The Price of Tumor Control: An Analysis of Rare Side Effects of Anti-CTLA-4 Therapy in Metastatic Melanoma from the Ipilimumab Network

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Abstract

Background: Ipilimumab, a cytotoxic T-lymphocyte antigen-4 (CTLA-4) blocking antibody, has been approved for the treatment of metastatic melanoma and induces adverse events (AE) in up to 64% of patients. Treatment algorithms for the management of common ipilimumab-induced AEs have lead to a reduction of morbidity, e.g. due to bowel perforations. However, the spectrum of less common AEs is expanding as ipilimumab is increasingly applied. Stringent recognition and management of AEs will reduce drug-induced morbidity and costs, and thus, positively impact the cost-benefit ratio of the drug. To facilitate timely identification and adequate management data on rare AEs were analyzed at 19 skin cancer centers.

Methods and Findings: Patient files (n = 752) were screened for rare ipilimumab-associated AEs. A total of 120 AEs, some of which were life-threatening or even fatal, were reported and summarized by organ system describing the most instructive cases in detail. Previously unreported AEs like drug rash with eosinophilia and systemic symptoms (DRESS), granulomatous inflammation of the central nervous system, and aseptic meningitis, were documented. Obstacles included patients delay in reporting symptoms and the differentiation of steroid-induced from ipilimumab-induced AEs under steroid treatment. Importantly, response rate was high in this patient population with tumor regression in 30.9% and a tumor control rate of 61.8% in stage IV melanoma patients despite the fact that some patients received only two of four recommended ipilimumab infusions. This suggests that ipilimumab-induced antitumor responses can have an early onset and that severe autoimmune reactions may reflect overtreatment.

Conclusion: The wide spectrum of ipilimumab-induced AEs demands doctor and patient awareness to reduce morbidity and treatment costs and true ipilimumab success is dictated by both objective tumor responses and controlling severe side effects.

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† For the ipilimumab network.
Introduction

Ipilimumab has been shown to enhance pre-existing immune responses, including antitumor responses, by directly blocking cytotoxic T-lymphocyte antigen-4 (CTLA-4) mediated T cell inhibition [1,2] and is now FDA and EMA approved as treatment modality in patients with metastatic melanoma. One treatment cycle consists of four infusions at approximately $30,000 each for a total of $120,000 drug costs per treated patient. In general, tumor responses are long-lasting [3], yet relatively limited with responses in only 10–15% of patients [4,5]. However, its association is with immune-related adverse events (irAEs) in up to 64% of patients [6] and detailed treatment algorithms for the management of commonly reported side-effects are provided by the manufacturer. Since CTLA-4 is inducibly expressed on virtually all T cells, ipilimumab has the potential to induce irAEs in a wide variety of tissues and organs. Single cases of unpredictable, in part astonishing, and difficult to treat, life-threatening or even fatal side-effects, have been reported including cases of nephropathy [7], myopathy [8], sarcoidosis [9], Guillain-Barré syndrome [10], uveitis, and leucopenia [11].

Since ipilimumab is increasingly being applied, the medical community will be confronted with new ipilimumab-induced side effects. To limit ipilimumab-related morbidity, stringent identification and immediate treatment of side-effects is crucial. Therefore, we summarized rare and difficult-to-treat ipilimumab-induced side effects among 19 skin cancer centers. In addition, we address specific hurdles, which we feel are critical for the success of CTLA-4-based immunotherapy.

Methods

Ethics Statement

This retrospective study was approved by the local institutional review board of the Friedrich-Alexander-Universität Erlangen-Nürnberg. In addition, all clinical protocols (44 in total among 19 participating study centers) were reviewed and approved by the local institutional review boards of each participating center and were performed according to Good Clinical Practice (GCP) and the Helsinki Declaration. In agreement with the local institutional review board of the Friedrich-Alexander-Universität Erlangen-Nürnberg, no written consent was obtained from included patients since the study was conducted completely anonymous.

Study Centers and Treatment Settings

Participating study centers screened patient files for ipilimumab-associated AEs and reported them on a template. Common AEs (e.g., rash, colitis) were excluded. Based upon the authors’ discretion, additional information was requested for the 15 most compelling and instructive cases. Study centers and treatment settings are summarized in Table S1.

Results

A total of 752 melanoma patients were treated with ipilimumab at 19 skin cancer centers and 120 AEs were reported. These included fatigue, flu-like symptoms, rigor/chills, cosinophilia and rashes (38 patients), which were not further evaluated. A total of 88 rare AEs in 82 patients affecting skin (23 patients), endocrine system (14 patients), nervous system (11 patients), liver (11 patients), respiratory tract (8 patients), gastrointestinal tract (6 patients), pancreas (3 patients), sinuses (3 patients), renal system (2 patients), musculoskeletal system (2 patients), heart (1 patient), eyes (1 patient), and upper extremities (1 patient) were observed. In addition, a systemic grade IV anaphylactoid reaction and a fatal case of tumor mass liquefaction were reported.

Skin

Ipilimumab-induced skin reactions are common, yet rarely severe (1–4% grade 3/4 reactions). Maculopapular rashes occur in 10–50% of patients independently of dosage and pruritus has been documented in up to 29.6% [5]. Rarely, Sweets syndrome or Stevens-Johnson syndrome [10–50%] has been observed (product monography). Importantly, melanoma-associated hypopigmentation (MAH) has been reported and postulated to be prognostically favorable [12,13]. In our study eight cases of MAH were reported (Figure 1A) and associated with one complete response (CR), one partial response (PR), one mixed response (MR), four stable diseases (SD) and one progressive disease (PD).

Other reported skin reactions included prurigo, acneiform rash, lichenoid exanthema, pyoderma gangraenosum-like ulcerations, skin toxicity in irradiated area, photosensitivity reaction and a drug rash with eosinophilia and systemic symptoms (DRESS) and detailed in Table 1 and Table 2.
diarrhea (up to 10 times/day; treated with i.v. and subsequently oral steroids) 37 weeks after treatment initiation. In addition, circumscribed depigmented, non itchy areas below both knees, a pronounced itchy rash, erythematous macules and infiltrated plaques affecting the lumbar and inguinal region, the flexural areas, scalp, both palms and the lower right leg, were reported. Laboratory blood tests showed no signs of inflammation. Histological evaluation demonstrated a spongiotic epidermis with parakeratosis and acanthosis, a discrete edema in the dermal papillae and a perivascular infiltrate of lympho-histiocytes with some eosinophils. Based on these findings, a pruritic exanthema and/or a drug eruption was postulated, which completely subsided under treatment with topical steroids.

**Gastrointestinal Tract and Pancreas**

Diarrhea/colitis is a common ipilimumab-induced AE [14,15]. Severe colitis can result in bowel perforation or intractable bleeding requiring colectomy [4,5,16–18] and is associated with high mortality [19]. In our study, three bowel perforations, three cases of pancreatitis and one case of asymptomatic elevation of amylase and lipase (lipase 860 U/L; normal range 13–60 U/L, amylase 337 U/L; normal range <110 U/L) were reported (Table 2). In one patient, pancreatitis was preceded by an amenorrhea with hyperprolactemia (Table 3 patient 3).

**Patient 4 - Sigma perforation.** A 55-year old man with lung, bone and lymph node melanoma metastases and a history of diverticular disease developed diarrhea (9–10 times/day) and cramp-like abdominal pain two weeks after the third ipilimumab infusion (3 mg/kg body weight). Intravenous steroids were given and symptoms improved. Since the abdomen was soft without signs of rebound tenderness, steroids were continued orally (2 mg/kg body weight). However, after switching to oral steroids, acute abdominal pain with signs of peritonitis developed. Computed tomography (CT) imaging demonstrated pneumoperitoneum highly suspicious for a perforation. The perforated sigmoid was resected and a colostomy was performed. Histological findings demonstrated an exacerbated purulent diverticulitis (positive for *Prevotella intermedia*, streptococci and *Escherichia coli*) and perforation. Steroids were continued after surgery and subsequently tapered.

In this case the colitis initially seemed completely controlled by intravenous (i.v.) steroid treatment, yet rapidly deteriorated under steroid reduction. This implicates incomplete suppression of ipilimumab-triggered autoimmune effects or masking of symptoms under steroid treatment. Importantly, a known inflammatory condition like diverticular disease might represent a relative contraindication for ipilimumab. This may require special caution with prior ultrasound examination and/or prophylactic steroid treatment.

**Patient 5 - Colonic perforation.** A 74-year old melanoma patient with progression of disease despite prior polychemo- and radiotherapy showed a partial remission six weeks after initiation of ipilimumab treatment (3 mg/kg body weight). Five days later she reported diarrhea (10 times/day) that had been ongoing for five days. Treatment with i.v. prednisolone (2 mg/kg body weight) and loperamide (2 mg after each defecation) was initiated. Symptoms subsided within five days and she was continued on oral steroids (1 mg/kg body weight). After three days of oral steroids diarrhea recurred and i.v. prednisolone treatment (2 mg/kg body weight) was reinitiated. However, symptoms now were steroid-refractory and despite additional therapy with infliximab (300 mg absolute i.v.) an acute abdomen developed. A hemicolectomy with colostomy was performed due to perforation of the colon. Treatment with infliximab was continued every 6 weeks with final amelioration of the colitis.

**Patient 6 - Toxic megacolon.** A 44-year old woman with stage IV melanoma developed diarrhea (up to 30 times/day) and a subsequent acute abdomen sixteen weeks after initiation of ipilimumab treatment. X-ray showed ballooned bowels. A
Table 1. Ipilimumab-induced cutaneous reactions.

| side effect | onset (weeks after start of ipilimumab) | treatmenta of side effect | outcome of side effect | age (years) | gender | primary tumor | stagebc | previous systemic therapies | metastases before ipilimumab | remission after ipilimumab | clinical response |
|-------------|----------------------------------------|---------------------------|------------------------|-------------|--------|---------------|---------|-----------------------------|-----------------------------|---------------------------|-----------------|
| drug rash with eosinophilia and systemic symptoms (DRESS)* | 4 | steroids (1 mg/kg) | resolved | 77 | M | skin | IV | none | lung, skin, LN | lung, skin, LN | PR |
| photosensitivity reaction* | 2 | none | resolved | 47 | F | skin | adjuvant | n/a | n/a | n/a | SD |
| skin toxicity in radiotherapy field* | 1.5 | none | resolved | 59 | F | skin | IV | none | lung, skin, adrenal gland, none | none | SD |
| pyoderma gangraenosum-like ulcerations, diarrhea, cold clammy skin, fatigue | 12 | steroids (200 mg), antibiotics | permanent changes | 57 | F | skin | IV | IFN-γ, DTIC, TKI (E7080) | liver, bone, spleen | none | PD |
| acneform rash décolleté | 12 | local steroids | resolved | 69 | M | skin | IV | IFN-α | lung, liver, bone, soft tissue | none | SD |
| acneform rash décolleté | 14 | local steroids | ongoing | 50 | M | skin | adjuvant | n/a | n/a | n/a | SD |
| acneform rash décolleté | 4 | local steroids | resolved | 38 | M | skin | adjuvant | n/a | n/a | n/a | SD |
| lichenoid exanthema | 12 | local steroids | resolved | 59 | F | skin | IV | IFN-α | lung, skin | none | PD |
| prurigo | 8 | local steroids | resolved | 64 | M | skin | adjuvant | n/a | n/a | n/a | SD |
| prurigo and maculopapular exanthema | 4 | steroids, antihistamines | resolved | 65 | F | ocular | IV | DTIC | stomach, LN, mesenterial, lungs | none | PD |
| prurigo | 11 | local steroids | resolved | 73 | M | skin | IV | DTIC | soft tissue, LN, pnicard, lung, bone | n/a | PD |
| prurigo | 11 | local steroids | resolved | 72 | F | skin | IV | IFN-γ, DTIC, vaccination† | GIT, soft, tissue, skin | n/a | SD |
| prurigo | 5 | local steroids, antihistamines | resolved | 81 | M | skin | IV | DTIC, paclitaxel | GIT | n/a | PD |
| prurigo | 6 | local steroids | resolved | 71 | F | skin | IV | temozolomide | LN, brain | LN | MR |
| MAH | 9 | none | permanent changes | 81 | M | skin | IV | DTIC, paclitaxel | GIT | n/a | PD |
| MAH | 14 | local steroids | permanent changes | 71 | F | skin | IV | temozolomide | LN, brain | LN | MR |
| MAH | 41 | none | permanent changes | 64 | M | skin | adjuvant | n/a | n/a | n/a | SD |
| MAH | 14 | none | permanent changes | 47 | F | skin | adjuvant | n/a | n/a | n/a | SD |
| MAH | 24 | none | permanent changes | 70 | F | skin | adjuvant | n/a | n/a | n/a | SD |
| MAH | 52 | none | permanent changes | 56 | F | skin | IV | DTIC, sorafenib | lung | lung | CR |
| MAH | 9 | none | permanent changes | 41 | M | skin | IV | DTIC, gemcitabine-treosulfan | liver, suprarenal gland, LN, brain | n/a | SD |
| MAH and pruritic eczema | 11 | local steroids, antihistamines | permanent changes | 65 | M | skin | IV | DTIC | LN, skin, bone | LN, bone, skin | PR |

*Case is detailed in the result section.

†listed treatments are systemic treatments unless otherwise specified.
perforation was feared and a colostomy was performed to relieve pressure. Interestingly, the colostomy showed no signs of healing and a revision was performed two weeks later. Histological findings showed ulcers and a granulocytic infiltrate in the mucosa. No bacterial or viral pathogens were found and the patient fully recovered from this AE.

**Patient 7 - Small bowel perforation.** Ipilimumab treatment (3 mg/kg body weight) was initiated in a 67-year old male with multiple melanoma metastases. Since the patient developed abdominal pain a colonoscopy was conducted but showed no signs of ulceration or colitis. However, the small intestine could not be investigated. Twelve weeks after the first ipilimumab treatment, the patient was admitted with acute abdomen and an emergency small bowel resection was performed. At this time, he also suffered from purulent peritonitis, thus no steroids were given. Staging showed a PR with regression of all metastases except bone lesions.

Detailed treatment algorithms for the management of ipilimumab-induced diarrhea/colitis exist [20]. Whereas in previous reports three out of four patients with colonic perforation were refractory to initial treatment with high-dose steroids [21], perforations in our study occurred after initial symptom improvement and steroid reduction. Steroids should be slowly tapered (30–60 days) and in cases of symptom recurrence, steroids should immediately be administered i.v. and if symptoms do not improve within 24 hours of therapy, additional immunosuppressive therapy (e.g. infliximab) should be initiated.

**Patient 8 - Ischemic gastritis.** A 72-year old female received ipilimumab (3 mg/kg body weight) due to progressive metastatic melanoma affecting lymph nodes, subcutaneous tissue and the gastrointestinal tract (cecum and jejunum without signs for passage disorders). Shortly after the first infusion, the patient underwent a complete surgical resection of all metastases with histological confirmation of the excised lesions. After the third infusion, the patient developed generalized pruritus including eyes and genital mucosa, which responded to antihistamines. Staging after the fourth treatment showed no metastases but a new strong diffuse fluorodesoxyglucose (FDG)-enhancement in the gastric wall (most intense in the gastric corpus). Since the patient was asymptomatic with normal S100 values, radiologic follow-up without further action was advised. The next positron emission tomography (PET)-CT scan demonstrated no enhancement in the stomach mucosa. However, three months later, a strong FDG-enhancement in the corpus of the stomach was detected again, suggesting the presence of gastritis without further evidence of metastases. Radiological findings and an ongoing anemia eventually causing dyspnea, prompted a gastroscopy. Biopsy showed an ischemic gastritis compatible with the endoscopic findings (Figure 2). No helicobacter pylori (HP) or metaplasia were detected. Symptoms spontaneously resolved and the last PET-CT scan detected no enhancement of the gastric wall.

**Hepatitis**

Hepatotoxicity is reported in 3–9% of ipilimumab patients and usually manifests as an asymptomatic increase of transaminases and bilirubin. Hepatitis has been reported in up to 0.8% of patients in the first [4] and up to 1.6% in the second phase III study [5]. Importantly, this AE can be life-threatening since one patient with fatal liver failure has been reported [4]. Thus, high dose steroids are recommended in case of grade 3/4 hepatotoxicity. In our study, 11 cases of liver-related irAEs were reported (Table 2, Table 3 patient 12; Table 4 last patient).

**Patient 9 – Fatal autoimmune hepatitis and nephritis.** A 71-year old man with stable stage IV B-cell non-Hodgkin lymphoma suffered from metastatic melanoma. After the second
Table 2. Ipilimumab-induced gastrointestinal, pancreatic and hepatic reactions.

| Side effect                          | Onset (weeks after start of ipilimumab) | Treatment* of side effect | Outcome of side effect | Age (years) | Gender | Primary tumor | Stageb,c | Previous systemic therapies | Metastases before ipilimumab | Remission after ipilimumab | Clinical response |
|-------------------------------------|----------------------------------------|---------------------------|------------------------|-------------|--------|---------------|----------|-----------------------------|-----------------------------|-----------------------------|---------------------|
| Sigma perforation                   | 8                                      | steroids (2 mg/kg), bowel resection | permanent changes      | 56          | M      | Mucosal       | IV       | Nilotinib, imatinib          | Lung, bone, LN              | None                        | PD                  |
| Colonic perforation                 | 7                                      | steroids (2 mg/kg), infliximab (300 mg), surgery | permanent changes      | 74          | F      | Skin          | IV       | DTIC, vindesine, cisplatin, gemcitabine, treosulfan | Lung, bone, soft tissue | Lung, bone, soft tissue | PR                  |
| Toxic megacolon                     | 16                                     | Colostomy                | permanent changes      | 44          | F      | Mucosal       | IV       | None                        | Lung, LN                   | None                        | PD                  |
| Small bowel perforation             | 12                                     | Small bowel resection    | permanent changes      | 67          | M      | Skin          | IV       | IFN-α                        | Skin, muscle, bone, retroperitoneal | PR                          |                     |
| Ischaemic gastritis                 | 54                                     | None                     | Ongoing                | 72          | F      | Skin          | IV       | None                        | Intestinal, soft tissue, skin | None                        | SD                  |
| Diarrhea, maculo-papular exanthesm, pruritus, fever, chills, dizziness | 3                                      | None                     | Resolved              | 60          | M      | Skin          | IV       | TVP                         | Skin, brain, lung, soft tissue | Brain                       | PR                  |
| Pancreatitis                        | 8                                      | Steroids                | Resolved               | 41          | F      | Skin          | Adjuvant | N/A                         | N/A                         | N/A                         | SD                  |
| Pancreatitis                        | 12                                     | Steroids                | Resolved               | 68          | F      | Skin          | Adjuvant | N/A                         | N/A                         | N/A                         | SD                  |
| Fulminant hepatitis and capillary leak, nephritis* | 4                                      | Steroids                | Fatal                  | 71          | M      | Skin          | IV       | DTIC                        | LN, lung, liver             | None                        | N/A                 |
| Elevation of lipase/amyrase         | 3                                      | None                     | Resolved               | 45          | M      | Skin          | IV       | IFN-α                        | Lung, skin, brain            | None                        | PD                  |
| Elevation of AST/ALT/GGT           | 9                                      | Steroids                | Resolved               | 73          | F      | Skin          | IV       | IFN-α, TKI (RAF265), temozolomide, LN, bone | None                        | SD                          |                     |
| Elevation of AST/ALT               | 6                                      | Steroids                | Resolved               | 31          | M      | Skin          | Adjuvant | N/A                         | N/A                         | N/A                         | SD                  |
| Hepatitis                           | 6                                      | Steroids                | Resolved               | 66          | M      | Skin          | Adjuvant | N/A                         | N/A                         | N/A                         | SD                  |
| Hepatitis                           | 3                                      | Steroids                | Resolved               | 31          | M      | Skin          | Adjuvant | N/A                         | N/A                         | N/A                         | SD                  |
| Hepatitis                           | 38                                     | Steroids                | Resolved               | 47          | F      | Skin          | Adjuvant | N/A                         | N/A                         | N/A                         | SD                  |
| Hepatitis                           | 6                                      | Steroids, imurek         | Resolved               | 39          | F      | Skin          | Adjuvant | N/A                         | N/A                         | N/A                         | SD                  |
| Icterus                              | 6                                      | Steroids, UV-therapy     | Resolved               | 66          | M      | Skin          | Adjuvant | N/A                         | N/A                         | N/A                         | SD                  |

* Case is detailed in the result section.
* Listed treatments are systemic treatments unless otherwise specified.
* Tumor free high-risk stage III melanoma (AJCC 2009); adjuvant administration of ipilimumab.
* Stage IV metastatic disease (AJCC 2009).
  M indicates male; F, female; TVP, polychemotherapy with temozolomide + vinblastin + carboplatin; TKI, tyrosine kinase inhibitor RAF265; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; LN, lymph nodes; IFN-α, interferon-α; DTIC, dacarbazine; PR, partial response; SD, stable disease; PD, progressive disease.

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| Side Effect                                      | Onset (weeks after start of ipilimumab) | Treatment of Side Effect | Outcome of Side Effect | Age (years) | Gender | Primary Tumor | Stage of Tumor | Previous Systemic Therapies | Metastases Before Ipilimumab | Remission After Ipilimumab | Clinical Response |
|-------------------------------------------------|----------------------------------------|--------------------------|------------------------|-------------|--------|---------------|----------------|-----------------------------|-----------------------------|-----------------------------|-------------------|
| Hypophysitis                                    | 7                                      | Steroids                | Permanent changes      | 74          | M      | Skin          | IV             | IFN-α, cisplatin, vindesine+DTIC, DTIC+ sorafenib, paclitaxel+ carboplatin | Skin, liver, lung, brain, LN | None            | PD                |
| Hypophysitis with symptoms of brain edema*      | 14                                     | Steroids                | Permanent changes      | M           | 67     | Skin          | IV             | IFN-α, cisplatin, vindesine+DTIC, DTIC+ sorafenib, paclitaxel+ carboplatin | Skin, liver, lung, brain, LN | None            | PD                |
| Amenorrhea, hyperprolactinemia, hypophysitis, with normal TSH/cortisol, pancreatitis | 8                                       | None                    | Resolved              | 41          | F      | Skin          | Adjuvant       | IFN-α, allovectin+DTIC, nilotinib | Soft tissue, LN, lung | None            | PD                |
| Increased hypophysal insufficiency (hypophysitis, hypothyreosis, hypogonadism) | 15                                      | Steroids                | Ongoing               | 59          | F      | Skin          | IV             | IFN-α, allovectin+DTIC, nilotinib | Soft tissue, LN, lung | Skin            | PR                |
| Hypophysitis                                    | 10                                     | Steroids                | Resolved              | 57          | M      | Skin          | IV             | Temozolomide     | Brain            | None            | PD                |
| Hypophysitis                                    | 8                                       | Steroids                | Resolved              | 56          | F      | Skin          | IV             | DTIC            | Skin, peritoneal,        | None            | PD                |
| Hypophysitis                                    | 9                                       | Steroids, levothyroxine | Resolved              | 60          | F      | Mucosal       | IV             | IFN-α, DTIC, paclitaxel, docetaxel | Parotis, LN, skin, lung | None            | PD                |
| Hypophysitis                                    | 10                                      | Steroids, levothyroxine | Permanent changes      | 31          | M      | Skin          | IV             | DTIC, MEX-inhibitor | LN, skin          | LN, skin         | PR                |
| Hypophysitis                                    | 12                                      | Steroids, levothyroxine, testosterone | Ongoing               | 72          | M      | Skin          | IV             | DTIC, limb perfusion* | LN, spleen, lung, liver, muscle, skin | None            | PD                |
| Hypophysitis + hepatitis                        | 12                                      | Steroids                | Permanent changes      | M           | 54     | Skin          | Adjuvant       | IFN-α, allovectin+ DTIC, nilotinib | None            | None            | PD                |
| Hypophysitis, pronounced fatigue, flu like symptoms | 23                                      | Steroids                | Resolved              | M           | 64     | Skin          | Adjuvant       | IFN-α, allovectin+ DTIC, nilotinib | None            | None            | PD                |
| Hypophysitis (hyperprolactinaemia, low IGF-1, hypornatamia, hypokaliaemia, hypophosphataemia) | 11                                      | Steroids                | Resolved              | F           | 49     | Skin          | IV             | Bevacizumab, temozolomide, DTIC, eldesine, platino, paclitaxel, sorafenib | Lung, liver, soft tissue, pancreas, LN, bone, skin, GIT | Liver, LN        | MR                |

*a case is detailed in the result section.
*Listed treatments are systemic treatments unless otherwise specified.
*Tumor-free high-risk stage III melanoma (AJCC 2009); adjuvant administration of ipilimumab.
*Stage IV metastatic disease (AJCC 2009).
*Limb perfusion with melphalan.
M indicates male; F, female; LN, lymph nodes; IFN-α, interferon-α; DTIC, dacarbazine; GIT, gastrointestinal tract; IGF-1, insulin-like growth factor-1; TSH, thyroid-stimulating hormone; PR, partial response; SD, stable disease; PD, progressive disease; MR, mixed response.

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ipilimumab treatment, the patient presented in a reduced general condition and with massive increase of liver transaminases and creatinine. Systemic steroids induced a slight improvement in liver transaminases within 24 hours. However, creatinine levels further increased and the patient required dialysis. In addition, his neurologic condition rapidly deteriorated with reduced responses to his environment and reduced respiration (spontaneous oxygenation below 60%). An inflammatory-induced capillary leak syndrome completely abolished renal function. Despite full symptomatic supportive treatment in the intensive care unit the patient died three days after admittance. Autopsy showed necrotic metastases and septal infiltration of the liver with CD3+ lymphocytes. This finding supports an ipilimumab-induced reaction and is less likely induced by lymphoma progression.

Unfortunately, this hepatotoxicity described above was steroid-refractory and resulted in fatal outcome. Importantly, successful treatment of a fulminant hepatitis refractory to treatment with steroids and mycophenolate mofetil with antithymocyte globulin (1.5 mg/kg for four times) has been reported [22].

Endocrine System

IrAEs affecting the endocrine system include euthyroid Graves ophthalmopathy [23] and thyroid dysfunction with both hypothyroidism and hyperthyroidism, which can manifest as thyroiditis [24,25]. Hypophysitis is a rare, yet serious complication of ipilimumab treatment. Incidence varies between 1.8% and 17% of patients at the 1–3 and 10 mg/kg doses, respectively [26,27]. Symptoms include loss of libido, fatigue, headache, memory difficulties, dizziness, vision changes and constipation. When suspected, a complete work-up including serum potassium, sodium, morning cortisol, luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, insulin-like growth factor (IGF)-1 and free thyroxine (fT4), as well as a brain magnetic resonance imaging (MRI) – also to exclude brain metastases - is necessary. Pituitary enlargement can precede clinical or laboratory evidence of an autoimmune-mediated hypophysitis. In contrast, hypophysitis cannot be ruled out by normal MRI findings. Upon diagnosis, prompt steroid therapy and regular follow-up with blood tests (serum potassium, sodium, testosterone (in men) and fT4) are indispensable. Cortisol measurements are not informative if steroid treatment is ongoing.

A newly diagnosed hyponaetremia or a secondary amenorrhoe seen in a premenopausal patient (patient 3, Table 3) are suspicious for newly developed corticotropin deficiency due to pituitary deficiency, whereas normal menses exclude gonadotropic deficiency in premenopausal women. Therapy consists of hormone replacement. Within this study, 14 patients with endocrinological AEs are reported including 12 cases of hypophysitis (Table 3).

**Patient 10– Hypophysitis.** A 74-year old male with no history of brain metastases received ipilimumab (3 mg/kg bodyweight) due to progressive metastatic disease affecting both adrenal glands. Shortly after the second treatment, he showed progressive ataxia and aphasia. MRI ruled out newly developed brain metastases and hypophysitis was suspected. Except for a slightly decreased testosterone, no hormonal changes were observed. Oral steroids (dexamethasone) were started and tapered. After two weeks, a decrease in thyroid hormones and testosterone was noted. Due to steroid therapy, the likewise decreased cortisol levels could not be used for interpretation. A second MRI demonstrated improvement of the morphological changes. Thyroid-hormone substitution was initiated and steroids tapered. After discontinuing steroid treatment, progressive ataxia and aphasia developed again. At this time, laboratory findings revealed hyponaetremia (serum sodium

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**Figure 2. Ipilimumab-induced ischemic gastritis.** Hematoxillin eosin staining showed edematous hypervascularized lamina propria mucosae, foveolar hyperplasia and regenerative basal crypts at 10× magnification (A) and 50× magnification (B). Endoscopic narrow band imaging (NBI) showed signs of reactive chronic inflammation of the gastric corpus mucosa with prominent vascular pattern consistent with an ischemic gastritis (C). Positron emission tomography (PET) scan illustrated high level tracer uptake in the gastric wall consistent with inflammation (D) and its spontaneous resolution after four months with a remaining thickening of the gastric wall (E).

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### Table 4. Ipilimumab-induced miscellaneous reactions.

| side effect                                         | onset (weeks after start of ipilimumab) | treatment* of side effect | outcome of side effect | age (years) | gender | primary tumor | stage b,c | previous systemic therapies | metastases before ipilimumab | remission after ipilimumab | clinical response |
|-----------------------------------------------------|----------------------------------------|---------------------------|------------------------|-------------|--------|---------------|-----------|-----------------------------|-----------------------------|---------------------------|---------------------|
| tumor mass liquefication*                           | 9                                      | antibiotics, surgery     | fatal                  | 66          | M      | skin          | IV        | IFN-α, limb perfusion, DTIC, temozolomide + vindesine, cisplatin, fotemustine | LN, skin, intraabdominal, bone | LN, skin, intraabdomial | PR                  |
| grade IV anaphylactoid reaction                     | 3                                      | steroids, antihistamines | resolved               | 54          | M      | skin          | adjuvant  | n/a                         | n/a                         | n/a                       | SD                  |
| swollen and numb hand                               | 21                                     | n/a                      | resolved               | 36          | F      | skin          | adjuvant  | IFN-α, sorafenib            | lung, adrenal glands, soft tissue, liver | lung                    | MR                  |
| conjunctivitis                                      | 12                                     | sodium hyaluronate eye gel | resolved               | 57          | M      | skin          | IV        | DTIC, sorafenib              | LN, skin, LN, intraabdominal, brain | lung                    | MR                  |
| non-septic arthritis                                | 11                                     | local steroids           | resolved               | 41          | F      | skin          | adjuvant  | n/a                         | n/a                         | n/a                       | SD                  |
| non-septic arthritis                                | 19                                     | steroids                 | resolved               | 32          | M      | skin          | adjuvant  | n/a                         | n/a                         | n/a                       | SD                  |
| purulent sinusitis, VZV-infection                   | 6, 16, 13                             | antibiotics, valacyclovir | resolved               | 43          | F      | unknown primary | IV     | sorafenib, temozolomide, fotemustine | lung, liver spleen, brain | lung                    | MR                  |
| sinusitis                                           | 14                                     | antibiotics              | resolved               | 38          | M      | skin          | adjuvant  | n/a                         | n/a                         | n/a                       | SD                  |
| sinusitis                                           | 3                                      | antibiotics              | resolved               | 53          | M      | skin          | adjuvant  | n/a                         | n/a                         | n/a                       | SD                  |
| myocardial fibrosis, portal liver necrosis with granulocytic infiltrates | 16                                     | n/a                      | fatal                  | 61          | F      | skin          | IV        | IFN-α, vaccination, vaccination | skin, LN, lung, brain, epicardial, liver | n/a                       | PD                  |

*case is detailed in the result section.

*listed treatments are systemic treatments unless otherwise specified.

* tumor-free high-risk stage III melanoma (AJCC 2009); adjuvant administration of ipilimumab melanoma.

*stage IV metastatic disease (AJCC 2009).

*sinusitis.

*VZV-infection.

*limb perfusion with melphalan.

*MAGE-A3 vaccination by GSK; NCT 00796445.

*PRAME; vaccination with GSK2302025A.

M indicates male; F, female; LN, lymph nodes; IFN-α, interferon-α; DTIC, dacarbazine; VZV, varicella-zoster virus; SD, stable disease; MR, mixed response; PD, progression of disease.

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125 mmol/l, normal range 135–145 mmol/l), and reduced levels of cortisol (2.6 µg/dl, normal range 5–25 µg/dl), testosterone (0.5 ng/ml, normal range 2.0–0.5 ng/ml) and dehydroepiandrosterone (DHEAS) (<150 ng/ml, normal range 800–5000 ng/ml). Adrenocorticotropic hormone (ACTH), LH, prolactin, free triiodothyronine (fT3) and FT4 (under treatment with thyroid hormones) were normal. A third MRI showed similar results to the second MRI scan, but pituitary impairment persisted. Under i.v. steroids (dexamethasone 4 mg/day) ataxia and aphasia subsided and serum sodium levels normalized. In conclusion, the observed hyponatraemia appeared due to an acute Addison crisis after steroid withdrawal because of persisting corticotropin deficiency. Although the clinical course was further complicated by a steroid-induced psychosis and a bacterial infection of unknown origin, the patient is now free of symptoms under hormonal substitution with thyroid hormones, hydrocortisone and testosterone.

**Patient 11 - Hypophysitis with brain edema.** A 67-year old man with multiple melanoma metastases received stereotactic irradiation for a single parietooccipital brain metastasis (4 mm diameter) and developed a new cerebellar metastasis. Four consecutive treatments with ipilimumab (3 mg/kg body weight) were well tolerated apart from a slight decrease in thyroid-stimulating hormone (TSH) (0.3 mU/l; normal range 0.4–4.9 mU/l) with normal FT4 before the third treatment. After the third treatment the cerebellar metastasis was stereotactically irradiated. Fourteen weeks after initiation of ipilimumab, the patient presented with acute onset of nausea, dizziness and ataxia. On examination, a blurred speech was noticed, prolactin was 4.9 mU/l) with normal fT4 before the third treatment. After the third treatment (dexamethasone 16 mg/day) symptoms ameliorated.

In agreement with previous reports, 4 out of 12 hypophysitis patients require ongoing hydrocortisone replacement and in some cases additionally thyroid hormones and testosterone/estradiol.

**Nervous System**

Ipilimumab-associated neurological symptoms are rare, but may be life-threatening. Common symptoms include headache, dizziness, lethargy, and asthenia. Rarely, patients present with cranial neuropathy and optic nerve ischemia, ataxia, tremor, myoclonia, dysarthria and peripheral neuropathy. Importantly, Guillian-Barré syndrome was described twice [30] with fatal outcome in one patient [4]. In addition, meningoradiculoneuritis [31], enteric neuropathy [32] and cerebral edema with convulsions [33] have been reported.

Six out of eleven rare neurological AEs reported in this study required immunosuppression (Table 5, last patient Table 6). In general, steroid treatment was effective although one patient experienced a therapy-refractory neuropathy and eventually died despite additional treatment with intravenous immunoglobulin (IVIG).

**Patient 12– Granulomatous inflammation of the central nervous system (CNS).** A 30-year old man with stage IIIA melanoma experienced transient chills without fever eight weeks after initiation of ipilimumab. In addition, two weeks later right-sided facial paresthesia and muscle weakness in both legs were observed. Paresthesia in his face deteriorated and extended to the whole face. Additionally, singulus and nausea appeared and persisted. Twelve weeks after start of ipilimumab, his neurological condition suddenly worsened resulting in sensoric ataxia and a disabling progressive paraparesis affecting both legs. All examinations for bacterial, fungal and viral causes were negative. No melanoma cells could be detected by lumbar puncture, and a marked lymphocytic pleocytosis was seen. Blood CD4/CD8 ratio was markedly increased at 4.3 (normal range 1.1–3.0) with an increase of absolute CD4-lymphocytes (1.0/µl; normal range 0.5–1.2/µl). The MRI showed an enhancement in both trigeminal nerves and, additionally, three parenchymal lesions with no indication for meningoencephalitis or pituitary enlargement. Because of suspected ipilimumab-induced granulomatous disease high dose steroids were initiated and quickly improved symptoms. Brain lesions disappeared in a subsequent MRI and symptoms completely resolved.

**Patient 13– Tolosa-Hunt-Syndrome.** A 65-year old male patient with primary skin melanoma without brain metastases or thyroid disease received ipilimumab (3 mg/kg body weight) due to progressive metastatic melanoma. Eighteen weeks after the first dose of ipilimumab, the patient presented with acute onset of strong pain above his right eye that radiated to the paranasal sinus, epiphora and double vision. In addition, dizziness and nausea was reported. The ophthalmologist recorded a mydriasis of the right pupil and a ptosis with paresis of the oculomotorius nerve leading to limited mobility and inability to completely open the right eye, compatible with a Tolosa-Hunt-Syndrome. Brain MRI revealed a markedly prominent neural sheath of the right optic nerve, no alteration in the cavernous sinus and no metastases. Except for a stable anemia and an increased CRP (54 mg/l, normal range <5 mg/l), laboratory values were within normal range with normal TSH and negative thyroid autoantibodies. Interestingly, an unclear FDG-enhancement in the left thyroid lobe had been previously detected by FDG-PET/CT. High-dose steroids (initially i.v. methylprednisolone and subsequently oral dexamethasone) and local radiotherapy (10×3 Gray) was simultaneously started. This treatment combination markedly and promptly reduced the ocular pain and paresis but showed only little impact on the vision disturbances. Staging showed progressive disease with new liver metastases.

**Patient 14– Aseptic meningitis.** A 52-year old female with melanoma metastases affecting lymph nodes, soft tissue, liver, and bones (sacral vertebra, 2 ribs and temporal bone) presented with nausea, vomiting, chills and rash, three weeks after the first ipilimumab infusion. Therapy included rehydration, antiemetics, novaminsulin and topical steroids. One week later, she presented with agitation, disorientation, aggressive behaviour and the inability to make contact with other people. Since she was screaming and physically attacking the physicians, a blood draw required four people holding the patient in addition to sedation with esketamin (50 mg) and midazolam (15 mg). Body temperatures had risen to 39°C but no signs of meningitis were observed. Treatment with i.v. ceftriaxon, ampicillin as well as acyclovir and dexamethasone was initiated. Liquor analyses revealed mostly lymphomonocytic cells (mainly CD3-positive lymphocytes) and excluded a meningoencephalitis or herpes simplex infection. Brain MRI showed four new brain metastases (<1 cm in diameter each) and an accentuation of the temporal bone metastasis. After electroencephalography (EEG), transcranial ultrasound, and repeated liquor examinations, an aseptic ipilimumab-induced meningitis was suspected. Ipilimumab treatment was permanently discontinued and symptoms resolved completely. Unfortunately, the patient demonstrated disease progression despite subsequent gamma knife and fotemustine treatment.
Table 5. Ipilimumab-induced reactions of the nervous system.

| Side effect                                      | Onset (weeks after start of ipilimumab) | Treatment of side effect | Outcome of side effect | Age (years) | Gender | Primary tumor | Stage | Previous systemic therapies | Metastases before ipilimumab | Remission after ipilimumab | Clinical response |
|-------------------------------------------------|----------------------------------------|--------------------------|------------------------|-------------|---------|--------------|-------|-----------------------------|----------------------------|-------------------------|-----------------|
| Tolosa-Hunt-Syndrom*                             | 18                                     | steroids                 | ongoing                | M           | 65      | skin         | IV    | IFN-α, TKI (RAF265), DTIC   | LN, soft tissue, GIT     | PD                      | PR              |
| Granulomatous inflammation of the central nervous system* | 10                                     | steroids, fotemustine    | resolved               | M           | 50      | skin         | adjuvant | none                        | brain, LN              | n/a                     | PD              |
| Aseptic meningitis*                              | 4                                      | steroids, acyclovir, antibiotics | resolved               | F           | 52      | skin         | IV    | DTIC                        | LN, soft tissue, liver, bones | none                    | PD              |
| Dysgeusia                                        | 8                                      | n/a                      | ongoing                | M           | 66      | skin         | adjuvant | n/a                         | n/a                      | n/a                     | SD              |
| Dysgeusia                                        | 35                                     | n/a                      | ongoing                | F           | 44      | skin         | adjuvant | n/a                         | n/a                      | n/a                     | SD              |
| Facial nerve paralysis                           | 7                                      | steroids (0.65 mg/kg)    | resolved               | M           | 61      | mucusosal    | IV    | IFN-α                       | lungs                   | none                    | PD              |
| Neuralgiform pain                                | 31                                     | pregabalin               | ongoing                | M           | 53      | skin         | adjuvant | n/a                         | n/a                      | n/a                     | SD              |
| Therapy refractory neuropathy                    | 2                                      | steroids, venlaflaxin, hydromorphone, IV, pregabalin | ongoing (till death)   | M           | 50      | skin         | IV    | IFN-α, DTIC                  | LN, skin, liver, kidney | none                    | PD              |
| Tinnitus, acute hearing loss, chills/shivering, diarrhea, generalized pruritus | 1                                      | betahistin, loperamide  | resolved               | M           | 50      | skin         | IV    | IFN-α, DTIC                  | LN, soft tissue, brain   | LN, soft tissue, brain | PR              |
| Generalized epileptic seizure                    | 2                                      | steroids, levetiracetam | resolved               | M           | 72      | unknown primary | IV    | paclitaxel, vemurafenib     | lung, skin, brain       | none                    | PD              |

*a case is detailed in the result section.

*Listed treatments are systemic treatments unless otherwise specified.

*Stage IV metastatic disease (AJCC 2009); adjuvant administration of ipilimumab.

M indicates male; F, female; LN, lymph nodes; IFN-α, interferon-α; DTIC, dacarbazine; TKI, tyrosine kinase inhibitor RAF265; GIT, gastrointestinal tract; PR, partial response; SD, stable disease; PD, progressive disease.

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| Side effect | Onset (weeks after start of ipilimumab) | Treatment of side effect | Outcome of side effect | Age (years) | Gender | Primary tumor | Stage | Previous systemic therapies | Metastases before ipilimumab | Remission after ipilimumab | Clinical response |
|-------------|----------------------------------------|--------------------------|------------------------|-------------|--------|---------------|-------|---------------------------|-----------------------------|---------------------------|---------------------|
| Barkly rhinitis | 11 | Steroids | Resolved | F | 49 | Skin | IV | Bevacizumab, temozolomide, DTIC, eldesine, platinol, paclitaxel, sorafenib | Lung, liver, soft tissue, pancreas, LN, bones, skin, GIT | Liver, LN | MR |
| Alveolitis | 3 | Steroids | Resolved | M | 59 | Unknown primary | IV | Temozolomide | Brain, lung | None | PD |
| Persistent bronchitis | 17 | Antibiotics | Resolved | M | 38 | Skin | Adjuvant | N/A | N/A | N/A | SD |
| Dyspnea | 14 | Inhalative steroids | Resolved | F | 44 | Skin | Adjuvant | N/A | N/A | N/A | SD |
| Intermittent dyspnea | 39 | Steroids | Resolved | M | 64 | Skin | Adjuvant | N/A | N/A | N/A | SD |
| Cough, dyspnea, arthritis, myalgia, diarhrea, sweating, papular exanthema | 1 | Acetylcysteine | Resolved | M | 48 | Skin | Adjuvant | IFN-α, DVP | LN, lung, liver, bone | LN, liver | PR |
| Acute renal failure, interstitial nephritis, atypical pneumonia | 6, 10 | Steroids, Antibiotics | Resolved | F | 72 | Unknown primary | IV | DTIC, Vaccination (PRAME) | Skin, LN | LN | PR |
| Acute renal failure, atypical pneumonia, iridocyclitis/keratitis, deafness | 8<sup>th</sup>-10<sup>th</sup> | Steroids (1 mg/kg) | Resolved, permanent changes<sup>5</sup> | F | 53 | Mucosal | IV | IFN-α, DTIC, sorafenib, carboplatin+paclitaxel, fotemustine | Kidney, skin, paracolic area, spinal cord | Kidney, skin, paracolic area, spinal cord | PR |

<sup>*</sup>Case is detailed in the result section.
<sup>a</sup>Listed treatments are systemic treatments unless otherwise specified.
<sup>b</sup>Tumor-free high-risk stage III melanoma (AJCC 2009); adjuvant administration of ipilimumab.
<sup>c</sup>Stage IV metastatic disease (AJCC 2009).
<sup>d</sup>PRAME study; vaccination with GSK2302025A.
<sup>e</sup>Atypical pneumonia.
<sup>f</sup>Acute renal failure.
<sup>g</sup>Renal failure/atypical pneumonia.
<sup>h</sup>Iridocyclitis/keratitis, deafness.
<sup>i</sup>Renal failure/atypical pneumonia/iridocyclitis/keratitis.
<sup>j</sup>Deafness.

M indicates male; F, female; LN, lymph nodes; IFN-α, interferon-α; DTIC, dacarbazine; DVP, polychemotherapy with dacarbazine/vindesine/paclitaxel; GIT, gastrointestinal tract; PR, partial response; SD, stable disease; MR, mixed response; PD, progressive disease.

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Physicians should be aware that neurologic symptoms can exacerbate unexpectedly at any time. The patient described above would have died of meningitis if no relatives would have been around. Importantly, it has been reported that prior therapy with neurotoxic agents may increase the risk of neurological-related adverse reactions [30], however, these findings are not confirmed in our study population.

Respiratory Tract and Renal System

Reported ipilimumab-induced respiratory tract-related adverse reactions include a life-threatening pneumonitis after allogeneic hematopoietic cell transplantation [34], fatal acute respiratory distress syndrome (product monography), pulmonary granulomatosis [35] and sarcoidosis [9,36,37]. Observed rare respiratory-tract irAEs, included barky rhinitis, alveolitis and atypical pneumonia (Table 6). Ipilimumab-induced respiratory tract-related irAEs have significant clinical implications, since they may be life-threatening. In addition, radiological signs of sarcoidosis can be confused with pulmonary metastases [38] resulting in inadequate change of therapy.

Adverse reactions affecting the renal tract are rare [7]. In this study two cases of acute renal failure were reported (Table 6). Both responded well to steroid treatment and resolved without sequelae.

Miscellaneous

It remains unclear why different targets are affected during ipilimumab treatment. In the literature, hemophilia A [39] and autoimmune polyposis [40] have been reported.

In our study, eleven patients presented with AEs not related to skin, liver, endocrine system, respiratory tract, kidney, or nervous system (Table 4). Interestingly, one patient showed myocardial fibrosis in conjunction with hepatitis (Figure 3). Importantly, one patient experienced an anaphylactoid reaction with flush, diffuse muscle contractions, chest tightness, hypertension, tachycardia and tachypnoe after infusion of 20% of the total ipilimumab volume of the second infusion. Symptoms resolved upon treatment with i.v. steroids and antihistamines. To the best of our knowledge, this is the first reported grade IV anaphylactoid reaction to ipilimumab.

Patient 15 - Tumor mass liquefication. A 66-year old male patient presented with progressive left-sided iliac lymph node metastases and bulky tumor growth on the left-sided abdomen, the groin and left thigh despite lymph node dissection, hyperthermala limb perfusion, dacarbazine monochemotherapy, polychemotherapy with temozolomide, vindesine and cisplatin and fotemustine. In addition, arterial and venous compression, infiltration and thrombosis led to a massive increase of the pre-existing lymph edema of the left leg. An attempt of brachytherapy failed. Upon the second treatment with ipilimumab, the patient reported a considerable reduction of the tumor mass and lymph edema. At this time, the tumor bulk on the left-sided abdomen showed two ulcerations with minimal foetid secretion (Figure 4A). Four weeks later, tumor infiltration and lymph edema of the left leg were nearly resolved. Both ulcerations were still present and sore. Abdominal examination revealed a ballottement and a highly inflammatory induration of the entire lower abdominal wall. No hyperphosphatemia, hyperkalemia, hyperuricemia or hypocalcemia were observed. An ultrasound-guided tumor incision on the right side of the abdomen released approximately 400 ml of a liquefied, partially necrotic and putrefied tumor bulk. Further GT-controlled drainage of the abdominal mass did not result in any improvement and the patient unfortunately died shortly after the third treatment despite anesthetic treatment including broad spectrum antibiotics, presumably as a consequence of septicemia.

This case illustrates ipilimumab’s potential to induce strong antitumor immune responses with rapid and sustained tumor destruction (Figure 4B-G). Tumor destruction releases antigens, including non-self antigens as well as endogenous danger signals, which trigger inflammation, cytokine release and the infiltration of immune cells in the tumor-affected organs. Extended inflammation, tumor destruction and necrosis may induce multi-organ failure similar to a tumor lysis syndrome.

Discussion

This study summarizes unexpected and rare ipilimumab-induced AEs. To our knowledge, we report for the first time on (i) rare skin reactions, including a DRESS, a photosensitivity reaction and skin toxicity in a previously radiated area, (ii) a case of ischemic gastritis, (iii) rare neurological reactions, including granulomatous CNS inflammation, a Tolosa-Hunt-Syndrome and aseptic meningitis and (iv) a case of tumor mass liquefication with fatal outcome. Furthermore, we report on three intestinal perforations of which one was masked by ongoing steroid therapy, one occurred despite therapy with steroids and infliximab and one occurred in the small intestine outside the endoscopic examination range. In addition, different courses of ipilimumab-induced hypophysitis are described. Time course of specific side effects differed with e.g., much earlier onset of hepatitis than previously reported [20].

Since ipilimumab-induced irAEs can virtually affect any organ system physicians have to consider all symptoms as potentially ipilimumab-associated. In turn, patients have to be instructed to report all symptoms even if deemed unrelated. Several centers experienced difficulties in patient compliance with reporting AEs. For example, patients with severe diarrhea mostly reported symptoms several days after onset, which in one case resulted in a colonic perforation. As compliance of patients is limited when they fear their treatment will be stopped due to AEs, the fact that treatment efficacy is not abrogated despite steroid treatment should be stressed [41,42]. In the case of ipilimumab-induced irAEs prompt steroid treatment reduces intensity and duration of symptoms [43]. Even conditions that classically do not respond to steroid treatment, like Guillain-Barré syndrome [30] or hypophysitis, readily respond if ipilimumab-induced. Among all 120 evaluated patients in this study, side effects resolved in 82 cases. In 22 patients, side effects are ongoing and in 13 patients permanent changes remained. Unfortunately, three ipilimumab-induced fatalities occurred despite treatment.

Side effects caused by other drugs have to be differentiated from ipilimumab-induced irAEs as seen in patient 10 with psychological symptoms, first due to an ipilimumab-induced hypophysitis and later due to steroid treatment. Similarly, steroid-induced myopathy in a colitis patient can resemble an irAE. Thus, in order to avoid steroid-induced side effects in prolonged autoimmune reactions, a switch to other immunosuppressants, like etanercept, infliximab, or mycophenolat moleftil may be advisable. Furthermore, skin- and gut-related adverse reactions may reflect immune activation in response to signals from commensal organisms [44]. However, in the skin immunoreactivation through commensal microflora seems less likely, since most ipilimumab-induced skin reactions morphologically show exanthema, rather than eczema. In patients with colitis, detailed studies addressing antigen-specificity of ipilimumab-induced immune reactions are needed to distinguish autoimmunity from an enhanced reaction to resident flora.

Frequency and severity of irAEs seem to be dose-dependent [12,13]. However, immunotherapeutics in general do not show
linear dose response curves since induction of immunity depends on the host's immune system. Limited data exists to predict response or identify patients who are likely to develop severe irAEs. CTLA-4 polymorphism may play a role [45] although clear evidence is still pending [46]. Importantly, genetic predisposition for the development of autoantibodies is postulated since mice expressing specific CTLA-4 isoforms developed spontaneous autoimmunity, including elevated production of autoantibodies [47]. In addition, anti-CTLA-4 antibodies have been shown to induce autoantibodies in mice [48] and CTLA-4 specific autoantibodies have been found in sera from patients suffering from various autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Behçet’s disease and Sjogren's syndrome [49]. Although autoantibodies may be generated in vivo by an antigen-dependent mechanism and are postulated to modulate immune responses by interfering with CTLA-4 on T cells, it remains unclear whether CTLA-4 specific autoantibodies contribute to or protect against autoimmune reactions. Future studies in patients undergoing ipilimumab-treatment will be necessary to elucidate this question.

Interestingly, several large studies reported increased efficacy in patients affected by irAEs [21,42,50–52] with responses in 26% of patients experiencing any irAE compared to 2% in patients, who did not experience any irAE [42]. There also was a ‘severity-response-effect’ with response rates of 22% and 28%, in patients with grade 1/2 and grade 3/4 adverse reactions, respectively [42]. Nevertheless, clinical responses are

Figure 3. Ipilimumab-induced myocardial fibrosis in conjunction with hepatotoxicity. Hematoxillin eosin staining at 50× magnification (A), 200× magnification (B) and 400× magnification (C) and chloracetate esterase staining at 50× magnification (D), 200× magnification (E) and 400× magnification (F) revealed neutrophilic granulocytes (black arrow) mostly around the central vein (asterisk). Portal fields were almost normal (white arrows). Some necrotic hepatocytes (black arrow heads panel C) and cholestasis of hepatocytes (white arrow heads panel C) indicating liver insufficiency, were detected pericentrally. Slightly elevated myocardial fibrosis (white arrow heads panel F) surrounded by structural changes of cardiomyocytes were detected (black arrow heads panel F). doi:10.1371/journal.pone.0053745.g003
also seen in patients treated with ipilimumab without any irAE [50]. Furthermore, it is unclear whether four infusions as currently approved for treatment are necessary to induce tumor response. In patients where treatment was interrupted due to irAEs, clinical benefit was already observed after 1–3 ipilimumab infusions. This suggests that in a subgroup of patients fewer infusions might be sufficient to boost pre-existing anti-tumor immunity. A reduced number of infusions - if proven effective - could reduce costs as well as associated irAEs.

In our study, tumor regression was observed in 30.9% (21 out of 69) and tumor control in 61.8% (42 out of 69) of stage IV patients. No association was observed between organ system affected by the side effect and organ system that responded to therapy. Since ipilimumab can only augment existing T-cell responses, a previous tumor-specific immune response is indispensable [53] and could be induced by prior antigen-specific immunotherapy.

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**Supporting Information**

**Table S1 Participating centers, number of patients treated, dosages administered, and treatment settings.**

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**Author Contributions**

Conceived and designed the experiments: LMH SP DG CJV. Analyzed the data: LMH CJV. Contributed reagents/materials/analysis tools: SMG CL CR KB TB C. Berking TE MF CG RG AH RH GH AJ UK CM PM SP IS DS BS UT JU RV RM PW TD GD. Wrote the paper: L. Meliha K. Camiran M. Gamba M. Fettouh. Critical review of the manuscript: L. Meliha C. Berking G. Sanders.

**Figure 4. Ipilimumab-induced tumor mass liqueification.** Ulcerated, partially liquefied tumor mass inguinal left (A). Histologic examination confirmed an abundance of necrotic tumor cells with leukocytic infiltration and residual highly pleomorphic tumour cells, haemorrhage and fibrosis (images in hematoxillin eosin staining, magnification 200 x; B+C). doi:10.1371/journal.pone.0053745.g004
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