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Parathyroid hormone related protein (PTHrP)-mediated hypercalcemia in malignancy associated with anti-PD-1 immune checkpoint inhibitor treatment and related inflammatory reactions

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ABSTRACT

Over the last decade, the breakthrough of immune checkpoint inhibitors has revolutionized cancer therapeutics, an enterprise not devoid of a novel constellation of unique immune-related adverse events. In this article, we present the first two patients, one with metastatic urothelial bladder cancer and another one with inoperable non-small squamous cell lung carcinoma, with immune-related parathyroid hormone related protein (PTHrP)-mediated hypercalcemia concurrent with immune-related pneumonitis following administration of anti-PD-1 monoclonal antibody nivolumab. The second patient present immune-related colitis as well. In both patients the hypercalcemia developed when cancer was in remission, rendering unlikely the diagnosis of humoral hypercalcemia of malignancy. The time of onset of PTHrP-mediated hypercalcemia–11 weeks and 15 weeks after initiation of nivolumab for the first and second patient respectively– insinuated the immune-related origin of PTHrP. The concurrent immune-related pneumonitis raised the question of whether the immune-related inflammatory milieu in the context of pneumonitis could be the source of the immune-related PTHrP. In conclusion, increased awareness of nivolumab-related hypercalcemia –an extremely rare immune-related adverse event– could enable the identification of immune-related elevation of PTHrP. Moreover, our cases provide the rationale for further research in pursuit of not only the source of immune-related PTHrP expression, but also of a causative link connecting the inflammatory milieu of immune-related pneumonitis and/or immune-related colitis with PTHrP-mediated hypercalcemia. Finally, the correlation of immune-related adverse events observed herein with response to nivolumab is in line with previous reports, necessitating further consolidation.

1. Introduction

With the advent of era of cancer immunotherapy, immune checkpoint inhibitors have been endorsed in anticancer arsenal, imposing a shift paradigm in cancer therapeutics. Exploiting the host immune system, immune checkpoint inhibitors hold a great promise for durable responses and long-term survival. However, this milestone might be accomplished at the expense of a constellation of immune-related adverse events with a unique dynamic, affecting virtually every organ and system.

The cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and the programmed death-1 (PD-1) are the most investigated immune checkpoints assigned to inhibit T cell differentiation, activation, and survival [1,2]. Confined to T cells, CTLA-4 abrogates principally the priming phase of T-cell activation and the immunosuppressive function of T regulatory (Treg) cells [2]. PD-L1, expressed on B, T, dendritic cells, macrophages, mast and tumor cells in a broad range of tissues interfaces with its receptor PD-1 on T cells, creating an “immune shield” that halts autoimmunity, acting mainly in the effector phase of T cell activation [3].

Cancer cells, overexpressing CTLA-4 and PD-L1, hijack the immune checkpoint pathways to evade antitumor immunity. Consequently, CTLA-4 and PD-1 blockade can unleash an anti-tumor immune response, inevitably coupled with immune-related adverse events [4], which display a global incidence of any and of severe grade of, respectively, 26.8% and 6.1% [5]. Endocrine immune-related adverse

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events present a wide range of incidence –0% to 63%− while, recently, an incidence of 12.9% was reported related with ipilimumab (CTLA-4 immune checkpoint inhibitor), nivolumab, and/or pembrolizumab (PD-1 inhibitor) [6]. Immune-related endocrine toxicity encompasses mainly hypophysitis, hypothyroidism, hyperthyroidism, ophalmopathy Graves, diabetes mellitus type 1, and primary adrenal insufficiency. A median time of onset of adverse events related with ipilimumab and nivolumab of, respectively, 3–11 weeks and 6–15 weeks has been reported [7,8].

Nivolumab is a humanized monoclonal IgG4 anti-PD-1 antibody approved for the treatment of unresectable, metastatic or completely resected (adjuvant) melanoma, metastatic non-small-cell lung carcinoma (NSCLC), urothelial cancer, advanced renal cell carcinoma, head and neck cancer, Hodgkin Lymphoma, colorectal carcinoma and hepatocellular carcinoma [9]. It acts through binding to the PD-1 receptor and intercepting its interaction with both PD-L1 and PD-L2, thereby restoring a PD-1 pathway mediated antitumor immune response. Ongoing clinical trials are currently addressing the therapeutic potential of inhibition of PD-1/PD-L1 axis in cancer types that are conventionally considered less immunogenic [9]. The PD-1/PD-L1 axis inhibitors show generally a milder toxicity profile compared with other immunotherapy drugs such as CTLA-4 inhibitors and chemotherapy, comprising significantly lower risk of any all-grade and high-grade adverse events but increased risk of all-grade rash, pruritus, colitis, hypothyroidism, hyperthyroidism, and pneumonitis [10].

2. Case presentation

2.1. Patient #1

A 53 year-old male was diagnosed in May 2017 with urothelial bladder cancer at inoperable stage (IVA; AJCC/TNM staging system) due to paraortic and iliac lymph node infiltration. The patient was treated with combination therapy with cisplatin and gemcitabine for 6 cycles and subsequently he was treated with loco-regional radiation therapy. Four months later he presented disease progression with liver metastatic disease. Then, second line treatment with anti-PD-1 active immunotherapy, with nivolumab at dosage of 3 mg/kg of body weight every 14 days was initiated.

The patient 11 weeks after immunotherapy initiation (he had received five 2-week cycles) presented fatigue, confusion, loss of appetite, nausea, constipation, shortness of breath, cough, and mild grade of dyspnea. He underwent blood and biochemical tests, as well as detailed imaging control with computed tomography (CT) and FDG-positron emission tomography (PET)/CT scans. The primary blood results showed leukocytosis, serum hypercalcemia (calcium level at 14.8 mg/dL) with normal albumin levels (4.3 g/dL) and high levels of C-reactive protein (CRP) at 43.9 mg/L (Upper Limit at 1 mg/L) (Table 1). CT scan showed bilateral areas of consolidations with ground-glass opacities. A moderate (grade 2) checkpoint inhibitor pneumonitis (CIP) was diagnosed. No metastatic or other nature of osteolytic bone lesions were revealed. Cancer restaging showed non-PD (progressive disease) and partial response (PR) of liver metastases (overall response PR), according to the revised RECIST guideline (version 1.1) [11].

No elevation of the patient's serum antibody titers against Mycoplasma pneumoniae and Chlamydia pneumoniae was observed. Tests for pneumococcal and Legionella pneumophila serotype 1 antigens were negative. Serologic work-up was not indicative of any collagen vascular disease. Anti-neutrophil cytoplasmic antibody (ANCA) were negative. No microorganisms grew on a sputum culture. Fiberoptic bronchoscopy was performed and the bronchoalveolar lavage fluid (BALF) analysis showed a cell count of 3.2×10^5 cells·mm^{-3} with 17% lymphocytes and 13% neutrophils. Results on microbiological testing of BALF were negative (including staining and culture for bacteria, fungi, viruses, mycobacteria and Pneumocystis jirovecii).

In order to identify the nature of hypercalcemia, specific biochemical tests related to calcium metabolism were performed. A significant increase of plasma Parathyroid Hormone Related Protein (PTHrP) concentration at 58 pmol/L was diagnosed, while parathyroid hormone (PTH) was decreased at the concentration of 3.6 ng/L and 1,25-dihydroxy vitamin D [1,25$(OH)_2$vitamin D] was also reduced at 21.4 pmol/L (Table 1). For PTHrP detection in EDTA-plasma an ELISA PTHrP kit was used (Cloud-Clone Corp., USA; the detection range of this kit is typically 4.5–290 pmol/L and the minimum detectable dose less than 1.6 pmol/L). Normally PTHrP should be undetectable or minimally detectable in plasma and the reference value in adults according to Mayo Clinic laboratories is ≤4.2 pmol/L. The biochemical work-up showed hypercalcemia attributed to elevation of PTHrP as presented in Table 1.

Once the diagnosis of immune checkpoint inhibitor-induced interstitial lung disease was set, oral prednisone was started at the dosage of 60 mg daily as well as intravenous (IV) fluids and diuretics were administrated. Amelioration of patient's symptoms was observed following treatment. The patient's symptoms and the hematological abnormal indices were improved rapidly and resolved totally within 4 weeks (Table 1). The dose of oral steroids was gradually decreased and discontinued after 3 months.

2.2. Patient #2

A 72 year-old male was diagnosed in January 2018 with NSCLC of squamous histology at inoperable stage IIIC (T3N3M0; AJCC/TNM staging system). The patient was treated with combination therapy with cisplatin and docetaxel for 4 cycles and subsequently he was treated with loco-regional radiation therapy. Three months later he presented disease progression of the primary lung tumor and mediastinal lymph nodes. A second line treatment with anti-PD-1 active immunotherapy, with nivolumab at the dosage of 240 mg IV every 14 days was initiated.

The patient 15 weeks after immunotherapy initiation (he had received six 2-week cycles) presented fatigue, loss of appetite, moderate abdominal pain, diarrhea (5–6 mucous stools / day), and cough. He underwent blood and biochemical tests, as well as detailed imaging control with computed tomography (CT) and FDG-positron emission tomography (PET)/CT scans. The primary blood results showed serum leukocytosis, hypercalcemia (13.9 mg/dL) with low albumin levels (3.3 g/dL), resulting in corrected calcium levels equal to 14.46 mg/dl, and significantly high levels of C-reactive protein (CRP) at 61.2 mg/L (Upper Limit at 1 mg/L) (Table 2). Lung CT scan showed areas of consolidations with ground-glass opacities compatible to pneumonitis. Also, PET/CT demonstrated inflammatory activity along colon including small bowel. No metastatic or other nature of osteolytic bone lesions were revealed. Cancer restaging showed partial response (PR) of the cancer disease, according to the revised RECIST guideline (version 1.1) [11].

No elevation of the patient's serum antibody titers against Mycoplasma pneumoniae and Chlamydia pneumoniae was observed. Tests for pneumococcal and Legionella pneumophila serotype 1 antigens were negative. Serologic work-up was not indicative of any collagen vascular disease. Anti-neutrophil cytoplasmic antibody (ANCA) were negative. No microorganisms grew on a sputum culture. Fiberoptic bronchoscopy was not performed because patient's cough was resolved rapidly after treatment initiation with corticosteroids and the patient remained asymptomatic. A mild to moderate (grade 1–2) checkpoint inhibitor pneumonitis (CIP) was diagnosed.

Due to diarrhea the patient underwent an endoscopy/colonoscopy. Diarrhoea was scored according to CTCAE version 4.03. Routinely, stools were tested for microorganisms, including Salmonella, Shigella, Yersinia, Cambylobacter (SSYC), Clostridium difficile and viral pathogens and found negative. For the assessment of endoscopic activity and severity of colitis the Mayo score was used. The endoscopic findings showed a grade 2 colitis (moderate) with a diffuse component of inflammation, marked erythema, absent vascular pattern, friability and

Table 1
Diagnostic work-up values for patient 1 at diagnosis and 4 weeks later after treatment.

Variable	zero-time (at diagnosis)	4-weeks later	Laboratory Reference values
White blood cell count (/μL)	**15,300**	7200	4.000–11.000
Neutrophils (%)	53	72	40–75
Lymphocytes (%)	38	25	20–45
Monocytes (%)	8	2	2–10
Eosinophils (%)	1	1	1–6
Basophils (%)	0	0	0–1
Hemoglobin (g/dL)	13.1	12.8	13.00–18.8
Hematocrit (%)	41.2	39.5	40–54
Platelet count (/μl)	345,000	268,000	150.000–400.000
C reactive protein (mg/L)	**43.9**	5.2	< or = 1
Total protein (g/dL)	6.8	7.0	6.0–8.3
Albumin (g/dL)	4.3	4.4	3.2–5.5
Urea (mg/dL)	36	45	20–50
Creatinine (mg/dL)	0.8	1.0	0.7–1.5
Sodium (mEq/L)	141	140	135–147
Potassium (mEq/L)	4.1	4.5	3.5–5.3
Calcium (mg/dL)	**14.8**	8.8	8.1–10.5
Magnesium (mg/dL)	2.1	1.9	1.5–2.4
Aspartate aminotransferase (SGOT) (U/L)	36	35	15–37
Alanine aminotransferase (SGPT) (U/L)	38	42	20–55
γ-Glutamyl-transferase (γ-GT) (U/L)	42	40	15–85
Alkaline phosphatase (U/L)	**34**	102	40–130
Total bilirubin (mg/dL)	1.0	0.9	0.2–1.2
Lactate dehydrogenase (U/L)	246	175	100–190
Fasting blood glucose (mg/dL)	102	115	70–110
LDL cholesterol (mg/dL)	114	93	< 100
HDL cholesterol (mg/dL)	31	35	> 40
Triglyceride	192	186	< 150
Free thyroxine (pmol/L)	15	14	10–20
Thyroid stimulating hormone (miu/L)	1.9	2.5	0.2–4.0
Cortisol (μg/dL)	21	29	7–28 (morning)
Adrenocorticotropin (ACTH) (pmol/L)	8.8	7.2	1.55–11.1 (morning)
Parathyroid Hormone (PTH)(ng/L)	**3.6**	47	10–65
Parathyroid Hormone Related Protein (PTHrP) (pmol/L)	**58**	Undetectable	< or = 4.2
1,25-dihydroxy vitamin D [1,25$(OH)_2$vitamin D] (pmol/L)	**18.4**	72	48–108
Serum Angiotensin converting enzyme (ACE) (nmol/mL/min)	19	12	< 40
quantiFERON-TB test	negative	–	–

erosions.

In order to identify the nature of hypercalcemia, specific biochemical tests related to calcium metabolism were performed. A significant increase of plasma PTHrP concentration at 74.4 pmol/L was diagnosed, while PTH was decreased at the concentration of 2.2 ng/L and 1,25$(OH)_2$vitamin D was also reduced at 29.1 pmol/L (Table 2). For PTHrP detection in EDTA-plasma an ELISA PTHrP kit was used (Cloud-Clone Corp., USA; the detection range of this kit is typically 4.5–290 pmol/L and the minimum detectable dose less than 1.6 pmol/L). Normally PTHrP should be undetectable or minimally detectable in plasma and the reference value in adults according to Mayo Clinic laboratories is ≤4.2 pmol/L. The biochemical work-up showed hypercalcemia attributed to elevation of PTHrP as presented in Table 2.

Once the diagnosis of immune checkpoint inhibitor-induced interstitial lung disease and related colitis was set, oral prednisolone was started at the dosage of 80 mg daily as well as IV fluids and diuretics were administrated. Amelioration of patient's symptoms was observed following treatment. The patient's symptoms and the hematological abnormal indices were improved rapidly and resolved totally within 6 weeks (Table 2). The dose of oral steroids was gradually decreased and discontinued after 4 months.

3. Discussion

Although calcium homeostasis is tightly regulated via a finely tuned balance among skeleton, kidneys, parathyroid glands, and intestine, neoplasms might tilt this balance towards hypercalcemia. In hospitalized patients, hypercalcemia exhibits a prevalence extending from 0.17% to 2.92% [12] attributed principally to malignancy and secondarily to primary hyperparathyroidism –the culprits behind 90% of hypercalcemia in general population [13].

Clinical manifestations of hypercalcemia, affecting renal function, central nervous, gastrointestinal, cardiovascular, and musculoskeletal system, may provide a clue for the etiology. The mild (calcium serum < 12 mg/dL) and the moderate (calcium serum 12 to 14 mg/dL) chronic hypercalcemia are usually asymptomatic, while the acute moderate type and the severe type (calcium serum > 14 mg/d) are symptomatic. Hypercalcemia of malignancy –often severe and acute– may be symptomatic and a herald of poor prognosis [14]. The evaluation of intact serum PTH levels is a step pivotal in the differential diagnosis of hypercalcemia distinguishing the PTH-dependent causes, characterized by high or inappropriately not suppressed intact serum PTH levels, from the PTH-independent ones [15,16], as presented in Table 3. It should not be ignored that the primary hyperparathyroidism in cancer patients is incriminated for one third of hypercalcemia in this population [17]. In our patients, the serum PTH levels were not elevated, excluding any PTH-dependent etiology. Among the PTH-independent causes, a thorough history, the physical examination and the laboratory work-up indicated the diagnosis of hypercalcemia of malignancy. In the context of the differential diagnosis of hypercalcemia of malignancy [16,18–26], as depicted in Table 4, the co-evaluation of increased serum PTHrP levels with suppressed serum PTH levels and decreased serum 1,25-dihydroxy vitamin D (1,25(OH)2D) levels observed in our patients could point to humoral hypercalcemia of malignancy.

Encountered in 20–30% of cancer patients, humoral hypercalcemia of malignancy accounts for almost 80% of hypercalcemia of malignancy complicating mainly squamous cell, renal cell, breast and genitourinary

Table 2
Diagnostic work-up values for patient 2 at diagnosis and 4 weeks later after treatment.

Variable	zero-time (at diagnosis)	6-weeks later	Laboratory Reference values
White blood cell count (/μL)	**16,400**	6500	4.000–11.000
Neutrophils (%)	46	66	40–75
Lymphocytes (%)	45	28	20–45
Monocytes (%)	9	5	2–10
Eosinophils (%)	0	1	1–6
Basophils (%)	0	0	0–1
Hemoglobin (g/dL)	13.1	12.8	13.00–18.8
Hematocrit (%)	41.2	39.5	40–54
Platelet count (/μl)	345,000	268,000	150.000–400.000
C reactive protein (mg/L)	**61.2**	3.2	< or = 1
Total protein (g/dL)	5.8	6.6	6.0–8.3
Albumin (g/dL)	3.3	4.0	3.2–5.5
Urea (mg/dL)	48	34	20–50
Creatinine (mg/dL)	1.2	0.9	0.7–1.5
Sodium (mEq/L)	137	139	135–147
Potassium (mEq/L)	3.2	4.1	3.5–5.3
Calcium (mg/dL)	**13.9**	8.3	8.1–10.5
Magnesium (mg/dL)	1.7	1.8	1.5–2.4
Aspartate aminotransferase (SGOT) (U/L)	44	29	15–37
Alanine aminotransferase (SGPT) (U/L)	51	38	20–55
γ-Glutamyl-transferase (γ-GT) (U/L)	108	46	15–85
Alkaline phosphatase (U/L)	**38**	95	40–130
Total bilirubin (mg/dL)	0.7	0.6	0.2–1.2
Lactate dehydrogenase (U/L)	284	148	100–190
Fasting blood glucose (mg/dL)	147	98	70–110
LDL cholesterol (mg/dL)	78	84	< 100
HDL cholesterol (mg/dL)	42	51	> 40
Triglyceride	123	147	< 150
Free thyroxine (pmol/L)	18	13	10–20
Thyroid stimulating hormone (miu/L)	3.8	1.7	0.2–4.0
Cortisol (μg/dL)	25	34	7–28 (morning)
Adrenocorticotropin (ACTH) (pmol/L)	5.5	3.3	1.55–11.1 (morning)
Parathyroid Hormone (PTH)(ng/L)	**2.2**	52	10–65
Parathyroid Hormone Related Protein (PTHrP) (pmol/L)	**74.4**	Undetectable	< or = 4.2
1,25-dihydroxy vitamin D [1,25$(OH)_2$vitamin D] (pmol/L)	**12.1**	55.6	48–108
Serum Angiotensin converting enzyme (ACE) (nmol/mL/min)	34	16	< 40
quantiFERON-TB test	negative	–	–

carcinomas [27]. Grounded in 1941 by Fulah Albright, humoral hypercalcemia of malignancy was revolutionized in 1987 by the discovery of its orchestrator, the Parathyroid Hormone Related Pepide (PTHrP), a 16 Kda protein of 141 amino acids, showing homology with PTH in the first 20 residues. Alternative splicing of the PTH-Like Hormone (PTHLH) gene, located on the short arm of chromosome 12, generates three isoforms of PTHrP consisting of 139, 141, or 173 amino acids. Binding to PTHR1, PTHrP regulates the mammary development, the chondrocytes biology, the normal osteoblast function, the transport of calcium across the placenta from mother to fetus during pregnancy, the fetal development, the lactation, the smooth muscle relaxation and cell growth, and stimulates the RANKL-induced bone resorption [28,29].

However, in patient #1 the hypercalcemia developed at a time of absence of disease progression and partial response of liver metastases. Likewise, in patient #2 the hypercalcemia developed at a time of partial response of cancer disease. Consequently, we postulate that in our cases humoral hypercalcemia of malignancy –a paraneoplastic syndrome associated most often with advanced cancer and unfavorable prognosis– is an unlikely scenario [30].

A diagnostic challenge posed by our cases is to identify the origin of the PTHrP expression. The time of onset of hypercalcemia –11 weeks and 15 weeks for the patient #1 and the patient #2 respectively following initiation of nivolumab– is consistent with literature concerning immune-related adverse events. Therefore, it is reasonable to presume that the hypercalcemia described herein pertains to immunotherapy.

Notably, immune-related hypercalcemia is an extremely rare event and in most cases the levels of PTHrP were not evaluated. Only two female patients were shown to develop hypercalcemia, defined as a serum calcium level > 11 mg/dl, in the review of the ipilimumab-induced endocrine immune-related adverse events retrospectively recognized in 13 Bristol-Myers Squibb-sponsored clinical trials enrolling patients with advanced and/or metastatic melanoma at the Memorial

Table 3
Differential diagnosis of hypercalcemia according to PTH status.

PTH-dependent causes	PTH-independent causes
Primary hyperparathyroidism	Hypercalcemia of malignancy
Familial benign hypocalciuric hypercalcemia	Granulomatous diseases (e.g., sarcoidosis, tuberculosis)
Lithium therapy	Endocrinopathies (e.g., thyrotoxicosis, adrenal insufficiency, pheochromocytoma, VIPoma)
Tertiary hyperparathyroidism in chronic renal failure	Drugs (e.g., vitamin D intoxication, vitamin A intoxication, thiazide diuretics, milk alkali syndrome, aluminum, theophylline)
Parathyroid carcinoma	Immobilization
	Acute renal failure
	Paget's disease

PTH: parathyroid hormone.

Table 4
Differential diagnosis of hypercalcemia of malignancy based on hormonal and biochemical profile.

Causes of HM	Intact PTH	PTHrP	$1,25(OH)_2$vitamin D	Calcium
HHM	Decreased/undetectable	Elevated	Decreased	Elevated
Osteolytic bone disease	Decreased/undetectable	Decreased/undetectable	Decreased	Elevated
$1,25(OH)_2$vitamin D-mediated HM	Decreased/undetectable	Decreased/undetectable	Elevated	Elevated
Simultaneous $1,25(OH)_2$vitamin D-mediated HM and PTHrP-mediated HM	Decreased/undetectable	Elevated	Elevated	Elevated
Simultaneous ectopic PTH- and PTHrP-mediated HM	Elevated	Elevated	Decreased	Elevated
Ectopic PTH-mediated HM	Elevated	Decreased/undetectable	Elevated	Elevated

$1,25(OH)_2$vitamin D: 1,25-dihydroxy vitamin D, HHM: Humoral hypercalcemia of malignancy, HM: hypercalcemia of malignancy, PTH: parathyroid hormone, PTHrP: Parathyroid Hormone Related Protein.

Sloan-Kettering Cancer Center (MSKCC) between 2007 and 2013. PTH levels were low but detectable in both patients [31]. A patient with metastatic renal cell carcinoma has been reported to develop grade 4 hypercalcemia (calcium level: 16.3 mg/dl) that manifested as consciousness disorder 3 days after initiation of nivolumab. Despite the correction of hypercalcemia, the patient died due to rapid progression of cancer [33]. In a randomized open-label phase III trial enrolling patients with recurrent squamous-cell carcinoma of head and neck receiving nivolumab after progression of disease within 6 months following platinum-based chemotherapy, hypercalcemia of any grade, grade 3–4, and grade 5 was observed in, respectively, 3 cases (1.3%), 1 case (0.4%), and 1 case (0.4%). In this study, in patients undergoing standard treatment only 1 case of hypercalcemia (0.9%), being grade 3–4, was reported [34].

In 2015, Mills et al reported the development of PTHrP-mediated hypercalcemia and cachexia in a 49-year-old female four months after initiation of ipilimumab for treatment of metastatic melanoma. Notable was the elevation of serum PTHrP level: 273 pcg/mL (normal range: 14–17 pcg/mL). Aggressive hydration and administration of zoledronic acid led to normalization of calcium levels. However, the patient experienced a new episode of hypercalcemia unresponsive to calcitonin, zoledronic acid, and denosumab. Unfortunately, the tumor progressed, and the patient died. Biopsy indicated that melanoma cells expressed higher levels of PTHrP. Interestingly, the melanoma cells showed also higher expression of monocarboxylate transporter 1 as opposed to reduced expression of Beta-galactosidase at hypercalcemia as compared to pre-hypercalcemia status, markers linking PTHrP to cachexia which in turn was related to high mortality [32].

In both our patients, the previously unreported concurrence of immune-related PTHrP-mediated hypercalcemia with immune-related pneumonitis gives rise to an unforeseen diagnostic dilemma: is it possible that the immune-related pneumonitis constitutes the source of the immune-related PTHrP elevation, resulting in PTHrP-mediated hypercalcemia?

Immune-related pneumonitis, defined as a focal or diffuse inflammation of the lung parenchyma related with immunotherapy [35], is considered a rare adverse event following treatment with CTLA-4 and PD1-inhibitors with a median time to onset of 5 months [8]. An incidence rate of pneumonitis of 5% has been described in advanced solid cancers and melanoma following anti–PD-1/PD-L1 antibodies [35], while grade 3–4 pneumonitis associated with nivolumab and pembrolizumab shows an overall incidence of less than 1%. Symptoms may be non-specific, often including new cough or dyspnea, in patients receiving these agents, necessitating pulmonary function tests and radiographic imaging (e.g., CT scan), whereas other patients may be asymptomatic presenting only radiologic findings [35]. Bronchoscopy serves to rule out other etiologies. High-dose corticosteroids with a slow taper are often effective, although fatal events have also been described [8].

So far, PTHrP expression has not been described in the context of pneumonitis. Nevertheless, the designation of PTHrP as crucial player of inflammatory process rationalizes the assumption that immune-related pneumonitis could be the source of PTHrP inducing hypercalcemia in our patients. Indeed, PTHrP has been shown to be expressed by the inflammatory synovium in streptococcal cell wall-induced arthritis in a way reminiscent of its expression in human rheumatoid synovium induced by tumor necrosis factor a (TNFa) [36,37]. Moreover, PTHrP has been demonstrated to be a feature of granulomatous inflammation in coccidioidomycosis and a response in endotoxinemia stimulating the hepatic acute phase response. Further, the emerging role of PTHrP signaling in chronic pancreatitis has been considered as a potential therapeutic target [38].

Within this framework, the potential contribution of the inflammatory milieu of immune-related pneumonitis to immune-related PTHrP-mediated hypercalcemia merits further investigation. Moreover, it is uncertain if the immune-related colitis observed in the patient #2 contributes to the elevation of PTHrP.

In the light of the information explosion concerning cancer immunotherapy, we highlight the most recent discoveries that represent the path forward for cancer treatment. It is acknowledged that integrating immune checkpoint inhibitors with conventional anticancer therapies –surgery, chemotherapy, and radiotherapy– and other landmark targeted therapies, such as mammalian target of rapamycin (mTor) inhibitors [39], has led to important milestones in cancer treatment [9,40–42]. Whereas immune checkpoint inhibitors are considered mainstay treatment for various cancer types, the primary and acquired resistance to this modality prompt research on novel strategies of immunotherapy, namely the molecular therapy, the cellular therapy, and the vaccines [42].

Beyond the immune checkpoint inhibitors, the molecular therapy comprises: (i) the cytokine IL-2, which has been proven to promote T cell growth; (ii) agonists of co-stimulatory receptors, leading to T cell activation; (iii) inhibitors of immune suppressive factors secreted by tumor cells into tumor microenvironment; and (iv) agonists of T cell metabolism [42]. The main component of the cellular therapy is the adoptive T cell therapy– ex vivo genetic engineering and expansion of a patient's T cells that are re-infused into patient– involving adoptive transfer of tumor infiltrating lymphocytes and use of blood T cells to generate genetically engineered T cells. The latter are mainly the T cells expressing transgenic receptors, known as TCR T cells, and the chimeric antigen receptors (CAR) T cells expressing CARs, both targeting tumor cells [42]. Additional strategies of cellular therapy target the Treg cells –key suppressive cells– resulting in depletion and abrogation of Treg, blockade of Treg differentiation and trafficking, and reprogramming of Treg to effector T cells [42]. Another type of cancer immunotherapy is the vaccination in the context of either prevention or treatment of cancer [42].

With approximately 2000 immunotherapeutic agents currently in development [9], more than 250 clinical trials on CAR T cells [42], and more than 200 vaccines under investigation in clinical trials [42], the cancer therapy landscape is evolving rapidly. First and foremost, ensuring an acceptable and manageable toxicity profile is an overarching goal.

4. Concluding remarks

To the best of our knowledge, this is the first report of two patients presenting immune-related PTHrP-mediated hypercalcemia concurrent with immune-related pneumonitis. Though described rarely, the immune-related PTHrP-mediated hypercalcemia should not be ignored in patients receiving anti-PD1 treatment. Further studies are needed to unravel the source of PTHrP attributed to immunotherapy, especially nivolumab. The intriguing concurrence of pneumonitis with PTHrP-mediated hypercalcemia related with nivolumab merits further exploration in search of not only the origin of PTHrP, but also a causative link connecting these two immune-related adverse events. Such a link could be translated in designation of immune-related pneumonitis as a risk factor for immune-related hypercalcemia. Intriguingly, the inflammatory milieu of immune-related colitis in patient #2 as a potential source of PTHrP merits further study. Finally, the correlation of immune-related adverse events with response to nivolumab observed in our patients is in line with pertinent literature [43,44], a notion awaiting consolidation. Our cases highlight the importance of active surveillance of patients receiving immune checkpoint inhibitors in order to yield the optimal therapeutic benefit mitigating the risk of harm. Five principles –prevention, anticipation, detection, treatment, and close monitoring– have been suggested to guide this surveillance [45].

Declaration of Competing Interest

None.

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