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# Progression of vertebral fractures in patients with adrenocortical carcinoma undergoing mitotane therapy

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#### 1 Progression of vertebral fractures in patients with adrenocortical carcinoma undergoing

#### 2 mitotane therapy

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22 Vertebral fractures in patients with adrenocortical carcinoma.

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#### 25

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- 40

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#### 49 Abstract

50 *Context:* patients with adrenocortical carcinoma (ACC) are frequently on mitotane therapy for a long-time period. The 51 drug exerts an adrenolytic activity requiring glucocorticoid supplementation, which can be potentially detrimental for 52 bone.

53 *Objectives:* to explore whether mitotane plus/minus chemotherapy is associated with an increased proportion of 54 morphometric vertebral fractures (VFs) in ACC patients. Secondary objectives were: proportion of patients with VF 55 progression, or worsening of the spinal deformity index (SDI) during mitotane therapy; predictive factors of VF 56 progression and prognostic role of VF progression.

57 *Design and setting:* multicenter, retrospective cohort study of patients with ACC who received mitotane alone or in 58 association to chemotherapy, recruited from January 2010 to January 2020 in two reference centers in Italy and France.

59 *Results:* a significant increase in the frequency of VFs before and after mitotane therapy was seen both in Italian (28.3%

60 vs 47.8%, p: 0.04) and French (17.8% vs 35.6%, p 0.04) series. VF progression was observed in 39.1%, and 28.9% of 61 patients, respectively. Baseline VFs and increased patient body mass index, but not the dose of cortisol 62 supplementation, showed an independent association with VF progression at multivariate analysis. Among the 72 63 advanced ACC patients, progression of VFs was associated with a poorer survival.

64 *Conclusions:* the administration of mitotane plus/minus chemotherapy in ACC patients impairs bone health 65 independently from cortisol supplementation. Appropriate preventive measures to decrease the fracture risk should be 66 implemented in these patients.

#### 68 Introduction

Adrenocortical carcinoma (ACC) is a rare and aggressive malignant disease with an incidence of 0.7-2 new cases per million populations per year (1). The majority of ACC is functioning at presentation and Cushing syndrome is the most frequent clinical manifestation (2).

72 Radical surgery represents the mainstay of treatment of localized tumors (1) and it is the only treatment strategy that is 73 able to offer a chance of cure to the patients. Despite radical resection, however, most patients are destined to relapse, 74 often within the first 2 years. Based on the results of a retrospective cross-sectional study (3,4), whose results have been 75 confirmed by other retrospective studies (1), adjuvant mitotane therapy for at least 2 years is currently prescribed and 76 recommended by available guidelines (1,5). Single agent mitotane or the combination of mitotane plus chemotherapy 77 are the treatment strategies adopted in patients with locally advanced or metastatic disease, not suitable for surgery (6). 78 Etoposide, doxorubicin and cisplatin administered in association of mitotane (EDP-M) is the standard chemotherapeutic 79 regimen (7,8). The efficacy of this regimen is overall limited, although a subgroup of patients can obtain a long-term 80 survival (9). Non progressing patients after 6 cycles of EDP usually continue mitotane therapy till progression.

81 Mitotane exerts an adrenolytic activity requiring glucocorticoid supplementation, which is tapered on the basis of 82 clinical assessment (10). Cortisone acetate or hydrocortisone are the recommended drugs for steroid replacement (1,5). 83 Due to increased steroid clearance and increased cortisol-binding globulin levels induced by mitotane, a higher 84 replacement dose is usually required than that prescribed for the management of Addison disease (1). Glucocorticoid 85 replacement in patients taking mitotane is empirically done and may lead to either mild hypo- or hypercortisolism (11). 86 Glucocorticoid administration, even at low doses, is associated with rapid bone loss and fragility (12). Glucocorticoids, 87 in fact, negatively affect bone health via a complex mechanism that includes both a decrease in bone formation and an 88 increase in bone resorption. Glucocorticoid-induced inhibition of bone formation involves multiple pathways, including 89 a reduction in osteoblast proliferation via suppression of the growth factors BMP2 and TGFb1, upregulation of Wnt 90 antagonists (Dkk-1, Wif-1, and Sost), and downregulation of the Wnt receptor complex (frizzled 4, 7, Dsh1, and Axin1). 91 This results in strong suppression of osteoblast differentiation, maturation, and activity (13). Skeletal fragility is a 92 frequent complication of Cushing syndrome, and fragility fractures may be the first clinical manifestation of the disease. 93 However, improvement of bone mineral density was reported after resolution of hypercortisolism (14).

On these premises, a detrimental effect of steroid replacement on bone deserves attention in ACC patients on mitotane therapy. This issue is relevant since many patients are receiving mitotane after surgery in adjuvant setting and a number of patients with advanced disease are on mitotane therapy for a long time period. To the best of our knowledge, the effect of mitotane (and associated steroid replacement) on fracture risk has never been explored. Vertebral fractures (VFs) are the classical hallmark of glucocorticoid-induced osteoporosis and bone fragility (15,16), and are associated

99 with impaired quality of life and an increased risk of future fractures (17). The majority of VFs are mild and 100 asymptomatic, and are diagnosed through a radiological exam. Vertebral morphometry is a quantitative method to 101 identify VFs. It is based on the measurement of dorsal and lumbar vertebrae on lateral radiographic images 102 (Morphometric X-ray Radiography, MRX) or on DEXA scans (Morphometric X-Ray Absorptiometry, MXA) (18). With 103 the purpose to evaluate the frequency of VFs in ACC patients during mitotane therapy, we conducted a retrospective 104 case-control study in 2 reference institutions for the management of ACC patients in Italy and France, respectively. The 105 primary aim was to assess the frequency of VFs before and after mitotane treatment, secondary aims were: assessment 106 of the proportion of patients with progression of VF or worsening of the spinal deformity index (SDI) during mitotane 107 therapy, identification of risk factors for progression of VF and the impact of VFs on patient prognosis.

108

#### 109 Patients and methods

#### 110 Study design and objectives

111 This was a retrospective, multicentric, case-control study. Data of patients from the Azienda Socio Sanitaria Territoriale 112 (ASST) Spedali Civili in Brescia (Italy) and Institute Gustave Roussy in Villejuif (France), meeting the eligibility 113 criteria and recruited from January 2010 to January 2020, have been collected. Italian patients were the primary cohort 114 and French patients the validation cohort. To be included in the study, the patients should have met the following 115 eligibility criteria: age > 18 years; histological diagnosis of adrenocortical carcinoma; mitotane treatment alone or in 116 association with chemotherapy for at least 6 months; availability of CT scans with sagittal view performed at baseline 117 and during treatment. Exclusion criteria were the following: other malignancies except basal cell carcinoma or in situ 118 cervical cancer diagnosed within 5 years before the diagnosis of ACC, concomitant diseases known to affect bone, 119 previous or concomitant bisphosphonate therapy or other bone-active drugs except for vitamin D supplements, bone 120 metastases. All patients received steroid supplementation, which consisted in cortisone acetate in Italian patients and 121 hydrocortisone in the French patients. The equivalence between the 2 steroids is: hydrocortisone 20 mg = cortisone122 acetate 25 mg. The primary objective was to evaluate the prevalence of morphometric VFs during at least 6 months of 123 treatment with mitotane  $\pm$  chemotherapy in the overall series, and in the primary and validation cohorts. Secondary 124 endpoints were the following: evaluation of proportion of patients with VF progression and worsening of the spinal 125 deformity index during mitotane therapy, identification of bone fragility factors at baseline (i.e. hormone 126 hypersecretion, age, sex, BMI, menopausal status, defined as age  $\geq 60$  years or no menses for 1 year in the absence of 127 prior chemotherapy or tamoxifen use, or ovariectomy), and dosage of cortisol replacement therapy as independent risk 128 factors of VF progression; prognostic role of VFs on overall survival in the subgroup of patients with metastatic disease.

129 In addition to ACC patients on mitotane, two different control groups were also included: 1) ACC patients not treated

with mitotane and 2) breast cancer (BC) patients treated with aromatase inhibitors (AI) in adjuvant setting for at least 2 years. BC patients had normal renal function, none of them were receiving previous or current treatment with antiosteoporotic drugs (except for calcium and vitamin D) or glucocorticoids. Previous chemotherapy was permitted, but tamoxifen use was not. In the group 1) volumetric spine CT scans obtained at ACC diagnosis and during the follow up period were evaluated for VFs (at least every 6 months); in the group 2) CT scans were prospectively evaluated at baseline conditions, before the beginning of AI treatment, and at subsequent follow-up assessments.

All the ACC patients enrolled in the present study are registered in the ENS@T ACC registry approved by the local ethics committees of both Brescia and Villejuif. BC patients were included in a clinical study conducted at Medical Oncology and Breast Unit of ASST-Spedali Civili in Brescia (19, 20). All patients included had given their written informed consent.

140

#### 141 Data collection and assessment of vertebral fractures

Demography data, comorbidities, clinical and pathological tumor characteristics and treatment information have been collected. Volumetric spine CT scans obtained before the beginning of mitotane treatment have been evaluated and compared to those obtained after at least 6 months of treatment. The sagittal reformatting view with bone window has been used for the analysis performed by the same experienced radiologists, following a standardized semi-quantitative morphometric approach applied to dorsal and lumbar vertebrae.

Vertebrae T4 to L4 were assessed for the presence of VFs according to the validated Genant's semi-quantitative method (21). The Genant Grades were defined as following: percentage reduction of the anterior, middle, and/or posterior height: grade 1 (mild fracture) 20-25%; grade 2 (moderate fracture) 25-40%; grade 3 (severe fracture) >40%. Wedge, biconcave and concave fractures were also separately identified. Prevalent VFs were analyzed at baseline. VFs of any grade present at follow up, but not at baseline, were considered new VFs. Progression of VFs was defined as the development of new/incident VFs (in patients with no VFs or in patients with previous VFs in other vertebrae) and/or the documented minimum 1-point increase in the Genant scoring in preexisting VFs during the period of follow-up.

154 A spinal deformity index (SDI), which has been demonstrated to be a good predictor of incident vertebral fractures, was

155 calculated by summing in each patient the grade of each vertebra from T4 to L4 (22).

156 Radiographs were individually assessed simultaneously by a team of three experienced observers (MF, RG and CV),

157 who were blinded for any patient characteristics. In case of a discrepancy in assessment, a consensus opinion was 158 obtained.

- 159
- 160 Statistical analysis

161 Descriptive statistics were used to analyze patient clinical characteristics. Differences between categorical variables 162 were assessed by chi-square or Fisher test when indicated. Whereas, continuous variables were compared through 163 parametric (T-test) and non-parametric (Wilcoxon-test, Mann-Withney U-test) when indicated. Logistic regression 164 model was employed to assess the odd ratios (ORs) and 95% confidence intervals (95% CIs) both in the univariate and 165 multivariate analysis, with the lowest risk group as the reference group. Only factors that obtained p < 0.10 in univariate 166 analysis entered the multivariate model. OS curves have been calculated with Kaplan-Meier method and compared with 167 log-rank test. Statistical significance has been set at p < 0.05. SPSS v23.0 software has been used for the statistical 168 analyses (SPSS Inc., Chicago, IL).

169

#### 170 Results

#### 171 Patient characteristics

172 A total of 91 ACC consecutive patients, meeting the eligibility criteria and treated with mitotane entered the study: 46 173 were enrolled at the Medical Oncology Unit at ASST-Spedali Civili in Brescia (Italy) and 45 at the Endocrine Oncology 174 and Nuclear Medicine Department, Gustave Roussy Cancer Institute in Villejuif (France). Patient and tumor 175 characteristics are summarized in Table 1. Median age at the beginning of mitotane therapy was 51 years (range 18-72) 176 in the Italian group, and 44 (range 17-78) in the French group. Median BMI was 24.3 (range 16.4-31.2) and 23.8 (range 177 18.9-36.9) in the two groups, respectively. Although not statistically significant, a higher percentage of Italian women 178 had a postmenopausal status at diagnosis: 43.5%, versus 26.7% of the French counterpart (p = 0.24). Tumor 179 hypersecretion at diagnosis was present in 29 Italian patients (63.0%) and 36 French patients (80.0%) (p = 0.10). In 180 particular, cortisol hypersecretion was higher in the French cohort (68.9%) than in the Italian cohort (47.8%, p 0.05). 181 Nine (18%) patients in the Italian and 19 (38%) in the French cohort had cortisol hypersecretion at the time of starting 182 systemic antineoplastic therapy (p = 0.04).

At the time of the first evaluation, the proportions of patients with limited (stage I-II), locally advanced (stage III) or metastatic disease (stage IV) were 19.6%, 15.2% and 65.2% in the Italian group and 24.4%, 40.0% and 35.6% in the French series (p = 0.01). During the study period, 7 Italian and 19 French patients progressed from non-metastatic to metastatic disease.

187 In the whole population, 31 patients (34.1%) were treated with single agent mitotane (27 as adjuvant therapy 4 as 188 palliative therapy), 43 patients (47.2%) with mitotane in association with EDP or EP (EDP/EP-M). Seventeen patients 189 (18.7%), initially treated with mitotane in adjuvant setting, received also the EDP or the EP regimen at disease 190 progression while continuing mitotane (these patients were counted in both groups). Comparing the two cohorts, a

- 191 larger number of patients was treated with mitotane monotherapy in the French cohort, whereas in the Italian one a
- 192 higher number received concomitant mitotane and chemotherapy (p = 0.008 and p = 0.0001, respectively).
- 193 Hydrocortisone dose was converted to the equivalent cortisone acetate dose and the median supplementation dose was
- 194 cortisone acetate 62.5 mg/day (range 12.5-150).
- 195 Patients were followed for a median time of 30.6 months. At the last follow-up examination, 40 patients (43.9%) were

196 died. The median OS of the overall series was 24.2 months (range: 6.2-194.7).

- 197 Table 1 also describes the characteristics of patients not receiving mitotane therapy (control group 1). In particular,
- 198 median age at diagnosis was 44 years (range 21 74), 71.4% of patients were females and tumor hypersecretion was 199 present in 61.4% of cases.
- 200 The control group of BC patients consisted in 176 women (control group 2). Their median age was 64 years (range 30-201 74), all of them was in post-menopausal status (physiological or drug-induced) and the median BMI was 25.0 (range 202 16.5-42.2). Regarding histological subtype, 150 patients (85.2%) had a HR positive/HER2 negative whereas 26 patients 203 (14.8%) had a HER2 positive/HR positive tumor. All of the considered patients (100%) underwent surgery for BC. 204 Neoadjuvant or adjuvant chemotherapy were used in 6.3% and 36.4% of patient respectively. All of the patients 205 received adjuvant hormone therapy, in particular 155 patients (88.1%) received AI alone, whereas 21 patients (11.9%) 206 were treated with AI plus Luteinizing Hormone Releasing Hormone (LHRH). CT scans performed in each patient at 207 baseline and every 6 months were prospectively evaluated.
- 208

#### 209 Vertebral fractures in ACC patients at baseline

210 At baseline conditions, 13 out of 46 patients (28.3%) in the Italian group, 8 out of 45 patients (17.8%) in the French 211 group, who subsequently received mitotane therapy and 3 out of 21 (14.3%) ACC patients not treated with mitotane had 212 at least one prevalent VF. Among ACC patients treated with mitotane, 12 (26.1%) and 4 (8.9%) patients, respectively, 213 had  $\geq 1$  mild VF, 4 (8.7%) and 2 (4.4%) had  $\geq 1$  moderate VF and 1 (2.2%) and 3 (6.7%) had  $\geq 1$  severe VF. Five Italian 214 and 1 French patients had multiple VFs with different grades. Considering all patients together, VFs were found in 24 215 patients (21.4%) at baseline, 13 (11.6%) classified as mild, 7 (6.2%) moderate and 4 (3.6%) severe. Seven (6.2%) 216 patients had multiple VFs with different grades. As depicted in the Table 2, baseline VFs significantly correlated with 217 older age and sex, while no significant relationship was found with BMI, cortisol excess and, among women, 218 menopausal status.

219

#### 220 Vertebral fractures in the follow-up

- In the patient subset treated with mitotane  $\pm$  chemotherapy assessed after a median treatment time of 13.2 months (range 6.3-48.3) in the Italian patients and 24.4 months (range 7.0-41.0) in the French ones, a significant increase in the frequency of VFs was seen in both series (p = 0.043 and p = 0.047, respectively) (Table 3). In particular, progression of VFs was observed in 18 Italian patients (39.1%), and in 13 French patients (28.9%).
- 225 Considering together the 2 series, 53 patients (58.2%) remained free from VF during the study period; baseline fractures
- 226 remained stable in 7 (7.7%) patients, whereas a VF progression was seen in 31 (34.1%) patients (Table 3). Moreover,
- the total number of VFs of 37 at baseline (22 G1, 9 G2, 6 G3) increased to 113 after treatment (38 G1, 42 G2, 33 G3).
- 228 The distribution of VF severity and SDI before and after mitotane  $\pm$  chemotherapy is given in Figure 1. In particular, the
- 229 proportions of moderate and severe VFs were 5.5% and 4.4% at baseline and 11% and 15.4% after mitotane. A
- worsening in SDI was seen in 18 (39.1%) Italian patients and in 13 (28.9%) French ones. In the whole series 34.1% of
- 231 patients had a worsened SDI.
- 232 Conversely, in ACC patients not treated with mitotane there was only one new fracture in the follow-up and the increase
- 233 in the proportion of patients with vertebral fractures was not statistically significant (p = 0.50) (Table 3). In the BC
- patient subset, the treatment with AIs led to a statistically significant rise in the proportion of patients with at least one
- 235 VF (13.5% vs 20.2%, p = 0.04). Moreover, 13 patients developed new VFs (7.4%) and 5 patients had worsened VFs
- 236 (2.8%). Figure 1 gives the distribution of VF severity and SDI before and after AIs in BC patients. The proportions of
- 237 moderate and severe VFs were 5.6% and 0.6% at baseline and 8.4% and 1.7% after AI therapy and 11.9% of patients
- had a worsened SDI (Table 3).
- 239

#### 240 Risk factors of VF progression in ACC patients receiving mitotane therapy

- At univariate analysis, the following baseline characteristics: age  $\geq$ 48years, BMI  $\geq$ 24 and the presence of a VF were
- 242 associated to an increased risk of vertebral fractures progression. Both the presence of baseline VFs and BMI
- 243 maintained an independent association at multivariate analysis (odd ratio [OR] 4.2, 95%CI: 1.3-12.9, p = 0.01 and OR
- 244 2.6, 95% CI: 1.0-7.1, p = 0.05, respectively) (Table 4).

#### 245 Relationship between VF progression and patient outcome

- Among the 72 metastatic patients, 28 (38.9%) had VF progression after treatment. VF progression was associated with a
- 247 poorer survival (median 20.9 vs 41.5 months, Hazard Ratio 0.50, 95%CI 0.26-0.95, p = 0.032) (Figure 2).
- 248
- 249 **Discussion**
- 250 This multicentric, retrospective study was designed to obtain data on the impact on bone fragility of systemic treatment

administered in patients with ACC. Patients with ACC undergoing mitotane +/- chemotherapy from 2 reference centers for this rare disease were included in the study. We also evaluated a control group of patients with ACC not receiving mitotane therapy and a series of breast cancer patients undergoing aromatase inhibitors, a treatment known to be associated with an increased frequency of fragility fractures.

255 This study demonstrates for the first time a progression of VFs in 34% of patients with ACC treated with mitotane 256 during a median follow-up period of 30 months. A recent multicenter, multinational, retrospective study, showed that 257 bone metastases in ACC patients are associated with high risk of adverse skeletal related events (23). In this patient 258 series without bone metastases, we showed that also oncological treatment may contribute to an increased bone fragility 259 and elevated fracture risk. The Italian and French patients were similar in terms of sex distribution and BMI at baseline 260 conditions, however Italian patients were older and had a greater proportion of menopausal women than the French 261 counterpart. With regard to tumor characteristics, ACCs in the Italian population were more advanced and less 262 frequently hormone secreting than in the French one. Consequently, a greater proportion of Italian patients received 263 chemotherapy in association with mitotane. These differences notwithstanding, a significant progression of VFs was 264 demonstrated in both series considered separately.

265 About 23% of patients of patients treated with mitotane had at least one VF at baseline. Although this proportion is comparable to the VF frequency in the general population of healthy individuals (men and women) with >50 yr age 266 267 (24), it was superior to that in the control patients with ACC who did not receive mitotane. Most of the patients treated 268 with mitotane had advanced disease and this condition, even in the absence of bone metastases, predisposes for a higher 269 bone turnover (25). The frequency of VFs consistently increased in patients treated with mitotane + chemotherapy 270 (42%) but not in untreated patients. Mitotane therapy was also associated to a consistent increase in the VF severity: 271 grade 2-3 VFs according to the Genant scale increased from 40.5% at baseline to 66.4% after treatment and this 272 observation is noteworthy, since moderate-severe VFs are clinically relevant (26). It is known that AI administration in 273 women with breast cancer is associated with an increased risk of bone fragility and fractures and a bone loss preventive 274 therapy with bisphosphonates or denosumab is currently recommended by international guidelines in this setting (16, 275 27, 28). Noteworthy, the absolute increase in VF after AI in breast cancer patients evaluated in this study (+6.7%) was 276 lower than that observed in ACC patients submitted to mitotane (+18.7%) and VF progression occurred in 2.8% versus 277 6.6% of patients, respectively. This observation is relevant and provides a measure of the considerable deterioration of 278 bone health observed in patients on mitotane therapy.

We also performed some exploratory analyses to evaluate the impact of other well-known parameters associated with bone fragility on the fracture risk of the patients included in the study. At baseline, only age and sex were associated with the presence of VFs. Other bone fragility parameters such as BMI, menopause and cortisol hypersecretion at diagnosis failed to show a significant relationship. Older age and the presence of at least one VF at baseline 283 significantly correlated with an increased risk of VF progression after mitotane treatment in univariate analysis. The 284 presence of fractures at baseline maintained the statistical significance in multivariate analysis while age just failed to 285 enter the model. These data are consistent with what is observed in postmenopausal osteoporosis where both age and 286 history of previous fractures strongly predict fracture risk regardless of bone mineral density (29). However, in this 287 series high BMI appeared to be associated with an increased fracture risk after mitotane therapy and this is the opposite 288 of what is observed in postmenopausal osteoporosis where low BMI is an independent risk factor (30,31). The 289 association between adiposity and bone fragility is complex (32). On one hand, obesity has a protective role in relation 290 to the higher concentrations of estrogens due to higher aromatase activity, on the other hand it is associated with 291 detrimental effects on bone quality via several mechanisms, including alteration of bone regulating hormones, increased 292 oxidative stress and inflammation, and altered bone cell metabolism (32). In a recent cross-sectional study, elevated fat 293 body mass was found to have a direct relationship with fracture risk in women under AI therapy (19). A plausible 294 mechanism is that AIs induce a profound inhibition of estrogen synthesis leading to a loss of estrogen protection against 295 fragility-related fracture and allowing the deleterious effect of adiposity to prevail. Mitotane has been shown to inhibit 296 the aromatase enzyme (33) and, similarly to what we observed in women with breast cancer under AIs, this mechanism 297 may explain the correlation between high BMI and VF. Another possible alternative is that the deleterious effect of 298 adipose tissue on bone quality in patients with high BMI may be synergistic with steroid supplementation associated to 299 mitotane therapy. However, the dose of steroids did not show any correlation with VF and this suggests that steroid 300 supplementation does not play a major role on the bone fragility of patients on mitotane therapy.

Finally, the present study also showed that, in patients with advanced ACC without overt bone metastases, the occurrence of VFs was associated with poorer prognosis. This observation is in line with what observed both in the general population (34) and in cancer patients undergoing specific antineoplastic therapy (35).

The relatively high number of patients enrolled and the significant increase in the number and severity of VFs, which occurred after treatment both in the exploratory and validation series, are strengths of this study. The retrospective nature and the different observation period to which patients were subjected are the main limitations.

307 In conclusions, this study demonstrated for the first time that ACC patients, which are submitted to mitotane therapy, 308 are at high risk of developing fragility fractures. Previous fractures and high BMI are independent risk factors. These 309 data should be taken into consideration in order to adopt appropriate preventive measures, such as the prescription of 310 bone resorption inhibitors.

311

#### 312 Data availability

313 Some data generated and analyzed during this study are included in this published article.

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#### Tables

#### Table 1. Patient characteristics.

	Patients treated		Patients NOT			
	Italian	French	<i>p</i> -value	Total	treated with	
					mitotane	
N°	46	45		91	21	
Age						
Median (range)	51 (18-72)	44 (17-78)	0.14	48 (17-78)	44 (21 - 74)	
Sex, N (%)						
Male	18 (39.1)	18 (40.0)	1.00	36 (39.6)	6 (28.6)	
Female	28 (60.9)	27 (60.0)		55 (60.4)	15 (71.4)	
BMI						
Median (range)	24.3	23.8	0.59	24.0		
	(16.4-31.2)	(18.9-36.9)		(16.0-37.0)		
	0 (0.0)	3 (6.6)		3 (3.3)	21 (100%)	
Not available, N (%)						
Menopausal status						
N (%)	20 (43.5)	12 (26.7)	0.24	32 (35.1)	32 (35.1)	
Not available, N (%)	0 (0.0)	3 (6.7)		3 (3.3)	3 (3.3)	
Tumor secretory status at diagnosis, N (%)						
Secreting tumors,	29 (63.0)	36 (80.0)	0.10	65 (71.4)	11 (52.4)	
Cortisol excess						
$(\pm \text{ other hormones})$	22 (47.8)	31 (68.9)	0.05	53 (58.2)	11 (52.4)	
Androgens only	5 (10.9)	5 (11.1)	1.00	10 (11.1)	0 (0.0)	
Other	1 (2.2)	0 (0.0)		1 (1.1)	0 (0.0)	
Non secreting tumors	17 (37.0)	9 (20)		26 (28.6)	10 (47.6)	
Cortisol hypersecretion at the time of mitotane	e					
starting, N (%)	9 (18.0)	19 (38.0)	0.04	28 (56.0)		
ENSAT stage, N (%)						
I-II	9 (19.6)	11 (24.4)	0.62	20 (22.0)	14 (66.7)	
III	7 (15.2)	18 (40.0)	0.01	25 (27.5)	7 (33.3)	
IV	30 (65.2)	16 (35.6)	0.006	46 (50.5)	0 (0.0)	
Treatment administered, N (%)						
Only mitotane	10 (21.7)	21 (46.7)	0.008	31 (34.1)		
Adjuvant mitotane and then chemotherapy plus	7 (15.2)	10 (22.2)	0.10	17 (18.7)		
mitotane						
Concomitant chemotherapy plus mitotane	29 (63.0)	14 (31.1)	0.0001	43 (47.2)		
Mitotane therapeutic range attainment, N (%)	28 (60.9)	29 (64.4)	0.50	57 (62.6)		
Not available	0 (0.0)	3 (6.7)		3 (3.3)		
Cortisone acetate supplementation *, N (%)	46 (100)	45 (100)	1.00	91 (100)		
Median mg/day	50 (range 12.5-	62.5 (range 25-	0.037	62.5 (range 12.5-		
	150)	100)		150)		

BMI, Body Mass Index; NA not available.

416 417 \*Italian patients were supplemented with cortisone acetate whereas French patients with hydrocortisone. Hydrocortisone dose was converted in the equivalent cortisone acetate dose.

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#### Table 2. Correlations between risk factors of bone fragility and vertebral fractures at baseline.

VFs	No	Yes	<i>p</i> -value
Age at mitotane start, N/Tot (%)			0.004
<48 years	51/112 (45.5)	6/112 (5.4)	
≥48 years	37/112 (33.0)	18/112 (16-1)	
Sex, N/Tot (%)			0.05
Male	29/112 (25.9)	13 (11.6)	
Female	59/112 (52.7)	11 (9.8)	
BMI, N/Tot (%)			0.07
<24	37/88 (42.0)	7/88 (8.0)	
≥24	30/88 (34.1)	14/88 (15.9)	
Cortisol excess*, N/Tot (%)			0.11
Yes	44/112 (39.3)	16/112 (14.3)	
No	44/112 (39.3)	8/112 (7.1)	
Menopausal status, N/Tot (%)			0.62
Yes	33/66 (50.0)	6/66 (9.1)	
No	23/66 (34.8)	4/66 (6.1)	

433 VFs Vertebral Fractures.

434 \*at the time of starting mitotane therapy

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## 436 437 438 Table 3. Assessment of vertebral fractures on CT scan at baseline and after at least 6 months of mitotane±

#### chemotherapy.

	ACC patients treated with Mitotane± chemotherapy					ACC patients NOT		Breast cancer patients		
	Italian pa	tients	French pat	ients	IT+FR pat	tients	treated with mitotane		treated with AI	
	Baselin	After	Baseline	After	Baseline	After	Baseline	After	Baseline	After AI
	e	M±CHT		M±CHT		M±CHT				
VFs, N/Tot (%)										
No VFs	33/46	24/46	37/45	29/45	70/91	53/91	18/21	17/21	154/176	141/176
VEa	(71.7)	(52.2)	(82.2)	(64.4)	(76.9)	(58.2)	(85.7)	(80.9)	(87.5)	(80.1)
VFS	13/46	22/46	8/45	16/45	21/91	38/91	3/21	4/21	22/176	35/176
	(28.3)	(47.8)	(17.8%)	(35.6)	(23.1)	(41.8)	(14.3)	(19.1)	(12.5)	(19.9)
	<i>p</i> 0	.04*	<i>p</i> 0.	04*	р 0.0	005*	<i>p</i> 0.50* <i>p</i> 0.		.04*	
New VFs,										
N/Tot (%)		17/46		13/45		30/91		1/21		13/176
		(36.9)		(28.9)		(32.9)		(4.7)		(7.4)
Worsened VFs,						6 /0 A				
N/Tot (%)		4/46		2/45		6/91		0/21		5/176
D 4		(8.7)		(4.4)		(6.6)		(0.0)		(2.8)
Both new										
worsened VEc		3/46		2/45		5/01		0/21		2/176
N/Tot (%)		(6.5)		(4 4)		(5.5)		(0, 0)		(1 1)
Stable VFs		(0.5)		(+.+)		(5.5)		(0.0)		(1.1)
N/Tot (%)		4/46		3/45		7/91		3/21		19/176
( )		(8.7)		(6.7)		(7.7)		(14.3)		(10.8)
SDI, mean (±SD)	0.89	2.20	0.38	2.67	0.64	2.43	0.19	0.29	0.25	0.39
	(±3.16)	(±4.63)	(±0.91)	(±5.29)	(±2.34)	(±4.94)	(±0.51)	(±0.64)	(±0.74)	(±0.95)
	p (	).03	р 0	.03	<i>p</i> 0.	002	p 0.3	3	p 0.0001	
SDI <u>&gt;</u> 2, N/Tot (%)										
No	40/46	31/46	40/45	31/45	80/91	62/91	20/21	19/21	161/176	156/176
	(87.0)	(67.4)	(88.9)	(68.9)	(87.9)	(68.1)	(95.2)	(90.5)	(91.5)	(88.6)
Yes	6/46	15/46	5/45	14/45	11/91	29/91	1/21	2/21	15/176	20/176
	(13.0)	(32.6)	(11.1)	(31.1)	(12.1)	(31.9)	(4.7)	(9.5)	(8.5)	(11.4)
	р (	0.02	р 0	.02	<i>p</i> 0.001		<i>p</i> 0.1	0	<i>p</i> 0.24	
Worsened SDI,										
N/Tot (%)		18/46		13/45		31/91		1/21		21/176
		(39.1)		(28.9)		(34.1)		(4.7)		(11.9)

\*One tailed chi square. Pt: patients, VFs: vertebral fractures. CHT: chemotherapy

# Table 4. Correlations between VF progression and risk factors of bone fragility according to univariate and multivariate analyses.

	VF progression		Univariate analysi	s	Multivariate analysis		
	Yes	No	OR (95% CI)	<i>p</i> -value	OR (95% CI)	<i>p</i> -value	
Age, N/Tot (%)							
<48years	9/31 (29.0)	35/60 (58.3)	1	0.010	1	0.07	
≥48years	22/31 (71.0)	25/60 (41.7)	3.4 (1.4-8.7)		2.5 (0.9-7.0)		
Sex, N/Tot (%)							
Male	12/31 (38.7)	24/60 (40.0)	1	0.90			
Female	19/31 (61.3)	36/60 (60.0)	1.1 (0.4-2.6)				
BMI, N/Tot (%)							
<24	10/31 (32.2)	23/57 (40.4)	1	0.02	1	0.05	
≥24	21/31 (67.8)	34/57 (59.6)	3.1 (1.2-7.8)		2.6 (1.0-7.1)		
Menopausal status (total							
number of patients 51),							
N/Tot (%)				0.11			
Yes	14/18 (77.8)	18/33 (54.5)	2.9 (0.8-10.8)				
No	4/18 (22.2)	15/33 (45.5)	1				
Mitotane in range, N/Tot (%)				0.33			
Yes	18/31 (58.1)	39/57 (68.4)	1				
No	13/31 (41.9)	18/57 (31.6)	1.6 (0.6-3.9)				
Steroid replacement dose,							
N/Tot (%)				0.52			
≤62.5 mg/day	18/31 (58.1)	39/60 (65.0)	1				
>62.5 mg/day	13/31 (41.9)	21/60 (35.0)	1.3 (0.6-3.3)				
Chemotherapy, N/Tot (%)				0.23			
Yes	23/31 (74.2)	37/60 (61.7)	1.8 (0.7-4.7)				
No	8/31 (25.8)	23/60 (38.3)	1				
VFs at baseline, N/Tot (%)				0.001		0.01	
Yes	14/31 (45.2)	7/60 (11.7)	6.2 (2.2-18.0)		4.2 (1.3-12.9)		
No	17/31 (54.8)	53/60 (88.3)	1		1		

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Figure 1. A: Proportion of ACC patients divided on the bases of the worst VF grade (mild, moderate, severe) at baseline and after at least 6 months of mitotane±chemotherapy. B: Proportion of BC patients divided on the bases of the worst VF grade (mild, moderate, severe) at baseline and after a median time of AI treatment of 18 months. C: Distribution of Skeletal Deformity Index (SDI)\* at baseline and after treatment with mitotane±chemotherapy in ACC patients (proportion of patients). D: Distribution of SDI\* at baseline and after treatment with AI in BC patients (proportion of patients).



CHT chemotherapy.

\*SDI was calculated by summing in each patient the grade of each vertebra from T4 to L4. In theory, the SDI value can vary between 0 (no fracture) and 39 (all the assessed vertebrae are grade 3) (20).



