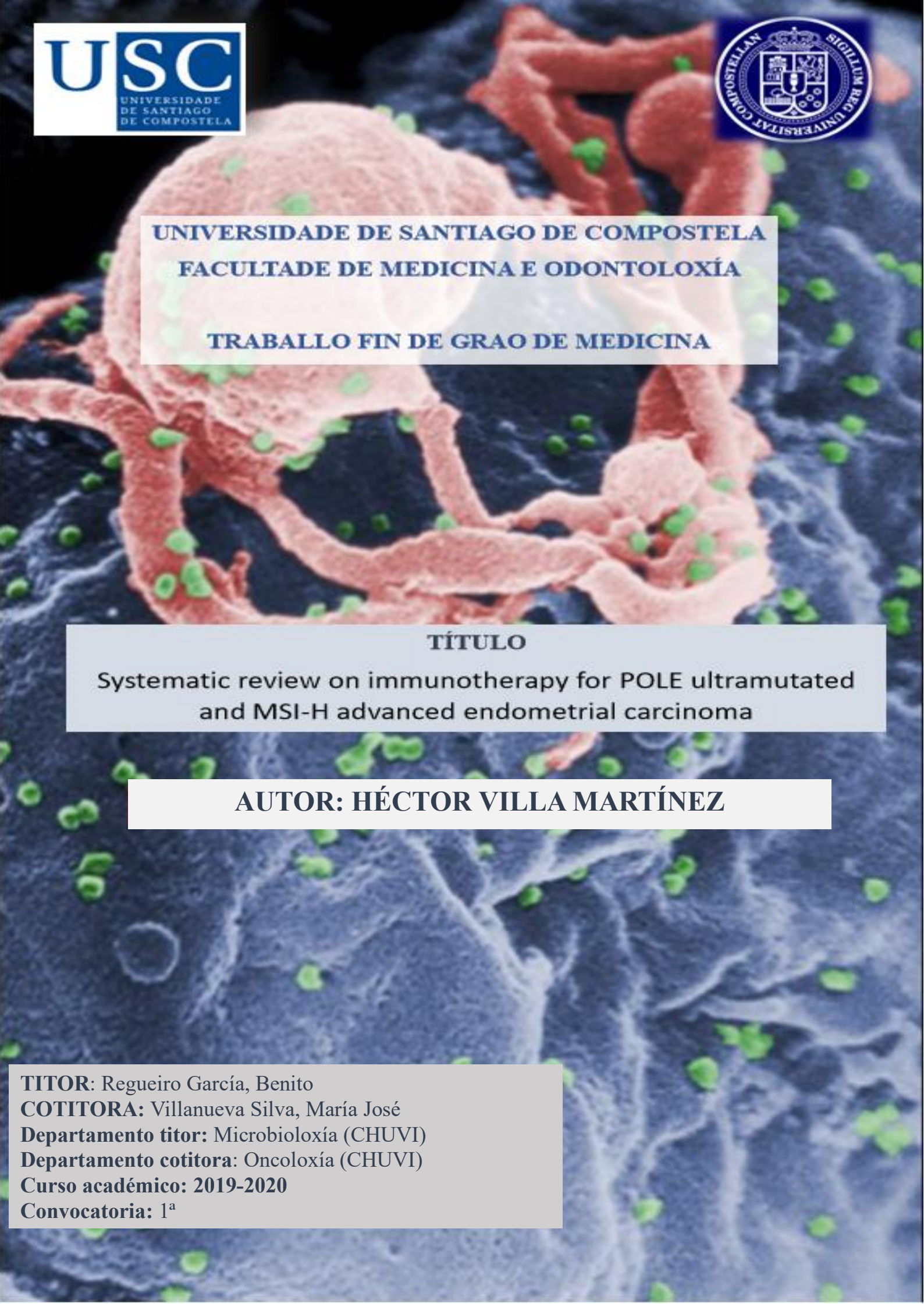

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TÍTULO

Systematic review on immunotherapy for POLE ultramutated and MSI-H advanced endometrial carcinoma

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ABSTRACT

Key Words: Endometrial cancer, POLE ultramutated, microsatellite instability (MSI), Mismatch-Repair Deficiency, immunotherapy, checkpoint inhibitors.

BACKGROUND: Recent advances in molecular biology have enabled identification of microsatellite instability high (MSI-H), DNA polymerase epsilon mutation (POLE) as two of four endometrial carcinoma (EC) subtypes, plausibly amenable to treatment with immune checkpoint inhibitors (ICI).

OBTECTIVE: To perform a systematic review of all available evidence with ICI in MSI-H and POLE advanced EC subtypes to assess efficacy and tolerability.

MATERIAL AND METHODS: An electronic search of clinical trials with MSI-H and POLE advanced EC, published as a research article or in abstract form between 2010-2020, was performed. No language restrictions were applied. A predefined PROPERO-LIKE was designed in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. Population was defined as POLE, mismatch repair deficiency (MMRd), MSI-H advanced EC; intervention as ICI, anti-PD1, Anti PDL1, PD1/PDL1 axis, immunotherapy, clinical trial. Response rate (RR), clinical benefit rate (CBR) progression free survival (PFS) and overall survival (OS), where available, as well as adverse events were assessed.

RESULTS: The search identified 573 records. After screening phase and eligibility process, eleven phase I or II trials were included for final analysis, with 9 monotherapy trials (140 MSI-H patients in total, 1 POLE, 113 MSI-H/MMRd evaluable) and 2 in combination therapy trials (9 MSI-H/MMRd patients, 0 POLE)**.** A pooled analysis in monotherapy MMRd/MSI-H patients yielded 48% RR (10% complete response; 38% partial response, some long lasting), with 57% CBR. Lack of results on PFS or OS in most trials preclude a pooled analysis on survival. Adverse events were as expected.

CONCLUSION: ICI showed promising activity in MSI-H advanced EC, some of them long lasting. PFS and OS results are pending. Future investigation is needed to establish the role of these agents in monotherapy and/or in combination.

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BACKGROUND

Uterine cancer is the most common gynecologic cancer in developed countries, of which endometrial carcinomas account for >90% of cases (1). Most cases are detected at an early stage (2). With a median age at diagnosis of 60 years, and closely related to elevated body mass index, five-year survival rate is over 95% in those tumors confined to the uterus at diagnose (1), which they account for 85% of cases, plummeting to 17% in patients with distant metastatic disease (1).

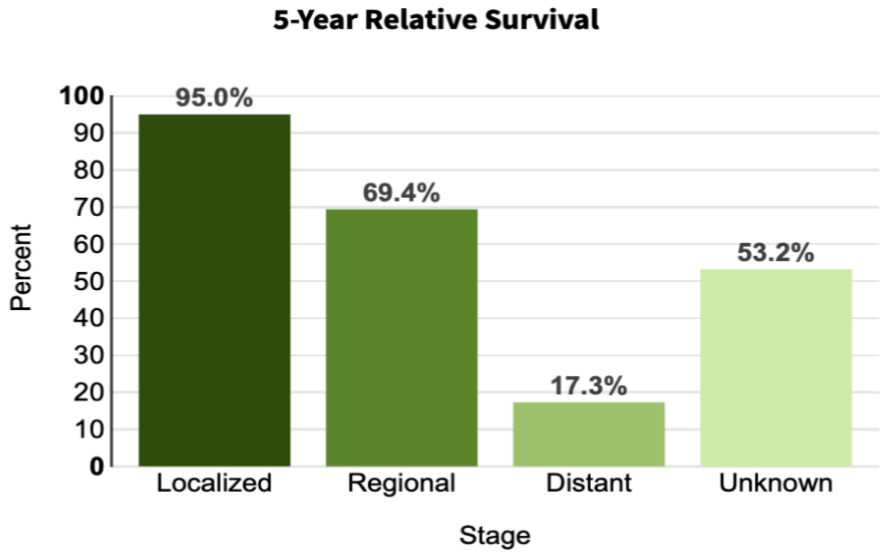

Figure 1. SEER 18 2010-2016, All Races, Female Summary Stage 2000

In 1983 a first endometrial carcinoma subtype classification, based on clinical and hormonal features, was proposed by Bokhman, distinguishing between type I endometrioid (well and poorly differentiated) and type II (including serous, clear cell, carcinosarcoma, dedifferentiated and undifferentiated subtypes) (3).

Recent advances in molecular biology, mainly in genome-wide analyses, have revealed a wide range of genomic alterations in endometrial carcinomas, providing valuable insight into the pathogenesis of these tumors. As thus, The Cancer Genome Atlas classification delineated in 2013 four subgroups groups of endometrial cancer (EC) as follows: microsatellite instability high (MSI-H), DNA polymerase epsilon (POLE), copy number high, and copy number low (4).

Carcinomas with a hotspot mutation of the catalytic unit DNA polymerase epsilon (POLE), the one in charge of nuclear DNA replication and repair, display an ultramutated sequence (5). This sequence is recognizable to the immune system, and confers a good prognosis, among high-risk early endometrial cancer (6)(7)(5)(8). Likewise, MSI high tumors are hypermutated due to their inability to repair replicational mutations related to alteration of MLH1, MSH2, MSH6, PMS2 genes, developing characteristic microsatellite repeats which make these tumors immunologically active and susceptible to treatment with immunotherapy (IT) (9)(10).

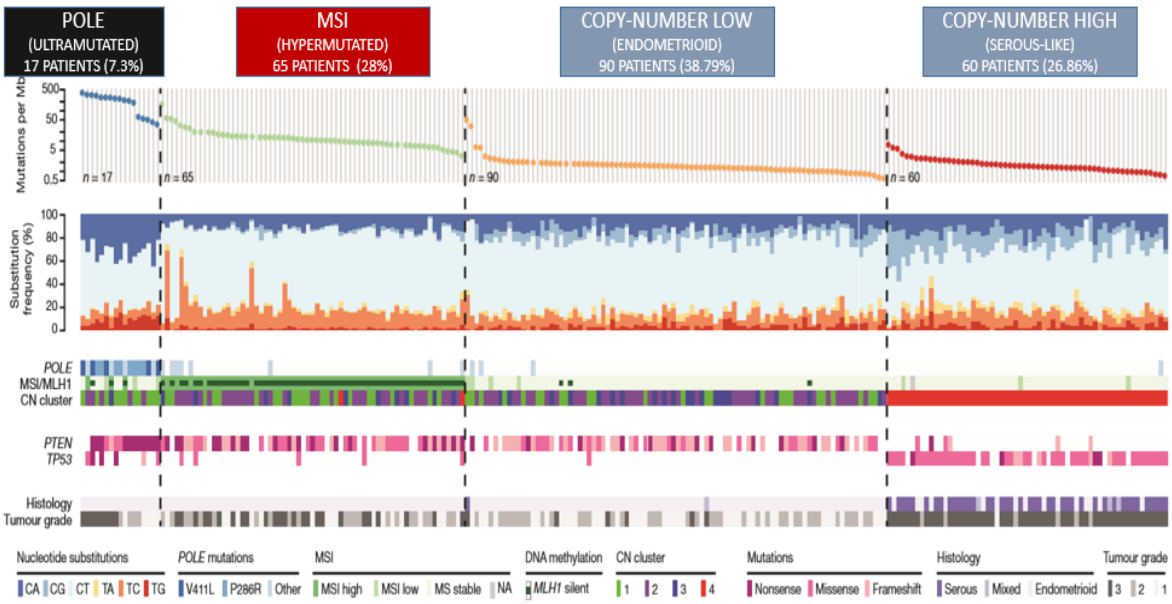

Figure 2. TCGA MOLECULAR CLASSIFICATION Kandoth C. *et al*. *Nature 2013:* 497, 67-73

Most endometrial cancers (EC) are detected at early stages and cured by local treatments, either surgery, radiotherapy or both. Upon recurrence, the prognostic is dismal, with no cure possible with current systemic treatments and short median survival time is expected (5). Even though the two higher mutational subtypes, (POLE)-ultra-mutated and microsatellite instability-hyper-mutated (MSI-H), usually convey a better prognosis, life expectancy is short upon recurrence. Previous studies have evidenced that MSI-H and POLE endometrial carcinoma exhibit a high mutational rate and neoantigen load, increased tumor infiltrating lymphocytes (TILs) as well as high expression of PDL1, rationale for a potential activity of checkpoint inhibitors (11) (12) (13).

Neoantigens are presented to inactive T-cells by antigen-presenting-cells (APC, such as dendritic cells or macrophages) through interaction of the major histocompatibility complex (MHC) and T-cell receptors, the so-called priming phase, i.e. primary signal for T-cell activation. To fully achieve lymphocyte activation, another costimulatory signal is required, through interaction between B7 on APCs and CD28 on T-cells (14). In order to preclude indefinite T-cell activation (which would otherwise implicate enhance autoimmunity), a co-inhibitory signal, known as Checkpoint 1, stops lymphocyte stimulus, once adequate activation is achieved. As such, CTLA-4 is produced in T-cells and transported to the cell surface in a proportional way to antigen stimulation; it binds to B7 with greater affinity than CD28, resulting in specific T-cell inactivation (checkpoint 1)(15).

Once activated, activated T-cells move to peripheral tissues to eradicate neoantigen-harboring cells, i.e. tumor cells, the so-called effector phase. Mission accomplished, activated T cells undergo apoptosis through programmed cell death receptor (PD-1) activation (Checkpoint 2, at peripheral tissue level). Tumor cells can express PDL-1 (PD-1 ligand) to prevent T-cell attack (15).

Immune checkpoint blockage through CTLA-4 (checkpoint 1 inhibitors) or PD1/PD1-L1 (checkpoint 2 inhibitors), by means of monoclonal antibodies, increase cytotoxic T-cell activity, resulting in both, increased tumor response and immune-related adverse events.

Pembrolizumab, a checkpoint 2, anti-PD1 inhibitor has been proved to have clinical utility in MSI tumors regardless tumor type, granting the first Food and Drug Administration (FDA) drug approval relying on molecular diagnosis alone, based on NCT0187651 trial (16). In endometrial cancer, with MSI high tumors prevalence being as high as 40%, according to The Cancer Genome Atlas data (only 2% in serous tumors), this indication could be of the upmost importance, even though acknowledging the fact that only two endometrial MSI High where included in the aforementioned trial (6).

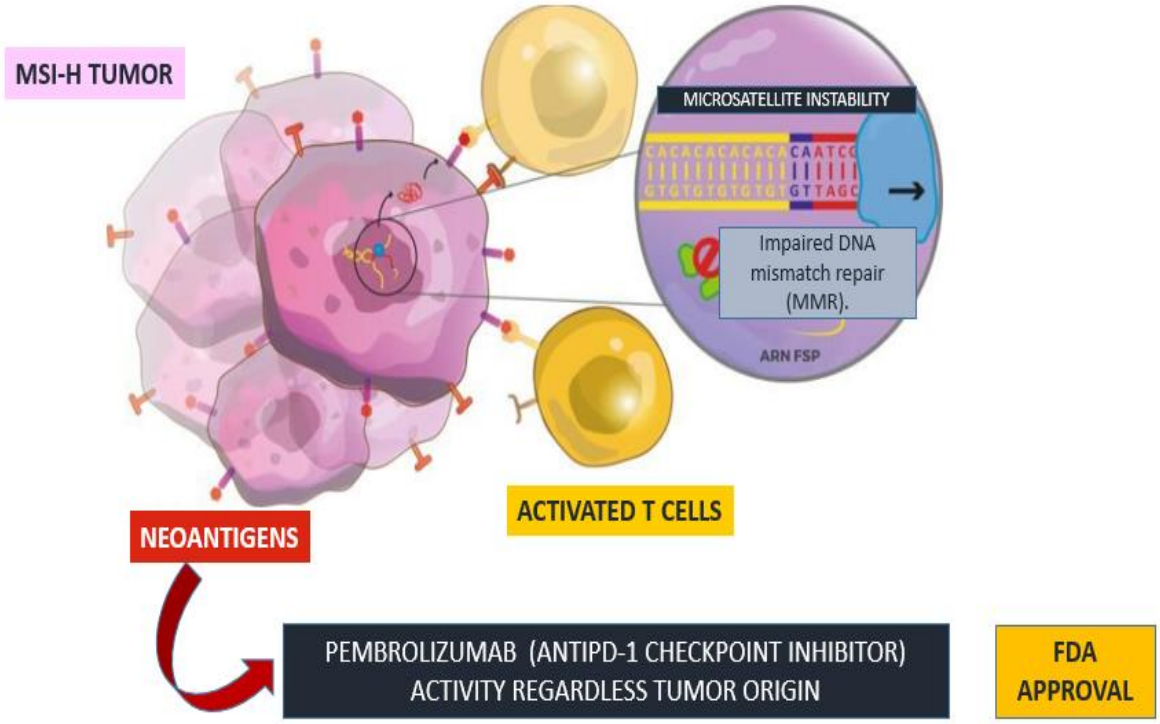

Figure 3. NEOANTIGEN RELEASE BY MSI-H TUMOR OF AGNOSTIC ORIGIN

OBJECTIVE

To perform a systematic review in order to identify and summarize all available evidence with immune checkpoints inhibitors in both mismatch deficient highly mutated and POLE-ultramutated advanced endometrial carcinoma, to assess efficacy and tolerability.

MATERIAL AND METHODS

Inclusion criteria were clinical trials with highly mutated and ultramutated endometrial carcinoma, published as a research article or in abstract form between 2010 and 2019. No language restrictions were applied (Table 1). A predefined protocol PROSPERO-like was designed and subsequently followed in accordance with the PRISMA guideline for systematic review (see protocol on Appendix 1). Population was defined as Pole-ultramutated, mismatch deficient, highly mutated or MSI-H endometrial carcinoma. Intervention was defined as checkpoint inhibitor, anti-PD1, Anti PDL1. PD1/PDL1 axis, nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, immunotherapy, in clinical trial. Pooled response rate (RR) and clinical benefit rate (CBR) were estimated as outcomes as were progression free survival (PFS) and overall survival (OS), duration of response (DOR) and time to response (TTR), where available as well as adverse events.

SEARCH STRATEGY

An comprehensive electronically search was performed in PubMed, Embase, Clinicaltrials.Gov, Web of Science for articles as well as ASCO and ESMO meeting database for abstracts reporting the use of immunotherapy in advanced/recurrent endometrial carcinoma (Table 1), using a combination of broad terms related to endometrial carcinoma and immunotherapy, checkpoint inhibitors, POLE*, MSI*, clinical trial. A thorough manual search in the reference section of the retrieved articles as well as in the appendix was also conducted.

Inclusion criteria were clinical trials trial with both subtypes (POLE and MSI) between January 2010 and March 2020. Trial selection was performed by author of the present graduation work with double check with the librarian, supervised by the cotutor. There was no restriction about the language used in the publications.

TRIAL SELECTION

Clinical trials were selected on the basis of advanced endometrial carcinoma focusing on or including POLE mutated and/or MSH-High subgroups, treated with immunotherapy directed to PD1 and PD-L1. Therefore, the terms applied in the search include checkpoint inhibitors, POLE, MSI-H, recurrent or advanced endometrial carcinoma, immunotherapy, clinical trial, PD1, PDL1, Pembrolizumab, Nivolumab, Atezolizumab, Avelumab, Durvalumab. No restriction on type or number previous line was applied. Ongoing studied with published results were included as well. Apart from monotherapy trials, combination therapies studies were included, although evaluated in a separate section. In addition, endometrial carcinomas whose molecular characteristics were mismatch repair deficient (MMRd) were considered as highly mutated (MSI-H) and finally included.

Table 1. Search criteria for the selection process

Type of study	Immunotherapy Clinical trial
Condition or domain being studied	Advanced/recurrent endometrial carcinoma
Participants/Population	Adult population (>18 years of age) included in population-based studies
Timeline criteria	Studies published between January 2010 and March 2020
Linguistic criteria	Any language

Exclusion criteria were: (i) patients with no advanced/recurrent endometrial carcinoma, (ii) clinical trials with no POLE or MSI subtype tumors reported, (iii) studies that matched different databases, (iv) completed trials with no published results, (v) ongoing trials with no published results, (vi) phase III trials, (vii) case reports, narrative reviews, editorials, news articles, commentaries or letters. In the event of multiple publications reporting the same trial, only the most recent data were considered.

DATA EXTRACTION

The studies retrieved during the search were screened for relevance. Those defined as being potentially eligible were fully evaluated to find out if they met the requirements for inclusion criteria. They were accepted or rejected based on the predefined inclusion and exclusion criteria.

For each included study the following information was extracted: number of patients enrolled, design, type of trial, age, stage, performance status, sample size, treatment type, type of immunotherapy delivered, other drugs if applied, median follow-up, response type according to RECIST/iRECIST criteria (17) (complete response, partial response, stable disease, progression), overall response rate (ORR, including partial response plus complete response), clinical benefit rate (complete response plus partial response plus long lasting stable disease), time to progression (TTR, defined as the time elapsed from the first cycle to response demonstration), duration of the response (DOR, defined as the time elapsed from the first response documentation of partial or complete response to progression, last follow-up or death,

whichever came first), progression free survival (PFS), defined as the time elapsed from the first cycle to progression, last follow-up or death, whichever came first, overall survival (OS), defined as the time elapsed from the inclusion in the study to death or last follow-up), and toxicity. Adverse Events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0.

RESULTS

A search on three major medical and scientific databases articles and oncology meeting abstracts yielded 573 records, through database searching. After identification, 502 were rejected, 481 based on title, and 21 for being case reports, narrative reviews, editorials or letters. Of the 71 remaining for screening, 47 were excluded either based on abstract (20), because of being an ongoing trial without efficacy results available (22), or for duplication (5). Twenty-five trials were assessed for eligibility, of which 14 were rejected because no on mutated or ultramutated endometrial subtype were included, leaving a total of 11 phase I or II trials on immune checkpoint inhibitors dealing with advanced endometrial carcinoma and reporting data on survival (overall or progression-free survival), tumor response, or adverse events, meeting the inclusion criteria and included in this systematic review for final analysis (Figure 4).

In 2015, Le and colleagues published a phase II study showing, for the first time, efficacy of immune checkpoint blockade with pembrolizumab in different treatment-refractory progressive cancer types with a mismatch-repair deficiency (MMRd), as compared with mismatch-repair proficient (MMRp) colorectal patients (16). Coprimary end points were the 20-week immune-related progression-free survival rate and the immune-related objective response rate. The MMR proficient group showed no response as compared with 40% objective response rate in the MMRd colorectal group (95% CI 12-74) and 71% (95% CI 29-96). Amongst MMRd patient non-colorectal cases (7 evaluable for response/9 recruited), 2 were endometrial carcinoma showing 1 complete response and 1 partial response by RECIST (Appendix 2 of the trial publication). Median time to response was 12 weeks in the latter group. Moreover, after a median follow up of 21 weeks, median PFS was 5.4 months, with median OS not reached in MMR deficient (MMRd) non colorectal cancer patients, two of which were endometrial cancer patients (2/7) (16).

Following those results, on May 23 2017, the Food and Drug Administration (FDA) granted accelerated approval to pembrolizumab for tumor-agnostic treatment, i.e. regardless of solid tumor tissue/site of origin, provided they are metastatic or unresectable, they express microsatellite instability-high (MSI-H) or DNA mismatch repair deficiency (MMRd), and they have progressed to at least one previous line and have no satisfactory treatment alternative [FDA home page].

Based on previously reported programmed death ligand 1 (PDL-1) positivity as a plausible predictor of response to immune checkpoint inhibitors in solid tumors (16), a multicohort phase Ib basket trial was launched (KEYNOTE-028) to evaluate safety and efficacy of pembrolizumab in advanced solid tumors with PDL1 positivity, the results from the advanced endometrial cancer cohort being released in 2017 (18). Skilled patients were treated with

intravenous pembrolizumab 10 mg/kg every 2 weeks for a maximum of 24 months. A small cohort of 24 advanced endometrial cancer patients was enrolled and analyzed (18).

Most of the patients had endometroid adenocarcinoma subtype (n=17), being the other histologies serous, adenocarcinoma other and carcinosarcoma. Regarding previous line treatment, 15 patients (62.5%) had received at least 2 previous lines of treatment and 2 patients had not prior treatment. Median follow up was 76.2 weeks. The ORR (partial + complete response rate, by RECIST 1.1) was 13% (95% Confidence Interval CI, 2.8% to 33.6%), with 3 patients (13%) achieving partial response. Three additional patients presented stable disease (13%) and the remaining 13 cases showed progressive disease as best response (56.5%, 95% CI). Median duration of stable disease was 24.6 weeks. Obtained results were promising, with a median PFS of 1.8 months at data cutoff, 19% at 6 months and 14.3% at 12 months (18).

Even though not predefined, subgroup analysis per microsatellite instability, stability and POLE mutation was retrospectively performed in responders. Of the three patients who obtained partial response, one was POLE+, the other one was non-MSI-H, the remainder being unknown MSI status. Of the 19 samples evaluated for TCGA molecular subtype, there was a unique case POLE+, which achieved a partial response, accounting for an ORR of 100% in this subgroup. It´s time to response was 8 weeks, sustained for over 14 months. The patient improved while on pembrolizumab.

Regarding adverse events, 54.2% of patients experienced some degree of treatment-related toxicity, with no grade 4 not treatment discontinuation observed. The most common adverse events observed were fatigue, pruritus, pyrexia and decrease appetite. Grade 3 TRAEs were observed in four patients (16.7%) (18).

Even though not predefined, subgroup analysis per microsatellite instability, stability and POLE mutation was performed in responders. Of the three patients who obtained partial response, one was POLE+, the other one MSI-Low and the remaining one had unknown MSI status. Of the 19 samples evaluated for TCGA molecular subtype, there was a unique case POLE+, which achieved a partial response, accounting for an ORR of 100% in this subgroup. It´s time to response was 8 weeks, sustained for over 14 months. The patient improved while on pembrolizumab.

Published preliminary results of a phase II clinical trial evaluating the clinical efficacy of pembrolizumab in patients with previously treated MMRd carcinomas, to a maximum of 4 prior regimens. The drug study dose was 10 mg/Kg, delivered every two weeks. The trial enrolled 9 patients with EC, all of them with endometrioid histology. The primary endpoint was response rate, assessed every 8 weeks. The median follow-up was 9.1 months (range 7 to 18 months) (19).

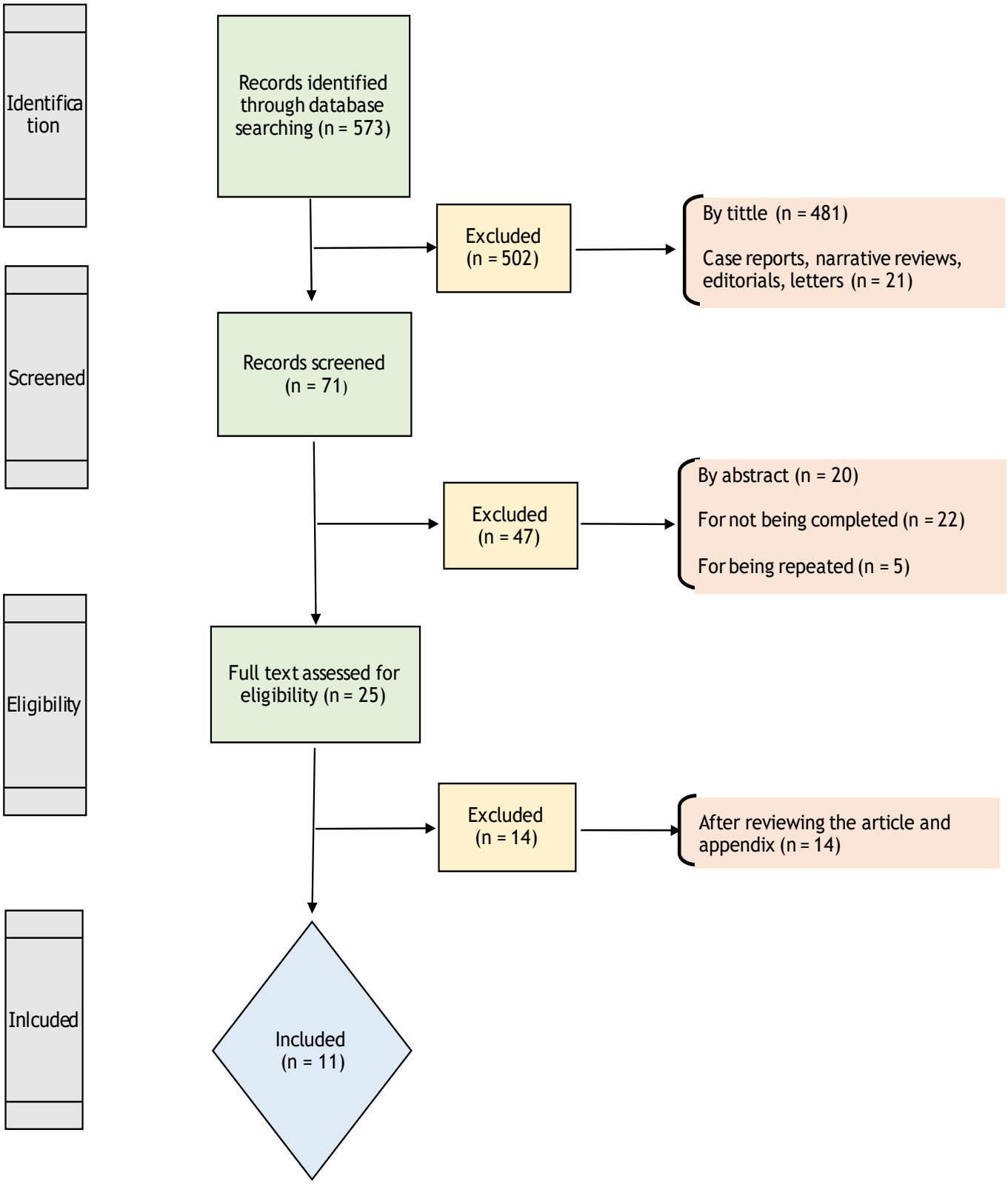

Figure 4. EVIDENCE SYNTHESIS FLOWCHART ACORDING TO PROSPERO-LIKE PROTOCOL (PRISMA GUIDELINES

The results posted in 2016 showed an objective response rate by iRECIST of 56% (95% CI, 21%-86%) with 4 partial responses and 1 complete response in EC (5/9). The patient developing CR had previously undergone 3 lines of treatment and 3 surgeries and had remained disease free for over 17 months. Three additional patients had stable disease for over 2 assessments, accounting for a clinical benefit rate/disease control rate of 88.9% (8/9). One patient developed a mixed response, with small volume increase in the liver and peritoneum. Twelve-month OS was 89% and the median OS had not been reached. (19). There were no toxicities greater that grade 3 at date cutoff (19).

A phase II clinical trial of Nivolumab in Japanese patients with uterine cervical cancer, uterine corpus cancer or soft tissue sarcoma demonstrated that Nivolumab has clinical activity with acceptable toxicity in those patients. A flat dose of 240mg of Nivolumab was given at 2-week intervals in 23 patients with uterine corpus cancer, most of them having endometrioid carcinoma (15 out of 23), being the remaining histologies serous (5/23), carcinosarcoma (2/23), and undifferentiated (1/23). All corpus cancer had received prior treatment, 60% of which with two or more lines. Median duration of the treatment was 2.4 months in corpus cancer, and the median duration of the follow up was 6.8 months in this cohort. Median ORR (RECIST 1.1) was 23% (80% CI: 11%-38%), median OS, 8.7 months (80% CI 7.1-not estimable) and median PFS, 3.4 months (80% CI 2.0-5.4 months) in the corpus cancer cohort (20).

In this study, PD-L1 expression and microsatellite-instability (MSI) status were assessed as plausible efficacy biomarkers. As such, MSI status was ascertained in hindsight in eight patients with corpus cancer, of which 2 were proved to be MSI-H, one with PDL1 < 1%, the other one with PDL-1 expression > 1%. Both of them responded, accounting for an ORR in MSH-I of 100% (2 out of 2; 80% CI: (32%-100%) as compared as no response in MSS patients (0/6, 0%). OS at 6 months was 100% and median PFS was considered non estimable (20). Both MSI-H patients obtained partial responses.

Sixty-one percent of patients experimented any treatment-related AEs, and four patients were grade 3-4. There were not any grade 5 AE. The most common treatment-related adverse events in the corpus cancer cohort was pruritus, hypothyroidism, increased AST, diarrhea and increased lipase (20).

Azad and colleagues reported the Z1D subprotocol of the screening master protocol NCI-MATCH (EAY131), an open-label, single-arm, a phase II trial basket trial recruiting in 42 relapsed/refractory MMRd/MSI-H, non-colorectal cancer patients, treated with Nivolumab, 13 out of which were endometrial cancer of varying histologies. Nivolumab was administered intravenously 3 mg/Kg every two weeks and 480 mg every 4 weeks after cycle 4. Median follow up was 17.3 months (21).

There were 7 patients with endometrioid endometrial adenocarcinoma type and 6 additional cases with adenocarcinoma variants, for a total of 13 patients, 11 of which evaluable for response. The primary endpoint was objective response rate (ORR) per RECIST 1.1, with secondary endpoints being PFS6, time to progression, evaluation of predictive biomarkers, and toxicity.

The ORR was 36% (90% CI, 23,5%-49,5%). In this heavily treated population study, with 74% of patients having two or more previous lines, median PFS was 6.3 months, and

median OS, 17.3 months. Regarding the endometrial cancer cohort the following results were obtained: 4 partial responses and 2 complete responses, accounting for an ORR of 55% (6/11, CI 90%). (21). Four patients initially progressed, and one additional patient had stable disease. Two patients had ongoing partial responses at 20 months. One CR was ongoing at 18 months (21).

Treatment related adverse events (NCI adverse events criteria 4.0) were generally moderate. The most common adverse events ere fatigue, anemia, rash and hypoalbuminemia. There were no grade 5 toxicities. There were two grade 4 toxicities in three patients, 2 sepsis and 1 pneumonitis.

Liu et al reported a cohort of 27 advanced gynecologic patients enrolled in a phase Ia clinical trial, treated with 1200 mg or 15 mg/m2 of atezolizumab in monotherapy, given intravenously every 3 weeks for 16 cycles or 1 year of treatment, unless progression or unacceptable toxicity. Primary endpoint was safety and tolerability of atezolizumab. Secondary endpoints were ORR, DOR and PFS. Exploratory objectives included preliminary assessment of potential biomarkers (PDL-1, tumor mutational burden (TMB), MSI status) and OS. Microsatellite instability (MSI) was assessed by means of the Foundation One NGS panel, yielding in the uterine cohort 1 MSI-H (1/15), 12 MSS (12/15) and 2 MSI unknown because of lack of tissue (22).

The duration of the treatment in the study was 4.1 months. All but one uterine patient had received prior chemotherapy, 8 of them treated with two or more lines (53.3%), and 10 cases (66.7%) had prior radiotherapy. On the whole, the best ORR was 13,3 % (95% CI; 1.7, 40.5) per RECIST 1.1. Two patients obtained partial response, with no confirmed complete response. Of the two responding patients, one had unknown MSI status, endometrioid subtype, unknown TMB (tumor mutational burden), and expressed 70% PDL-1 positivity. The remainder responding case was the only MSI-High case identified by NGS in the entire cohort, showed 10% PDL-1 positivity, harbored ATM mutation, had endometrioid subtype and high TMB (221 mut/Mb), and achieved a clinical complete response, although defined as unconfirmed complete response because of non-target nodal lesions, i.e. partial response per RECIST 1.1. This figure accounts for an ORR of 100% among MSI-H patients (95% CI). Duration of response in the 2 responders in the uterine cohort was 7.3 and 16.6+ months (22).

In the uterine cohort, the median follow-up of survival was 20.4 months (range: 0.6-38.2 months), median PFS was 1.7 months (95% CI: 1.3-4.0), (range, 0.6-11+ months); and the median OS was 9.6 months (95% CI: 6.8-13.8). Among C2-C3 PDL-1 positive patients (the two responders belong to this group along with 2 additional non responding cases), median PFS was 4.2 months (5.5-38.2+ months), and median OS was 38.2 months (5.5-38.2+ months) (22).

No new safety signals were identified in this study. The adverse effects were mostly grade 1 or 2, with no grade 4 or 5 drug-related AE identified. In the corpus cancer cohort, the most common adverse effects were diarrhea and fatigue in three and two patients respectively. Grade 3 diarrhea, colitis and rash occurred in three patients respectively (22).

Avelumab was also tested in an ongoing phase II trial published by Konstantinopoulos et al. This study evaluated avelumab in two cohorts of endometrial cancer: MMRd (by immunochemistry)/POLE (polymerase epsilon, harbouring documented mutation in the

exonuclease domain of POLE) and MMR proficient patients (MMRp). Avelumab 10 mg/Kg IV was given every two weeks until inacceptable toxicity or progression. Co-primary outcomes in this trial were objective response rate (ORR) and PFS at 6 months (PFS6). Secondary outcomes were duration of PFS, duration of OS, number of participants with TRAEs and immune related response rate (23).

Of the 33 patients enrolled, 16 were MMRp, cohort closed after the first stage because of futility (only one response). The remaining 17 patients were MMRd (no POLE case was identified), two of whom did not initiate protocol treatment and were subsequently excluded for analysis.

All patients in the MMRd had received at least one previous chemotherapy line, with 40% having been treated with 3 or more lines. The ORR rate in this cohort was 4 out of 12 patients, 33% (95% CI), with 1 complete response (ongoing at 22 months+) and 3 partial responses (2 of them ongoing at 22 and 21 months). Four patients had stable disease as best response, two of them lasting over 6 months -one for 18 months and the other one for 12 months and still ongoing), accounting por a clinical benefit rate (CR + PR + long lasting SD) of 40%, described as PFS6 in this study. Four patients had progression disease. All patient had endometrial subtype (23). TMB and TILs did not correlate with response to avelumab in this trial. Of note, three of nonresponding MMRd patients exhibited both JAK1 or B2M mutations, which have been associated to immunotherapy resistance (24).

PFS6 was 40.0% (95% CI, 16.3% to 66.7%) in the MMRd cohort. Interestingly enough, 5 of 6 (83,3%) PFS6 responses were seen in patients with more than three lines of prior therapy and PD-L1 negative tumors, a subgroup of otherwise especially grim prognosis.

Twenty-two patients (71%) experienced any treatment-related adverse event of any grade, (including the two cohorts), 6 of them (19%) were grade 3 toxicities. There were no grade 4 and 5 toxicities (23).

A phase II clinical trial of Durvalumab 1500 mg IV Q4W in advanced endometrial carcinoma (PHAEDRA trial), according to mismatch repair (MMR) status, was reported at 2019 ASCO meeting. The primary endpoint was objective response rate (ORR) by iRECIST, and secondary objective were disease control rate at 16 weeks (DCR16w) and immune related adverse events (25).

Of the 71 patients recruited from Feb 2017 to Sep 2018, 35 had MMRd (deficient MMR) and 36 MMRp (proficient MMR). Durvalumab was the first, second and subsequent line of non-hormonal therapy in 15, 14 and 6 patients with MMRd, respectively. Treatment response rate by treatment libre were, 40%, 43% and 33% at first, second and third or subsequent lines, respectively.

In the MMRd cohort, median follow up were 8.3 months. In this subgroup, 94% were endometrioid type and there was no serous subtype. The ORR was 40 % (14/35, 95% CI 26-56) with 10 partial responses and 4 complete responses. Additionally, 7 stable disease for lasting 16 weeks or longer were observed, accounting for a DCR16w of 60% (21/35, 95% CI 44-74) (25).

Treatment related adverse event occurred in 14 patients (40%), consisting of hyperthyroidism in 6, hypothyroidism in 6, pneumonitis in 1 and hepatitis in 1, although no grade has been provided in the abstract form (25).

Preliminary safety and efficacy results from PART B GARNET TRIAL, ongoing phase I-II clinical trial with TSR-042 (dostarlimab), an anti PD-1 monoclonal antibody, were first presented at 2018 ESMO annual meeting and further updated at the 50th Annual Meeting of the Society of Gynaecologic Oncology, on March 2019. Study population was recurrent or advanced, with 2 cohorts MSI-H and MSS endometrial cancer who progressed on or after cisplatin therapy, limiting to ≤2 prior lines of treatment for recurrent or advanced disease. Patients received the TSR-042: 500 mg every three weeks for the first 4 cycles and 1000 mg every six weeks thereafter (26).

In the phase I trial, primary outcomes were safety and tolerability parameters. Secondary outcomes were ORR, DOR and disease control rate, i.e. patients achieving CR, PR or SD by RECIST 1.1, among others. Immune related objective response rate (IrORR) by irRECIST will be evaluated too (26). The primary endpoints in the endometrial expansion cohorts (Part 2B) were ORR and duration of response (DOR) in MSI-H (n = 65) and MSS (n = 125) patients, as well as safety and tolerability (26) (27).

A total of 110 EC patients received at least 1 dose. Overall, 79 patients had at least 1 tumor assessment and 15 patients discontinued treatment prior to week 12. Roughly 50% of patients had received at least 2 prior lines of chemotherapy. Remarkably, only 56.7% of patients had stage IV disease (27)

ORR (including confirmed and unconfirmed responses per irRECIST) was 29.6% (95% CI, 21.8-38.4) in the assessed population, 48.8% (95% CI, 32.9-64.9) in the MSI-H cohort, and 20.3% (95% CI, 12.0-30.8) in the MSS subgroup. Six patients (2 MSI-H and 4 MSS) achieved a complete response (4.8%), and 31 patients (18 MSI-H and 12 MSS) experienced partial responses (24.8%) in the overall population. The disease control rate was 52.8% in the assessed population; being 63.4% in MSI-H patients (95% CI, 46.9-77.9) and 46.8% in MSS patients (95% CI, 35.5-58.4) (26)(27).

The disease control rate was 52.8% on the whole, 63.4% in MSI-H patients (95% CI, 46.9-77.9) and 46.8% in MSS patients (95% CI, 35.5-58.4). At the time of data cut off, responses were ongoing 83.8% of the entire population, including 85.0% of the MSI-H cohort, 81.3% of the MSS cohort, and 100% in unknown MSI status patients (n = 5) (27).

Median DOR has not been reached after a median follow-up of 10 months. Notably, 89% of patients remained on treatment for >6 months and 49% for >1 year. In addition, 84% of responders are still on treatment.

In terms of safety, sixty-eight EC patients (68%) had at least 1 treatment-related adverse. Grade ≥3 drug related AE were reported in 13 patients (11.8%), the most common grade ≥3 being aspartate 26 aminotransferase increase (2.7%) (26).

Table 2 summarizes the obtained results in the eight monotherapy studies included in this systematic review, showing clinical trial reference, trial type, primary endpoint, secondary

endpoint, overall population type, MSI-H/POLE cohort, and results, above all ORR, and PFS and OS, when available.

Table 3 displays the retrieved results on efficacy in terms of response within MSI-H/MMRd EC population included in the 11 trials phase I/II prospective trials with monotherapy immune checkpoint inhibitors resulting in an overall response rate (ORR, Complete response + Partial response, by RECIST 1.1) of 48% in MSI-H/MMRd population. AntipPD-1 therapy resulted in an overall response rate of 53.85% (35/65) in MSI-H/ MMRd cohort. AntiPDL1 therapy resulted in an overall response rate of 39.6% (19/48).

With a total study population of 141 patients (140 MSI-H/MMRd and 1 POLE), and 113 MSI-H assessed for response per RECIST 1.1, a pooled analyses was performed for response rate (Figure 4) as well as for clinical benefit rate (Figure 5) in MSI-H endometrial cancer included in the 11 aforementioned checkpoint inhibitors monotherapy trials.

COMBINATION THERAPIES

Makker et al. presented at 2018 ASCO annual meeting the results of a multicentre, open-label, single-arm, phase I/II trial (NCT02501096) in patients with advanced metastatic endometrial cancer (with a maximum of 2 previous lines in the phase II part), irrespective of microsatellite instability (MSI) or mismatch repair (MMR) status, treated with pembrolizumab in combination with lenvatinib, a multikinase inhibitor with antiangiogenic activity. Pembrolizumab 200 mg was administered intravenously every 3 weeks, and 20 mg oral lenvatinib daily. The results were later published in The Lancet Oncology in 2019 (28).

The primary outcome was the objective response rate at week 24 according to irRECIST. Secondary endpoints were OS, DOR and PFS assessed by investigators.

The median follow-up time was 13.3 months. The study enrolled 54 patients, the most common subtypes were endometroid and serous carcinoma. 53% had received two o more treatment lines (43% and 13%, respectively). Regarding microsatellite status, 4 patients (8%) were MSI-H, 45 cases (85%) were MSS and 4 (8%), unknown. (28)

After evaluation, 53 patients were assessed, 21 patients responded, accounting for an ORR 39.6% (CI 95%: 26.5-54.0) at week 24, including 1 complete response and 20 partial responses assessed by investigators. After independent review, 3 complete responses (5.7%) and 22 partial response (41.5%) were identified ORR (47.5%). Twenty-two patients had stable disease (35.8%), 5 progressed (9.4%) and 4 (7.5%) were unknown/not assessable

Table 2. Clinical trial including MSH-I/MMRd patients treated with checkpoint inhibitors 2 monotherapy

Trial, references	Trial type	Agent	Primary endpoint	Secondary endpoint	Overall population type	MSI-H/POLE EC	Results
Le et al	Phase II, MMR status stratification. 3 cohorts: A: colon MMRp; B: colon MMRd C: non-colon MMRd	Pembrolizumab	IrPFS, IrORR	OS, ORR, DCR	Colon MMR stratification and non- colon MMRd (2 EC)	2 MSI-H	ORR: 100%
Otte et al/Keynote 0-28	Multicohort phase Ib basket trial	Pembrolizumab	ORR	PFS, OS, DOR	PDL-1 positive advanced solid tumors (24 EC)	1 POLE (Retrospectively assessed in responders, 3/23)	ORR;100% (CI 95%), PFS 14 months +
Fader et al.	Multicohort phase II basket trial	Pembrolizumab	ORR	NA	MMRd solid tumors	9 MMRd	ORR: 56 % (CI 95%), OS at 12 months: 89%, OS NR
Tamura et al.	Multicohort phase II basket trial	Nivolumab	ORR	PFS, OS, DOR	Uterine corpus cancer, cervical cancer, STS	2 MSI-H Retrospectively assessed in 8/23 (2MSI-H; 6MSS)	ORR 100% (CI 80%); OS6: 100 %
Azad et al. Z1D subprotocol of NCI-MATCH (EAY131)	Multicohort phase II basket trial	Nivolumab	ORR	PFS6, time to progression, toxicity	MMRd non-colorectal cancer (13 EC)	13 MMRd 11 MMRd evaluable	ORR: 45% (CI 90%),
F. Liu et al.	Multicohort phase Ia basket trial	Atezolizumab	Safety and tolerability	ORR, PFS, DOR. Exploratory: OS, Biomarkers	Solid or hematologic malignancies	1 MSI-H Biomarkers: PDL-1, MSI status, TMB	ORR 100% (CI 95%); OS 9.5 months (CI 95%),
Konstantinopoulos et al.	Phase II umbrella trial	Avelumab	PFS6 DOR	PFS, OS TRAEs, IrORR	EC 2 COHORTS MMRd/POLE-MMRp	15 MMRd 0 POLE	ORR: 26.7 % (CI 95%); CBR: 40% PFS6: 40% (CI 95%)
Antill et al. PHAEDRA trial	Phase II umbrella trial	Durvalumab	ORR IRECIST	DCR16w IrAE	MMRd or MMRp EC	35 MMRd	ORR: 40% (CI 95%), DCR16w: 60% (CI 95%)
Oaknin et al.	Multicohort phase I-II basket trial	TSR-042 (Dostarlimab)	ORR, DOR (Part 2B)	ORR, DOR, disease control rate, IrORR	Solid tumors Part 2B: EC Cohort A1: MMRd/MSI-H Cohort A2: MMRp/MSS	65 MSI-H	ORR: 48.8% (CI 95%),

CBR: clinical benefit rate; DCR16w: Disease control rate at 16 weeks; Duration of response; EC: endometrial cancer; IrAE: immune related adverse event; IrORR: Immune related objective response rate; IrPFS: Immune related progression free survival MMRd: Mismatch repair deficiency; MMRp: Mismatch repair proficient; MSI-H: Microsatellite instability high; NR: not reached; DOR: ORR: Objective response rate; OS: Overall survival; OS6: Overall survival at 6 months; PFS 6: PFS at 6 months; PFS: Progression free survival; STS: Soft tissue sarcoma; TRAEs: Treatment related adverse events.

Table 3. POOLED ANALYSIS IN MSI-H EC WITH CHECKPOINT INHIBITORS IN THE ASSESSED POPULATION

Trial, references	Agent	MSI-H/ POLE cohort	ORR	CR	PR	SD	LG	PD	CBR
Le et al	Pembrolizumab	2 MSI-H	100% (2/2)	1	1	-	-	-	
Keynote 028	Pembrolizumab*	1 POLE	100% (1/1)	-	1	-	-	-	
Fader et al	Pembrolizumab*	9 MMRd	56 % (5/9)	1	4	3	(3)	1	
Tamura et al	Nivolumab*	2 MSI-H	100% (2/2)	-	2	-	-	-	
Azad et al	Nivolumab*	11 MMRd	55% (6/11)	2	4	1	-	4	
F. Liu et al	Atezolizumab ‡	1 MSI-H	100% (1/1)	-	1	-	-	-	
Konstantinopoulos et al	Avelumab ‡	15 MMRd (12 evaluated)	33% (4/12)	1	3	4	(2)	4	
Antill et al	Durvalumab ‡	35 MMRd	40% (14/35)	4	10	7	(7)	14	
Oaknin et al	TSR-042*	65 MMRd; (41 evaluated)	48.8% (20/41)	2	18	6	-	15	
Total		1 POLE 140 MSI-H/MMRd (113 with results available)	ORR MSI-H: 54/113: 47.79%	11 (9.73%)	43 (38.05%)	22 (19,47%)	(12) (10,62%)	38 (33.63%)	62/113 (58.4%)

*: Anti-PD1 therapy; ‡: Anti PD-L1 therapy; CBR: clinical benefit rate (CR+PR + LG); CR: Complete response; LG: long lasting stable disease; MMRd: Mismatch repair deficiency; MSI-H: Microsatellite instability high; ORR: Objective response rate; PD: Progression disease; PR: Partial response; SD: Stable disease.

Among the 4 MSI-H patients, 3 had stable disease, 2 of them ongoing at 30 and 90 weeks, and one progressed, accounting for an ORR of 75% (28).

Median progression-free survival was 7.4 months (95%, CI: 5.0-Not estimable). DOR of at least 6 months was present in 11 patients 79.3 % (95% CI, 48.5-92.9), and 8 patients maintained response at 12 months, 79.3 % (95% CI) by independent review.

As far as treatment related adverse events, are concerned, fifty patients (94%) experienced some grade of AE, with 36 cases presenting grade 3 treatment-related adverse events (68%) and no grade 4 were registered. Five patients (9%) discontinued the study because of TRAEs, 2 because of grade 3 acute renal failure (one of them associated with grade 2 ischemic colitis), one grade 3 hypertransaminasemia and 1 due to the only grade 5 toxicity in the study, an intracranial hemorrhage damned related to treatment (28).

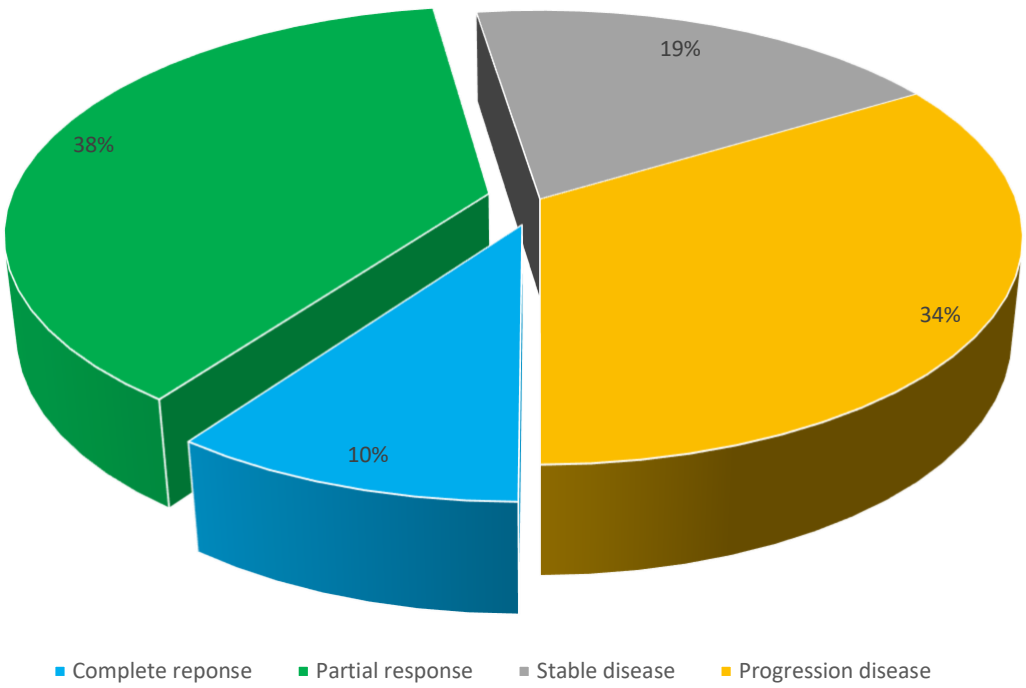

Figure 4. POOLED ANALYSIS OF RESPONSE RATE IN MSI-H EC WITH CHECKPOINT INHIBITORS-2 MONOTHERAPY

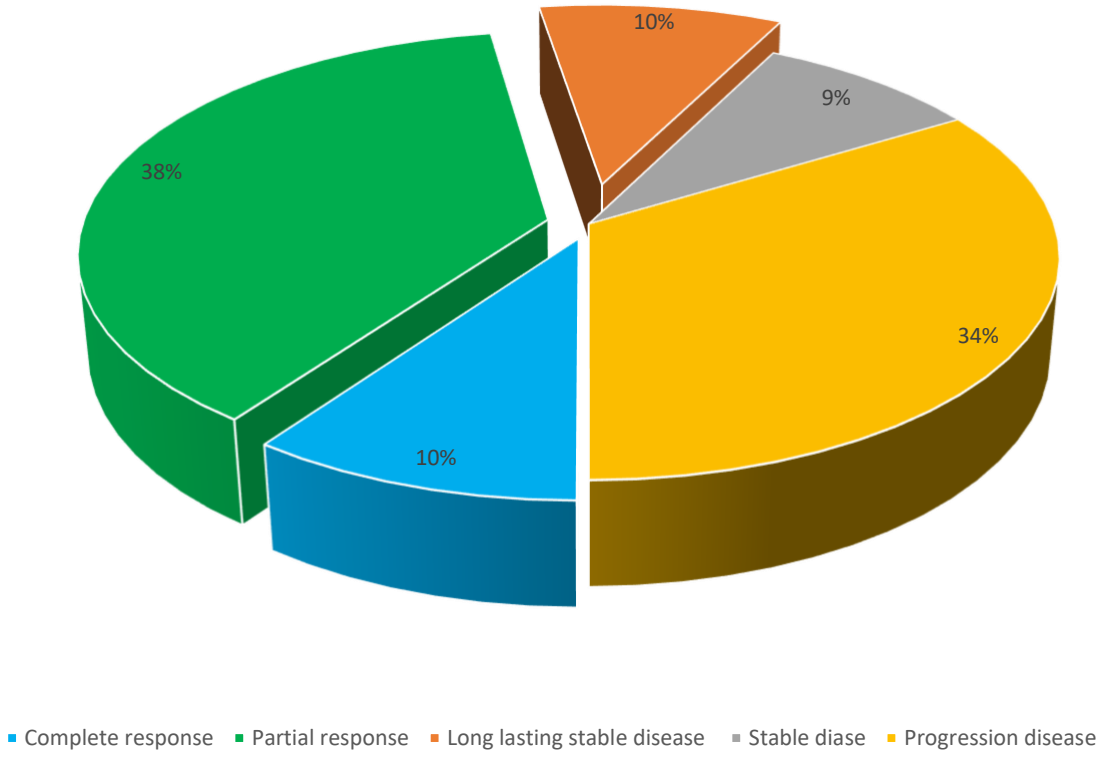

Figure 5. POOLED ANALYSIS OF CLINICAL BENEFIT RATE IN MSI-H EC WITH CHECKPOINT INHIBITORS-2 MONOTHERAPY

Overall, the most frequent adverse events were hypertension (58%), fatigue (55%), diarrhea (51%), and hypothyroidism (47%) (28).

Rubinstein et al presented preliminary results of a randomized phase II trial of Durvalumab (D), a PDL1 inhibitor, with or without Tremelimumab (T), a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor in advanced endometrial carcinoma and endometrial carcinosarcoma (ClinicalTrials.gov number, NCT03277482) at 2019 ASCO annual meeting. Patients were randomly assigned to receive D 1500 mg intravenously (IV) every 4 weeks in monotherapy or in association with T 75 mg IV every 4 weeks for 4 cycles, followed by D1500 mg intravenously every 4 weeks (29). The primary outcome was objective response rate (ORR) by RECIST 1.1.

Fifty-six patients (28 per arm) were enrolled at planned interim analysis, with two patients excluded per evaluation (one on each arm) due to early death. There were 15 patients with endometrioid histology, 15 with carcinosarcoma, 14 with serous subtype and 12 patients with another histology (29). Most tumors were MSS (48 patients, 86%). There were 5 MSI-H cases (9%), and the remaining 3 (5%) were unknown.

In the monotherapy group, 3 patients obtained partial response (2 MSS, 1 MSI-H) and 1 patient had a complete response (MSS), with an ORR of 14.8% (CI: 6,6-100%). The median PFS in the monotherapy arm was 7.6 weeks and PFS at 24 weeks 13.3% (CI 6.1-100%). Median duration of response (DOR) was 16 weeks. (29)

In the combination treatment arm, 2 patients obtained CR (1: MSI-H, 1: MSS) and 1 achieved PR (MSS). The ORR was 11.1% (CI: 4.2-100%). Median PFS was 8.1 weeks, and PFS at 24 weeks was 18.5% (CI 10.1-100%). DOR was 8 weeks.

Thus, of the 5 MSI-H patients, one obtained a partial response (1/5, 20%) and another achieved a complete response (1/5, 20%), accounting for an ORR in the MSI-H population of 40% (2/5).

Grade 3 and 4 TRAEs were more common in the combination arm than in the monotherapy arm, namely 7% vs 32% and 4% vs 11%, respectively. Two patients discontinued treatment due to a TRAE. Most common AEs related to treatment were diarrhea (20%) fatigue (23%), nausea (14%), pruritis (11%) and vomiting (13%) (29)

Table 4. **Clinical trial including MSH-I/MMRd patients treated with combination therapies**

Trial, references	Trial type	Agent	Primary endpoint	Overall population type	MSI-H/POLE EC	Results
Makker et al	Multicohort phase I/II basket trial	Pembrolizumab + Lenvatinib	ORR	Advanced EC	45 MSS, 4 MSI-H	75% (CI 95%), ORR
Rubinstein et al	Phase II umbrella trial	Durvalumab + Tremelimumab	ORR	Persistent or recurrent EC	48 MSS, 5 MSI-H	40% (CI 90%), ORR

CI: confidence interval; EC: endometrial carcinoma; MSI-H: Microsatellite instability high; MSS: Microsatellite stable; ORR: objective response rate;

SIDE EFFECTS ASSOCITED WITH IMMUNE CHECKPOINT INHIBITOR THERAPY

Immune checkpoint blockade has shown good benefit in the treatment of many types of cancer. It works blocking intrinsic down-regulators of immunity like CTLA-4 or programmed cell death (PD-1) or its ligand (PDL-1). These drugs increase the activity of the immune system. Immune checkpoint blockade can produce inflammatory side effects, witch of them are usually denominated immune-related adverse events. This side effects can occur in any organ system but gastrointestinal tract, skin, endocrine glands and liver are most commonly involved. (30). Although it is less frequent, cardiovascular system, central nervous system, pulmonary and hematologic systems can also be affected (30).

Immune checkpoint inhibitors are not directed only to tumor-specific T cell, they can also activate non-tumor-specific responses because of the antigens expressed on non-tumor tissue. It would be an undesirable response. IrAE can occur in a variety of organs.

Even though the clinical benefits of immune checkpoint inhibitor therapy, intolerable adverse effects can happen during the treatment. In single agent trials, the incidence of any grade adverse event is described to range from 15 to 90%. The rate of severe adverse events demanding withdrawal of treatment is 0.5-13%. (31).

From all studies included in this systematic review, toxicities associated with each treatment have been extracted, as specified in the protocol. Eight single agent trials have been included in this systematic review (two studies of pembrolizumab, two of nivolumab, one of atezolizumab, one of durvalumab and an anti-PD-1 named TSR-042).

Figures 6-11 show most common side effects and grades reported in the different trials grouped by agents, when available. Each diagram shows the most frequent adverse effects, as well the most severe, reported in the trials and grouped by drug.

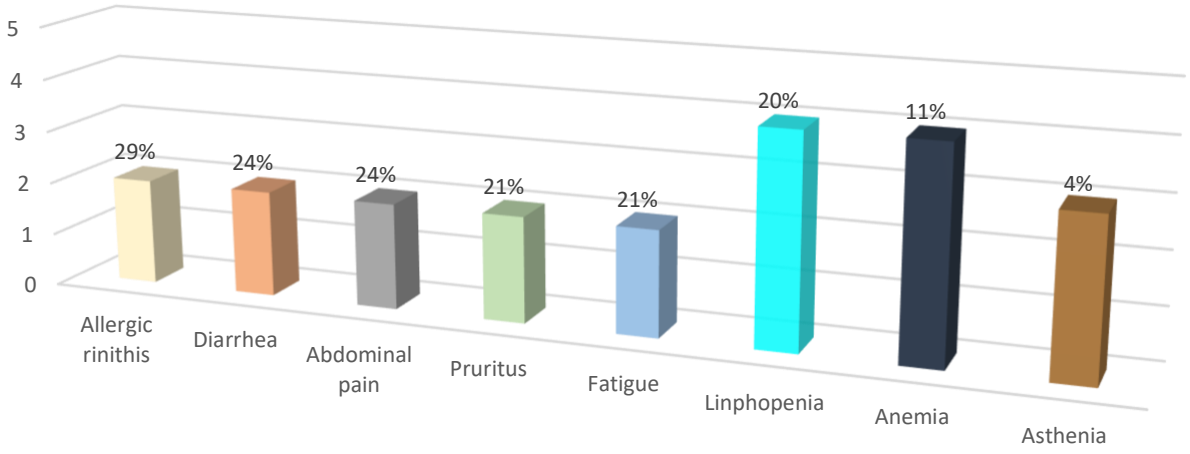

Figure 6. Side effects by frequency and grades, reported in pembrolizumab trials

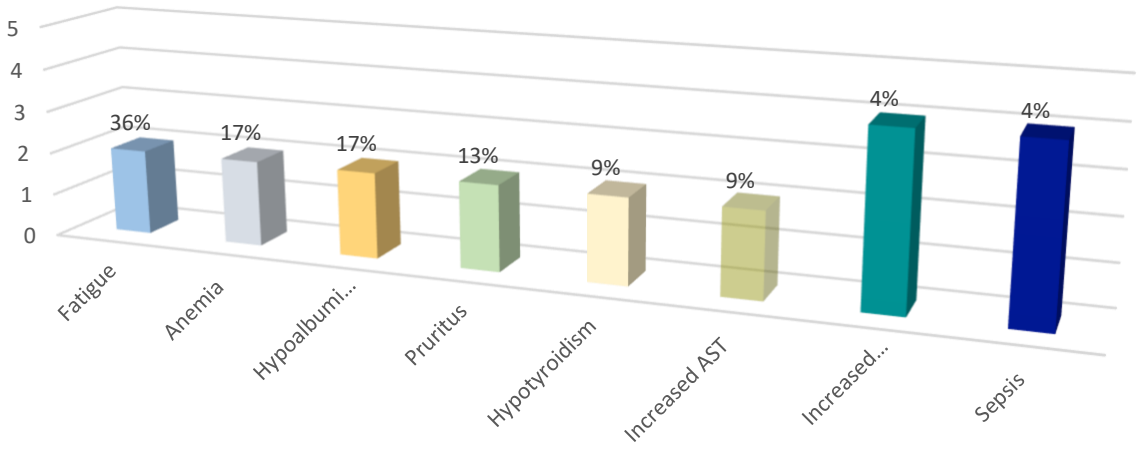

Figure 7. Side effects by frequency and grades, reported in nivolumab trials

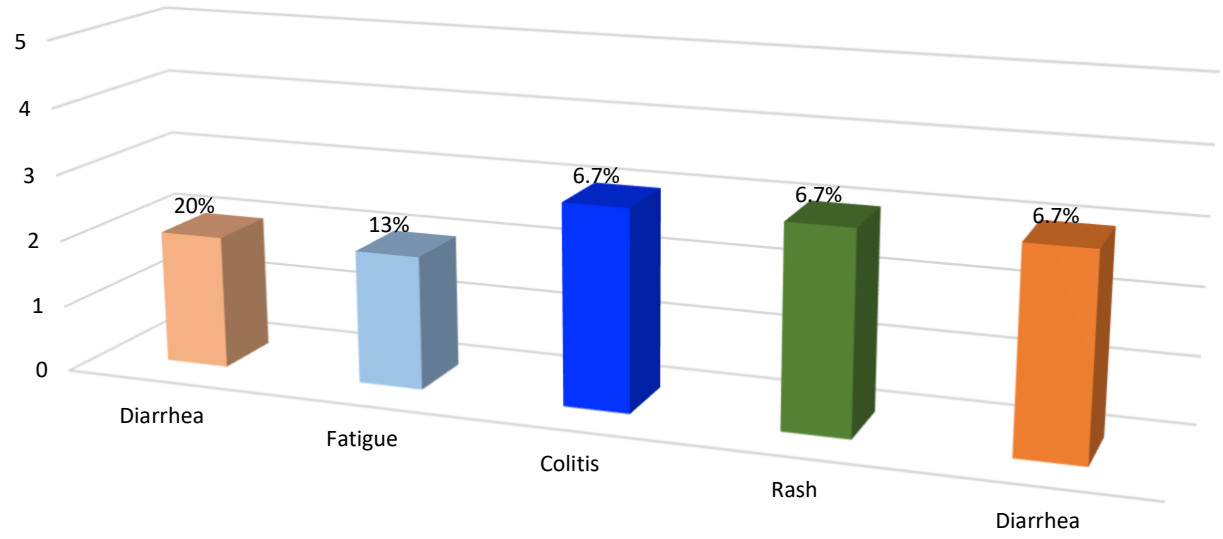

Figure 8. Side effects by frequency and grades, reported in atezolizumab trial.

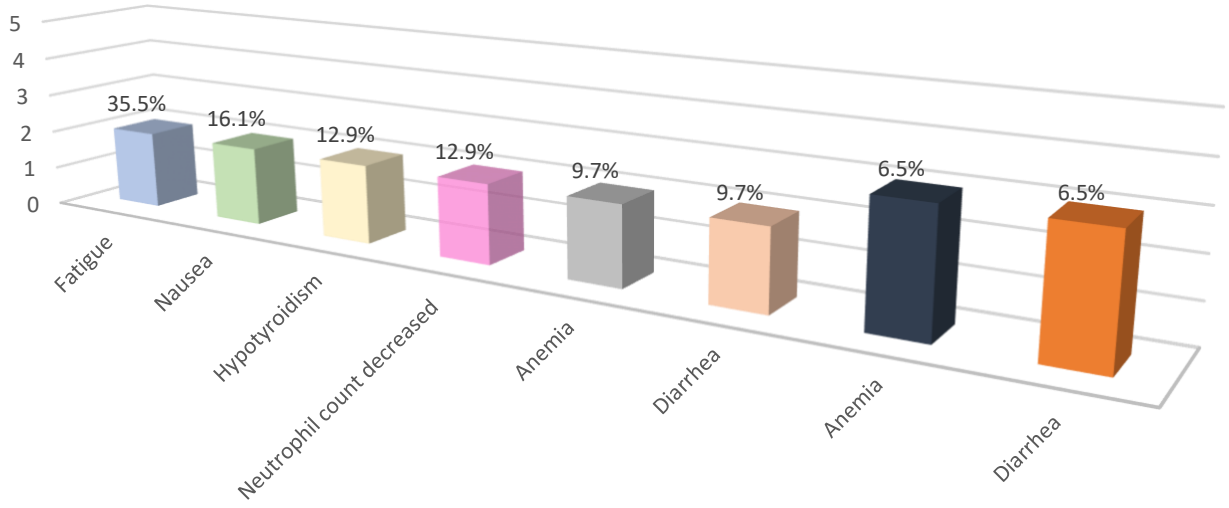

Figure 9. Side effects by frequency and grades, reported in avelumab trial

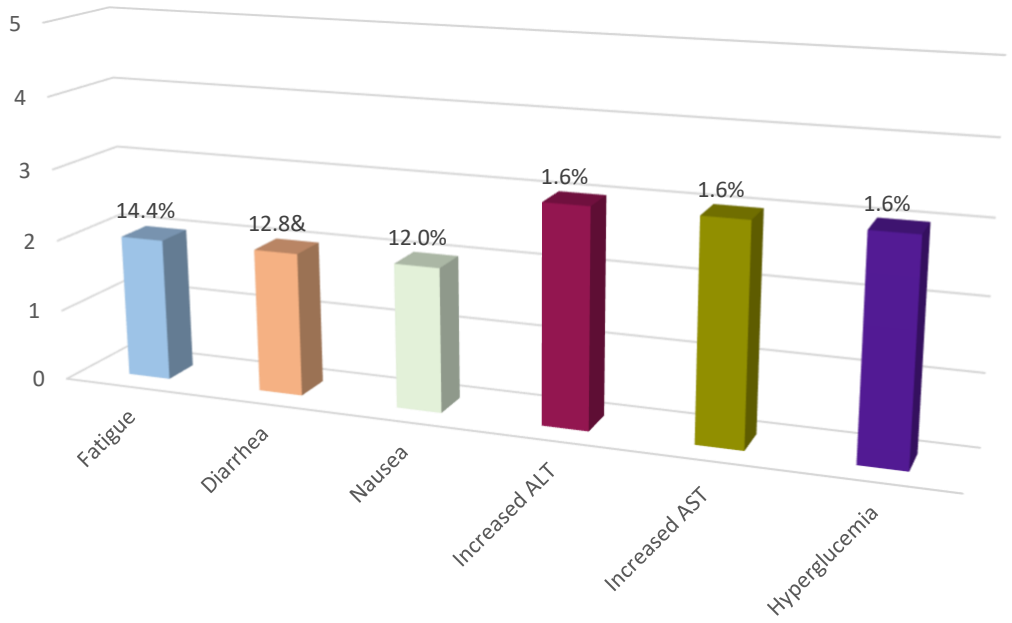

Figure 10. Side effects by frequency and grades, reported in dostarlimab, TSR 042 trial

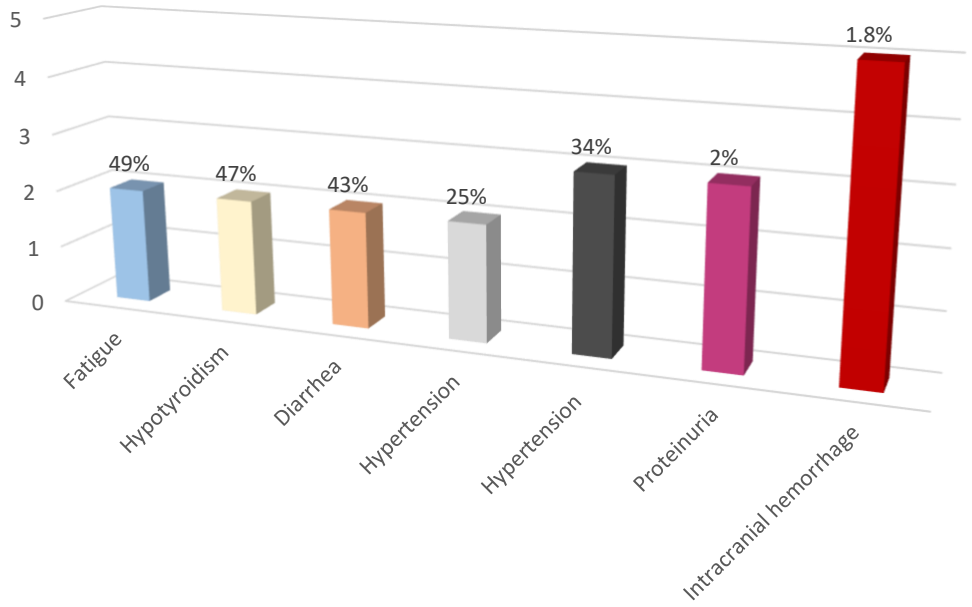

Figure 11. Side effects by frequency and grades, reported Pembrolizumab + Lenvatinib trial

DISCUSSION

Firstly, described in familial Lynch syndrome, mismatch repair deficiency (MMRd), results from germline mutations located in one of four MMR genes (*MLH1*, *MSH2*, *MSH6*, or *PMS2*). Less frequently, it is caused by deletions in the vicinity of the *MSH2* gene on chromosome 2p21, at the epithelial cell adhesion molecule–locus (EpCAM) (32). Since then, other genetic causes resulting in MMRd have been identified, mainly inherited mutations in DNA damage repair (DDR) genes such as the ATM mutation found in one response reporter in the Liu et al. study (22).

In addition to germline mutation, somatic sporadic causes of dMMR/MSI-H tumors have also been recognized, mainly in colorectal cancer (CRC), at approximately a 15% prevalence, while acknowledging its existence at a lower prevalence through many common cancer types, including endometrial cancer (33). In these somatic non-germline cases, MMRd usually results from epigenetic hypermethylation of the MLH1 promoter or, unfrequently due to either somatic structural rearrangements or homozygous somatic mutations in an MMR gene.

As a failure to repair mutations in short repetitive DNA sequences, the so-called microsatellites, Both, genetic or somatic causes of loss of function in a MMR gene give rise to hypermutation and high microsatellite instability (MSI-H) (33). Many patients across the retrieved trial in this systematic review presented MMRd with no associated mutations and plausibly due to somatic mutations.

Mismatch repair pathways are so important identifying and repairing mismatched bases during DNA replication in normal and cancer cells. Defects in DNA mismatch repair proteins and following microsatellite instability-high causes the accumulation of mutation in cancer related genes. It supposes the generation of neoantigens that will stimulate the anti-tumor response of the host (34).

In 2017, following the results of Pembrolizumab efficacy, with an ORR of 39.6%, with 78% of responses lasting over 6 months in refractory MMRd tumors (16). The FDA granted accelerated drug approval to a pembrolizumab for any advanced o recurrent solid tumor regardless of tissue or origin of the tumor, i.e. the first tumor-agnostic treatment authorization, provided they are dMMR/MSI-H tumors, with no satisfactory therapeutic options after first line (35).

Apart from anecdotal cases reports showing activity of Checkpoint inhibitors in advanced/recurrent endometrial carcinoma (36), 11 phase I-II trials having reported preliminary results including some or focusing in MMRd/MSI-H, have being identified across this systematic review, showing meaningful long-term immunotherapy-related responses in dMMR or MSI-H EC.

However, aside from Le and colleagues' trial, focused on MMRd colorectal and non-colorectal tumors, which gave raised to FDA approval, only 5 monotherapy trials were specifically designed to evaluate the activity of immune-checkpoint monotherapy in MMRd tumors, with only three of them with specific endometrial cancer cohorts. In an additional trial, MMRd was assessed as a biomarker, along with PDL-1 and TMB, as a predefined exploratory endpoint. In the remaining two mono-immunotherapy trials MSI status was assessed

retrospectively, either in the 3 responding patients or in 8 out of 23 treated patients regardless of response, plausibly due to the lack of specimen.

Nonetheless, given the similarities of patient populations, a pooled analysis results were performed that showed an ORR of 47.79% (9.73% complete response; 38.05% partial response), a clinical benefit of 58.4 in heavily pretreated patients with no satisfactory treatment options, some of them long lasting. Owing the immaturity of most available results and like of survival data in most of the trial, a pooled analysis of PFS or OS was not feasible.

The finding in Konstantinopoulos trial that all responses and all but one long lasting response were observed in heavily pretreated patients, with three or more therapy lines is challenging and requires validation. A similar trend was observed with pembrolizumab in ovarian cancer (37). The priming effect of neoantigen release, upregulation of PD-L1, activation of type I interference response, to name a few, have been evidenced after chemotherapy, radiotherapy or other targeted therapy (38)(39)(40)(41). Whether type, number, intensity or duration of previous therapies may boost immunotherapy response warrants additional investigation. However, Durvalumab monotherapy trial showed promising activity and safety in EC with MMRd regardless of prior lines of chemotherapy, where accumulative refractory mutations are expected. That said, is also undeniable that patients who live longer because of a lest aggressive tumor time are also more likely to undergo more treatment lines. Hence, timeline of prior therapy matters.

As far as combination therapy trials is concerned, the two combination trials I/II and II trial, respectively, both focusing in endometrial carcinoma, reported preliminary results only included 9 MMRd/MSI-H patients on the whole. In the first trial, pembrolizumab was combined with lenvatinib, a multiple kinase inhibitor, achieving high ORR irrespective MSI status, 47.5% ORR in the overall population, with impressive 75% ORR among the 4 MSI-H patients included. This regimen is being compared to standard of care chemotherapy in an ongoing randomized phase III trial in recurrent EC (ClinicalTrials.gov number, NCT03517449, KEYNOTE 775). Another ongoing phase II trial of bevacizumab and atezolizumab is being conducted in this same population. In the second trial, with checkpoint 1 and 2 combination achieved 40% ORR in the 5 MSI-H as compared with 11% ORR in the overall patient population.

These results provide proof of concept and add further evidence and support to MSI or MMRd status testing in metastatic endometrial carcinoma as well as the use of checkpoint inhibitors as a therapeutic option for patients upon progression after first or later chemotherapy lines, reinforcing the FDA approval for MMRd endometrial carcinoma. This cumulative evidence has led to excitement in medical community and the public. Ultimately, the choice between drugs when treating patients with an MSI-H/MMR advanced endometrial carcinoma in that setting will depend on the patient medical history and prescriptions, the experience of the clinician with the different drugs not to mention financial costa, and availability of the drug.

Yet, results are preliminary, median follow up short in most of them, immature PFS with unreached OS, precluding any evidence-based conclusion on survival. With no randomized control trials having been published to date, comparing immunotherapy to chemotherapy in the first line setting, it is impossible to foreseen whether or not this drug will replace chemotherapy as first line treatment option. Even more, whether certain cases would be

better served with chemotherapy-immunotherapy combination o either one separately will depend on the results of eagerly awaited randomized clinical trials as well as other ongoing trials (Table 5).

On the other hand, It is important to point out that the method used to assess MMRd status, identified MMRd by using an IHC assay in order to detect MMR gene nuclear expression or the one used to evaluate MSI status, by quantifying the presence of microsatellites by polymerase chain reaction (PCR), matters. The combination of both gives the best estimation of the prevalence of MSI-H/MMRd. The low percentage of MSI-H tumors identified in the different endometrial cancer trials illustrates the rarity of a condition that apparently benefits from immunotherapy as well as the need of optimal identification of MMRd status.

As not all MSI-H patients did not respond to immunotherapy, further research into microbiome, host immune microenvironment, other signaling pathways is needed to improve results.

Regarding POLE ultramutated population there is an absolute lack of data due to the rarity of this endometrial carcinoma subgroup. In fact, even though inclusion of POLE mutation EC was preplanned in Konstantinopoulos study, no patients of the 31 included in the trail harbored the mutation.

Table 5. Ongoing phase II/III trials in MSI-H/MMRd tumors

NCTC03914612	First line stage III/IV recurrent endometrial metastatic or recurrent uterine cancer	Phase III carboplatin + paclitaxel +/- Pembrolizumab
NCTC03241745	Metastatic o recurrent uterine cancer	Nivolumab phase II
NCTCN4014530	Metastatic colon/ endometrial (separate arms)	Pembrolizumab + ataluren (small molecule ribosome modulator) phase I/II
NCTC04082572	Locally advanced solid high recurrence risk (> 20%)	
NCT04197219	Pembrolizumab + Axitinib	Phase II
NCT03914612	Pembrolizumab + Carboplatin + Paclitaxel versus Carboplatin + Paclitaxel	Phase III
NCT03603184	Atezolizumab + Carboplatin + Paclitaxel versus Carboplatin + Paclitaxel	Phase III
NCT03572478	Nivolumab + Rucaparib	Phase I-II
NCT03526432	Atezolizumab + Bevazizumab	Phase II
NCT03503786	Avelumab + Carboplatin + Paclitaxel	Phase II
NCT03277482	Durvalumab + Tremelimumab	Phase I
NCT03015129	Durvalumab + Tremelimumab	Phase II
NCT02912572	Avelumab + Talazoparib	Phase II
NTC02549209	Pembrolizumab + Carboplatin + Paclitaxel	Phase II

As far as adverse events are concerned, they were in line with those reported previously with immunotherapy, with gastrointestinal tract, skin, endocrine glands and liver are most commonly involved (30). The grade 5 intracranial hemorrhage reported with pembrolizumab-lenvatinib combinations is plausibly attributable to lenvatinib due the common vascular side effects with this drug.

CONCLUSION

Immunotherapy for MSI-H/dMMR tumor-agnostic treatment is still in its infancy. Even though a consistent objective response rate has been shown through different trials, none of the retrieved RCTs have reported mature results in OS or PFS.

Nonetheless, in absence of therapeutic options upon progression after first line in POLE/MSI-H/dMMR advanced/recurrent endometrial carcinoma patients, immunotherapy appears to be an attractive option on an individualized decision-making basis, for those with good performance status, with the intent to obtain responses to palliate the disease, acknowledging the debatable fact whether outcomes such as treatment response and/or quality of life could serve as treatment rational while awaiting ongoing trial mature results on overall survival and PFS.

Whether or not immunotherapy is expected to replace chemotherapy in the first line setting will depend on results on an ongoing phase III trial (clinicaltrial.gov, number NCT03914612).

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APPENDIX

Prospero like protocol according to PRISMA guidelines

ADMINISTRATIVE INFORMATION

Tittle	Systematic review on immunotherapy for POLE ultramutated and MSI-H advanced endometrial carcinoma.
Registration	The protocol of this systematic review will not be recorded.
Authors	Héctor Villa Martínez supervised by the cotutor, María José Villanueva Silva.

INTRODUCCTION

Rationale	Recent advances in molecular biology have revealed a wide range of genomic alterations in endometrial cancers. The Cancer Genome Atlas classification delineated four subgroups of EC: microsatellite instability high (MSI-H), DNA polymerase epsilon (POLE), copy number high and copy number low. Hotspot mutation of the catalytic unit DNA polymerase epsilon (POLE) display and ultramutated sequence that is recognizable to the immune system and confers a good prognosis. MSI-H tumors are hypermutated because of their inability to repair replicational mutations, which make these tumors immunologically active to treatment with immunotherapy.
Objectives	To perform a systematic review to summarize all available evidence with checkpoint inhibitors in highly and ultramutated endometrial cancer subtypes to asses efficacy and tolerability.

METHODS

Eligibility criteria	Inclusion criteria will be phase I trial and phase II trial in both subtypes (POLE and MSI-H) between January 2012 and March 2020, in advanced/recurrent endometrial carcinoma. There will be no restrictions on the language used in the publications.
Information sources	An electronically search will be performed in PubMed, Embase, Clinicaltrials.gov, Web of Science for articles as well as ASCO and ESMO meeting database for abstracts.
Search strategy	Search strategy will include a combination of broad terms related to endometrial carcinoma and immunotherapy, checkpoint inhibitors, POLE, MSI-H, clinical trial, PD1, PD-L1, Pembrolizumab, Nivolumab, Atezolizumab, Avelumab, Durvalumab.
Exclusion criteria	Exclusion criteria will be: (i) no advanced endometria carcinoma, (ii) clinical trials with no POLE or MSI-H subtype tumors reported, (iii) studies that matched different databases, (iv) completed trials with no published results, (v) ongoing clinical trials with no published results (vi) phase III trials, (vii)

	case reports, narrative reviews, editorials, news articles, commentaries or letters.
DATA	
Data items	For each included study we will extract the following data: number of patients included, design, type of trial, age, stage, performance status, simple size, treatment type, type or immunotherapy, other drugs if applied, primary outcome, secondary outcomes, median follow-up, overall response rate (ORR), including partial response (PR) and complete response (CR), progression free survival (PFS), overall survival (OS), duration of response (DOR), time to response (TTR) and toxicity.