td>
<td>2 weeks after the second dose</td>
<td>ACTH 2.64, no cortisol, TSH 0.7, no FT&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Thinning and prominence of sella and infundibulum</td>
<td>Not assessed</td>
<td>Yes</td>
<td>Dead</td>
<td>None</td>
<td>NCT00495066</td>
</tr>
<tr>
<td>Case</td>
<td>Age, gender</td>
<td>mg/kg (no. of doses)</td>
<td>Symptoms</td>
<td>Hormone axes affected</td>
<td>Onset of hypophysitis</td>
<td>Biochemistries</td>
<td>Imaging, MRI</td>
<td>Recovery of pituitary function</td>
<td>Received high-dose steroids</td>
<td>Status</td>
<td>Non-dermatological irAEs</td>
<td>Protocol no.</td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td>----------------------</td>
<td>----------</td>
<td>-----------------------</td>
<td>----------------------</td>
<td>------------------</td>
<td>-------------</td>
<td>-------------------------------</td>
<td>--------------------------</td>
<td>--------</td>
<td>------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>10</td>
<td>58, f</td>
<td>10 (4)</td>
<td>Headache, blurred vision, nausea, and emesis</td>
<td>Probable ACTH and TSH</td>
<td>1 day after the third dose</td>
<td>Cortisol 1.58 (48 h prednisone therapy), no ACTH, TSH 0.12, FT4 14.80, T3 1.77 TSH 0.05, FT4 13.12, TPO Ab &lt; 10, thyroglobulin antibody (Tg Ab) &lt; 20 LH 25.8, FSH 53.9, and prolactin 0.31</td>
<td>Homogenous enlargement</td>
<td>No symptoms recurred after prednisone dose tapered</td>
<td>Yes</td>
<td>Dead</td>
<td>None</td>
<td>NCT00920907</td>
</tr>
<tr>
<td>11</td>
<td>74, m</td>
<td>10 (8)</td>
<td>Fatigue and weakness</td>
<td>ACTH</td>
<td>2–3 weeks after the fifth dose</td>
<td>Cortisol 0.25, ACTH &lt; 1.1, TSH 1.51, FT4 16.34, LH 2.16, FSH 7.17, and testosterone 159</td>
<td>Normal</td>
<td>No</td>
<td>Yes</td>
<td>Unknown</td>
<td>None</td>
<td>NCT00920907</td>
</tr>
<tr>
<td>12</td>
<td>69, f</td>
<td>10 (7)</td>
<td>Severe fatigue and poor appetite</td>
<td>ACTH</td>
<td>3 months after the first dose</td>
<td>Cortisol am &lt; 0.05, ACTH 2.2, TSH 3.60, FT4 11.58, T3 2.76, and prolactin 1.34</td>
<td>Not done</td>
<td>No</td>
<td>Yes</td>
<td>NED</td>
<td>Colitis</td>
<td>NCT01323517</td>
</tr>
<tr>
<td>13</td>
<td>71, f</td>
<td>10 (6)</td>
<td>Arthralgias, fatigue, weight loss, and dehydration</td>
<td>LH and FSH not assessed</td>
<td>8 months after the first dose</td>
<td>Cortisol 0.275, ACTH 1.54, and TSH 2.93</td>
<td>Normal sella</td>
<td>No</td>
<td>Yes</td>
<td>Alive</td>
<td>Hypercalcemia</td>
<td>NCT01323517</td>
</tr>
<tr>
<td>14</td>
<td>77, f</td>
<td>10 (3)</td>
<td>Unknown</td>
<td>TSH and possibly ACTH</td>
<td>2 months after the first dose</td>
<td>Cortisol 0.875 (had been on prior steroid therapy), ACTH evaluation not done, TSH 0.01, and FT4 7.72</td>
<td>Not done</td>
<td>No</td>
<td>Yes</td>
<td>NED</td>
<td>Colitis</td>
<td>NCT01323517</td>
</tr>
<tr>
<td>15</td>
<td>78, m</td>
<td>10 (4)</td>
<td>Headaches and fatigue</td>
<td>Panhypopituitarism</td>
<td>3 months after the first dose</td>
<td>Cortisol 0.25, ACTH 2.86, TSH 0.99, FT4 9.01, and testosterone &lt; 6.94</td>
<td>Patchy enhancement of sella with thickening of stalk</td>
<td>Not done</td>
<td>No</td>
<td>Yes</td>
<td>NED</td>
<td>1) Thyroiditis 2) Pneumonitis</td>
</tr>
<tr>
<td>16</td>
<td>47, f</td>
<td>3 (4)</td>
<td>FEVERS, chills, and fatigue, arthralgias</td>
<td>ACTH and LHFSH not assessed</td>
<td>3.5 months after the first dose</td>
<td>Cortisol am &lt; 0.125, ACTH &lt; 1.1, and TSH 4.80</td>
<td>Not done</td>
<td>No</td>
<td>Yes</td>
<td>NED</td>
<td>1) Thyroiditis 2) Pneumonitis</td>
<td>NCT01024231</td>
</tr>
<tr>
<td>17</td>
<td>73, f</td>
<td>3 (4)</td>
<td>Headache, visual changes, fatigue, nausea and emesis, diarrhea, altered mental status</td>
<td>ACTH and LHFSH not assessed</td>
<td>3.5 months after the first dose</td>
<td>Cortisol pm 0.6, ACTH &lt; 1.1, TSH 1.53, FT4 14.03, FSH 18.9, and LH 5.2</td>
<td>Enlargement of sella with heterogeneous enhancement and focal nodular area of hypointensity</td>
<td>Not assessed</td>
<td>Yes</td>
<td>NED</td>
<td>Pancreatitis</td>
<td>NCT01024231</td>
</tr>
</tbody>
</table>
Table 2 Continued

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, gender</th>
<th>mg/kg (no. of doses)</th>
<th>Symptoms</th>
<th>Hormone axes affected</th>
<th>Onset of hypophysitis</th>
<th>Biochemistry</th>
<th>Imaging, MRI brain/sella</th>
<th>Recovery of pituitary function</th>
<th>Received high-dose steroids</th>
<th>Status</th>
<th>Non-endocrine irAEs</th>
<th>Protocol no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>52, f</td>
<td>3 (5)</td>
<td>Fatigue</td>
<td>ACTH and TSH, LH/FSH not assessed</td>
<td>5 months after 1st dose</td>
<td>Cortisol, am 0.675, ACTH, am 1.1, TSH 2.59, and FT4 15.06</td>
<td>Not done</td>
<td>Not assessed</td>
<td>Yes</td>
<td>SD</td>
<td>1) Hypothyroidism</td>
<td>NCT01024231</td>
</tr>
<tr>
<td>19</td>
<td>74, m</td>
<td>3 (7)</td>
<td>Severe fatigue, arthralgias, and mental status changes</td>
<td>ACTH and TSH and preserved LH/FSH</td>
<td>6 months after first ipilimumab infusion</td>
<td>Cortisol, am &lt;0.05, ACTH &lt; 1.1, TSH 3.02, and FT4 11.12</td>
<td>Normal</td>
<td>Not assessed</td>
<td>Yes</td>
<td>SD</td>
<td>1) Thyroiditis</td>
<td>NCT01024231</td>
</tr>
</tbody>
</table>

*Cortisol*: reference ranges am = 1.25–6.25 nmol/l; pm = 0.75–3 nmol/l.
*Adrenocorticotropic (ACTH)*: reference range 1.3–10.56 pmol/l.
*Thyroid-stimulating hormone (TSH)*: reference range 0.5–4.78 mIU/l.
*Total T4*: reference range 0.924–2.772 nmol/l.
*Luteinizing hormone (LH)*: reference range 2–9 IU/l.
*Follicle-stimulating hormone (FSH)*: reference range 1–18 IU/l.
*Total testosterone*: reference range 62.80–263.03 pmol/l.
*Prolactin*: reference range 0.087–0.78 nmol/l.
*Thyroglobulin antibody (TPO Ab)*: reference range 0–35 IU/l.
*Thyroid peroxidase antibody (Tg Ab)*: reference range 0–20 IU/l.

*aCortisol*: reference ranges am = 1.25–6.25 nmol/l; pm = 0.75–3 nmol/l.
*bAdrenocorticotropic (ACTH)*: reference range 1.3–10.56 pmol/l.
*cThyroid-stimulating hormone (TSH)*: reference range 0.5–4.78 mIU/l.
*dFree T4*: reference range 11.58–23.17 pmol/l.
*eTotal T4*: reference range 0.924–2.772 nmol/l.
*fLuteinizing hormone (LH)*: reference range 2–9 IU/l.
*gFollicle-stimulating hormone (FSH)*: reference range 1–18 IU/l.
*hTotal testosterone*: reference range 62.80–263.03 pmol/l.
*iProlactin*: reference range 0.087–0.78 nmol/l.
*jThyroglobulin antibody (TPO Ab)*: reference range 0–35 IU/l.
*kThyroid peroxidase antibody (Tg Ab)*: reference range 0–20 IU/l.

*1) Hypothyroidism
2) Hepatitis
3) Colitis
4) Thyroiditis
5) Myocarditis

SD: stable disease.
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 gonadotropin 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.

<table>
<thead>
<tr>
<th>Hypophysitis following CTLA4 blockade</th>
<th>n=19 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Males, n=134 total</td>
<td>11 of 134 (8)</td>
</tr>
<tr>
<td>Females, n=77 total</td>
<td>8 of 77 (10)</td>
</tr>
<tr>
<td>Dose of ipilimumab</td>
<td></td>
</tr>
<tr>
<td>3 mg/kg (n=119)</td>
<td>10 (8)</td>
</tr>
<tr>
<td>10 mg/kg (n=92)</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Median time to onset of hypophysitis after the first dose of ipilimumab</td>
<td>4 months</td>
</tr>
<tr>
<td>Hormone axes involved (confirmed and/or suspected)</td>
<td></td>
</tr>
<tr>
<td>Corticotrophs (could not be assessed in three patients)</td>
<td>16</td>
</tr>
<tr>
<td>Thyrotrophs (definite and/or possible; could not be assessed in one patient)</td>
<td>11</td>
</tr>
<tr>
<td>Gonadotrophs (could not be assessed in six patients)</td>
<td>5</td>
</tr>
<tr>
<td>Definite preservation of corticotroph secretion</td>
<td>0</td>
</tr>
<tr>
<td>MRI brain/sella completed</td>
<td>12</td>
</tr>
<tr>
<td>Normal/near normal</td>
<td>3 (25)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>9 (75)</td>
</tr>
<tr>
<td>Received high-dose steroids at diagnosis</td>
<td>18 (95)</td>
</tr>
<tr>
<td>Recovery of hormone function</td>
<td></td>
</tr>
<tr>
<td>Not assessed</td>
<td>5</td>
</tr>
<tr>
<td>No recovery of axis/axes</td>
<td>9</td>
</tr>
<tr>
<td>Recovery of ACTH secretion</td>
<td>3</td>
</tr>
<tr>
<td>Recovery of TSH secretion</td>
<td>0</td>
</tr>
<tr>
<td>Recovery of LH and FSH secretion</td>
<td>2</td>
</tr>
<tr>
<td>Developed additional non-dermatological irAEs</td>
<td>8</td>
</tr>
<tr>
<td>Overall status at last follow-up</td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>11</td>
</tr>
<tr>
<td>Dead</td>
<td>7</td>
</tr>
<tr>
<td>Unknown, lost to follow-up</td>
<td>1</td>
</tr>
</tbody>
</table>
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 FT₄ levels were evaluated at baseline and during follow-up in all patients. Of the 137 patients, 20 (15%) had mild abnormalities in TSH or FT₄ 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 FT₄ 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 μg/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
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 underestimated 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
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.

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

References

Received in final form 8 January 2014
Accepted 30 January 2014